

Sesamoids in Anurans: New Data, Old Issues

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ABSTRACT

Sesamoids are skeletal elements rarely considered in studies of the vertebrate skeleton. In this work, we integrate ontogenetic data of anuran sesamoids in two species (*Leptodactylus latinasus* and *Pleurodema* cf. *guayapae*), the related structures (tendons, muscles, and joints) in *L. latinasus*, and a survey of sesamoid distribution in 185 anuran taxa. Our main goals are: (1) to contribute to the knowledge of the comparative anatomy of sesamoids in tetrapods; (2) to provide additional developmental evidence to interpret the ontogenetic pattern of sesamoids in anurans, as a key to elucidate that of tetrapods in general; (3) to provide data about tendon development in relation to sesamoid development in anurans for the first time; and (4) to propose a pattern of anuran sesamoid distribution. The homologies of sesamoids across tetrapods are discussed here. Observations were made in cleared and stained skeletal whole-mounts. Fifty-four sesamoids were found in anurans, thirty-seven of which occur in *L. latinasus*. The traditional point of view of embedded sesamoids always resulting from biomechanical stimuli of a previously existing tendon is not sustained by our data. Many sesamoids arise before the differentiation of a tendinous tissue. Our survey results in a data set where the two big anuran clades, Hyloides (12 families) and Ranoides (14 families), were represented. The matrix has 38% missing entries. Most of the surveyed sesamoids have multiple origins, with only three of them (about 19%) having one origin. *Anat Rec*, 293:1646–1668, 2010. © 2010 Wiley-Liss, Inc.

Key words: anuran anatomy; ontogeny; histology; phylogeny; homologies

Sesamoids are skeletal elements usually ignored in studies of the vertebrate skeleton. They have often been considered extra skeletal structures, possibly because their origins and causes of existence are still under debate. Sesamoids were recently 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 and Olson, 2007). There are some articles focusing on sesamoid occurrence, development, and/or distribution (Pearson and Davin, 1921a,b; Haines, 1942, 1969; Hudson et al., 1965; Nussbaum, 1982; Le Minor, 1987; Vanden Berge and Storer, 1995; Olson, 2000; Maisano, 2002a,b,c; Hoyos, 2003; Vickaryous and

Olson, 2007; Kim et al., 2009; Jerez et al., in press) but in general, they are absent from skeleton descriptions.

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One of the most interesting things about sesamoids is the pervasive idea that their genesis is related mainly to extrinsic factors, because they seem to represent a response to the intense mechanical stresses involved in the angulated part of tendons (e.g., Le Minor, 1987; Giori et al., 1993; Carter et al., 1998; Sarin et al., 1999). In 1921, Pearson and Davin criticized the idea that sesamoids arise in response to mechanical stress as a “dogma” of medical anatomy (Pearson and Davin, 1921a; p. 136). They opposed it as they considered that this dogma only gave the sesamoid a “use value:” “ligaments of the articulations, or the firm tendons of strong muscles, or both, become bony by the violent compression they suffer in the situation they are” (Monro, 1726 in Pearson and Davin, 1921a, p. 137). More than 70 years later, most explanations about the origin of these structures remain rather unchanged (Sarin et al., 1999), although a new trend prefers to consider the sesamoid category a very artificial one, including all kinds of small and unusual skeletal elements (Vickaryous and Olson, 2007; Jerez et al., in press). In effect, the main point is the dichotomy, probably also artificial, between genetic and epigenetic factors, an issue that has been under discussion since the first extensive articles on sesamoids appeared (e.g., Pfitzner, 1892, 1896 in Pearson and Davin, 1921a). In fact, it has been demonstrated that most sesamoids can also develop without the intervention of extrinsic factors (Vickaryous and Olson, 2007). One of the strongest assertions in relation to sesamoid morphogenesis is that of Doherty (2007, p. 7): “sesamoids, like other bones of the skeleton, are genetically inherited and are found in most extinct and extant vertebrates, although in variant arrangements/numbers.” Despite the strength of this assertion, the actual stimulus eliciting sesamoid formation is still under discussion.

The development and evolutionary significance of sesamoids is usually disregarded when considering the appendicular skeleton. Sesamoids have been described in several tetrapod species, and they have been described as very common in reptiles and mammals (Hall, 2005; Jerez et al., in press). In anurans, specific data about their general aspect and development are scarce (Nussbaum, 1982; Olson, 2000; Hoyos, 2003; Hall, 2005; Vickaryous and Olson, 2007; Kim et al., 2009), and they are mainly mentioned in articles dealing with other anatomical issues (Laurent, 1941, 1942, 1961; de Sá and Trueb, 1991; Guayasamin, 2004; Avilan and Hoyos, 2006; Fabrezi, 2006; Lehr and Trueb, 2007; Maglia et al., 2007). A general interpretation of the origin, ontogeny, and distribution of these structures in anurans is still lacking.

In this work, we integrate ontogenetic data of anuran sesamoids in two species (*Leptodactylus latinasus* and *Pleurodema* cf. *guayapae*), the related structures (tendons, muscles, joints) in *L. latinasus*, and a survey of sesamoid distribution in 185 anuran taxa based on both our dissections and literature data. Our main goals are: (1) to contribute to the knowledge of the comparative anatomy of sesamoid in anurans; (2) to provide additional developmental evidence to interpret the ontogenetic pattern of sesamoids in anurans and, in this way, make a generalization to understand the ontogenetic pattern of tetrapods; (3) to provide data about tendon development in relation to sesamoid development in anurans for the first time; and (4) to propose a pattern of anuran

sesamoid distribution. The homologies of some sesamoids in the whole tetrapod clade are discussed here; this work analyzes the old issues related to the origin and evolution of tetrapod sesamoids.

MATERIALS AND METHODS

Observations were made in cleared and stained skeletal whole-mounts prepared following the protocol of Wassersug (1976). All observations and illustrations were made with a stereo dissection microscope Carl Zeiss Discovery V8.

Sesamoids were examined in the appendicular skeleton of 92 adult specimens—all cleared and stained—of *Centrolene robledoi* (DIAM 315), *Cochranella griffitsi* (DIAM 319), *Chacophrys pierotti* [FML 1019 (three specimens), 9012], *Gastrotheca gracilis* (FML 02209), *Hyalinobatrachium aureoguttatum* (DIAM 318), *Hypsiboas andinus* (FML 3812), *Hypsiboas cordobae* (FML 8851–8859), *Leptodactylus laevis* (FML 8928), *Leptodactylus chaquensis* (FML12097, 12098, 12100, 12101), *Leptodactylus bufonius* [FML 4410, 672 (five specimens), 3568 (three specimens), 4908 (five specimens), 9782] *L. latinasus* (FML 11912, 1429, 6284, 3539, 8583, 2410/2, 3, 5, 7, 9, 10; L 537, 665, 690, 700, 420, 599, 643), *Leptodactylus podicipinus* [FML 3577 (eight specimens); 4312 (three specimens), 760 (10 specimens)], *Oreobates discoidalis* [FML 462, 2120 (five specimens), 4405 (two specimens)], *Phyllomedusa azurea* (FML 4286), *Phyllomedusa sauvagii* (FML 3822, 3823) *Pleurodema borellii* (FML 2994, 4404), and *Rhinella granulosa* (FML 1052, 1060, 4408)]. On the basis of both this survey and on the literature, the possible maximum number of sesamoids in anurans was inferred. The muscular association of sesamoids was determined by examining incompletely cleared specimens of *L. latinasus* (L 677, 678, 740, 741, 762, 781), *Leptodactylus chaquensis* (L 738, 655), *Pleurodema borellii* (FML 3280), and *Physalaemus biligonigerus* (L 247, 248). We adopted the terminology and classification of muscles proposed by Ecker (1889), Duellman and Trueb (1994), Manzano (1996), and Manzano and Barg (2005). FML: Fundación Miguel Lillo collection. MACN: Museo Argentino de Ciencias Naturales collection. L: private collection of María Laura Ponssa. DIAM: CcyTTP- CONICET collection, Diamante, Entre Ríos, Argentina.

Sesamoid data of adult specimens of species not dissected for the present work were obtained from the literature (Laurent, 1941, 1942, 1961; de Sá and Trueb, 1991; Wild, 1997; Olson, 2000; Trueb et al., 2000; Fabrezi, 2001, 2006; Hoyos, 2003; Guayasamin, 2004; Avilan and Hoyos, 2006; Lehr and Trueb, 2007; Maglia et al., 2007) (Table 1). Because heterotopic elements are often overlooked or dismissed in morphological studies, the results presented in Table 1 must be interpreted with caution.

The ontogenetic pattern of sesamoids was analyzed in a growth series of *L. latinasus* (Leptodactylidae) and *Pleurodema* cf. *guayapae* (Leiuperidae). These species were selected because of the availability of densely sampled developmental series. Tadpoles were staged according to the developmental table of Gosner (1960). Postmetamorphic stages were selected on the basis of body size. Because both dissected species showed rather similar ontogenetic patterns, descriptions (see Results)

TABLE 1. Distribution of the sesamoids based on this study and the literature (details in Materials and Methods) (continued)

Sesamoids	%	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	
Sesamoid of the tendon of the m. gracilis major (graciella)	3	?	?	-	-	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
Subarticular carpus	2	?	?	?	?	?	?	X	-	-	?	?	?	?	?	?	?	?	?	?	?	
Glide sesamoids: Ventral to the distal head of proximal-phalange of digit IV and V feet	1	-	-	-	-	?	-	?	-	?	?	?	?	?	?	?	?	-	?	?	?	
Glide sesamoid: between phalange medial and distal digit IV feet	1	-	-	-	-	?	-	?	-	-	?	?	?	?	?	?	?	-	?	?	?	
Distal level of humerus	1	?	?	?	?	?	?	?	-	-	?	?	?	?	?	?	?	?	?	?	?	
Posterior lunula	1	?	?	?	?	?	?	?	-	-	?	?	?	?	?	?	?	?	?	?	?	
Glide sesamoids: Dorsal level of phalanges	0.5	?	?	?	?	?	?	?	-	?	?	?	?	?	?	?	?	?	?	?	?	
Os tibialis anticus	0.5	?	?	?	?	?	?	?	-	-	?	?	?	?	?	?	?	?	?	?	?	
Os sesamoide tarsal proximal	0.5	-	-	-	-	-	-	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
Patella ulnaris	0.5	-	-	-	-	-	-	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
Sesamoids	%	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61
Cartilago sesamoides	68	X	X	X	X	-	X	X	X-	?	-	-	X	X	X	X	-	-	X	-	-	X
Fabella	20	?	?	?	?	-	-	-	-	?	?	?	?	?	?	?	?	X	?	?	?	?
Os sesamoides tarsal	13	-	-	-	-	?	-	X-	?	-	-	-	-	-	-	-	-	-	-	-	-	-
Ventral to the distal head of metatarsals I-V	12	X	?	?	?	?	?	-	?	-	-	?	?	?	?	-	-	-	?	-	-	?
Glide sesamoids: S. ventral to the distal head of metacarpals II-V	10	-	?	?	?	-	?	-	-	-	-	?	?	?	-	-	-	-	?	-	-	?
Plantar	10	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
In the lateral surface of the lateral margin of each sacral diapophysis	10	?	?	?	?	?	?	?	X(2)	?	?	?	?	?	?	?	?	?	?	?	?	?
Dorsal to the articulation radius-ulna with radiale (pararadial)	9	?	?	?	?	?	?	?	X-	?	?	?	?	?	?	?	?	?	?	?	?	?
Palmar	8	?	?	?	?	?	?	-	X-	?	?	?	?	?	?	?	?	-	?	?	?	?
Glide sesamoids: S. ventral to the distal head of proximal phalange of digit IV and V manus	7	-	?	?	?	?	?	-	-	-	-	?	?	?	?	?	-	-	?	-	-	?
Sesamoid of the tendon of the m. gracilis major (graciella)	3	?	?	?	?	?	?	-	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Subarticular carpus	2	?	?	?	?	?	?	?	X	?	?	?	?	?	?	?	?	-	?	?	?	?
Glide sesamoids: Ventral to the distal head of proximal-phalange of digit IV and V feet	1	?	?	?	?	?	?	-	?	-	-	?	?	?	?	-	-	-	?	-	-	?
Glide sesamoid: between phalange medial and distal digit IV	1	?	?	?	?	?	?	-	?	-	-	?	?	?	?	-	-	-	?	-	-	?
Distal level of humerus	1	?	?	?	?	?	?	-	?	?	?	?	?	?	?	?	?	-	?	?	?	?

