# Intercalary Elements, Treefrogs, and the Early Differentiation of a Complex System in the Neobatrachia

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## ABSTRACT

Intercalary elements are additional skeletal structures of digits of many anuran amphibians. Twelve terminal clades in the neobatrachian lineage of frogs have intercalary elements revealing it is a homoplastic character with five to seven gains and two to four losses along a consensus phylogeny of the Neobatrachia. We analyzed anatomical variation of intercalary elements, related structures (distal phalanges, tendons, and muscles), and articulations of digits of 45 anuran species, representing eight suprageneric terminal taxa. The intercalary elements are integrated in a complex system that is probably related to different types of movements, which are produced by a similar set of muscles and tendons with limited variation among the studied taxa. Species in the clades Hyloides and Ranoides show distinctive patterns of morphostructural features in their intercalary elements that are usually wedge-shaped and composed of hyaline cartilage in Ranoides, and biconcave and composed of embryonic cartilage in Hyloides. Features derived from the typical hyloid condition may only be interpreted in some Hylidae (*Pseudis* and *Lysapsus*) and Centrolenidae. In Ranoides, the described features of the intercalary elements are found in all taxa examined with the exception of *Leptopelis*, which have an intercalary element similar to the other Ranoides but formed by connective tissue. Several features are shared by all taxa having intercalary elements: (1) the intercalary elements differ from the phalanges by lacking terminal epiphyses, (2) they are present in hands and feet, and (3) they appear in all digits. This finding suggests that the genetic basis for presence of intercalary elements may be homologous in all these taxa and may have evolved only once early in neobatrachian history. Anat Rec, 290:1551–1567, 2007. © 2007 Wiley-Liss, Inc.

Key words: intercalary elements; muscles; digits; treefrogs; anurans

The intercalary element is an additional skeletal structure located between the terminal and subterminal (penultimate) phalanges in the digits of many anuran amphibians. The presence of this element has been hypothesized to be related to other morphological specializations associated with arboreal habits, such as well developed adhesive digit pads (e.g., Paukstis and Brown, 1987, 1991; Wiens et al., 2005). The intercalary may be part of an integrated system of articulations allowing angular movements responsible for the attachment and detachment of the adhesive digit pads (Hanna and Barnes, 1991).

Analyses of the variation of the intercalary element in frogs were mainly focused on their presence or absence and resulted in different proposals on the monophyly of particular suprageneric groups (Lynch, 1973; Laurent, 1986; Duellman and Trueb, 1986; Ohler and Dubois, 1989; Ford and Cannatella, 1993; Duellman, 2001; Frost et al., 2006).

Only few studies were carried out on particular lineages analyzing the variation of the element itself. Drewes (1984) described four character states in hyperoliid frogs on the basis of structural features (juvenile cartilage without intracellular matrix, cartilaginous and unmineralized, cartilaginous and peripherally mineralized, completely mineralized). Vences et al. (2003) for the same taxon recognized intercalary elements formed by cartilage or not and concluded that the degree of mineralization can differ within a single species and represents only a gradual modification of basically identical structures. Scott (2005) in her analysis of ranoid relationships described some variation in the morphology of intercalary elements, and Faivovich et al. (2005) considered an elongated shape of intercalary elements in Neotropical treefrogs as a synapomorphy of the clade *Lysapsus* + *Pseudis*, and the partial mineralization of intercalary elements as a synapomorphy of the *Scinax catharinae* group.

Paukstis and Brown (1987), studying the intercalary elements in *Pseudacris*, recognized three morphological types (wide intercalaries with concave surfaces; cuboidal intercalaries; and very thin and reduced intercalaries), and concluded that reduction of this structure could be interpreted as a degenerative condition, further evolution toward burrowing habits possibly resulting in the loss of the intercalary. These authors, furthermore, analyzed the correlation between the morphology of the intercalary elements and the frog's habits, and hypothesized that the enlargement of the intercalary could lead to increased digit length for a better development of webbing in aquatic species (Paukstis and Brown, 1991).

Based on the most comprehensive reconstruction of amphibian phylogeny (Frost et al., 2006), the occurrence of the intercalary elements is known in five terminal taxa of Hyloides and five terminal taxa of Ranoides, which both are the major lineages in the clade Neobatrachia (Fig. 1). Frost et al. (2006) reconstructed intercalary elements to be synapomorphies for Centrolenidae (Centroleninae), Microhylidae (*Phrynomantis*), Artholeptidae (Leptopelinae), and Rhacophoroidea. These authors discussed intercalary element occurrence and include them as potential synapomorphies; nevertheless, in most of the cases, the character was not optimized and the branches were defined on molecular data.

Drewes (1984) noted that hyperoliid and leptopeline intercalary elements are histologically quite different from each other. Leptopelinae (*Leptopelis*) is distinguished morphologically by histological distinct intercalary phalangeal elements. Rhacophoroidea (Mantellidae and Rhacophoridae) is a sister group of Ranoidea (Nyctibatrachidae and Ranidae, *sensu stricto*), and one character that definitely optimizes on Rhacophoroidea is the intercalary element present.

Wiens et al. (2005) found evidence for a significant influence of homoplasy in the intercalary element on conflicts between morphological and molecular data sets. These authors characterized the Neotropical family Hylidae by several traits that presumably represent adaptations to the use of arboreal habitat (e.g., expanded toe pads and intercalary elements) and concluded that the placement of hemiphractines with other hylids may be caused by the convergent acquisition of traits associated with arboreality (e.g., offset terminal phalanges; claw-shaped terminal phalanges, intercalary elements; modified base of metacarpal III), in contrast to the strong molecular evidence that hemiphractines are not closely related to other hylids. Several other aspects in a phylogenetic reconstruction based on morphological traits were also seen as possible results of the misleading effects of this suite of traits: (1) the placement of the clade Centrolenidae + *Allophryne* with hylids, both groups containing taxa with intercalary elements; (2) the basal placement of *Pseudis*, *Lysapsus*, *Acris*, and some *Pseudacris* within hylids, which may be associated with a reversion to terrestrial and/or aquatic lifestyle in these clades, with a concomitant loss of one or more of the characters associated with arboreality (e.g., offset terminal phalanges, expanded toe pads); and (3) the loss of these traits in the terrestrial/fossorial hylid *Cyclorana* (i.e., terminal phalanges not offset, not claw-shaped, loss of intercalary elements, toe pads not expanded) which may contribute to the erroneous placement of this taxon outside of the Hylidae (Wiens et al., 2005).

Here we present a comparative analysis of different morphological traits related to intercalary elements in representative hyloid and ranoid taxa in which intercalaries are present. Because intercalary elements are morphologically and functionally integrated in anuran limbs, we describe and analyze both (1) the variation in shape and structure of the intercalaries and their articulations with phalanges, and (2) patterns of variation in associated muscles, in particular in the *Extensores breves profundus*, *Extensores breves distalis* tendons, and *Tendo superficialis*. Our goal in providing detailed comparative descriptions of these patterns is to obtain a basis for understanding their evolution in the light of recent phylogenetic hypotheses.

## MATERIALS AND METHODS

Specimens of 45 species of Neobatrachia with intercalary elements were studied. They belong to eight terminal suprageneric taxa following the classification proposed by Frost et al. (2006): Amphignathodontidae, Hylidae, Centrolenidae, Microhylidae, Mantellidae, Rhacophoridae, Arthroleptidae, and Hyperoliidae. The species examined, voucher specimens, and collection data are listed in Table 1. Three different kinds of data were gathered on these specimens: (1) Morphological
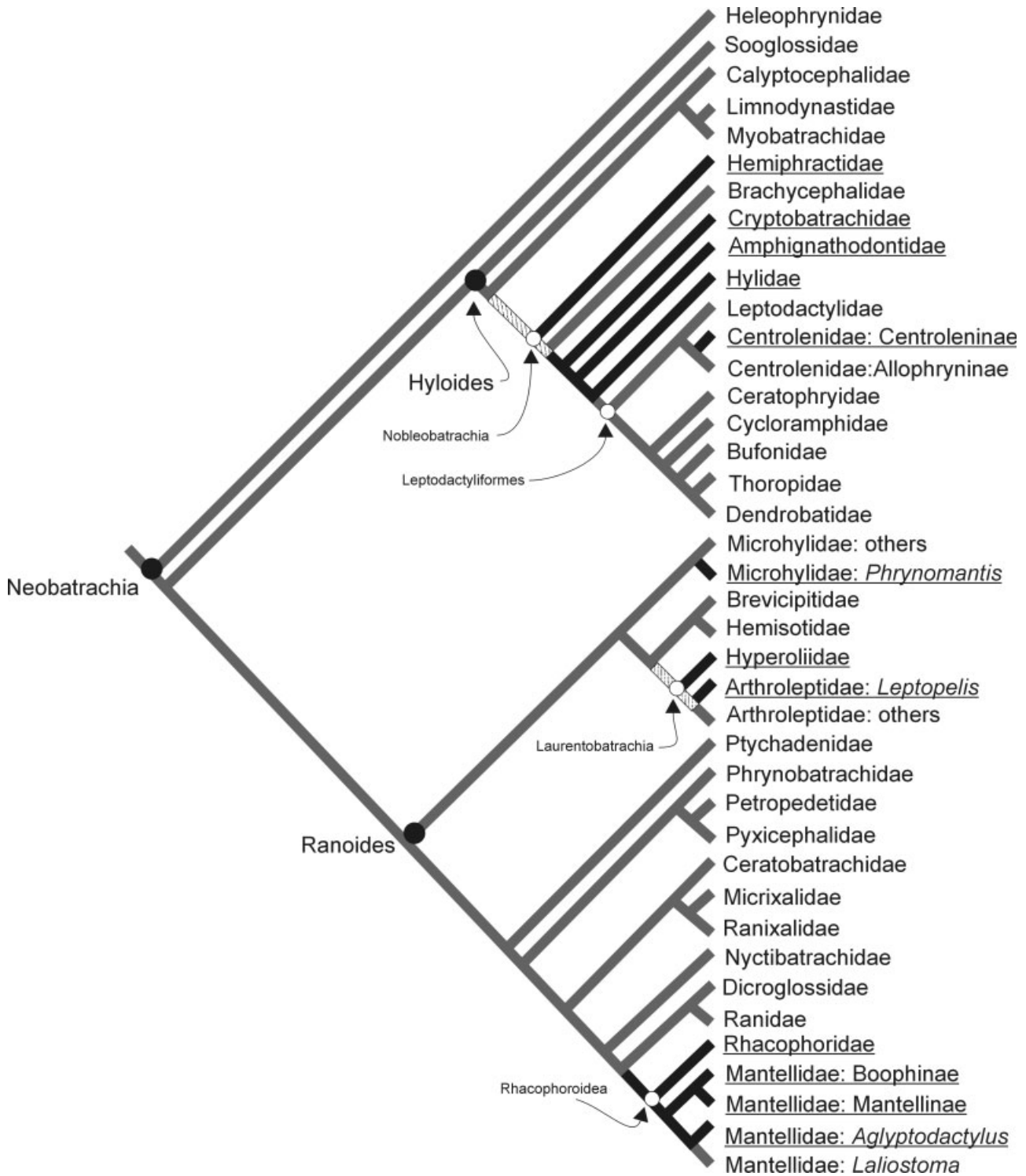
Fig. 1. Neobatrachian relationships based on recent molecular evidence. The tree topology in general follows Frost et al. (2006), with modifications in ranoid phylogeny following Van der Meijden et al. (2005, 2007) and Van Bocxlaer et al. (2006). Note that the nonmonophyly of marsupial frogs and their division into three families is controversial (Wiens, 2007). Similarly, the placement of *Phrynomantis* as most basal microhylid (Van Bocxlaer et al., 2006) is not supported (Van der Meijden et al., 2007) but its possible deeper nestedness within microhylids would only indicate more strongly a parallel evolution of the intercalary element in this taxon. Calyptocephalidae is the correct name for the taxon named Batrachophrynidae in Frost et al. (2006), according to Frost (2007). Terminal taxa are families except in cases where the presence or absence of the intercalary element in families is not universal. Underlined taxa are those having intercalary elements. Major clades are symbolized by black circles; secondary clades as used by Frost et al. (2006) and mentioned in the text are marked by white circles. Black branches are reconstructed as having an intercalary element, gray branches as lacking this element, and cross-hatched segments are differently reconstructed under ACCTRAN and DELTRAN transformations in MacClade.

