

Patterns of Carpal Development Among Anuran Amphibians

Marissa Fabrezi^{1*} and Mónica Barg²

¹CONICET, Museo de Ciencias Naturales, Universidad Nacional de Salta, Salta, Argentina

²CONICET, Laboratorio de Ecofisiología, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Mar del Plata, Argentina

ABSTRACT The unity and diversity of developmental processes in the vertebrate limb have singular importance in the interpretation of evolutionary hypotheses of tetrapod diversification. In anurans, the intraordinal diversity of forelimbs seems to be related to the fusion of distal carpals, whereas proximal carpals are invariable. However, there are different ontogenetic pathways involved in the differentiation of proximal carpals. This study presents a comparative analysis of early developmental features in one archeobatrachian and 23 neobatrachian species representing five families and explores the variability in the differentiation of carpal cartilages. We found new evidence supporting the presence of an embryonic inter-

medium that incorporates with the ulnare. Difference between the pipid *Xenopus* and the neobatrachians is interpreted as a change in the rate of differentiation of Distal Carpal 5 that does not affect the developmental pattern of digits. The developmental variability exhibited by the intermedium, radiale, and Element Y is combined in patterns that converge on the same adult carpal morphology among neobatrachians; these patterns appear to contain potentially useful phylogenetic information. *J. Morphol.* 249:210–220, 2001. © 2001 Wiley-Liss, Inc.

KEY WORDS: Anura; forelimb; carpal pattern; developmental variability

The differentiation, structure, and variation in tetrapod limbs were reviewed by Shubin and Alberch (1986), who proposed a general morphogenetic hypothesis to explain the tetrapod limb plan and its diversification. Fabrezi (1992) then applied the Shubin and Alberch model to an interpretation of the skeletal pattern of the forelimb of adult anurans.

Among adult anurans there are at least 10 different carpal morphologies defined by the number of individual elements present and their spatial relationships (Fabrezi, 1992). Six of these occur in so-called “advanced” anurans or neobatrachians (Ford and Cannatella, 1993). These carpal patterns are believed to have arisen by ontogenetic fusion of distal carpals (Holmgren, 1933; de Saint-Aubain, 1981; Shubin and Alberch, 1986; Fabrezi, 1992; Fabrezi and Alberch, 1996). Whereas intraordinal diversity is exhibited in the association of distal carpals, the more proximal—the ulnare, radiale, and Element Y—would seem to be conservative elements of the carpus. However, different underlying ontogenetic pathways are involved in carpal differentiation (Fabrezi, 1992; Fabrezi and Alberch, 1996). Fabrezi and Alberch (1996) reported carpal developmental variability among seven species from different families and discussed the homologies of the skeletal elements. They also proposed a scheme of hand development, criteria to identify the carpal elements, and a terminology useful for the anatomical descriptions of anuran forelimbs. These authors suggested that there is relevant intraordinal diversity in limb

development that needs more comprehensive examination.

In that regard we present here a comparative analysis of carpal development in one archaeobatrachian (the pipid *Xenopus laevis*) and 23 neobatrachian species representing five anuran families that have similar adult carpal morphology. The goals of this study are to: 1) assess intraordinal developmental variability in cartilage differentiation, and 2) examine developmental features in taxa having similar adult patterns and determine the phylogenetic extent of that variability in related taxa.

MATERIALS AND METHODS

Larval specimens of 23 anuran species were analyzed. Species and collection data are listed in the Appendix. Neobatrachian tadpoles were staged by Gosner’s table (1960) and *Xenopus* tadpoles by Nieuwkoop and Faber’s table (1956). The larvae were all selected between Stages 31 and 42 (Gosner, 1960) and 51 and 64 (Nieuwkoop and Faber, 1956), when

Contract grant sponsors: Consejo Nacional de Investigaciones Científicas y Técnicas and Consejo de Investigación de la Universidad Nacional de Salta.