TABLE 1. Distribution of the sesamoids based on this study and the literature (details in Materials and Methods) (continued)

Sesamoids	%	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61
Posterior lunula	1	?	?	?	?	?	?	-	?	?	?	?	?	?	?	?	?	-	?	?	?	?
Glide sesamoids: Dorsal level of phalanges	0.5	?	?	?	?	?	?	?	-	?	?	?	?	?	?	?	?	?	?	?	?	?
Os tibialis anticus	0.5	?	?	?	?	?	?	-	?	?	?	?	?	?	?	?	?	-	?	?	?	?
Os sesamoide tarsal proximal	0.5	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Patella ulnaris	0.5	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Sesamoids	%	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82
Cartilago sesamoides	68	X	X	X	?	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Fabella	20	?	?	?	?	-	-	-	-	-	-	-	X	X	X	X	-	-	-	-	-	-
Os sesamoides tarsal	13	-	-	-	?	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-
Glide sesamoids: ventral to the distal head of Metatarsals I-V	12	?	?	?	?	-	-	-	X ^c	-	-	-	X ^c	-	-	-	-	-	-	-	X ^c	-
Glide sesamoids: ventral to the distal head of Metacarpals II-V	10	?	?	?	?	-	X	-	?	-	-	-	-	-	-	-	-	-	-	-	-	-
Plantar	10	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
In the lateral surface of the lateral margin of each sacral diapophysis	10	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Dorsal to the articulation radius-ulna with radiale (pararadial)	9	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Palmar	8	?	?	?	?	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-
Glide sesamoids: S. ventral to the distal head of proximal phalange of digit IV and V manus	7	?	?	?	?	-	X	-	X	-	-	-	X	-	-	-	-	-	-	-	-	-
Sesamoid of the tendon of the m. gracilis major (graciella)	3	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Subarticular carpus	2	?	?	?	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glide sesamoids: Ventral to the distal head of proximal –phalange of digit IV and V feet	1	?	?	?	?	-	-	-	-	-	-	-	?	-	-	-	-	-	-	-	-	-
Glide sesamoid: between phalange medial and distal digit IV feet	1	?	?	?	?	-	-	-	-	-	-	-	?	-	-	-	-	-	-	-	-	-
Distal level of humerus	1	?	?	?	?	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-
Posterior lunula	1	?	?	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glide sesamoids: Dorsal level of phalanges	0.5	?	?	?	?	?	?	?	?	?	?	?	X	?	?	?	?	?	?	?	?	?
Os tibialis anticus	0.5	?	?	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Os sesamoide tarsal proximal	0.5	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Patella ulnaris	0.5	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Sesamoids	%	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103
Cartilago sesamoides	68	X	X	X	X	X	-	X	X	X	-	X	X	X	X	-	-	X	-	-	-	?
Fabella	20	?	-	?	?	?	?	?	?	?	?	?	?	-	?	?	X	-	?	X	?	?
Os sesamoides tarsal	13	-	-	-	-	-	-	-	?	-	-	-	-	-	-	-	-	-	-	-	-	?
Glide sesamoids: ventral to the distal head of Metatarsals I-V	12	-	-	-	?	?	?	?	?	?	?	?	-	X	-	-	-	-	-	-	-	?
Glide sesamoids: ventral to the distal head of Metacarpals II-V	10	?	-	?	?	X	?	?	?	?	X	X	?	?	X	-	-	-	-	-	-	?

TABLE 1. Distribution of the sesamoids based on this study and the literature (details in Materials and Methods) (continued)

Sesamoids	%	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185
Glide sesamoids: ventral to the distal head of Metacarpals II-V	10	-	-	-	-	-	-	-	?	?	-	-	?	?	?	?	?	?	?	?	-
Plantar	10	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	X(1)	X(1)	X(3)	?	-
In the lateral surface of the lateral margin of each sacral diapophysis	10	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	X	X	X	X	X
Dorsal to the articulation radius-ulna with radiale (pararadial)	9	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	-
Palmar	8	-	-	-	-	-	-	?	?	?	?	?	?	?	?	?	?	?	?	?	-
Glide sesamoids: S. ventral to the distal head of proximal phalange of digit IV and V manus	7	-	-	-	-	-	-	?	?	?	-	-	?	?	?	?	?	?	?	?	-
Sesamoid of the tendon of the m. gracilis major (graciella)	3	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Subarticular carpus	2	-	-	-	-	-	-	?	?	?	-	-	?	?	?	?	?	?	?	?	?
Glide sesamoids: Ventral to the distal head of proximal phalange of digit IV and V feet	1	-	-	-	-	-	-	-	?	?	-	-	?	?	?	?	?	?	?	?	-
Glide sesamoid: between phalange medial and distal digit IV feet	1	-	-	-	-	-	-	-	?	?	-	-	?	?	?	?	?	?	?	?	-
Distal level of humerus	1	-	-	-	-	-	-	?	?	?	?	?	?	?	?	?	?	?	?	?	-
Posterior lunula	1	-	-	-	-	-	-	?	?	?	?	?	?	?	?	?	?	?	?	?	-
Glide sesamoids: Dorsal level of phalanges	0.5	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	-
Os tibialis anticus	0.5	-	-	-	-	-	-	?	?	?	?	?	?	?	?	?	?	?	?	?	-
Os sesamoide tarsal proximal	0.5	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Patella ulnaris	0.5	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?

X: presence; -: absence; ?: missing data; (n) number of sesamoids when there are more than one.

^aTwo separate sesamoids.

^bFabrezi (2005) specifically refers to the presence/absence of sesamoid in the finger IV, she does not mention in which segments.

^cHoyos (2003) and Fabrezi (2006) do not specify the segments or finger.

Leptodactylidae: 1-*Leptodactylus latinus*, 2-*Leptodactylus chaquensis*, 3-*Leptodactylus podicipinus*; 4-*Leptodactylus podicipinus*; 5-*Pleurodema borellii*, 6-*Pleurodema bufoninum*, 7-*Physalaemus pustulosus*, 8-*Physalaemus biligonigerus*; 9-*Pseudopaludicola boliviana*; **Hyliidae:** 10-*Phyllomedusa azurea*, 11-*Phyllomedusa sauvaigi*, 12-*Hypsiboas andinus*, 13-*Hypsiboas cordobae*, 14-*Dendropsophus nanus* (*Hyla nana*), 15-*Isthmohyla rivularis* (*Hyla rivularis*), 16-*Pseudis platensis* (*Pseudis paradoxus*), 17-*Hylomantis lemur* (*Phyllomedusa lemur*), 18-*Scinax fuscovarius*, 19-*Aeris crepitans*; **Ceratophryidae:** 20-*Chacophrys pierotti*, 21-*Lepidobatrachus laevis*, 22-*Lepidobatrachus llanensis*, 23-*Telmatobius contrerasi*, 24-*Telmatobius oxycephalus*, 25-*Ceratophrys cornuta*, 26-*Ceratophrys pierotti welli*; **Arthroleptidae:** 27-*Arthrolepis wahlbergi* (Laurent, 1961); 28-*Arthrolepis poeciloneotus* (Hoyos, 2003), 29-*Astysternus occidentalis* (Hoyos, 2003), 30-*Leptopelis christyi* (Hoyos, 2003), 31-*Arthrolepis adolfi-friederici* (Fabrezi, 2006), 32-*Arthrolepis poeciloneotus* (Fabrezi, 2006), 33-*Arthrolepis variabilis* (Fabrezi, 2006), 34-*Astysternus diadematus* (Fabrezi, 2006), 35-*Cardioglossa cyaneospila* (Fabrezi, 2006), 36-*Cardioglossa leucomystax* (Fabrezi, 2006), 37-*Leptopelis christyi* (Fabrezi, 2006), 38-*Arthrolepis pyrrosocelis* (*Schoutedenella pyrrosocelis*) (Fabrezi, 2006), 39-*Arthrolepis sylvaticus* (*Schoutedenella sylvatica*) (Fabrezi, 2006), 40-*Arthrolepis schubotzi* (*Schoutedenella schubotzi*) (Fabrezi, 2006), 41-*Arthrolepis lameerei* (*Schoutedenella lameerei*) (Fabrezi, 2006), 42-*Arthrolepis stenodactylus* (Nussbaum, 1982), 43-*Arthrolepis tuberosus* (Nussbaum, 1982), 44-*Arthrolepis wahlbergi* (*Arthrolepis wageri*) (Nussbaum, 1982); **Bombinatoridae:** 45-*Bombina variegata* (Fabrezi, 2006); **Brachycephalidae:** 46-*Brachycephalus ephippium* (Nussbaum, 1982), **Brevicipitidae:** 47-*Breviceps mossambicus* (Hoyos, 2003); **Bufonidae:** 48-*Rhinella granulosa*, 49-*Atelopus varius* (Laurent, 1961), 50-*Inciilius nebulifer* (Fabrezi, 2006), 51-*Nannophryne variegata*