**TABLE 1. Specimens examined in this study**

| Family | Species | Collection data[a] | Number of specimens | Study |
|---|---|---|---|---|
| Amphignathodontidae | *Flectonotus fitzgeraldi* | MCN 017 | 2 | Skeleton |
| Amphignathodontidae | *Gastrotheca christiani* | MCN 437 | 1 | Skeleton, muscles, and histology |
| Amphignathodontidae | *Gastrotheca gracilis* | FML 2995 | 1 | Skeleton |
| Arthroleptidae | *Leptopelis christyi* | MCN 829 | 4 | Skeleton |
| | | MCN s/n | 1 | Muscles and histology |
| Centrolenidae | *Centrolene geckoideum* | DIAM 314 | 1 | Skeleton, muscles and histology. |
| Centrolenidae | *Centrolene grandisonae* | DIAM 0320 | 2 | Skeleton and muscles |
| Centrolenidae | *Centrolene robledoi* | DIAM 0315 | 2 | Skeleton and muscles. |
| Centrolenidae | *Centrolene notostictum* | UIS A 863 | 1 | Skeleton |
| | | UIS A 410 | 1 | Skeleton |
| Centrolenidae | *Cochranella griffithsi* | DIAM 0319 | 1 | Muscles |
| Centrolenidae | *Cochranella ignota* | DIAM 0321 | 2 | Skeleton and muscles. |
| Centrolenidae | *Cochranella savagei* | DIAM 0322 | 1 | Muscles |
| Centrolenidae | *Hyalinobatrachium aureoguttatum* | DIAM 0318 | 3 | Skeleton and muscles |
| Hylidae | *Argenteohyla siemersi* | FML 3954 | 1 | Skeleton |
| Hylidae | *Dendropsophus minutus* | DIAM 066 | 1 | Muscles |
| Hylidae | *Dendropsophus nanus* | MCN 791 | 1 | Skeleton |
| Hylidae | *Hylomantis lemur* | MCN 012 | 2 | Skeleton |
| Hylidae | *Hypsiboas andinus* | MCN 937 | 1 | Skeleton |
| | | MCN 092 | 1 | Histology |
| Hylidae | *Isthmohyla rivularis* | MCN 013 | 1 | Skeleton |
| Hylidae | *Litoria caerulea* | DIAM 313 | 1 | Skeleton, muscles, and histology. |
| Hylidae | *Lysapsus limellum* | FML 0716 | 1 | Skeleton |
| | | DIAM 019 | 2 | Skeleton and muscles. |
| Hylidae | *Phyllomedusa sauvagii* | MCN 795 | 1 | Skeleton, muscles, histology |
| | | MCN 392 | 1 | Histology |
| | | DIAM 0337 | 1 | Skeleton and muscles |
| Hylidae | *Pseudis minuta* | DIAM 0338 | 2 | Skeleton and muscles |
| Hylidae | *Pseudis paradoxa* | MCN 812 | 1 | Skeleton |
| | | MACN 37698 | 1 | Skeleton and muscles |
| | | MACN 37699 | 1 | Skeleton and muscles |
| | | MCN 567 | 1 | Histology |
| Hylidae | *Scinax acuminatus* | MCN 1006 | 1 | Skeleton and histology |
| Hylidae | *Scinax fuscovarius* | MCN 813 | 1 | Skeleton and histology |
| Hylidae | *Scinax nasicus* | MCN 1005 | 1 | Skeleton |
| | | DIAM 095 | 1 | Skeleton |
| Hylidae | *Scinax squalirostris* | DIAM 021 | 3 | Skeleton and muscles |
| Hylidae | *Trachycephalus venulosus* | FML 2712 | 2 | Skeleton |
| | | DIAM 024 | 1 | Skeleton and muscles |
| Hyperoliidae | *Afrixalus quadrivittatus* | MCN 943 | 2 | Skeleton |
| Hyperoliidae | *Afrixalus laevis* | MCN 993 | 2 | Skeleton |
| Hyperoliidae | *Afrixalus osorioi* | MCN 994 | 2 | Skeleton |
| Hyperoliidae | *Hyperolius castaneus* | MCN 833 | 5 | Skeleton |
| Hyperoliidae | *Hyperolius kivuensis* | MCN 804 | 2 | Skeleton, muscles, and histology |
| Hyperoliidae | *Kassina senegalensis* | MCN 823 | 2 | Skeleton |
| Hyperoliidae | *Opisthothylax immaculatus* | MCN 825 | 1 | Skeleton |
| Hyperoliidae | *Phlyctimantis verrucosus* | MCN 832 | 3 | Skeleton |
| Mantellidae | *Aglyptodactylus madagascariensis* | DIAM 0333 - 0324 | 2 | Skeleton, muscles, and histology |
| Mantellidae | *Boophis goudoti* | DIAM 0332 | 1 | Skeleton, muscles and histology |
| Mantellidae | *Boophis madagascariensis* | DIAM 0325 | 1 | Skeleton, muscles and histology |
| Mantellidae | *Mantella auriantica* | DIAM 0334 | 1 | Skeleton, muscles and histology |
| Mantellidae | *Mantidactylus curtus* | DIAM 0328 - 0335 | 2 | Skeleton, muscles, and histology |
| Mantellidae | *Guibemantis liber* | DIAM 0330 | 2 | Skeleton, muscles, and histology |
| Mantellidae | *Guibemantis timidus* | DIAM 0340 | 1 | Histology |
| Microhylidae | *Phrynomantis bifasciatus* | MCN 830 | 4 | Skeleton |
| Microhylidae | *Phrynomantis microps* | DIAM 0331 | 1 | Skeleton, muscles, and histology |
| Rhacophoridae | *Chiromantis rufescens* | MCN 831 | 4 | Skeleton |
| | | DIAM 0339 | 2 | Skeleton, muscles, and histology |

[a]Museum abbreviations: FML, Instituto de Herpetología, Fundación Miguel Lillo, Tucumán, Argentina; MCN, Museo de Ciencias Naturales, Universidad Nacional de Salta, Argentina; UIS, Colección Herpetológica, Escuela de Biología, Universidad Industrial de Santander, Colombia; MACN, Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Buenos Aires, Argentina; DIAM, Colección Herpetológica CICyTTP-CONICET, Diamante, Entre Ríos, Argentina.