*Correspondence to: Marissa Fabrezi, Museo de Ciencias Naturales, Universidad Nacional de Salta, Mendoza 2, 4400-Salta, Argentina. E-mail: museo@ciunsa.edu.ar

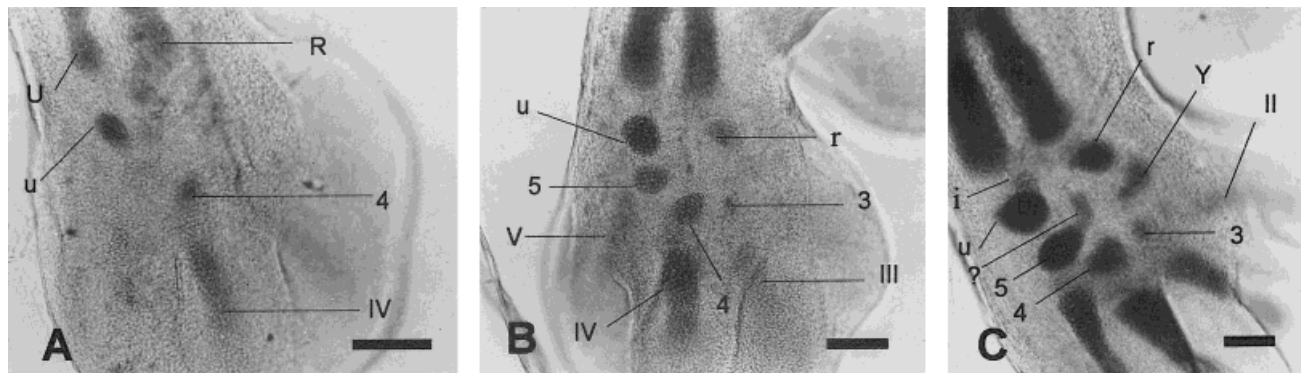


Fig. 1. Carpal development in *Xenopus laevis*. **A:** Larval Stage 51. The primary axis condensations are well defined and are the first to appear. This morphology is consistent with the original definition of the primary axis (Burke and Alberch, 1985). **B:** Larval Stage 54. Differentiation of the radiale and elements of Digits V and III has started. **C:** Larval Stage 58. Most carpal condensations are present. The ulnare and Distal Carpal 5 have conspicuous processes that suggest the participation of more than one embryonic element. In the ulnare, the process is interpreted as the intermedium, whereas the identification of the process of Distal Carpal 5 is undetermined. Scale bar = 0.1 mm. 5, 4, 3, distal carpals; i, intermedium; r, radiale; R, radius; u, ulnare; U, ulna; V, IV, III, II, metacarpals; Y, Element Y; ?, unidentified process of Distal Carpal 5.

limb chondrogenesis occurs. All specimens are deposited in Museo de Ciencias Naturales (MCN), Universidad Nacional de Salta, Argentina.

The specimens were cleared and double-stained for cartilage and bone as whole mounts (Wassersug, 1976). Observations and photographs were made using a compound microscope.

Terminology and criteria for identifying embryonic skeletal condensations follow those of Shubin and Alberch (1986) and Fabrezi and Alberch (1996).

RESULTS

Developmental Features in the Archaeobatrachian *Xenopus laevis*

The adult carpus of *Xenopus laevis* consists of the ulnare, radiale, Element Y, four distal carpals, and one prepollical element.

The first forelimb cartilages developed are the ulna, radius, ulnare, Distal Carpal 4, and Metacarpal IV (Fig. 1A). These condensations form a column called the "primary axis" (Burke and Alberch, 1985; Shubin and Alberch, 1986). Subsequent development involves the differentiation of Distal Carpals 5 and 3, Metacarpals V and III, and the radiale (Fig. 1B). Later, a cartilaginous process develops from the ulnare (identified as the intermedium) and Distal Carpal 5 has a lobular process. Element Y, Distal Carpal 2, and Metacarpal II also are differentiated (Fig. 1C). The radiale and Element Y seem to be formed by single condensations. The last carpal element to appear is the single prepollical element. The sequence of digit formation is IV-V-III-II.

Developmental Features in Selected Neobatrachians

Bufo. The carpal morphology of bufonids is characterized by the presence of the ulnare, radiale, Element Y, Distal Carpal 5-4-3, Distal Carpal 2, and

elements of the prepollex (Fabrezi, 1992). Adults of *Bufo fernandezae*, *B. paracnemis*, *B. spinulosus*, and *Melanophryniscus stelzneri* share this morphology.