(*Bufo variegatus*) (Fabrezi, 2006), 52-*Anaxyrus debilis* (*Bufo debilis*) (Nussbaum, 1982), 53-*Anaxyrus quercicus* (*Bufo quercicus*) (Nussbaum, 1982), 54-*Melanophryniscus stelzneri* (Nussbaum, 1982); 55-*Melanophryniscus rubriventris* (Fabrezi, 2006); **Centrolenidae**: 56-*Allophryne rufiventris* (Fabrezi, 2006); **Centrolenidae**: 56-*Allophryne rufiventris* (Fabrezi, 2006); **Ceratobatrachidae**: 57-*Platymantis vittensis* (Hoyos, 2003); 58-*Craugastor decoratus* (*Eleutherodactylus decoratus*) (Nussbaum, 1982); **Cycloramphidae**: 59-*Odontophrynus americanus* (Fabrezi, 2006); 60-*Rhinoderma darwini* (Fabrezi, 2006); **Dendrobatidae**: 61-*Hyaloxalus subpunctatus* (*Colostethus subpunctatus*) (Nussbaum, 1982); 62-*Hyaloxalus vertebralis* (*Colostethus vertebralis*) (Nussbaum, 1982); 63-*Phyllotates lugubris* (Nussbaum, 1982); 64-*Oophaga pumilio* (*Dendrobates pumilio*) (Nussbaum, 1982); **Dicroglossidae**: 66-*Euphylyctis cyanophlyctis* (Hoyos, 2003), 67-*Hoplobatrachus occipitalis* (*Dendrobates trivittatus*) (Laurent, 1961); **Dicroglossidae**: 66-*Euphylyctis cyanophlyctis* (Hoyos, 2003), 67-*Hoplobatrachus occipitalis* (Nussbaum, 1982); 68-*Hoplobatrachus rugulosus* (Hoyos, 2003), 69-*Ingerana tenasserimensis* (Hoyos, 2003), 70-*Limnonectes blythii* (Hoyos, 2003), 71-*Limnonectes kuhlii* (Hoyos, 2003), 72-*Limnonectes gyldestolpei* (*Limnonectes trivittatus*) (Hoyos, 2003), 73-*Fejervarya limnocharis* (*Limnonectes limnocharis*) (Hoyos, 2003), 74-*Nanorana parkeri* (Hoyos, 2003), 75-*Occidozyga lima* (Hoyos, 2003), 76-*Nanorana blanfordii* (Hoyos, 2003), 77-*Nanorana vicina* (*Paa vicina*) (Hoyos, 2003), 78-*Occidozyga laevis* (*Phrynoglossus laevis*) (Hoyos, 2003), 79-*Occidozyga magnapustulosa* (*Phrynoglossus magnapustulosa*) (Hoyos, 2003), 80-*Occidozyga martensii* (*Phrynoglossus martensii*) (Hoyos, 2003), 81-*Limnonectes hascheanus* (*Taylorana hascheana*) (Hoyos, 2003), 82-*Sphaerothera breviceps* (*Tomopterna breviceps*) (Hoyos, 2003), 83-*Hoplobatrachus occipitalis* (Fabrezi, 2006); **Eleutherodactylidae**: 84-*Eleutherodactylus martinicensis* (Hoyos, 2003); 85-*Eleutherodactylus marnockii* (*Syrnhophus marnockii*) (Nussbaum, 1982); 86-*Eleutherodactylus longipes* (*Syrnhophus longipes*) (Nussbaum, 1982), 87-*Eleutherodactylus* sp. (*Tomodactylus* sp.) (Nussbaum, 1982); **Hemiphractidae**: 88-*Flectonotus fitzgeraldi* (Fabrezi, 2006); **Hemisotidae**: 89-*Hemisus marmoratus* (Nussbaum, 1982); **Hyperoliidae**: 90-*Arixalus fornasini* (Nussbaum, 1982), 91-*Tachycnemis seychellensis* (*Megalixalus seychellensis*) (Nussbaum, 1982), 92-*Arixalus fulvovittatus* (Fabrezi, 2006), 93-*Hyperolius castaneus* (Fabrezi, 2006), 94-*Hyperolius kivuensis* (Fabrezi, 2006), 95-*Kassina senegalensis* (Fabrezi, 2006), 96-*Phlyctimantis verrucosus* (Fabrezi, 2006), 97-*Optisthoxyla immaculatus* (Fabrezi, 2006); **Mantelliidae**: 98-*Boophis brachyichir* (Hoyos, 2003), 99-*Mantella madagascariensis* (Hoyos, 2003); **Microhylidae**: 100-*Anodonthyla boulengerii* (Hoyos, 2003), 101-*Kaloula pulchra* (Hoyos, 2003), 102-*Phrynomantis bifasciatus* (Fabrezi, 2006), 103-*Hamptophryne boliviana* (de Sa and Trueb, 1991), 104-*Choerophryne rostetteri* (Nussbaum, 1982), 105-*Melanophryne barbatula* (Lehr and Trueb, 2007); **Myobatrachidae**: 106-*Pseudophryne guentheri* (Nussbaum, 1982); **Petropedetidae**: 107-*Conraua crassipes* (Hoyos, 2003; Fabrezi, 2006), 108-*Conraua alleni* (Hoyos, 2003); **Phrynobatrachidae**: 109-*Phrynobatrachus versicolor* (Fabrezi, 2006), 110-*Phrynobatrachus acutirostris* (Fabrezi, 2006), 111-*Phrynobatrachus petropedetoides* (Fabrezi, 2006), 112-*Phrynobatrachus acraensis* (Hoyos, 2003); 113-*Phrynobatrachus sulfureogularis* (Fabrezi, 2006), 114-*Phrynobatrachus latifrons* (*Phrynobatrachus acraensis*) (Hoyos, 2003); 115-*Phrynobatrachus batesii* (*Phrynobatrachus batesii*) (Nussbaum, 1982), 116-*Phrynobatrachus dendrobates* (Nussbaum, 1982), 117-*Phrynobatrachus gutturosus* (Nussbaum, 1982), 118-*Phrynobatrachus keniensis* (Nussbaum, 1982), 119-*Phrynobatrachus plicatus* (Nussbaum, 1982); **Pipidae**: 120-*Hymenochirus boettgeri* (Olson, 2000; Fabrezi, 2006), 121-*Xenopus laevis* (Fabrezi, 2006), 122-*Pipa pipa* (Trueb, 2000), 123-*Xenopus victorinus* (*Xenopus laevis victorinus*) (Nussbaum, 1982), 124-*Pipa carvalhoi* (Nussbaum, 1982), 125-*Pipa aspera* (Nussbaum, 1982), 126-*Pipa snethlageae* (Nussbaum, 1982), 127-*Pipa parva* (Nussbaum, 1982); **Ptychadenidae**: 128-*Ptychadena schillukorum* (*Ptychadena floweri*) (Hoyos, 2003), 129-*Ptychadena mascareniensis* (Hoyos, 2003), 130-*Ptychadena pujoli* (Hoyos, 2003), 131-*Ptychadena superiliaris* (Hoyos, 2003), 132-*Ptychadena tournieri* (Hoyos, 2003), 133-*Ptychadena uzunguensis* (Fabrezi, 2006), 134-*Ptychadena mascareniensis* (Hoyos, 2003), 138-*Ptychocephalus adspersus* (Hoyos, 2003), 139-*Amietia angolensis* (*Rana angolensis*) (Fabrezi, 2006), 140-*Tomopterna marmorata* (Hoyos, 2006), 141-*Aubria subsigillata* (Hoyos, 2003), 142-*Ptychocephalus adspersus* (Fabrezi, 2006), 143-*Amietia angolensis* (*Afrana angolensis*) (Fabrezi, 2006), 144-*Cacosternum boettgeri* (Nussbaum, 1982), 145-*Cacosternum capense* (Nussbaum, 1982), 146-*Natalobatrachus bonebergi* (*Arthroleptella hewitti*) (Nussbaum, 1982), 147-*Arthroleptella lightfooti* (Nussbaum, 1982), 148-*Cacosternum namaquense* (Nussbaum, 1982), 149-*Anhydrophryne hewitti* (Hoyos, 2003); **Ranidae**: 155-*Anolops monticola* (Hoyos, 2003), 156-*Hylarana albolabris* (*Rana albolabris*) (Hoyos, 2003), 157-*Rana arvalis* (Hoyos, 2003), 158-*Babina chapaensis* (*Rana chapaensis*) (Hoyos, 2003), 159-*Lithobates clamitans* (*Rana clamitans*) (Hoyos, 2003), 160-*Rana dalmatina* (Hoyos, 2003), 161-*Hylarana erythraea* (*Rana erythraea*) (Hoyos, 2003), 162-*Pelophylax ridibundus* (*Rana esculenta*) (Gaupp, 1896; Hoyos, 2003), 163-*Hylarana galamensis* (*Rana galamensis*) (Hoyos, 2003), 164-*Rana graeca* (Hoyos, 2003), 165-*Rana iberica* (Hoyos, 2003), 166-*Pelophylax lessonae* (*Rana lessonae*) (Hoyos, 2003), 167-*Hylarana nigrovittata* (*Rana nigrovittata*) (Hoyos, 2003), 168-*Pelophylax perezi* (*Rana perezi*) (Hoyos, 2003), 169-*Lithobates* (*Rana pipiens*) (Hoyos, 2003), 170-*Hylarana taipehensis* (*Rana taipehensis*) (Hoyos, 2003), 171-*Rana temporaria* (Hoyos, 2003), 172-*Hylarana albolabris* (*Amirana albolabris*) (Fabrezi, 2006), 173-*Rana boylii* (*Rana boylii*) (Nussbaum, 1982), 174-*Lithobates clamitans* (*Rana clamitans*) (Nussbaum, 1982), **Scaphiropodidae**: 175-*Spea bombifrons* (Fabrezi, 2006), 176-*Scaphiopus couchii* (Fabrezi, 2006); **Sooglossidae**: 177-*Sooglossus thomasseti* (*Nesomantis thomasseti*) (Nussbaum, 1982), 178-*Sooglossus sechellensis* (Nussbaum, 1982), 179-*Sechellophryne gardineri* (*Sooglossus gardineri*) (Nussbaum, 1982); **Strabomantidae**: 180-*Oreobates discoidalis*; 181-*Pristimantis bogotensis* (*Eleutherodactylus bogotensis*) (Avilan and Hoyos, 2006), 182-*Pristimantis huicundo* (*Eleutherodactylus huicundo*) (Guayasamin, 2004), 183-*Pristimantis ortizi* (*Eleutherodactylus ortizi*) (Guayasamin, 2004), 184-*Pristimantis racemus* (*Eleutherodactylus racemus*) (Guayasamin, 2004); **Amphignathodontidae**: 185-*Gastrotheca gracilis*.

TABLE 2. Location, identification, and quantity of sesamoids in anurans observed in this work, and comparison with those observed by Olson (2000)

Sesamoids	Unilateral in Anurans (Maximum number)	Unilateral in <i>Hymenochirus boettgeri</i> from Olson (2000)
1. Cartilago sesamoides	1 ^a	1
2. Fabella	1	1
3. Os sesamoides tarsal (distal)	1 ^a	1
4. Glide sesamoids: ventral to the distal head of Metatarsals I-V	10 ^a (two in each metacarpal)	
5. Glide sesamoids: ventral to the distal head of Metacarpals II-V	8 ^a (two in each metacarpal)	
6. Plantar	3 ^a	4
7. In the lateral surface of the lateral margin of each sacral diapophysis	1 ^a	
8. Dorsal to the articulation radius-ulna with radiale (pararadial)	1 ^a	1 ^b
9. Palmar	1 ^a	
10. Glide sesamoids: S. ventral to the distal head of proximal phalange of digit IV and V manus	4 ^a (two in each phalanx)	
11. Subarticular carpus	1 (Laurent, 1961)	
12. Glide sesamoid: ventral to the distal head of proximal –phalange of digit IV and V feet	4 ^a (two in each phalanx)	
13. Glide sesamoid between phalange media and distal digit IV feet	2 ^a	
14. Distal level of humerus	1 (Hoyos, 2003)	
15. Tibial lunula	1	1 ^c
16. Glide sesamoid: Dorsal level of phalanges	5 (one in each phalanx) (Hoyos, 2003)	
17. Os tibialis anticus	5 (Olson, 2000)	1
18. Os sesamoides tarsal proximal	1 (Olson, 2000)	1
19. Patella ulnaris	2 (Olson, 2000)	2
20. Sesamoid in the tendon of the m. gracilis major (graciella)	1 ^a	
TOTAL	54; 37^a	13

^aSesamoids present in *L. latinasus*.

^bRadial sesamoid in Olson (2000).

^cPosterior lunula in Olson (2000).

are based on the specimens of *L. latinasus*, with a brief comparison to *P. cf. guayanae*.

Histological preparations of the sesamoid in the ventral part of the femur-tibiofibula joint and the plantar sesamoids in *L. latinasus* were obtained to illustrate their relationships with the surrounding tendons. An important defining character of a sesamoid is that it is adjacent to an articulation or joint (see Vickaryous and Olson, 2007, p. 324). Because of that we decided to work with a structure located near a big articulation, whose movement could be considered an actual mechanobiological stimulus. To analyze development of the sesamoid in the femur-tibiofibula joint we include serial sections of the knee joint in larval specimens at Gosner Stages 37–43 and in a juvenile stage. The formalin-fixed specimens were dehydrated with graded alcohols and cleared in xylene. Serial sections were cut on an MSE sledge microtome, along the long axis of the tendon, and at right and sagittal angles to the bone. Histological serial sections (6 µm thick) of paraffin-embedded tissues were stained with Alcian blue-hematoxylin-eosin (modified from Totty, 2002).