**TABLE 2. Morphological variation of terminal phalanges among anuran species with intercalary elements**

| Species | Longitudinal axis | Distal tip | Apophyses | Shape of proximal epiphysis | Articulation phalange terminal–intercalary element |
|---|---|---|---|---|---|
| *Afrixalus laevis, A. osorioi, A. quadrivittatus* | Curved | Truncated | Two ventrolateral apophyses | Ellipsoidal | Plane and biaxial |
| *Aglyptodactylus madagascariensis* | Straight | V-shaped | Two ventrolateral apophyses | Ellipsoidal | Plane and biaxial |
| *Allophryne ruthveni* | Straight | V-shaped | One medioventral apophysis | Ellipsoidal | |
| *Argenteohyla siemersi* | Curved | Pointed | Absent | Rounded | Pivot and monoaxial |
| *Boophis goudotii* | Straight | Truncated | Absent | Ellipsoidal | Plane and biaxial |
| *B. madagascariensis* | Straight | V-shaped | Two ventrolateral apophyses | Ellipsoidal | Plane and biaxial |
| *Centrolene geckoideum, C. grandisonae, C. notostictum, C. robledoi* | Straight | V-shaped | One medioventral apophysis | Ellipsoidal | Condyloid and biaxial |
| *Chiromantis rufescens* | Straight | V-shaped | Absent | Ellipsoidal | Plane and biaxial |
| *Cochranella griffithsi, C. ignota* | Straight | V-shaped | One medioventral apophysis | Ellipsoidal | Condyloid and biaxial |
| *Dendropsophus minutus, D. nanus* | Curved | Pointed | Two ventrolateral apophyses | Rounded | Pivot and monoaxial |
| *Flectonotus fitzgeraldi* | Curved | Pointed | Absent | Rounded | Pivot and monoaxial |
| *Gastrotheca christiani, G. gracilis* | Curved | Pointed | Absent | Rounded | Pivot and monoaxial |
| *Hyalinobatrachium aureoguttatum* | Straight | V-shaped | One medioventral apophysis | Ellipsoidal | Condyloid and biaxial |
| *Hylomantis lemur* | Curved | Pointed | Absent | Rounded | Pivot and monoaxial |
| *Hyperolius castaneus, H. kivuensis* | Curved | Truncated | Two ventrolateral apophyses | Ellipsoidal | Plane and biaxial |
| *Hypsiboas andinus* | Curved | Pointed | Absent | Rounded | Pivot and monoaxial |
| *Isthmohyla rivularis* | Curved | Pointed | Absent | Rounded | Pivot and monoaxial |
| *Kassina senegalensis* | Curved | Truncated | Two ventrolateral apophyses | Ellipsoidal | Plane and biaxial |
| *Leptopelis christyi* | Curved | Pointed | Absent | Ellipsoidal | Plane and biaxial |
| *Litoria caerulea* | Curved | Pointed | Absent | Rounded | Pivot and monoaxial |
| *Lysapsus limellum* | Straight | Pointed | Absent | Ellipsoidal | Plane and nonaxial |
| *Mantella aurantiaca* | Straight | V-shaped | Absent | Ellipsoidal | Plane and biaxial |
| *Mantidactylus curtus, Guibemantis liber* | Straight | V-shaped | Absent | Ellipsoidal | Plane and biaxial |
| *Opisthothylax immaculatus* | Curved | Pointed | Two ventrolateral apophyses | Ellipsoidal | Plane and biaxial |
| *Phlyctimantis verrucosus* | Curved | Truncated | One medioventral apophysis | Ellipsoidal | Plane and biaxial |
| *Phrynomantis bifasciatus, P. microps* | Straight | V-shaped | One medioventral apophysis | Ellipsoidal | Plane and biaxial |
| *Phyllomedusa sauvagii* | Curved | Pointed | Absent | Rounded | Pivot and monoaxial |
| *Pseudis minuta, P. paradoxa* | Straight | Pointed | Absent | Plane | Plane and nonaxial |
| *Scinax acuminatus, S. fuscovarius, S. nasicus, S. squalirostris* | Curved | Truncated | Two ventrolateral apophyses | Rounded | Pivot and monoaxial |
| *Trachycephalus venulosus* | Curved | Pointed | Absent | Rounded | Pivot and monoaxial |

descriptions of distal phalanges and intercalary elements in skeletal whole-mounts cleared and double stained with Alcian Blue and Alizarin Red S (Wassersug, 1976); (2) anatomical descriptions of muscles and tendons based on dissections of whole specimens that were double stained with Alcian Blue and Alizarin Red S but not cleared. These preparations were preserved in 70% ethanol and, at the time of observation, temporarily stained with iodine solution to obtain a better contrast of the structures (Bock and Shear, 1972); and (3) histological serial sections of 7-μm thick of paraffin-embedded digit tips (Anderson and Bancroft, 2002) were stained with hematoxylin—eosin (Wilson and Gamble, 2002).

Most descriptions were based on features observed in finger and toe IV. Observations, illustrations, and photo-graphs were made with Nikon SMZ1000 and Olympus stereo dissection microscopes and with a Leica DM light microscope equipped with digital camera and camera lucida.

Movements of the articulations were interpreted by moving structures, pulling tendons, and muscles in each digit of preserved samples. Variation of terminal phalanges, intercalary elements, articulations, muscles, and tendons in fore- and hindlimbs are summarized in Tables 2–4. Standard muscle terminology follows Gaupp (1896) and Burton (1998a,b); arthrology nomenclature follows Dyce et al. (1996).

To reconstruct the phylogenetic history of intercalary elements, we compiled an informal consensus tree from recent molecular phylogenetic studies (Frost et al., 2006; Van Bocxlaer et al., 2006; Van der Meijden, 2005, 2007).

**TABLE 3. Morphological and structural variation of intercalary elements among anurans**

| Species | Shape | Structure | Articulation intercalary element–penultimate phalange |
|---|---|---|---|
| *Afrixalus laevis, A. osorioi, A. quadrivittatus* | Wedge-shaped | Hyaline cartilage | Sellaris and biaxial |
| *Aglyptodactylus madagascariensis* | Wedge-shaped | Hyaline cartilage fully mineralized | Sellaris and biaxial |
| *Argenteohyla siemersi* | Thick biconcave disc | Embryonic cartilage | Ginglymus and monoaxial |
| *Boophis goudoti, B. madagascariensis* | Wedge-shaped | Hyaline cartilage fully mineralized | Sellaris and biaxial |
| *Centrolene geckoideum, C. grandisone, C. notostictum, C. robledoi* | Thick biconcave disc | Embryonic cartilage with a large mineralized nucleus | Ginglymus and monoaxial |
| *Chiromantis rufescens* | Wedge-shaped | Hyaline cartilage with peripheral mineralization | Sellaris and biaxial |
| *Cochranella griffithsi, C. ignota* | Thick biconcave disc | Embryonic cartilage with a large mineralized nucleus | Ginglymus and monoaxial |
| *Dendropsophus minutus* | Thick biconcave disc | Embryonic cartilage | Ginglymus and monoaxial |
| *D. nanus* | Thick biconcave disc | Embryonic cartilage with a small mineralized nucleus | Ginglymus and monoaxial |
| *Flectonotus fitzgeraldi* | Thick biconcave disc | Embryonic cartilage | Ginglymus and monoaxial |
| *Gastrotheca christiani, G. gracilis* | Thick biconcave disc | Embryonic cartilage | Ginglymus and monoaxial |
| *Hyalinobatrachium aureoguttatum* | Thick biconcave disc | Embryonic cartilage with a large mineralized nucleus | Ginglymus and monoaxial |
| *Hylomantis lemur* | Thick biconcave disc | Embryonic cartilage with a small mineralized nucleus | Ginglymus and monoaxial |
| *Hyperolius castaneus, H. kivuensis* | Wedge-shaped | Hyaline cartilage with peripheral mineralization | Sellaris and biaxial |
| *Hypsiboas andinus* | Thick biconcave disc | Embryonic cartilage | Ginglymus and monoaxial |
| *Isthmohyla rivularis* | Thick biconcave disc | Embryonic cartilage with a small mineralized nucleus | Ginglymus and monoaxial |
| *Kassina angeli* | Wedge-shaped | Hyaline cartilage | Sellaris and biaxial |
| *Leptopelis christyi* | Wedge-shaped | Dense connective tissue | Sellaris and biaxial |
| *Litoria caerulea* | Thick biconcave disc | Embryonic cartilage | Ginglymus and monoaxial |
| *Lysapsus limellum* | Cylindrical | Hyaline cartilage fully mineralized | Plane and nonaxial |
| *Mantella auriantica* | Wedge-shaped | Hyaline cartilage fully mineralized | Sellaris and biaxial |
| *Mantidactylus curtus, Guibematis liber* | Wedge-shaped | Hyaline cartilage fully mineralized | Sellaris and biaxial |
| *Opisthothylax immaculatus* | Wedge-shaped | Hyaline cartilage | Sellaris and biaxial |
| *Phlyctimantis verrucosus* | Wedge-shaped | Hyaline cartilage | Sellaris and biaxial |
| *Phrynomantis bifasciatus, P. microps* | Wedge-shaped | Hyaline cartilage fully mineralized | Sellaris and biaxial |
| *Phyllomedusa sauvagii* | Thick biconcave disc | Embryonic cartilage with a small mineralized nucleus | Ginglymus and monoaxial |
| *Pseudis minuta, P. paradoxa* | Cylindrical | Hyaline cartilage fully mineralized | Plane and nonaxial |
| *Scinax acuminatus, S. fuscovarius, S. nasicus, S. squalirostris* | Thick biconcave disc | Embryonic cartilage | Ginglymus and monoaxial |
| *Trachycephalus venulosus* | Thick biconcave disc | Embryonic cartilage | Ginglymus and monoaxial |

Terminal taxa were families according to Frost et al. (2006) with some modifications, i.e., acceptance of Ranixalidae according to Van Bocxlaer et al. (2006) and usage of Calyptocephalidae instead of Batrachophrynidae according to Frost (2007). Families that contain some genera with and some genera without intercalaries were further split into terminal taxa to account for these differences. Ancestral states along the tree were traced using both *Acctran* and *Deltran* models in MacClade (Maddison and Maddison, 1998).

We used the concentrated changes test of Maddison (1990) to test the association of changes in the presence of intercalaries and arboreal habits, coding both as binary characters. This test determines the probability that various numbers of gains and losses of the dependent variable (terminal phalanx morphology) would occur in certain distinguished areas of the clade selected (defined by arboreal habits), given that a certain number of gains and losses occur in the whole clade, and given the null model that changes are randomly distributed among the branches of the clade. Because many groups of frogs contain species of somewhat climbing habits (e.g., on rocks), we used a rather strict definition of arboreality, and coded as arboreal only groups that contain a large proportion of real treefrogs that spend most of their life (except during breeding) on the vegetation,