The first, well-defined condensations appear when Digit IV is externally apparent on the forelimb bud. These condensations are the ulna, radius, radiale, Metacarpal IV, and the ulnare, which is synchronotically united to a large distal carpal (Fig. 2A). This latter is thought to represent a primary fusion of Distal Carpals 5 and 4 (Shubin and Alberch, 1986; Fabrezi and Alberch, 1996). Subsequently, ulnare and Distal Carpal 5-4 separate, Metacarpal V arises, and a lateral process develops from the ulnare. We interpret this process of the ulnare to be the intermedium (Fabrezi and Alberch, 1996). Progressive differentiation of three cartilaginous primordia distal to the radiale occurs while Distal Carpal 3 and Metacarpals III and II become defined (Fig. 2B). These condensations distal to the radiale fuse in two steps. The two condensations, found in the dorsal plane (Fig. 2B,C), form a bilobed cartilage that then incorporates the third condensation, located in the ventral plane. The resulting cartilage, which is Element Y, is pyramid-shaped with a concave base that articulates with Distal Carpal 2 (Fig. 2D). The proximal prepollical cartilage arises before the three condensations of Element Y fuse (Fig. 2C). Distal Carpal 2 appears before the condensations of the Element Y-complex are fully fused. Distal Carpal 3 fuses with Distal Carpal 5-4 before the differentiation of the distal prepollical element. The sequence of digit formation is IV-V-III-II.

Leptodactylidae. In most adult leptodactylids, the carpus is composed of the ulnare, radiale, Element Y, Distal Carpal 5-4-3, Distal Carpal 2, and elements of the prepollex; some taxa have a free Distal Carpal 3 and in others Distal Carpal 2 is fused to Element Y (Fabrezi, 1992). In adults of the species analyzed here the carpal morphology is of