On the basis of the observations presented in Table 1, a matrix was constructed, where 20 “major” elements (in the sense of Olson, 2000, p. 17) were included as characters; each one codified as present or absent. The charac-

ters were optimized or “mapped” onto a cladogram of amphibian relationships of Frost et al. (2006) reduced to family level. The tree was drawn with TNT software (Goloboff et al., 2003) and the optimization was done with Winclada software (Nixon, 1999).

In the context of our study, we used topology as the main argument to elucidate sesamoid homologies. Thus, two sesamoids are homologues if they are located in the same tendons of the same muscles, or in the same ligaments. This is in fact the conventional mode of inferring sesamoid homologies (Pearson and Davin, 1921a,b; Le Minor, 1987; Vanden Berge and Storer, 1995; Olson, 2000; Hall, 2005; Kawashima et al., 2007; Vickaryous and Olson, 2007; Kim et al., 2009; Jerez et al., in press, among others). On the basis of the literature data about the location of the sesamoids in the muscular tendons in other tetrapods (Flower, 1885; Pearson and Davin, 1921b; Davis, 1964; Evans, 1993; Jerez et al., in press), the homology of these bones across the tetrapod clade is proposed.

RESULTS

We found 12 major elements in *L. latinasus*; however, considering the other species surveyed and the literature data, this number rises to 20 in anurans (Table 2).

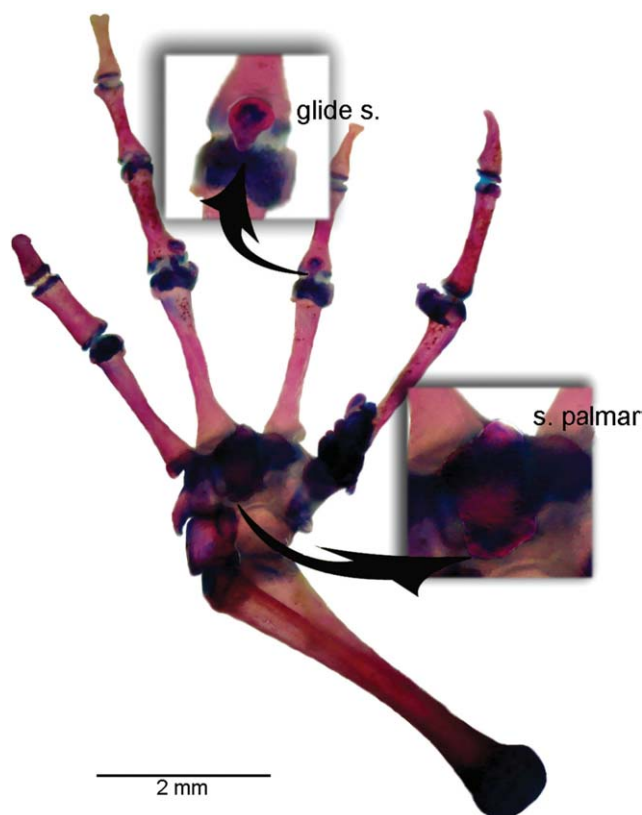


Fig. 1. Cleared and stained specimen of *L. latinasus* (L687), where the sesamoids of the ventral surface of the hand can be seen. Glide sesamoids, in the metacarpal-phalangeal joints; palmar sesamoid, ventral to the carpal bones. s.: sesamoid.

Nevertheless, no species shows all 20 (Table 1). The sesamoids of *L. latinasus* are described below.

Forelimb (Table 1; Figs. 1–3)

Proximodistally, the forelimbs present four groups of sesamoids.

Dorsal to the articulation of the radius-ulna with the radiale there is a small sesamoid, osseous in adults, which is embedded in the tendon of the *m. extensor carpi radialis* (Fig. 2). Because this sesamoid was not anteriorly described, we propose the name *pararadial* for it. Ventral to the carpal bones there is a big palmar sesamoid, osseous in adults, which is embedded in the tendon of the *m. flexor digitorum longus* (Figs. 1, 3). The flexor tendons of the digits arise directly from the palmar sesamoid (Fig. 3). Ventral to the distal heads of metacarpalia II–V, and to the distal head of the proximal phalanges of fingers IV and V, there are small elements that are referred to as the glide sesamoids of the hand. There are usually two per digit joint although sometimes both bones can be fused. They are small and osseous in adults. These are glide sesamoids because they provide a surface on which the over—underlying tendons can slide (Jerez et al., in press). The tendons correspond to the hand flexor tendons (Fig. 3).

Hindlimb (Table 1; Fig. 4)

Proximodistally, the hind limbs present six groups of sesamoids. Ventral to the femur-tibiofibula joint there is a small osseous sesamoid. It is embedded in the tendon of the *m. gracilis major*; because this sesamoid was not previously described, we propose the name *graciella* for it. In the ventral surface of the heel, there is a big and elongated sesamoid, osseous in adults. It is embedded in the Achilles tendon. This sesamoid is located near the tibiofibula-tibiale/fibulare joint and corresponds to the *os sesamoides tarsale* (Nussbaum, 1982) (Fig. 5). Deep to the *os sesamoides tarsale* there is a medium-sized sesamoid, osseous in adults, which corresponds to the *cartilago sesamoides* (Nussbaum, 1982). This sesamoid is located between tibiale-fibulare, near the tibiofibula joint, and is embedded in the tendon of the *m. plantaris profundus*. In the sole of the foot there are two medium-sized sesamoids; one between metatarsale IV, metatarsale V, distal tarsale III–II, and fibulare, and the other between metatarsale III, metatarsale IV, and distal tarsale III–II. In some specimens there is a third one, very small, located between both sesamoids. All these sesamoids are osseous in the adult. They are all embedded in the tendon that constitutes the plantar aponeurosis. The glide sesamoids of the foot are located ventral to the distal end of metatarsalia I–V, and to the distal head of the proximal phalanges of toes IV and V. There are usually two per joint of the digits, although sometimes both bones can be fused. These glide sesamoids (Jerez et al., in press) are small and osseous in adults. The tendons related to these sesamoids correspond to the *m. flexores digiti brevis*.

All observed sesamoids, except for the glide sesamoids and the tibial lunulae, belong to the embedded category of Jerez et al. (in press). This can be easily observed in the histological section of the *graciella* (Fig. 6) and the plantar sesamoids (Fig. 7). In both sesamoids, the connective tissue that will develop in tendon covers the bones on all their surfaces. The glide sesamoids of the digits present muscular fibers of the *flexores digiti brevis* inserted onto them and because of that we do not consider them as typical embedded sesamoids. We note that the tibial lunulae are the only interosseous sesamoids (Jerez et al., in press) found in anurans.

Development of Appendicular Sesamoids in *L. latinasus* (Table 3)

Forelimb. Stage 41: Humerus, radius-ulna, radiale-ulnare and metacarpalia III, IV, and V are about 80% ossified. The palmar sesamoid and the glide sesamoids ventral to the distal end of metacarpalia II–V are present. In *Pleurodema cf. guayanae* the palmar sesamoid arises a bit later, by Stage 42.

At juvenile stages (17.67 mm), all carpal bones and the palmar sesamoid present ossification centers. In larger specimens (27.32 mm), other sesamoids appear ventral to the distal end of the proximal phalanges of fingers IV and V. In adult specimens, an osseous sesamoid is visible dorsal to the articulation between radius-ulna with radiale (Fig. 2).

Hind limbs. Stage 37: The femur, the tibiofibula, the tibiale, the fibulare, and metatarsale IV exhibit ossification centers halfway through the shaft. Histology: Long

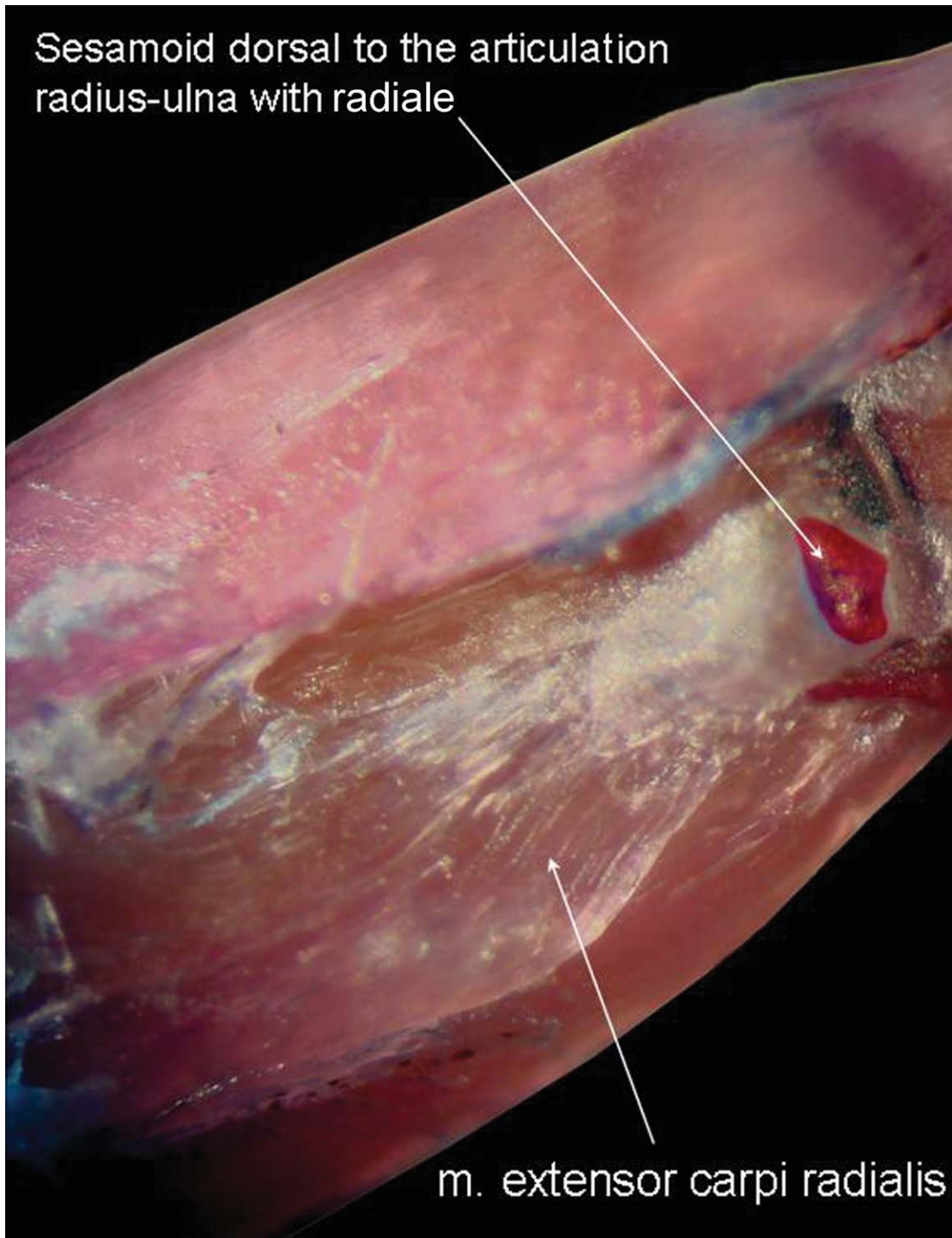


Fig. 2. Sesamoid dorsal to the articulation of the radius-ulna with radiale (pararadial) in the forelimb of *L. latinasus* (L741). m.: muscle.

bones epiphyses are composed of hyaline cartilage, consisting of chondrocytes immersed in a basophile matrix composed of fibers and ground substance (Fig. 6A). An extensive region of mesenchymal tissue surrounds the femur and the tibiofibula epiphyses, constituting a common tissue along the presumptive knee joint zone where the

long-bone articular surfaces begin their differentiation (Fig. 6A). The first morphological sign of this joint formation corresponds to the differentiation of the interzone, which is made of closely packed mesenchymal cells.