**TABLE 4. Observed variation in penultimate phalange, *Tendo Superficialis* and M. *extensor breves digitorium* among anurans with intercalary elements**

| Species | Longitudinal crests on distal epiphysis of penultimate phalange | Shape of *Tendo Sperficialis* at insertion | *Tendo Sperficialis* insertion | Relationships between intercalary element and *Tendo Superficialis* | Length of tendon of m. *extensor breves profundus* | Hand M. *extensor brevis distalis* | Foot M. *extensor brevis distalis* |
|---|---|---|---|---|---|---|---|
| *Aglyptodactylus madagascariensis* | Present | Truncated | On apophysis | Absent | Long | Present | Absent |
| *Boophis goudoti* | Present | Fan-shaped | medial | Absent | Long | Absent | Absent |
| *B. madagascariensis* | Present | Truncated | On apophysis | Few fibers on intercalary | Long | Present | Absent |
| *Centrolene geckoideum* | Present | Bifurcated | On apophysis | Through intercalary capsule | Some are short | Present | Present |
| *C. grandisonae, C. robledoi* | Present | Bifurcated | In front of apophysis | Few fibers on intercalary | Some are short | Present | Present |
| *Chiromantis rufescens* | Present | Fan-shaped | Medial | Few fibers on intercalary | Long | Present | Present |
| *Cochranella griffithsi* | Present | Truncated | On apophysis and in front of apophysis | Few fibers on intercalary | Some are short | Present | Present |
| *C. ignota* | Present | Truncated | In front of apophysis | Few fibers on intercalary | Some are short | Present | Present |
| *Dendropsophus minutus* | Present | Two distinct branches | On apophysis | Absent | Long | Present | Present |
| *Gastrotheca christiani* | Present | Fan-shaped | Medial | Few fibers on intercalary | Long | Present | Present |
| *Hyalinobatrachium aureoguttatum* | Present | Truncated | In front of apophysis | Few fibers on intercalary | Some are short | Present | Present |
| *Hyperolius kivuensis* | Present | Truncated | On apophysis | Few fibers on intercalary | Long | Present | Present |
| *Leptopelis christyi* | Present | Truncated | Medial | few fibers on intercalary | Long | Present | Present |
| *Litoria caerulea* | Present | Fan-shaped with an incipient bifurcation | On epiphysis | Absent | Long | Present | Present |
| *Lysapsus limellus* | Present | Two distinct branches | Medial | Few fibers on intercalary | Long | Present | Absent |
| *Mantella aurantiaca* | Absent | Fan-shaped | Medial | Absent | Long | Absent | Absent |
| *Mantidactylus curtus* | Present | Fan-shaped | Medial | Few fibers on intercalary | Long | Absent | Absent |
| *Guibemantis liber* | Present | Truncated | Medial | Few fibers on intercalary | Long | Present | Absent |
| *Phrynomantis microps* | Present | Fan-shaped | On apophysis | Absent | Largo | Absent | Absent |
| *Phyllomedusa sauvagii* | Present | Fan-shaped | Medial | Absent | Long | Present | Present |
| *Pseudis minuta, P. paradoxa* | Absent | Two distinct branches | Medial | Few fibers on intercalary | Long | Present | Absent |
| *Scinax nasicus* | Present | Two distinct branches | On apophysis | Absent | Long | Present | Present |
| *Scinax squalirostris* | Present | Two distinct branches | On apophysis | Absent | Long | Present | Absent |
| *Trachycephalus venulosus* | Present | Two distinct branches | On epiphysis | Absent | Long | Present | Absent |

namely, the families Hylidae, Hemiphractidae, Cryptobatrachidae, Amphignathodontidae, Hylidae, Centrolenidae, Hyperoliidae, Ceratobatrachidae, Rhacophoridae, mantelline and boophine Mantellidae; the Arthroleptidae genus *Leptopelis*; as well as nonphrynomerine Microhylidae (arboreal taxa in subfamilies Cophylinae and Asterophryinae).

## RESULTS
### Phylogenetic Character Tracing

According to character tracing along our informal consensus tree (Fig. 1) using parsimony criteria implemented in MacClade, intercalary elements have been independently gained 5 times and lost 4 times under ACCTRAN reconstructions, and gained 7 times and lost 2 times under DELTRAN reconstructions. Plotting these gains and losses onto trees where the evolution of arboreality (as defined here in the Materials and Methods section) is traced, 3 gains and 2 losses (ACCTRAN) and 6 gains and 1 loss (DELTRAN) occur in tree sections characterized by arboreality. The concentrated changes test indicated that changes indeed occur in significantly higher proportions in arboreal clades ($P < 0.05$) and, thus, supported a possible evolutionary correlation between arboreal habits and intercalaries.

### Morphology of Terminal Phalanges

Terminal phalanges are characterized by a cartilaginous proximal epiphysis, which sometimes is mineralized; and an ossified diaphysis. The shape of the tip is the most known feature of terminal phalanx variation among anurans (e.g., Liem, 1970; Lynch, 1971, Heyer, 1975; Clarke, 1981; Drewes, 1984; Laurent, 1986; Fabrezi, 1996; Kamermans and Vences, in press).

In the species examined here, we considered four aspects in the general morphology of the terminal phalanges (Fig. 2): (a) the body of the terminal phalanx is curved downward or straight; (b) the distal end is pointed, truncated, or bifurcated with two well differentiated processes; (c) a medioventral apophysis or two ventrolateral apophyses are present or absent; and (d) the shape of the proximal epiphysis is hemispherical, ellipsoidal (transversally enlarged) or planar. The observed variation can be summarized as follows (see also Table 2): (1) Terminal phalanx straight, pointed, with a planar epiphysis. No ventral apophyses. *Lysapsus* and *Pseudis* in Hylidae (Fig. 2A). (2) Terminal phalanx curved, with a truncated tip, a single ventral apophysis and hemispherical epiphysis. *Scinax* spp. in Hylidae (Fig. 2B). (3) Terminal phalanx curved, pointed, with a hemispherical epiphysis. A mid-ventral apophysis can be present. Amphignathodontidae and some Hylidae (Fig. 2C–E). (4) Terminal phalanx straight, distal tip with two well-developed lateral processes, a single medioventral apophysis, and an ellipsoidal epiphysis. Centrolenidae (Fig. 2F). (5) Terminal phalanx curved and pointed, a mid-ventral apophysis, and an ellipsoidal epiphysis. *Leptopelis* and *Opisthothylax* (Fig. 2G). (6) Terminal phalanx curved, with a truncated tip and an ellipsoidal epiphysis. Mid-ventral or ventrolateral apophyses are present. Some Hyperoliidae (Fig. 2H–K). (7) Terminal phalanx straight, distal tip with two well-developed lateral processes, and ellipsoidal epiphysis. No ventral apophyses. Some Rhacophoroidea (Fig. 2L). (8) Terminal phalanx straight, distal tip with two well-developed lateral processes, and ellipsoidal epiphysis. Ventrolateral apophyses are present. *Aglyptodactylus* and *Boophis madagascariensis* in Mantellidae. (9) Terminal phalanx straight, distal tip with two well-developed lateral processes, and ellipsoidal epiphysis. Mid-ventral apophysis present. *Phrynomantis* in Microhylidae.

### Morphostructural Features of Intercalary Elements

The occurrence of the variation observed is detailed in Table 3 and illustrated in Figures 3 and 4. There are three morphological types of intercalary elements based on their general shape: (1) The intercalary is long and cylindrical, with planar articular surfaces: *Lysapsus* and *Pseudis* in Hylidae (Fig. 3A). (2) The intercalary is a low element that could be described as a disc with variable thickness among taxa but the height is always lower than the radius, with both articular surfaces concave. Most Hyloides (Fig. 3B–G). (3) The intercalary is a cuboid or prismatic element, wedge-shaped in lateral view, with a proximal articular surface like a rider sitting in a saddle and a distal articular surface almost planar. The proximal articular surface includes a well-developed posterior process. Ranoides (Fig. 3H–L). We observed histological differences among the tissues that form the intercalary elements (Table 3):

***Hyaline cartilage.*** Characterized by a basophile matrix and isolated chondrocytes immersed in lacunae (Fig. 4A–C). Cartilaginous intercalaries are well stained in whole-mount preparations. They are found in Ranoides, *Lysapsus* and *Pseudis* in Hylidae. Differences in cartilaginous intercalaries are described as follows: (1) matrix of proteoglycans with abundant lacunae and chondroblasts, with nuclei strongly stained (Fig. 4A); (2) uniform and abundant matrix of proteoglycans with scarce lacunae. Only nuclei of chondrocytes are observed. Peripheral mineralization is also observed (Fig. 4B); and (3) few chondrocytes are evident. There is an abundant and homogeneous mineralized matrix (Fig. 4C).

***Embryonic cartilage.*** Numerous chondroblasts in lacunae with nuclei strongly stained (Fig. 4D,E). Matrix composed by a dense arrangement of connective fibers. Characteristic of most Hyloides. In whole-mounts, intercalary elements composed by embryonic cartilage do not accept Alcian Blue coloration, although there is some variation with respect to the Alizarin staining, revealing there are calcium deposits: (1) Alizarin staining negative, mineralization is absent; (2) with one or two small mineralized centers; and (3) with a large and internal mineralized focus.

***Connective tissue.*** With extracellular matrix composed by eosinophile fibers arranged in parallel, among these fibers basophile cells with enlarged nuclei can be observed (Fig. 4F). Characteristic of *Leptopelis chrystyi*.