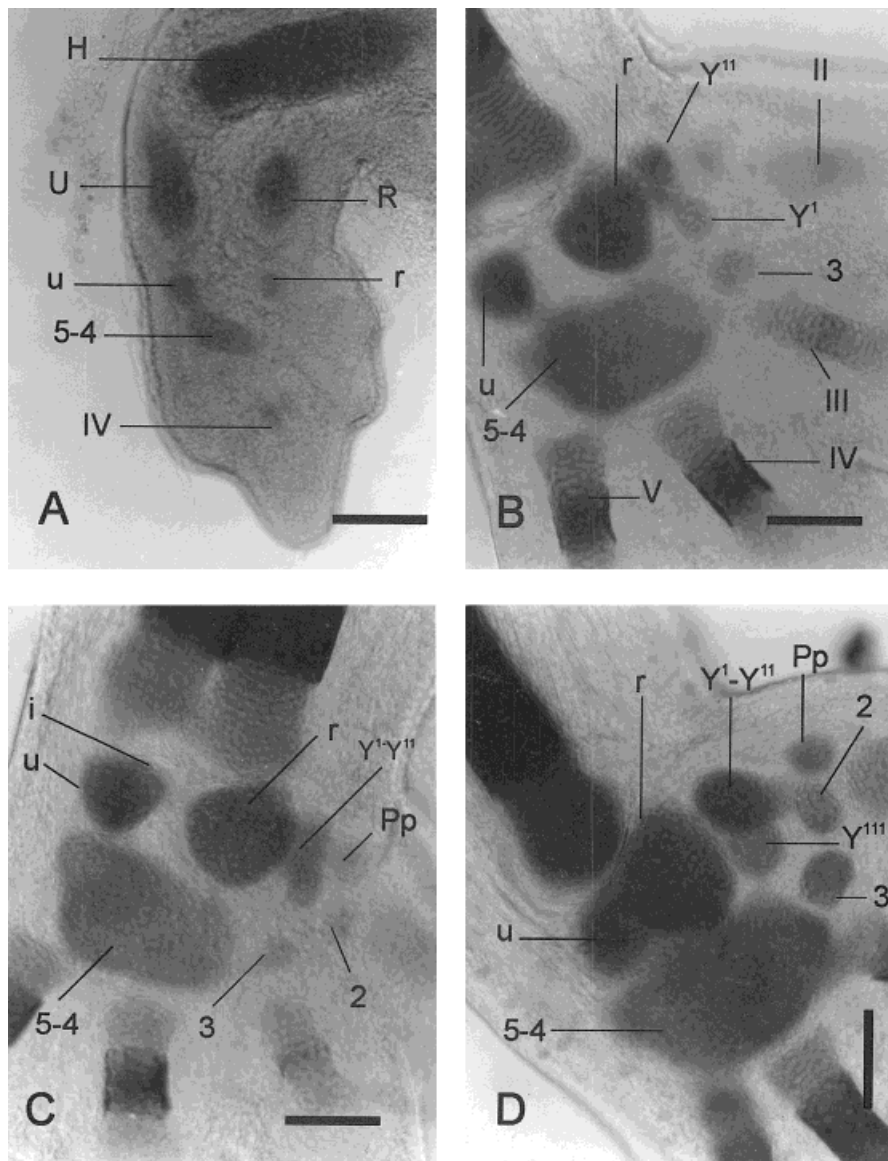


Fig. 2. Carpal development in selected bufonids. **A:** *Bufo fernandezae*, larval Stage 32. The primary axis and the radiale condensations are present; distal carpal (Carpal 5-4) has a cartilaginous connection with the ulnare. Digit IV is differentiated on the limb bud. **B:** *Bufo paracnemis*, larval Stage 35. Distal carpal 3 and two cartilaginous primordia distal to the radiale that will form Element Y are differentiated. **C:** *Bufo paracnemis*, larval Stage 36. The first fusion of condensations in the Element Y complex has begun and a bilobed cartilage is formed. Distal Carpal 2 is present. The ulnare has an intermediate process. **D:** *Bufo paracnemis*, larval Stage 37. Distal Carpal 2 and the proximal prepollical element are well differentiated. In the Element Y complex, the third embryonic cartilage that is placed in the ventral plane is not yet incorporated. Scale Bar = 0.1 mm. 5-4, 3, 2, distal carpals; i, intermedium; Pp, proximal prepollical element; r, radiale; R, radius; u, ulnare; U, ulna; V, IV, III, II, metacarpals; Y', Y'', Y''', cartilaginous condensations of Element Y.

the first type, except for *Leptodactylus bufonius* and *L. latinasus*, in which Distal Carpal 2 and Element Y are fused.

Well-developed condensations of the primary axis, a free intermedium, and a cartilaginous primordium (radiale) distal to the radius are present in early developmental stages (Figs. 3A, 4A). In all leptodactylids examined, the intermedium arises as a smaller, separate condensation from the ulnare (Figs. 3, 4). Distal Carpals 5 and 4 appear as separate condensations in *Ceratophrys cranwelli* (Fig. 3A), *Lepidobatrachus laevis*, *L. llanensis*, *Telmatobius cf. marmoratus* (Fig. 4A), *Leptodactylus bufonius*, *L. chaquensis*, and *L. latinasus*. Distal Carpal 4 is smaller than Distal Carpal 5 and Metacarpal IV is more developed than Metacarpal V. In *Odontophrynus lavillai*, Distal Carpals 5 and 4 originate as a single condensation that is interpreted as a primary fusion of these carpals. In the species with separate condensations of Distal Carpal 5 and Distal

Carpal 4, differentiation of Distal Carpal 3 takes place after the formation of Distal Carpal 5-4 (Fig. 3B). Lateral to the radiale, a free cartilaginous primordium differentiates in *C. cranwelli* (Fig. 3B), *Lepidobatrachus* spp. and *Odontophrynus lavillai* (Fig. 4D) and in the same location a lateral process is developed from the radiale in *T. cf. marmoratus* (Fig. 4B) and *Leptodactylus* spp. (Fig. 4C). The free condensation or the lateral process of the radiale ultimately is incorporated into the adult radiale. Three cartilages fuse to form Element Y in *C. cranwelli* (Fig. 3B,C), *Lepidobatrachus* spp., *O. lavillai*, and *T. cf. marmoratus*. In *Leptodactylus* spp., one condensation forms Element Y (Fig. 4C). In *Leptodactylus bufonius* and *L. latinasus* Distal Carpal 2 originates before the differentiation of the proximal prepollical condensation, and it fuses to Element Y after the fusion of Distal Carpal 3 with Distal Carpal 5-4. The sequence of digit formation is IV-V-III-II.