Stage 38: The central areas of the femur, tibiale, and fibulare are ossified, and the ossification zone occupies

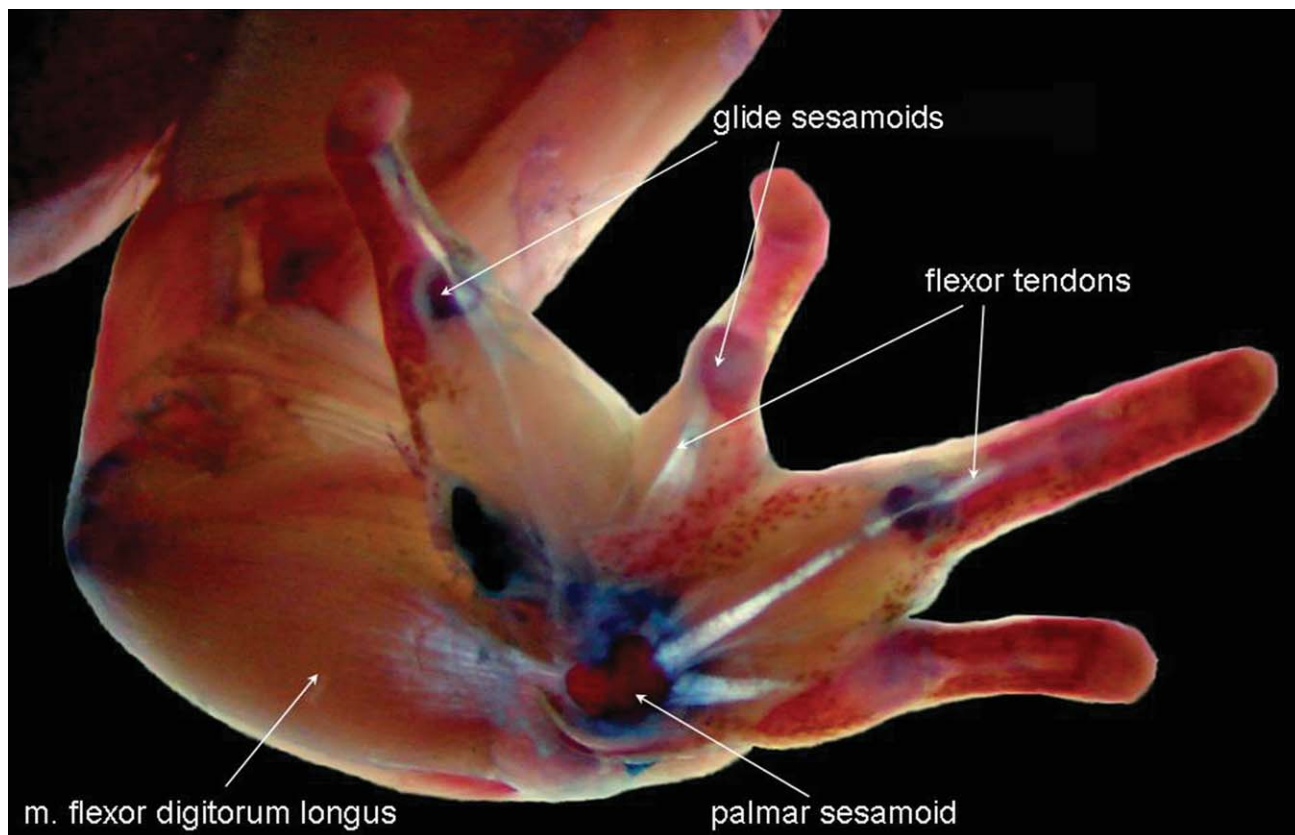


Fig. 3. Sesamoids of the ventral surface of the hand of *L. latinasus* (L741) showing the tendinous complex linking those sesamoids. The glide sesamoid, in the metacarpo-phalangeal joint, and the palmar sesamoid can be seen. m.: muscle.

the third of the length of the long bones. Histology: At this stage, the graciella is the one sesamoid to appear, as a cartilaginous condensation ventral to the presumptive femur-tibiofibula joint.

Stage 39: The ossification process in long bones diaphyses continues in a proximo-distal and postaxial-preaxial direction, while epiphyses and distal tarsal bones remain cartilaginous. Histology: The knee joint is defined as the cavitation process has just lead to the physical separation of both articular surfaces. The graciella appears as an oval condensation of hyaline cartilage composed of big unordered cells and embedded in dense connective tissue (Fig. 6B,C). The latter is in close contact with the presumptive tendon of the m. gracilis major on its proximal side and connected to the articular cartilage of the tibiofibula (Fig. 6C). In *Pleurodema* cf. *guayanae* the graciella is just appearing.

Stages 40–41: The diaphyses of stylopodial and zeugopodial long bones are almost completely ossified while the epiphyses and distal tarsal elements remain cartilaginous. Histology: The distinct components of the mature knee-joint form and include articular cartilage, capsule, and a full-fledged synovial cavity (Fig. 6D). Cells of the connective tissue surrounding the graciella are oriented more parallel to each other than in previous stages (Fig. 6D). From this connective tissue, at the proximal end of this sesamoid, there is a one-layered cell prolongation connecting it with the femur epiphysis (Fig. 6D).

Two cartilaginous plantar sesamoids appear in the ventral aspect of the foot between the proximal ends of metatarsalia II–V, within the tendon anlage of the m. plantaris longus (Fig. 7). Later, the cartilago sesamoides develops as a small cartilaginous condensation in the ventral aspect of the proximal end of the tibiale-fibulare.

Stage 42: In *Pleurodema* cf. *guayanae*, the cartilago sesamoides and the plantar sesamoid appear.

Stage 43: Metatarsalia and phalanges are about 90% ossified. Histology: The epiphyses of the femur and the tibiofibula show at least two differentiated regions: a central cartilaginous one, composed of small cells; and an articular cartilaginous zone with two lateral articular areas of hyaline cartilage, with bigger cells (lateral articular cartilages of Felisbino and Carvalho, 1999) (Fig. 6E). The lateral areas show a similar configuration in cell types and shape to the graciella. However, both differ in the surrounding tissues: the graciella is in the center of a connective tissue that links it with the tibiofibula epiphysis, whereas the lateral cartilages are separated from the periosteum by a fibrous region (osteochondral ligament, Felisbino and Carvalho, 2002). The tissue surrounding the graciella is almost differentiated in a mature tendon.

Juvenile Stages: In a juvenile stage of 17.67 mm, all distal tarsal bones remain cartilaginous. Femur epiphyses show signs of mineralization. In a bigger specimen (18.77 mm), the epiphyses of the long bones of the hind

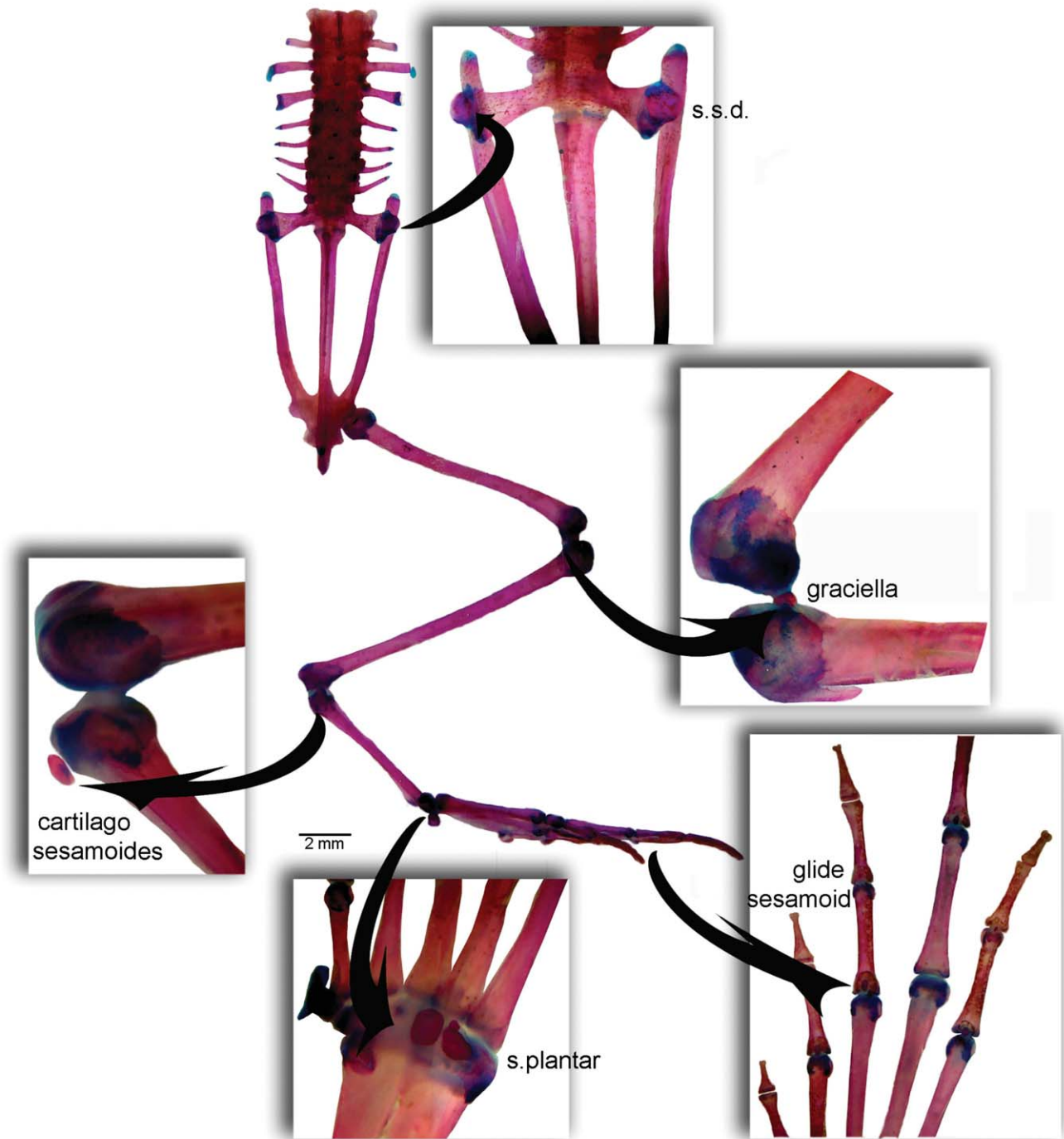


Fig. 4. Cleared and stained specimen of *L. latinasus* (L687) showing sesamoids of the hindlimb and the sacral diapophyses. In the hindlimbs can be seen: the graciella, ventral to the femur-tibiofibula joint; the cartilago sesamoides, between tibiale-fibulare, near the tibiofibula joint; the plantar sesamoids, three sesamoids in the ventral surface of

the pes; the glide sesamoids, in the metatarsal-phalangeal joint and between the proximal and medium phalanges of some digits. Also, a sesamoid between the sacral diapophyses and the ilial shafts can be seen. s.: sesamoid; s.s.d.: sesamoid of the sacral diapophyses.

limbs are almost fully ossified. In bigger specimens (21–22 mm), the cartilago sesamoides and the plantar sesamoid are ossified. The graciella shows the first signs of mineralization. Histology: The lateral articular epiphyseal cartilage appears in close contact with the perios-

teum of the diaphysis. A young tendon with many fibroblastic cells is anchored to an apophysis of the tibiofibula through a notably clear enthesion organ (Fig. 6F).