### Distal Epiphysis of the Penultimate Phalanx

The penultimate phalanx displays variation in the presence or absence of ventrolateral crests on its distal epiphysis. See Table 4 for the occurrence of this variation.
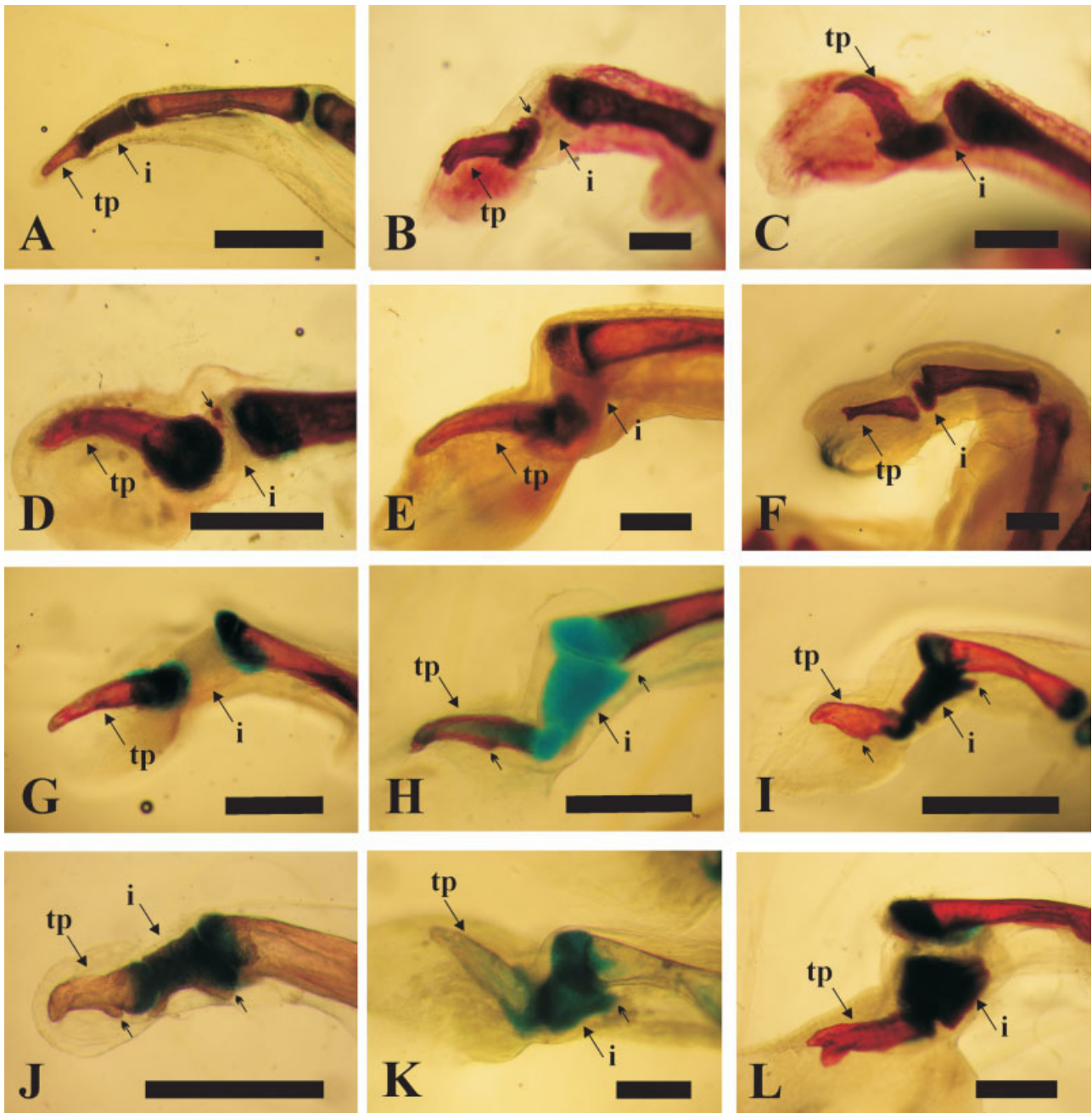
Fig. 2. Terminal phalanx and intercalary elements morphologies. **A:** *Pseudis paradoxa*. Pointed and straight terminal phalanx and cylindrical intercalary element. **B:** *Scinax fuscovarius*. Truncated and curved terminal phalanx, biconcave intercalary element. **C:** *Isthmohyla rivularis*. **D:** *Hylomantis lemur*, small arrow points mineralization. **E:** *Phyllomedusa sauvagii*. C–E: Pointed and curved terminal phalanx, biconcave intercalary element. **F:** *Centrolene notostictum*, terminal phalanx straight and T-shaped, biconcave intercalary element with a large mineralized nucleus. The small arrow indicates mineralization. **G:** *Leptopelis christyi*, curved and pointed terminal phalanx, wedge-shaped intercalary element. **H:** *Phlyctimantis verrucosus*, juvenile specimen; the adult has a mineralized intercalary element. Curved and pointed terminal phalanx, with a medioventral apophysis. The intercalary element is wedge-shaped. **I:** *Hyperolius castaneus*. Curved and truncated terminal phalanx with paired ventrolateral apophyses. The intercalary element is wedge-shaped. **J:** *Kassina senegalensis*. Curved and pointed terminal phalanx with paired ventrolateral apophyses. The intercalary element is wedge-shaped. **K:** *Afrixalus osorioi*. Curved and truncated terminal phalanx with paired ventrolateral apophyses. The intercalary element is wedge-shaped. **L:** *Chiromantis rufescens*. Terminal phalanx straight and T-shaped; the intercalary element is wedge-shaped. tp, terminal phalanx; i, intercalary element. Scale bar = 1 mm.
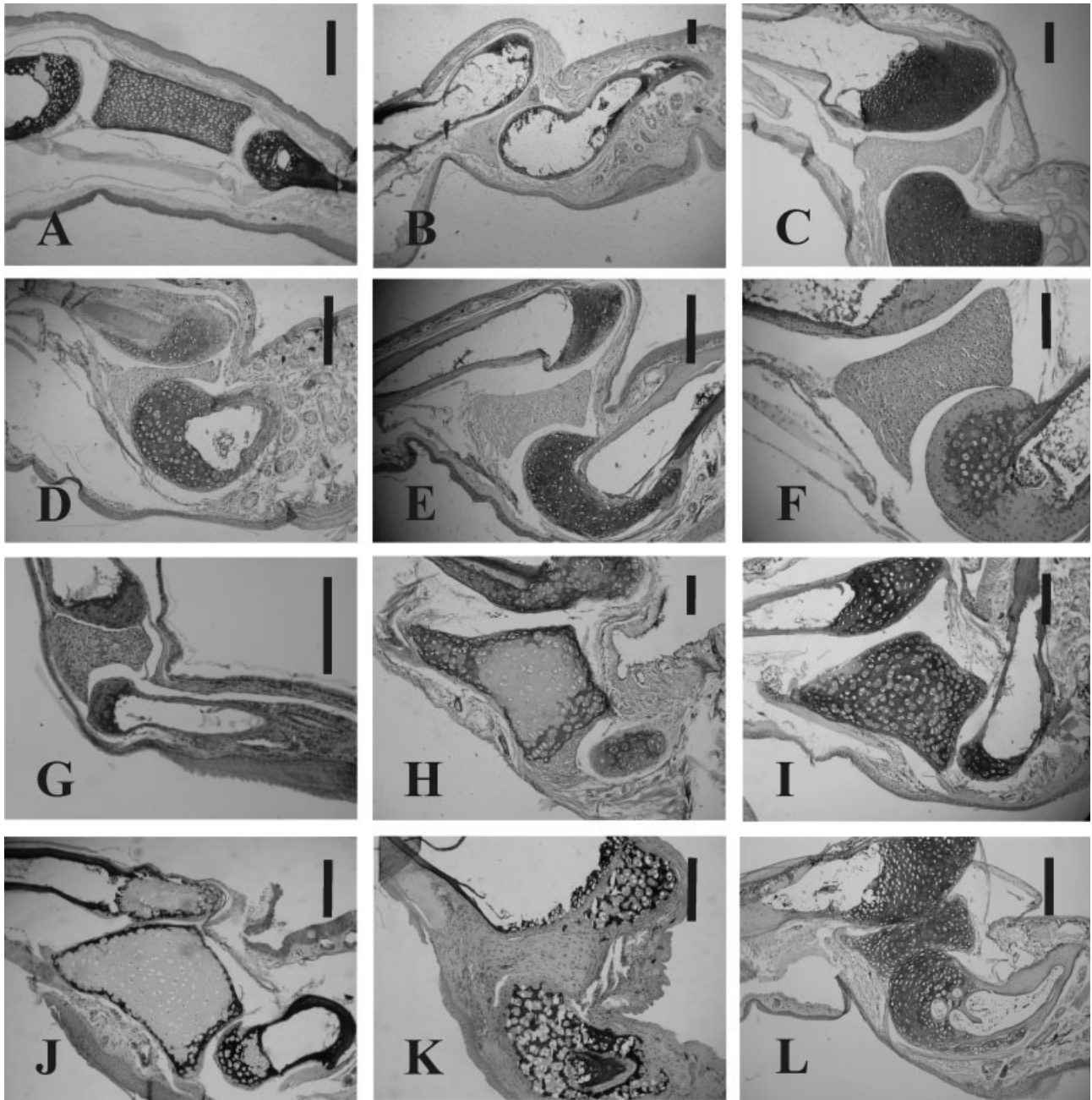
Fig. 3. Cross-longitudinal section of digit tips. **A:** *Pseudis paradoxa*. **B:** *Phyllomedusa sauvagi*. **C:** *Litoria caerulea*. **D:** *Gastrotheca christiani*. **E:** *Scinax fuscovarius*. **F:** *Hypsiboas andinus*. **G:** *Cochranella sauvagei*. **H:** *Chiromantis rufescens*. **I:** *Guibemantis timidus*. **J:** *Hyperolius kivuensis*. **K:** *Leptopelis christyi*. **L:** *Phrynomantis microps*. A,I,L: The cartilaginous intercalary fully mineralized. B–G: Embryonic cartilage with numerous chondrocytes in lacuna without matrix. H,J: Hyaline cartilage with peripheral mineralization. K: Intercalary element composed by connective tissue. Scale bar = 0.2 mm.