In a larger specimen (22.67 mm), a group of elements ventral to the distal end of metatarsalia I–V and a single

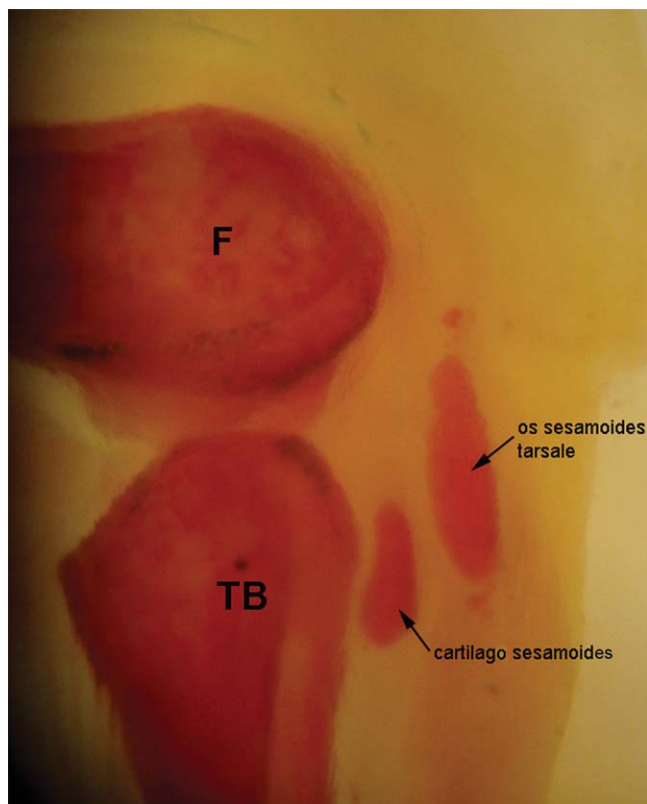


Fig. 5. Knee-joint of cleared and alizarin-stained specimen of *L. latinasus* (FML 11912) showing a small cartilago sesamoides and a larger os sesamoides tarsale. F: femur; TB: tibia-fibula.

element ventral to the distal end of the proximal phalanx of Toe IV appears. The latter appears as an ossified element in a bigger specimen (27.32 mm).

Sesamoids in the sacral diapophyses. At Stage 43, a cartilaginous sesamoid in the lateral surface of each sacral diapophysis appears. After metamorphosis, these sesamoids and the sacral diapophyses appear fully ossified (Fig. 4).

Phylogenetic Mapping

Our survey results in a data set where the two major anuran clades, Hyloides (12 families) and Ranoides (14 families), were represented. The matrix has 38% missing entries. Most of the surveyed sesamoids have multiple origins, with only three of them (about 19%) having one origin. All 20 major elements (Olson, 2000) (e.g., cartilago sesamoides, fabella, os sesamoides tarsale, glides, etc.) were mapped onto the Frost et al. (2006) phylogeny, but only one of them (cartilago sesamoides) was useful to diagnose a clade, the Neobatrachia.

DISCUSSION

Anuran sesamoids are as numerous as those in other vertebrate groups [54 pairs vs. for instance, 50 pairs in squamates (Jerez et al., in press)]. Thus, they are not as uncommon as was traditionally thought in amphibians (Haines, 1969; Olson, 2000). Among anurans, there are only two sesamoids present in 20% or more of the taxa examined, namely, the cartilago sesamoides, which appears in 68% of the taxa, and the fabella, which appears in 20% (Table 1). Olson (2000) described only

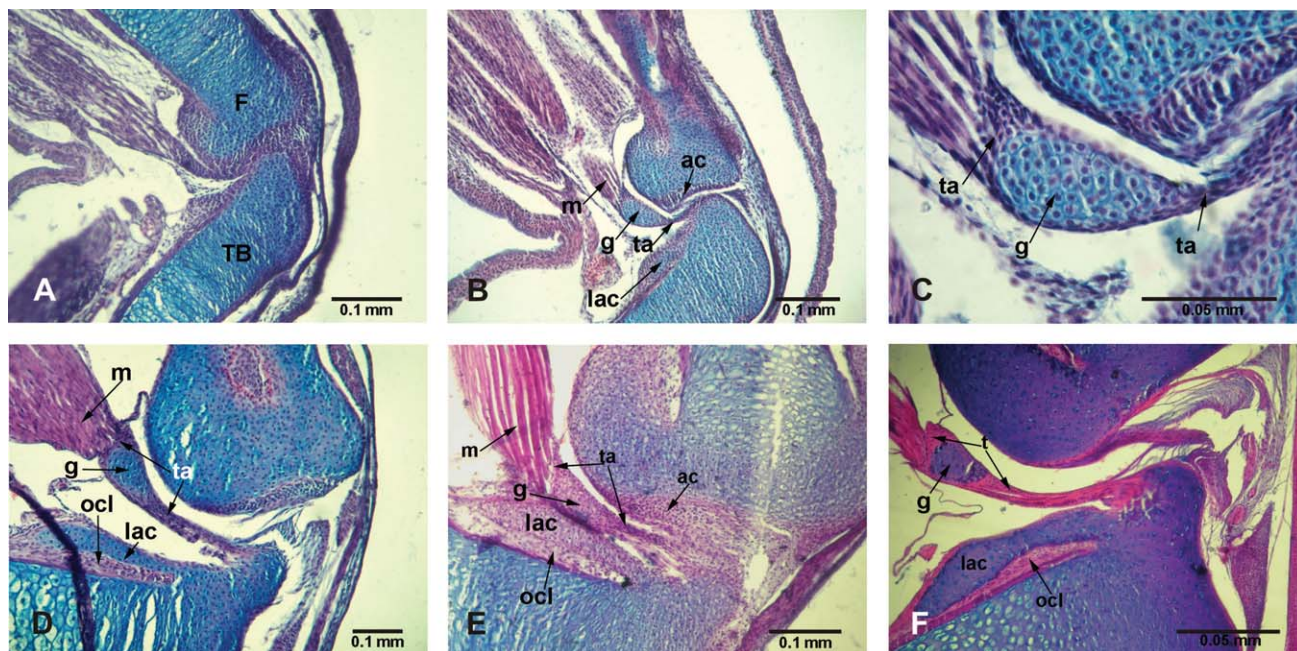


Fig. 6. Ontogenetic sequence of the sesamoid in the tendon of the muscle gracilis major (graciella), lateral articular cartilages, and tendons in the hindlimb of *L. latinasus*. **A:** Stage 37. **B:** Stage 39. **C:** Stage 39 detail: sesamoid still cartilaginous in a tendon anlage. **D:**

Stage 41. **E:** Stage 43. **F:** Juvenile of 18.77 mm: sesamoid embedded in differentiated tendon. F: Femur; TB: Tibiofibula; g: graciella; ac: articular cartilage; ta: tendon anlage; lac: lateral articular cartilage; m: muscle gracilis major; ocl: osteochondral ligament; t: tendon.

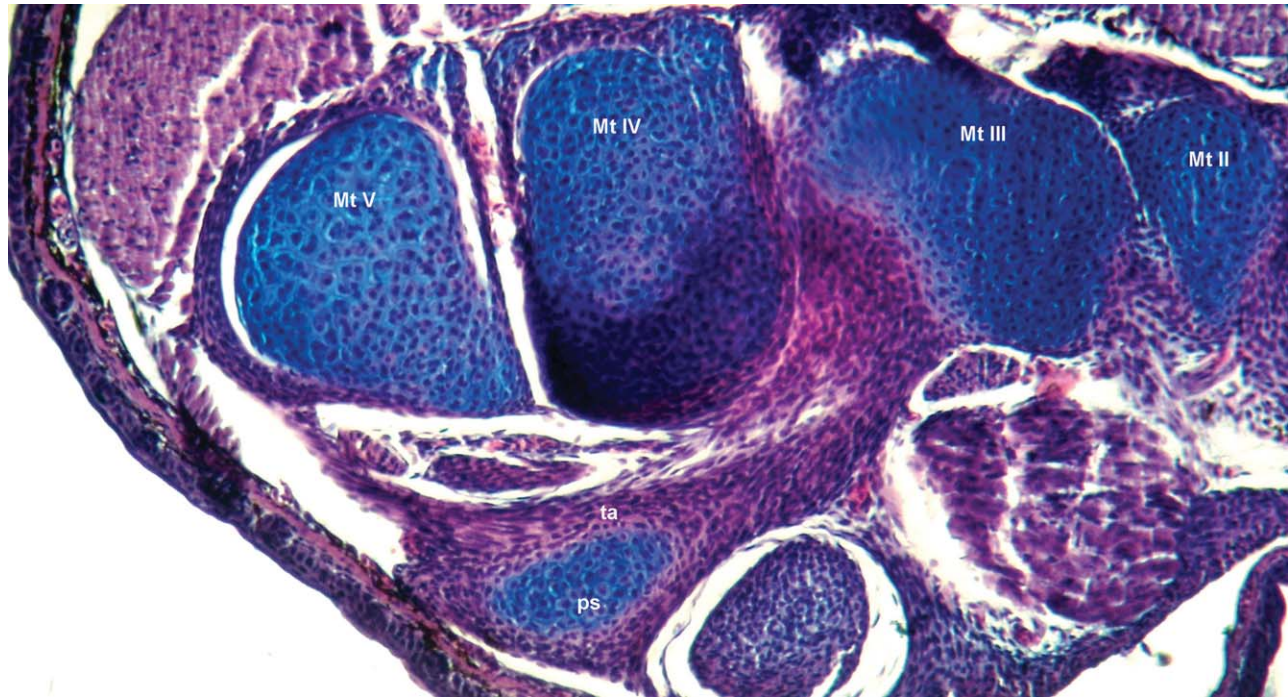


Fig. 7. Cartilaginous precursor of the plantar sesamoid, embedded in an undifferentiated connective tissue, which constitute a tendinous anlage. *L. latinasus*, Stage 41. ps: plantar sesamoid; ta: tendon anlage; Mt: metatarsal.

nine major elements in *Hymenochirus boettgeri*, and she interprets the hyperossification process typical of *Hymenochirus* as one of the possible reasons for the presence of so many heterotopic elements. Although the number of sesamoid described in *Hymenochirus* is high compared with other pipids (Olson, 2000), it is rather low in relation to all frogs.

Anuran sesamoids begin to appear in prometamorphic stages (Table 3). Olson (2000) stressed that those ancestrally present in *Hymenochirus boettgeri* appear early in its ontogeny (see her Table 3). In the present character mapping, it was also found that those sesamoids that can be traced to an ancestral state tend to arise earlier during their development. However, this trend is not as clear as in squamates, in which most of their constant sesamoids arise during the first ontogenetic stages (Jerez et al., in press). In *L. latinasus*, 70% of all sesamoids are present at Stage 43.

One of the biggest anuran sesamoids, the palmar sesamoid (Figs. 1, 3), appears at Stage 41 when the forelimbs are still within the branchial chamber, and therefore they are useless. Remarkably, it does not imply they are immobilized. Although tadpoles are not embryos, the nonfunctional hind limbs and forelimbs inside the branchial chambers can be compared to the nonfunctional limbs inside the amniote egg. Embryos do not passively await hatching from their eggs of amniotic containers but begin active movement very early on in their development, and embryonic motility represents an important epigenetic component of development (Carvalho and Felisbino, 1999; Müller, 2003; Hall, 2005). The formation of secondary cartilage on membrane bone and of constant sesamoids in tendons also depends on mechanical

stimulation (Sarin et al., 1999; Müller, 2003). Hence, if some extrinsic factor is necessary to trigger sesamoid formation, it must be comparable with the movement that normally occurs in amniote embryos. We postulate that probably the same kind of extrinsic factors could be active during the first stages of anuran limb development. Interestingly, Kim et al. (2009) showed that the denervation of the hind limb in *Hymenochirus boettgeri* strongly affect the development of some sesamoids, for example, the fabella, which was the only sesamoid that demonstrated complete regression or loss with paralysis. They inferred that location of the element within the head of the m. plantaris longus may also have made it more vulnerable to loss of muscle function. It could also be interpreted that, being the joint that the fabella is related to is extremely mobile, lack of movement during development results in regression or loss of the joint associated sesamoids. Thus, movement of the joints during development could be an extrinsic factor that is crucial to eliciting the genesis of some sesamoids but apparently, not all of them (Kim et al., 2009).

It was long thought that embedded sesamoids develop within tendons (Pearson and Davin, 1921a,b; Merrilees and Flint, 1980; Giori et al., 1993; Carter et al., 1998; Sarin et al., 1999; Summers and Koob, 2002; Müller, 2003; Hall, 2005; Doherty, 2007, among others). It was also believed that sesamoids functioned to improve the ability of tendon to respond to compressive load (Summer and Koob, 2002). Because the differentiation of many sesamoids begins well before the tendinous tissue is recognizable; and the only visible tissue connecting the muscle (and the sesamoid) with the bone is a dense connective one (see histological sequence in Figs. 5, 6),

TABLE 3. Sesamoid ontogenetic development in *Leptodactylus latinasus*

	Tadpoles (Gosner stages's)										Postmetamorphics (SVL in mm)						
	37	38	39	40	41	42	43	44	45	46	16.05	17.67	18.77	21.35	21.64	22.67	27.32
Graciella	-	X(c)	X(c)	X(c)	X(c)	X(c)	X(c)	X(c)	X(c)	X(c)	X(c)	X(c)	X(c)	X(c)	X	X	X
Plantar	-	-	-	X(c) (2)	X(c) (2)	X(c) (2)	X(c) (2)	X(c) (2)	X(c) (2)	X(c) (2)	X(c) (2)	X(c) (2)	X(c) (2)	X(c) (2)	X (2)	X (2)	X (2)
Palmar	-	-	-	-	X(c)	X(c)	X(c)	?	X(c)	X(c)	X(c)	X	X	X	X	X	X
Ventral to the distal head of Metacarpals II-V	-	-	-	-	X(c)	X(c)	X(c)	X(c)	X(c)	X(c)	X(c)	X(c)	X(c)	X(c)	X(c)	X(c)	X(c)
Cartilago sesamoides	-	-	-	-	X(c)	X(c)	X(c)	X(c)	X(c)	X(c)	X(c)	-	X(c)	X	X	X	X
In the lateral surface of the lateral margin of each sacral diapophysis	-	-	-	-	-	-	X(c)	X(c)	X(c)	X(c)	X(c)	X	X	X	X	X	X
Ventral to the distal head of Metatarsals I-V (feet)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X(c)
Ventral to the distal head of proximal phalange of digit IV and V (feet)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	IV
Ventral to the distal head of proximal phalange of digit IV (manus)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X
Dorsal to the articulation radius-ulna with radiale (pararadial)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Os sesamoides tarsal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

X: presence; -: absence; (c) cartilaginous; (n) number of sesamoids when there is more than one.

we postulate that the usual ideas regarding sesamoid origin must be reconsidered in light of the actual sequence of ontogenetic events, at least for some of sesamoids. The development of the graciella precedes the differentiation of the dense connective tissue surrounding it into the tendon of the m. gracilis major and therefore its functionality is restricted. Besides, at Stage 38, there is no knee-joint formed. Analyzing our histological data, it seems that the main stimulus eliciting the graciella formation should be genetic because the cartilage precursor is present before the dense connective tissue differentiates into a tendon. The same hypothesis can be supported by the plantar sesamoids, which can already be seen as hypertrophied cartilage within their tendon anlage (Fig. 7). When ontogenetic data for the embedded sesamoids of other tetrapod species are considered, for example, birds, rabbits, dogs, primates, etc., the same conclusion could be reached. In all those cases, the sesamoid under consideration is differentiated well before a mature tendon tissue is clearly recognizable (Walsmley, 1940; Le Minor, 1987; Clarck and Stechschulte, 1998; Müller, 2003). Although most of these authors mention the presence of tendon beginning in the early developmental stages, their figures and descriptions allow alternative interpretations. For example, Fig. 3a–c of Le Minor (1987) clearly shows an increasing organization of the connective tissue surrounding the os peroneum while the specimens grow. In fact, only in an adult specimen (see his Fig. 3c) is the classic alignment of the tendinous tissue visible. Collagen is a triple helical, rod-shaped protein that requires further modification in the plasma membrane of the cell before it is functional. Besides, the mature collagen fiber has a typical periodic banding pattern (64–70 nm) (Doherty, 2007). Thus, a tendon is recognizable by the organization of its fibers: regularly arranged, wavy appearing collagen bundles and fibrocyte nuclei (Turhan et al., 2004). According to our data, this organization is reached only in juvenile specimens of *L. latinasus*. Clarck and Stechschulte (1998) described a sequence of changes in the general conformation of the tendinous tissue in a 2-week-old rabbit that is very similar to those occurring in the tissues surrounding the graciella of *L. latinasus*. Felisbino and Carvalho (1999) reach the same conclusions in relation to the development of the calcaneal tendon in *Rana catesbeiana*. They stressed that mechanical loading is not a major factor in the primary events in the differentiation of the plantaris longus tendon, which seems to follow a programmed sequence of developmental steps. Müller (2003, Fig. 4) shows a cartilaginous sesamoid within its tendon anlage, which means that no mature tendinous tissue is present. The same images of sesamoid cartilaginous nodes embedded in tendon anlage can be seen in different taxa (Le Minor, 1987: os peroneum in primates), and of different sesamoids (this work, Fig. 7). It could be inferred that, at least for some sesamoids, the stimulus eliciting sesamoid formation is exerted earlier in the ontogeny than was previously thought, suggesting the process originating them is highly conservative.

When the distribution of sesamoids is considered, it is easily seen that the categories of Vickaryous and Olson (2007)—omnipresent and fluctuating—can be applied to all sesamoids: for example, the pisiform is present across the amniota clade. Likewise, the palmar sesamoid is present in many taxa of tetrapods: not all tetrapods

have one, but it is present in every major tetrapod clade (Abdala et al., 2009; Jerez et al., in press). Both, pisiform and palmar sesamoid belong to the constant or omnipresent sesamoids. Even when, for example, the palmar sesamoid has a scattered distribution among tetrapods, its presence in some anuran, squamate, and mammal (including primates) allows us to infer that it was present in the developmental program of the last common ancestor of all of them. There are other sesamoids, such as the os sesamoides tarsale, or the graciella, which vary even individually in those taxa having them (e.g., *L. latinasus*), and belong to the fluctuating category. It could be inferred that if the developmental program of a whole group includes a particular sesamoid, it will be constant, and its origin will be strongly linked to genetic stimulus, although the extrinsic factors can be never excluded. On the contrary, lack of regularity is clearly speaking of a preeminence of extrinsic factors over those genetic ones.

Sesamoid mineralization/ossification begins in postmetamorphic stages in anurans, in posthatching specimens in other tetrapods such as lizards (Jerez et al., in press), and some years after birth in mammals such as humans (Tortora and Derrickson, 2006). One might ask if the onset of mineralization is related to both limbs becoming functional and the dramatic change in the environment in which they move (see also Carvalho and Felisbino, 1999; Hall, 2005), or to the offset of the tendon differentiation in which the sesamoid is included. A third possibility should not be discarded: that the timing of their mineralization is genetically based. Further studies are necessary to elucidate these questions.

Comparing our developmental results with those of Olson (2000) and Vickaryous and Olson (2007), a few striking features can be mentioned. These studies agree that sesamoids generally appear in about Stages 37–38. Olson (2000) described nine major sesamoids in *Hymenochirus boettgeri*, whereas the present analysis comprises 12 major sesamoids in *L. latinasus*, of which only four are the same, and none of them appear at the same ontogenetic stage as in *H. boettgeri*. No similarity could be found in the developmental timing of the shared sesamoids. For instance, the first sesamoid appearing in *L. latinasus* is the cartilago sesamoides (Stage 38), whereas in *H. boettgeri* they are the os sesamoides tarsale, plantar sesamoids and lunula; the cartilago sesamoides appears at Stage 41 in *L. latinasus*, but in adult specimens in *H. boettgeri* (Olson, 2000; Vickaryous and Olson, 2007).

Müller and Wagner (1996) proposed that the fibular crest, a conspicuous osseous protuberance on the tibiotarsus of birds, is developmentally derived from a cartilaginous sesamoid through mechanical stimulation by muscle action. Similar ideas were expressed by Hall (2005), who stressed that the traction epiphyses (apophyses)—projections from long bones onto which tendons insert, which have their own secondary ossification centers—may have originated evolutionarily as sesamoids. The graciella anlage is conformed by the connective tissue located between the epiphyses of the femur and the tibiofibula (Fig. 6A,B). Close to the graciella anlage appear the epiphyseal lateral articular cartilages (Fig. 6B). During Stage 39, both structures are surrounded by connective tissue (Fig. 6B). In Stage 41, the attachment of the graciella to the tibiofibula epiphysis is different from the lateral articular cartilage of the tibiofibular-epiphysis

attachment: in the former, a connective tissue that later gives rise to tendon is found, whereas in the latter, a hypertrophied cartilage that gives rise to bone can be seen. Besides, according to Felisbino and Carvalho (2002), the region between the articular lateral cartilage and the periosteal bone of the epiphysis corresponds to a fibrous tissue that they called osteochondral ligament. This ligament is visible in the Stage 41 of *L. latinasus* (Fig. 6D). The similarity in shape and conformation of both structures, which vary only in the attachment with the bone epiphysis, could be explained by the sesamoid-traction-epiphysis hypothesis (Parsons, 1904, 1908; Barnet and Lewis, 1958). This hypothesis, revisited by Vickariou and Olson (2007), established that in some instances the long-bone osseous projection of insertion for a tendon or ligament develops independently of the limb element proper, and represents an incorporated sesamoid.

Phylogenetic Mapping

Nussbaum (1982) pointed out that the use of sesamoids in a phylogenetic context is restricted because they have a high probability of multiple origins. Olson (2000) considered some sesamoids, such as the patella ulnaris, the posterior lunulae, and the fabella, as phylogenetically informative among pipids. As a result of mapping our data onto the anuran phylogeny of Frost et al. (2006), some interesting findings arise, that is, almost 50% of the surveyed sesamoids have multiple origins, with only nine of them having one origin. For instance, the cartilago sesamoides appeared in the last common ancestor of Neobatrachia, and was independently lost in some taxa of both Hyloides and Ranoides (Frost et al., 2006) (Fig. 8). Thus, this sesamoid constitutes a synapomorphy of a clade, Neobatrachia, which includes 96% of frog diversity (Frost et al., 2006). However, the independent presence of this sesamoid in pipids indicates that it probably was an earlier acquisition in anurans (Fig. 8).

With respect to the glide sesamoids, their optimization on the tree of Frost et al. (2006) shows that the plesiomorphic condition is their absence (Fig. 9); also, they have been independently acquired in some families of Ranoides and Hyloides.