## Tendo Superficialis

The *Tendo Superficialis* is a flexor tendon that arises from the palmar/plantar aponeurosis, runs ventrally to the digit, and inserts on the ventral surface of the terminal phalange (Gaupp, 1896). It presents three types of variation at the insertion: (a) The *Tendo Superficialis* fibers are either parallel and end uniformly, diverge and expand in a fan-shaped end displaying an incipient division into two short branches, or diverge into two long branches. (b) The insertion of *Tendo Superficialis* is either positioned on the epiphysis of the terminal phalanx, at the base of the phalanx, on the mid-ventral surface of the phalanx, on the ventral/apophyses, or in front of the mid-ventral apophysis. (c) Fibers of the *Tendo Superficialis* may have a direct contact with the intercalary
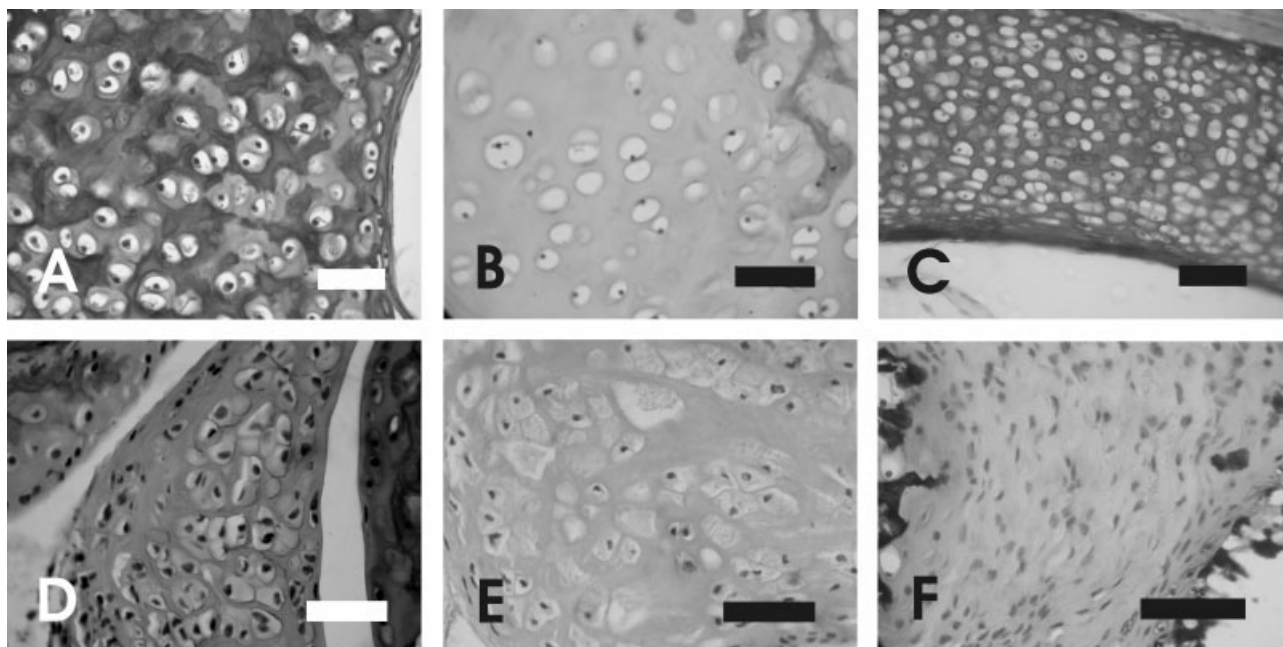
Fig. 4.   **A:** *Phrynomantis microps*. Hyaline cartilage with endochondral mineralization. **B:** *Chiromantis rufescens*. Hyaline cartilage. **C:** *Pseudis paradoxa*. Hyaline cartilage fully mineralized. **D:** *Hypsiboas andinus*. Embryonic cartilage. **E:** *Scinax acuminatus*. Embryonic cartilage. **F:** *Leptopelis christyi*. Connective tissue. Scale bar = 0.05 mm.

element, through the intercalary joint capsule (a fibrous connective layer surround the articulation limited by short ligaments, incorporating in these cases the intercalary inside, like a synovial joint), or there is not contact.

The occurrence of the variation observed is detailed in Table 4, defining the following morphological patterns: (1) *Tendo Superficialis* diverges into two short branches, inserts in front of the mid-ventral apophysis of terminal phalanx, and its fibers reach the intercalary. Centrolenidae (Fig. 5A). (2) *Tendo Superficialis* ends divided into two long branches, inserting on the mid-ventral apophysis of the terminal phalanx, and lacking of contact with the intercalary. Some Hylidae (Fig. 5B). (3) *Tendo Superficialis* ends divided into two long branches, inserting medially on the ventral surface of the terminal phalanx, and having contact with the intercalary. Some Hylidae (*Pseudis*) (Fig. 5C). (4) *Tendo Superficialis* ends uniformly, inserts on mid-ventral apophysis of terminal phalanx, and has contact with the intercalary. Mantellidae and Hyperoliidae (Fig. 5D). (5) *Tendo Superficialis* ends uniformly, inserts on mid-ventral apophysis of the terminal phalanx, and lacks contact with the intercalary. Mantellidae (Fig. 5E). (6) *Tendo Superficialis* ends uniformly, inserts medially on the ventral surface of terminal phalanx, and has contact with the intercalary. Mantellidae and Arthroleptidae. (7) *Tendo Superficialis* ends uniformly, inserts in front of the mid-ventral apophysis of the terminal phalanx, and its fibers reach the intercalary. Centrolenidae. (8) *Tendo Superficialis* ends fan-shaped, inserting medially on the ventral surface of the terminal phalanx, and lacks contact with the intercalary. Mantellidae and some Hylidae (*Phyllomedusa*). (9) *Tendo Superficialis* ends fan-shaped, inserting on the mid-ventral apophysis of the terminal phalanx, and

lacks contact with the intercalary. Microhylidae (*Phrynomantis*). (10) *Tendo Superficialis* ends fan-shaped, inserting medially on the ventral surface of the terminal phalanx, having contact with the intercalary. Amphignathodontidae. (11) *Tendo Superficialis* ends fan-shaped with an incipient division, inserting on the epiphysis of the terminal phalanx, and lacks contact with the intercalary. Some Hylidae (*Litoria*).

## Mm. Extensores Breves Profundus and Distales

All anurans have sets of deep and paired extensor muscles for the digits named the Mm. *Extensores breves profundi* (Figs. 5, 6). Fibers of each muscle arise proximally on the laterodorsal and mid-dorsal surface of each of the metapodia and converge into a long and thin tendon that runs along the mid-dorsal and laterodorsal surface of the phalanges and inserts on the dorsal surface of the terminal phalanx (Burton, 1998a,b). Sometimes, their fibers extend beyond the base of the proximal phalanx, and so the tendon is short as in *Eleutherodactylus* (Burton, 1998a,b). We observed variation in the length of the tendon (long or short), and the contact between the tendon and the intercalary element.

Some groups of anurans have an additional set of paired extensor muscles (Figs. 5, 6), the Mm. *Extensores breves distales* (Burton, 1996). Fibers of each muscle arise at the level of the distal epiphysis of the metapodia and run laterally along the digits, converge in a short tendon in the penultimate phalanx, which inserts on the dorsal surface of the terminal phalanx. Variation in the Mm. *Extensores breves distales* is related with its presence or absence in hand and foot, and the contact between the tendon and the intercalary element.
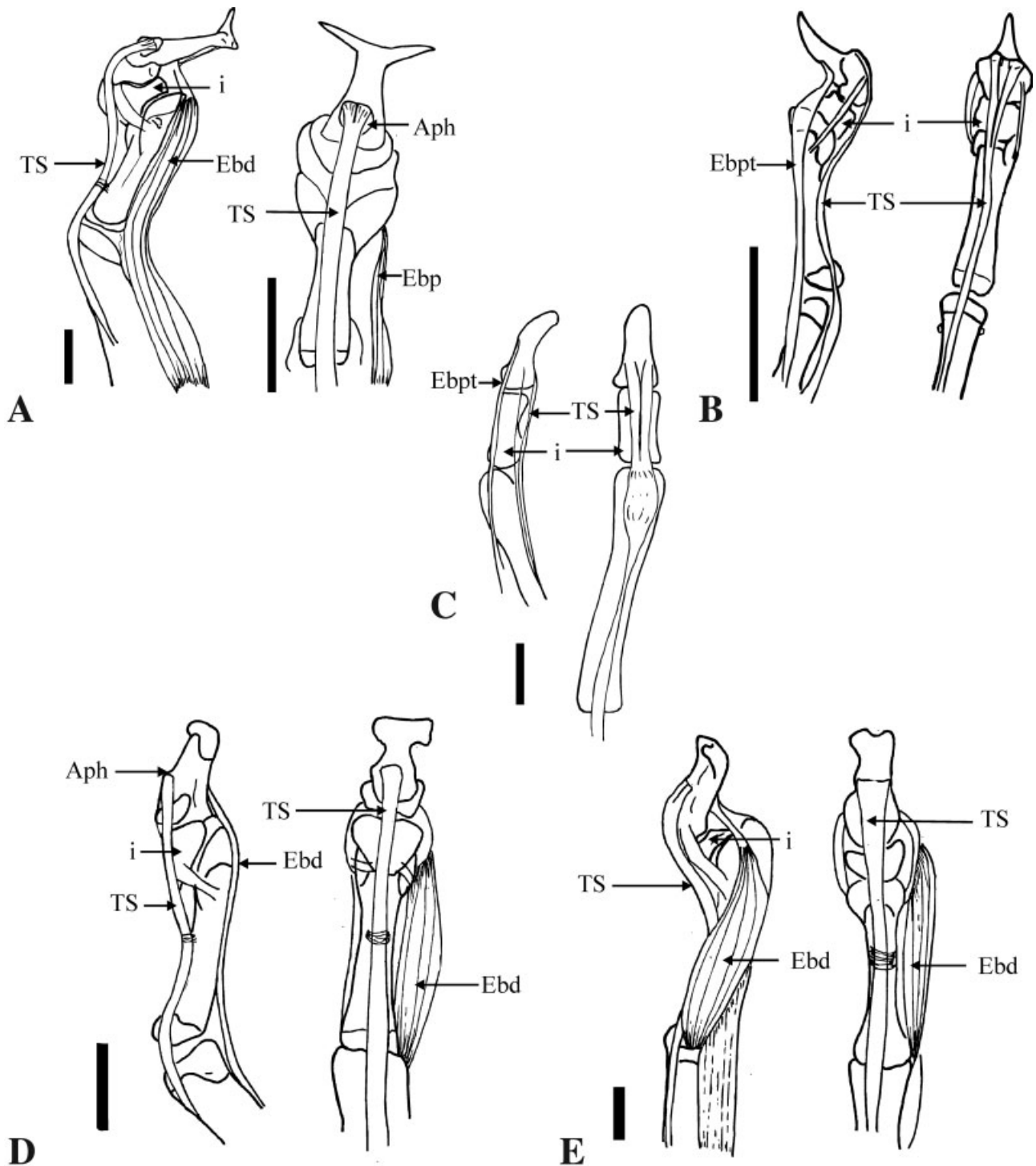
Fig. 5.　Lateral (on left) and ventral (on right) views of digits showing terminal phalanx, intercalary element, and subterminal phalanx. **A:** *Centrolene geckoideum*, digit IV, m. *extensor brevis distalis* is present, m. *extensor brevis profundus* has a short tendon, and *Tendo Superficialis* inserts on the ventral apophysis with two short branches. **B:** *Scinax squalirostris*, digit IV, m. *extensor brevis profundus* has a long tendon and *Tendo Superficialis* is divided in two long branches at the insertion. **C:** *Pseudis minuta*, digit II, m. extensor brevis profundus has a long tendon and *Tendo Superficialis* is divided in two long branches at the insertion. **D:** *Chiromantis rufescens*, digit IV, m. *extensor brevis profundus* has a long tendon, m. *extensor brevis distalis* is present and *Tendo Superficialis* is truncated at the insertion. **E:** *Aglyptodactylus madagascariensis*, digit IV, m. *extensor brevis profundus* has a long tendon, m. *extensor brevis distalis* is present and *Tendo Superficialis* is truncated at insertion. Aph, apophysis; i, intercalary element; Ebd, m. *Extensor brevis distalis*; Ebp, m. *Extensor brevis profundi*; Ebpt, m. *Extensor brevis profundi* tendon; TS, *Tendo Superficialis*. Scale bar = 1 mm.
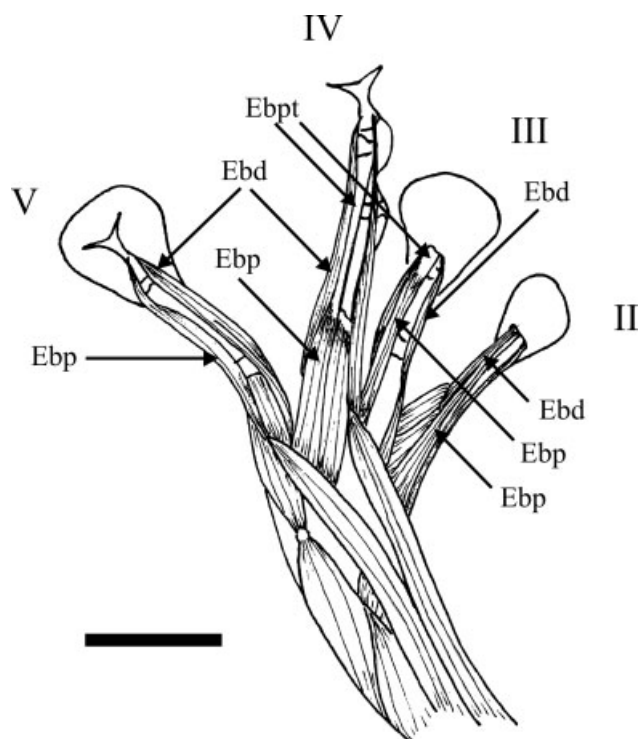
Fig. 6. *Centrolene geckoideum*, dorsal view of the left hand. Mm. *Extensores brevis profundus* have short tendons. Mm. *Extensores brevis distalis* are present. Ebd, m. *Extensor brevis distalis*; Ebp, m. *Extensor brevis profundi*; Ebpt: m. *Exensor brevis profundi tendon*. Scale bar = 50 mm.

The variation observed in these muscles is detailed in Table 4. From these data, the following main morphological character states can be distinguished: (1) Tendon of m. *Extensor brevis profundus* is long. Mm. *Extensores breves distales* are only present in hands. Some Mantellidae and some Hylidae. (2) Tendon of m. *Extensor brevis profundus* is long. Mm. *Extensores breves distales* are absent. Some Mantellidae and Microhylidae. (3) Tendon of m. *Extensor brevis profundus* is long. Mm. *Extensores breves distales* are present in hands and feet. Amphignathodontidae, some Hylidae, Hyperoliidae, Arthroleptidae. (4) The tendon of m. *Extensor brevis profundus* is short. Mm. *Extensores breves distales* are present in hands and feet. Centrolenidae (Figs. 5A, 6).

## Terminal Phalanx: Intercalary Element Articulation

Based on the shape of the articular surface of the intercalary, different kinds of movements of the terminal phalanx are observed in the studied taxa (see Table 2): (1) An intercalary with an ellipsoidal distal articular surface determines a condyloid articulation where the terminal phalanx has limited movements (lateral, forward, and backward). This is a biaxial articulation observed in Centrolenidae (Fig. 5A). (2) An intercalary element with a hemispherical distal articular surface results in an articulation in pivot, where the terminal

phalanx can rotate relative to the intercalary, allowing limited angular movements. This is a monoaxial articulation observed in Amphignathodontidae and most Hylidae (Fig. 5B). (3) An intercalary and a terminal phalanx, both with planar articular surfaces, result in a planar articulation allowing minimal lateral, forward, and backward movements. This is a nonaxial articulation observed in some Hylidae (*Pseudis* and *Lysapsus*) (Fig. 5C). (4) The distal articular surface of intercalary is almost planar and results in an articulation with limited movements (lateral, forward, and backward) of the terminal phalanx. This is a biaxial articulation typical of Ranoides (Fig. 5D,E).

## Subterminal Phalanx: Intercalary Element Articulation

Based on the shape of the intercalary, different kinds of movements of the intercalary and terminal phalanx relative to the subterminal phalanx are possible (see Table 3).

(1) The biconcave intercalary of most Hyloides results in a ginglymus type articulation with the distal epiphysis of the subterminal phalanx. This articulation allows flexion and extension movements of the intercalary–terminal phalanx complex. This monoaxial articulation is observed in Amphignathodontidae, most Hylidae, and Centrolenidae. (2) A wedge-shaped intercalary results in a sellaris or saddle type articulation with the epiphysis of the subterminal phalanx, where the intercalary articulation surface is shaped like a rider sitting in the saddle. This articulation allows lateral, forward, and backward movements of the intercalary–terminal phalanx complex and is biaxial. It is typical for Ranoides (Fig. 5D). (3) A cylindrical intercalary element defines a planar articulation with the epiphysis of the subterminal phalanx that allows reduced lateral, forward, and backward movements of the intercalary–terminal phalanx complex. It is nonaxial, and characterizes some Hylidae (*Pseudis* and *Lysapsus*) (Fig. 5C).

## DISCUSSION
## Function of the Intercalary Element

Distal phalanges, intercalary elements, muscles, and adhesive digit pads act as locally integrated units serving a common function: increasing the ability of climbing (Noble, 1931; Emerson and Diehl, 1980; Mc Allister and Channing, 1983; Paukstis and Brown, 1987, 1991; Burton, 1996, 1998; among others). It is possible to consider these units as modular parts of the limbs that integrate functionally related structures into units of evolutionary transformation (Wagner, 1996). Kamermans and Vences (in press) recently found statistical evidence for the concerted evolution of climbing habits and bifurcated terminal phalanges, although these two features were not obligatorily correlated. In this module, the intercalary element provides two additional articulations (penultimate phalanx–intercalary element and intercalary element–terminal phalanx) to the digit tip that allow additional movements (angular, side-to-side, back-and-forth, flexion and extension) that are produced by the action of the Mm. *extensores breves profundus* and *distalis* and the *tendo superficialis*. These movements increase the possibility to move the digit without detaching the digit

pad (Hanna and Barnes, 1991). Similar to the shape of terminal phalanges, there appears to be no obligatory correlation between the presence of intercalary elements and climbing habits. Several taxa, such as the mantellid genus *Aglyptodactylus*, do have intercalaries but are fully terrestrial. The microhylids of the genus *Phrynomantis*, with intercalaries, comprise species climbing on rocks such as *Phrynomantis annectens*, but no fully arboreal species. In contrast, there are many strictly arboreal taxa (e.g., in the ceratobatrachid genus *Platymantis* or the microhylid genera *Cophyla* and *Platypelis*) without intercalaries. In this context, it is interesting that Wu (1994), in a so far unpublished thesis, mentions the presence of weakly expressed, uncalcified intercalaries in some species of *Cophyla* and *Platypelis*. If confirmed by further studies, this finding would constitute a further instance of independent occurrence of intercalaries in concert with arboreal habits.

## Structural Variation of Intercalary Elements

Structural differences in the histology of the intercalary elements can be interpreted as different states of cartilage differentiation, including the states of mineralization. Intercalary elements of most Hyloides are formed by embryonic cartilage with numerous chondrocytes, with an extracellular matrix lacking chondroitin sulfate. On the contrary, most Ranoides have intercalaries formed by cartilage with a well-differentiated extracellular matrix with proteoglycans.

The extracellular matrix of the cartilage is a complex formed by glycosaminoglycans and collagen fibril/fibers, rates of synthesis of which are regulated independently. The appearance of type II collagen protein in limb mesenchyme coincides with differentiation of precursor cells into chondroblasts, whereas the synthesis of chondroitin sulfate neither is an exclusive property of, nor diagnostic for, chondrogenic cells (Hall, 2005). Hence, histological differences between the intercalaries of Hyloides and Ranoides seem to depend on the rates of differentiation of cells and of the extracellular matrix, which are slow in Hyloides and faster in Ranoides. Within Hyloides, the intercalary elements of *Pseudis* and *Lysapsus* would represent a derived condition resulting from fast differentiation rate of cartilaginous tissue as compared with most other Hyloides. Within the Ranoides, the connective intercalary element of *Leptopelis* would result from a slower differentiation rate, in which cells and matrix remain as a condensed mesenchyme. Data from cartilage in rats show a decrease in the number of chondrocytes, increase in glycogen and lipid contents, decrease in chondroitin sulfate but increase in keratan sulfate, an increase of thickness of collagen fibers, and increase of mineralization with increasing age of the animal (Hall, 2005). Some of these trends show remarkable parallels to the differences among the intercalary elements of Ranoides. The intercalary element of *Hyperolius* has few chondrocytes, whereas the intercalary elements of Mantellidae are strongly mineralized. Because several genes and cellular processes play a role in regulating pathways of cartilage differentiation (Hall, 2005), the structural differences exhibited in the intercalary elements of anurans, are probably results from differential differentiation rates that appear to be largely conserved in each lineage (Ranoides and Hyloides).