The plantar sesamoid appeared very early in anuran history. According to our data its presence is plesiomorphic for Anura, with independent losses in Hyloides.

Those sesamoids present in the distal extreme of the sacral diapophyses were described in a few anuran taxa (de Sá and Trueb, 1991; Wild, 1997; Guayasamin, 2004; Manzano and Barg, 2005; Avilan and Hoyos, 2006; Maglia et al., 2007). According to our optimizations, these sesamoids are plesiomorphic for Anura.

Previous character mapping (Olson, 2000) showed six sesamoids as autapomorphies of *Hymenochirus*. According to our data, the fabella, the cartilago sesamoides, and the posterior lunula appeared repeatedly throughout anuran history, the first two mentioned being distributed in most anuran taxa analyzed (see also Hoyos, 2003).

Sesamoid Homology in Tetrapods

Our data, together with those descriptions in the literature, allow us to propose the homology of some sesamoids across tetrapods.

The palmar sesamoid is homologous across tetrapods (Flower, 1885; Evans, 1993; Gilbert, 1997; Vickaryous

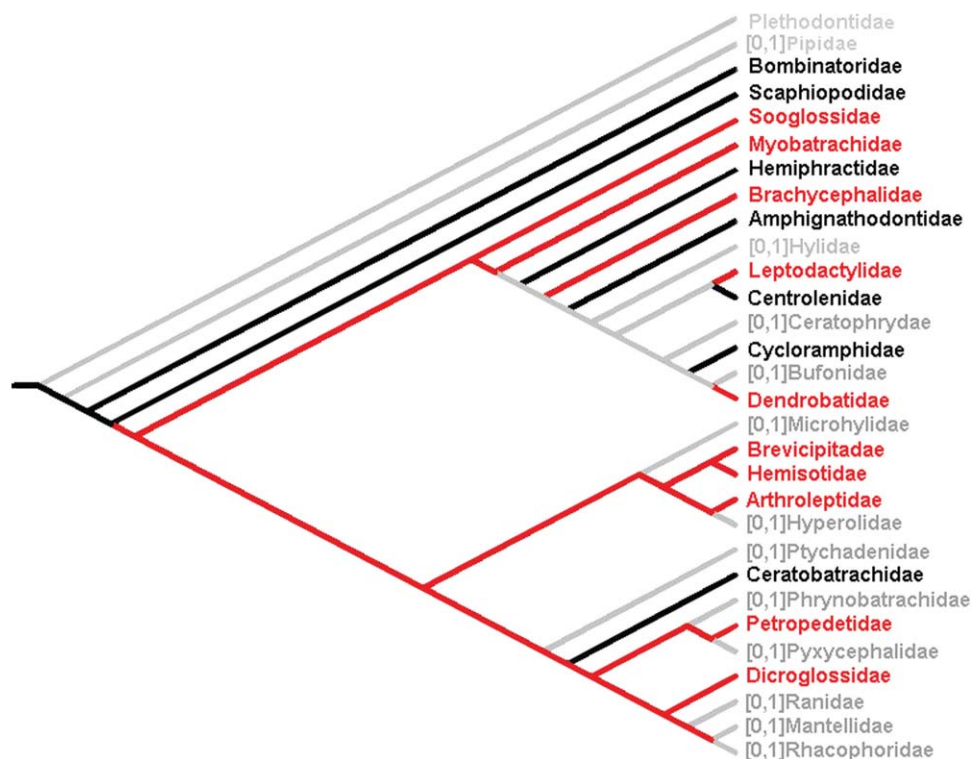


Fig. 8. Phylogenetic mapping of the cartilago sesamoides in the amphibian phylogenetic hypothesis of Frost et al. (2006), reduced to family level. Red branch: present; black branch: absent; gray branch: ambiguity. [0, 1] indicate that the character is polymorphic in the family, that is, both states are present.

and Olson, 2007; Jerez et al., in press), since in all of those taxa it is located within the tendon of the *m. flexor digitorum longus*.

Olson (2000) described as a radial sesamoid a lenticular element located in the dorsal surface of the hand of *Hymenchirus boettgeri*. We identified that structure as the sesamoid dorsal to the radius/ulna-radiale joint, which is embedded in the *m. extensor carpi radialis* tendon. In some mammals, a small sesamoid also called the radial sesamoid has been described but it is located in the insertion tendon of the *m. abductor pollicis longus* (Davis, 1964; Evans, 1993; Vickaryous and Olson, 2007). Le Minor (1994; in Vickaryous and Olson, 2007) named this element *os radiale externum* in most nonungulate placentals. On the other hand, Jerez et al. (in press) also found in some squamates an element dorsal to the radiale, embedded in the tendon of the *m. abductor pollicis longus*. On one hand, we propose to use the name radial sesamoid (as it is commonly used in mammals) for that element located within the tendon of the *m. abductor pollicis longus*. This sesamoid would be homologous within amniotes. On the other hand, we propose the name pararadial sesamoid for that element located within the tendon of the *m. extensor carpi radialis*.

The glide sesamoids of the hand seem to be homologous in anurans (this work), squamates (Jerez et al., in press), and mammals (Flower, 1885; Davis, 1964), because in all those groups they are associated with the *m. flexor digitorum longus* tendons of the digits (Vickaryous and Olson, 2007). However, Evans (1993) described the glide sesamoids embedded in the tendon of the *m.*

intermetacarpalis in dogs. On the other hand, the triassic fossil turtle *Proganochelys* presents what are interpreted as sesamoid ossifications in the extensor tendons of the digits (Gaffney, 1990). In life, sesamoids seem to have been directly dorsal to the medial phalange, between the ungual and metatarsalia or metacarpalia, near the probable position of the distal end of the ligament from the *m. extensor digitorum brevis*. Further research should be conducted to reach more extensive conclusions about this topic.

With respect to those sesamoids located in the pedes, we propose the homology of the glide elements with the two sesamoids linked to the hallux in man, which are also associated with the tendons of the flexor brevis complex of the foot (Davis, 1964; Netter, 2003). According to Vanden Berge and Storer (1995, p. 51), these specific ossifications are not described in birds, although a set of wedge-shaped bones is present in the metatarsophalangeal joint cavities of digits II, III, and IV in some taxa. Dorsal digital sesamoids have also been reported in some anuran taxa (Hoyos, 2003), in the fossil turtle *Proganochelys* (Gaffney, 1990) and also in some mammals such as dogs (Evans, 1993), and cats (Gilbert, 1997; Sebastiani and Fishbeck, 2005). Unfortunately, none of those authors mentioned the muscular tendons related to those sesamoids, although Gaffney (1990) mentioned the possible relationship between these sesamoids and extensor tendons of the toes. More data are also necessary to determine the homology of glide sesamoids in hind limbs.

The fabella is homologous in all tetrapod taxa, although it has not been described in birds (Vanden

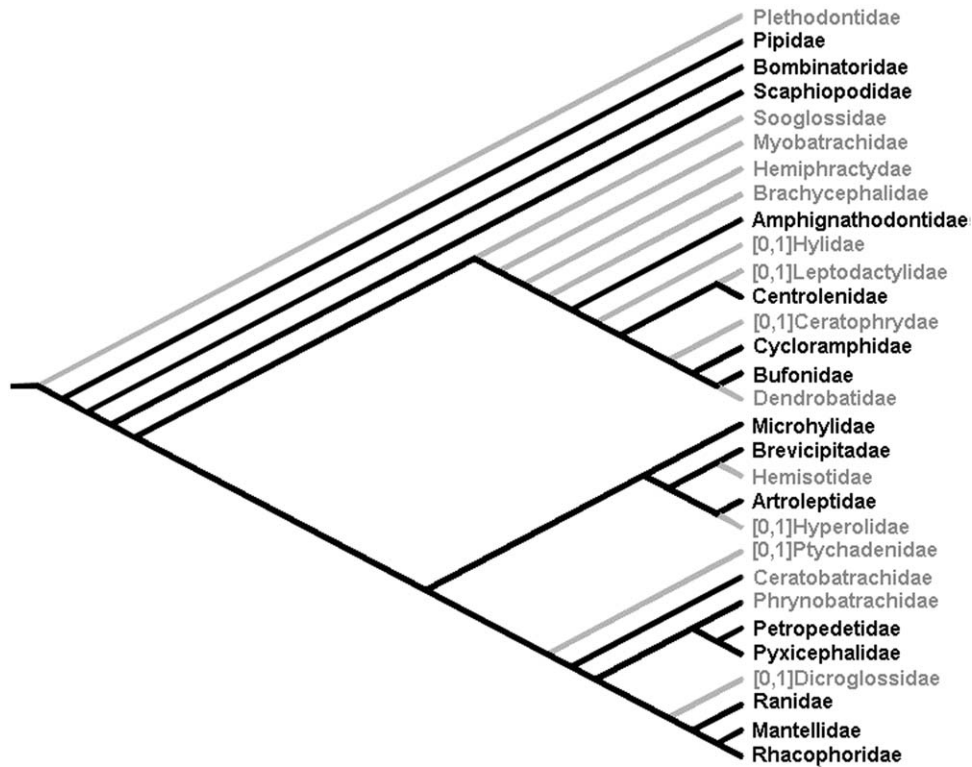


Fig. 9. Phylogenetic mapping of glide sesamoids ventral to the distal heads of metacarpalia II–V on the amphibian phylogenetic hypothesis of Frost et al. (2006), reduced to family level. Black branch: absent; gray branch: ambiguity. [0, 1] indicate that the character is polymorphic in the family, that is, both states are present.

Berge and Storer, 1995); it is always embedded in the tendon of the plantaris longus muscle in anurans, which is homologous with the gastrocnemius muscle in the rest of tetrapods (Pearson and Davin, 1921a,b; Olson, 2000). Reese et al. (2001) called this sesamoid the Vesalli bone in marsupials, but it is homologous to the fabella.

As far as we know this is the first time that a sesamoid embedded in the tendon of the *m. gracilis major*, the *graciella*, is described in tetrapods. It has a location very similar to the lateral fabella, and can be confused with it. Maybe a closer look at the tendon of the muscles in which these sesamoids are embedded can show that many sesamoids described as lateral fabella are in fact the *graciella*.

The *os sesamoides tarsale* is homologous to the lateral postaxial tarsal sesamoid of squamates (Jerez et al., in press), because it can be found in the tendon of the *m. plantaris longus-gastrocnemius* in both groups.

Remarkably, the plantar sesamoids are not homologous among tetrapods because the plantar aponeurosis of the feet arises from different muscles. In anurans, it is formed from the *m. plantaris longus*, whereas in squamates it comes from the *m. flexor digitorum longus* (Pers. Obs.).

We also propose the homology of the lunulae in all tetrapods, because those we found in *Hypsiboas andinus* are similar in location and description to those describe in other vertebrata (Pearson and Davin, 1921b; Haines, 1942b; Vickaryous and Olson, 2007; Jerez et al., in press, among others).

CONCLUSIONS

Anurans possess more sesamoids than previously thought. Both the presence and time of appearance of sesamoids among anurans are highly variable, suggesting that developmental constraints implied in their genesis are less rigid than in other tetrapod groups, for example, squamates.

Most anuran sesamoids are embedded, with only one interosseus element and usually 33 glide sesamoids.

The traditional point of view, that all embedded sesamoids arise as a result of biomechanical stimuli of previously existing tendons, is no longer sustained by our data. Some of them arise before any differentiation of the tendinous tissue.

The similarity in shape and conformation of both *graciella* and lateral articular cartilages of the long bones, structures which vary only in the attachment with the bone epiphysis, support the sesamoid-traction-epiphysis hypothesis.

Our general data support the idea of some sesamoids being useful characters for phylogenetic analysis.

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