## Intercalary and Terminal Phalanx Morphologies

In Hyloides, with the exceptions of the cartilaginous and cylindrical elements of *Pseudis* and *Lysapsus*, intercalary elements are morphologically and structurally very similar. In most representatives of this clade, the proximal and distal articulations of the intercalary element show the same pattern. In contrast, the centrolenids have a distal ellipsoidal articulation similar to that found in all ranoids. *Pseudis* and *Lysapsus* display distinctive patterns with planar articulations limiting the movements. Morphological variation of the terminal phalanx within Hyloides is limited to the following: Centrolenidae, having a straight terminal phalanx with two well-developed lateral processes; *Scinax* spp., with a curved and truncated terminal phalanx; and *Pseudis* and *Lysapsus*, with a straight and pointed terminal phalanx. The truncated terminal phalanx in *Scinax fuscovarius* arises from the reduction of two lateral processes during larval development, similar to the terminal phalanx development observed in some *Leptodactylus* spp. (Noble, 1917; Fabrezi, 1996). Many Hyloides have a curved and pointed terminal phalanx that was considered as a synapomorphy to join them with the Hylidae (Laurent, 1986; Ford and Cannatella, 1993). The observed variation in the morphology of terminal phalanges and articular surfaces of the intercalary in Centrolenidae suggests coordinated changes in both skeletal components.

The morphology of the intercalary within Ranoides displays scarce variation defining the same distal and proximal articulations. In this clade, the shape of the terminal phalanx with two lateral processes is widespread, although there are pointed and truncated terminal phalanx within Laurentobatrachia (Liem, 1970; Drewes, 1984; Kamermans and Vences, in press).

## Variation of Muscles and Tendons

The Mm. *Extensores breves profundus* are very uniform, and commonly their fibers extend along the metapodials up to form a tendon at the level of the proximal phalanx to insert dorsally on the terminal one. The only variation related to these muscles was observed in Centrolenidae (including *Allophrynae*), which displays elongated muscles forming a short tendon up to the level of the terminal phalanx. This condition has also been described for other anurans reported to be climbers or at least scansorials in their habits, such as the hyloid genus *Eleutherodacylus* (Burton, 1996, 1998a,b), which however, lack intercalaries. Differently from *Eleutherodactylus*, the Centrolenidae have also additional sets of muscles *Extensores breves distalis*. The presence of Mm. *Extensores breves distalis* in hands has been found to be strongly related to arboreality (Burton, 1996, 1998a,b, 2004). They are present in Hyloides and some Ranoides, their presence is variable within Mantellidae, and they are absent in *Phrynomantis*. When these muscles are present, there is no variation among the species. Summarizing, our results showed the morphological variation recorded in intercalary elements, terminal phalanx, *Tendo superficialis*, and muscles *Exensores digitorum* seems to be constrained to few combinations only.

## Phylogenetic Interpretations

In recent hypotheses of anuran phylogeny, the neobatrachians are composed of two sister clades, Hyloides and Ranoides (Frost et al., 2006), with 12 terminal taxa with intercalary elements (Fig. 1). As shown herein, there are morphostructural differences between the intercalary of Hyloides (patterns 1, 2, and 3) and Ranoides (patterns 5, 6, and 7). Within hyloids, intercalary elements are present in five taxa of Nobleobatrachia; they were probably lost in Brachycephalidae and twice within the Leptodactyliformes. Centrolenidae (including *Allophryne*) and also some leptodactylids have the set of muscles that integrate the articulation with intercalary elements even though the intercalary element is absent in some of them. The morphostructural characteristics of the intercalary elements have only been strongly modified in two hylid genera, *Pseudis* and *Lysapsus* (pattern 4). All Hyloides share a biconcave intercalary element formed by embryonic cartilage, except for these two genera. Variations in the morphostructural patterns described by us are mainly related to intercalary mineralization. The *Tendo superficialis* appears with a characteristic bifurcated pattern at insertion in Hylidae, and this same pattern is observed also in Centrolenidae. Among Hyloides, the articulation of the intercalary element and terminal phalanx, shape of terminal phalanx, and characteristics of the Mm. *Extensores breves profundus* and *distalis* observed in Centrolenidae seem to be derived in an integrated manner, which is in agreement with phylogenetic hypotheses placing centrolenids as not closely related to other Neotropical treefrogs (e.g., Frost et al., 2006).

Different from Hyloides, Ranoides share a wedge-shaped intercalary element composed of hyaline cartilage, with the exception of *Leptopelis*. Among the Ranoides, the intercalary would have appeared independently in three clades Laurentobatrachia, Microhylidae (genus *Phrynomantis*), and Rhacophoroidea. The intercalary elements of Rhacophoroidea have been interpreted as a synapomorphy for the clade (Frost et al., 2006). The genus *Laliostoma*, which is nested within Rhacophoroidea, has no intercalary element (Glaw et al., 1998) and its condition, therefore, is to be interpreted as secondary loss. In Laurentobatrachia, the intercalary of *Leptopelis* presents the same shape of the intercalary of Ranoides but is formed by dense connective tissue. Drewes (1984) suggested that the histological differences between the intercalary elements of *Leptopelis* and hyperoliids are arguments to reject their homology. Even if the intercalary elements of Rhacophoroidea appear as synapomorphy, their reappearance does not suggest changes from the ancient or plesiomorphic condition.

## Evolutionary Interpretations

From an evolutionary perspective, morphological novelty was defined as "… a structure that is neither homologous to any structure in the ancestral species nor homonymous to any other structure of the same organism." (Müller and Wagner, 1991, p. 243). In accordance with this definition, the intercalary element of anurans seems to be a morphological novelty that appeared in neobatrachians. No extant or fossil species belonging to any basal anuran lineage (i.e., not belonging to the Neobatrachia) is known to have intercalary elements. The intercalary element is not equivalent or homologous to other limb skeletal components and is not incorporated in the autopodia limb plan. Some invariable features characterize the intercalary elements: (1) they are always present in hands as well as feet, (2) they are always present between the penultimate and terminal phalanges, (3) they are present only in those taxa having a complete set of phalanges. These features suggest some level of homology and indicate they may have arisen only once, early in the neobatrachian history.

Paukstis and Brown (1987, 1991) explained quantitative variation of intercalary elements related to habitat or life style. They noticed a decrease in the thickness of the intercalary and size of digital pad in some species of Hyloides, and in *Mantella aurantiaca*, and the loss of digital pads in species of *Kassina* that may be associated with terrestrial habitat. The unusual features of the intercalary of the aquatic *Pseudis* and *Lysapsus* were interpreted as advantages to increase digit lengths for supporting extensive webbing on the feet, and for grasping aquatic vegetation with the hands. However, such a scenario fails to explain the presence of intercalary elements and well-developed digital pads in the aquatic *Litoria aurea* and the terrestrial *L. lesueuri*, where the loss of arboreal habits may be recent or the evolutionary rate of change in the intercalary is low (Paukstis and Brown, 1991). In our character tracing, under both ACCTRAN and DELTRAN reconstructions, more gains of intercalary elements than losses were reconstructed (Fig. 1). The most unequivocal loss is that of *Laliostoma*, a species that is phylogenetically nested in a clade (Rhacophoroidea) where otherwise intercalaries are universally present. However, there seem to be many instances of fully terrestrial or aquatic frogs with intercalaries, nested in otherwise largely arboreal clades, indicating that these elements do not cause any strong disadvantages to nonarboreal frogs and, therefore, are not under strong selective pressure to be lost to the evolutionary loss of arboreal habits.

The intercalary element is a modular part functionally integrated with other musculoskeletal structures and specializations of the tegument. Explaining the loss of its functional capability is not easy. The presence of intercalaries reveals strong constraints: (1) The intercalary elements are integrated in the developmental limb programs in those lineages in which they are present. This fact was demonstrated for experiments of digit regeneration in *Hyperolius viridiflavus* (Richards et al., 1975). (2) These elements show conservative patterns of variation, similar to other related structures. Occasional changes in this modular unit sometimes seem to be dissociated. For example: the absence of digital pads in *Pseudis paradoxus* is not related to the reduction of intercalary elements, and the absence of ventrolateral crests in the penultimate phalanx limits extension and flexion movements in the articulation in *Phyllomedusa*, *Mantella*, *Pseudis*, and *Lysapsus*, independently of the morphostructural features of the intercalary and development of the digital pad. Even if these taxa share the capability to oppose fingers, the absence of ventrolateral crests in the penultimate phalanx in all digits cannot be associated with this feature. In other cases, reappearances of this system, which are interpreted in Centrolenidae and Rhacophoroidea, may suggest coordinated

derived changes in distal articulation and muscles (Centrolenidae) or not (Rhacophoroidea); (3) *Allophryne ruthveni* lacks intercalary elements but presents a well-developed digital pad, short tendons of Mm. *extensores breves profundus*, a biaxial articulation between penultimate–terminal phalanges (Fabrezi and Langone, 2000), and Mm. *extensores breves distalis*. These features could be either interpreted as remnants from a condition with the intercalary element present ancestrally but that is today lost, or as a first step before a reappearance of an intercalary. However, both alternatives involve a similar level of speculation.

Due to the absence of intercalaries in possible outgroups, it is currently not possible to polarize any of the intercalary shapes typical for ranoids or hyloids (i.e., wedge-shaped hyaline cartilage vs. biconcave embryonic cartilage) as plesiomorphic or apomorphic. However, because in ranoids there are three phylogenetically independent origins of the intercalary and in each case wedge-shaped elements are observed, it is unlikely that this state represents a derived condition. In hyloids, it seems more likely that the intercalaries evolved only once and were subsequently lost in two lineages, re-evolving again in centrolenids. As a conclusion, if indeed early neobatrachians were characterized by intercalary elements or by the genetic basis to develop these, then it could by hypothesized that (1) the ranoid ancestor has lost this original neobatrachian state but this state was then re-acquired three times independently in Ranoides, and that (2) the biconcave element of hyloids is derived from this original neobatrachian state.

From our analysis, we conclude that the intercalary element is a morphological novelty and forms part of a modular unit in which other musculoskeletal structures and the tegument are functionally integrated. This modular unit, or its developmental genetic basis, may have originated early in the history of the Neobatrachia and has further evolved in parallel in Hyloides and Ranoides. We have here described morphological variation in this modular unit, pointing out those traits that could be useful for systematic analyses. However, variation in the modular unit formed by the intercalary elements is limited and suggests few opportunities of change.

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