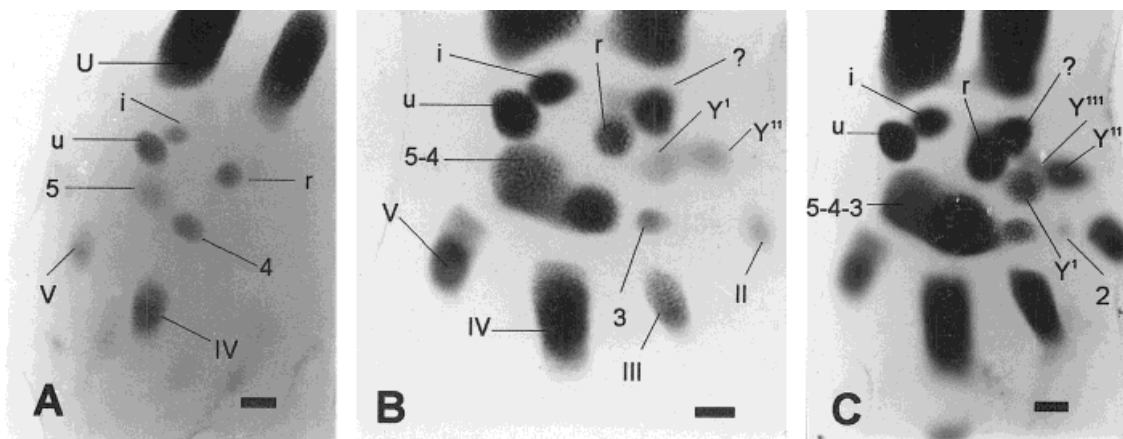


Fig. 3. Carpal development in the leptodactylid *Ceratophrys cranwelli*. **A:** Larval Stage 32. The ulna, ulnare, Distal Carpal 5 and 4, and Metacarpal IV form the primary axis. Also, the radiale, intermedium, and Metacarpal V are well defined. **B:** Larval Stage 34. Progressive differentiation of a separate element lateral to the radiale, two primordia of Element Y and Distal Carpal 3 takes place simultaneously with the fusion of Distal Carpal 5 and Distal Carpal 4. **C:** Larval Stage 37. Distal Carpal 2 and the third embryonic cartilage of Element Y are developed. Proximally, the ulnare and the intermedium are still separate and radiale condensations have started to fuse. Scale Bar = 0.1 mm. 5, 4, 3, 2, distal carpals; i, intermedium; r, radiale; u, ulnare; U, ulna; V, IV, III, II, metacarpals; Y¹, Y¹¹, Y¹¹¹, cartilaginous condensations of Element Y; ?, unidentified condensation of the radiale.

Pseudidae. The carpal morphology found in adult pseudids (represented by *Pseudis paradoxa*) is characterized by the presence of the ulnare, the radiale, Element Y, Distal Carpal 5-4-3, Distal Carpal 2, and elements of the prepollex (Fabrezi, 1992).

First condensations of the ulna, radius, and ulnare are present before Digit IV is evident externally on the limb bud (Fig. 5A). Subsequently, a round postaxial distal carpal and Metacarpal IV appear and two cartilages distal to the radius are well defined (Fig. 5B). Development then follows the same pattern described for hylids.

Hylidae. In adult hylids examined (i.e., *Hyla andina*, *Phrynohyas venulosa*, *Phyllomedusa boliviana*, *P. hypochondrialis*, *P. sawagii*, *Scinax acuminatus*, *S. fuscovarius*, and *S. nasicus*) the ulnare, the radiale, Element Y, Distal Carpal 5-4-3, Distal Carpal 2, and elements of the prepollex are present (Fabrezi, 1992).

Early larval stages possess well-developed condensations in the primary axis and two similar (in size and shape) condensations distal to the radius (Fig. 5C,D). The distal carpal in the primary axis is interpreted as the primary fusion of Distal Carpals 5 and 4 (Fig. 5C,D). Cartilaginous connections between the ulnare and this distal carpal occur in *Scinax* spp. (Fig. 5C,D), *Phrynohyas venulosa*, and *Hyla andina*. Metacarpal IV is always more developed than Metacarpals V and III, and Distal Carpal 3 appears as a distinct condensation (Fig. 5C-J). A cartilaginous process that we interpreted to be the intermedium develops from the ulnare (Fig. 5C-J). The two condensations distal to the radius start to fuse concomitant with the differentiation of the first condensation of Element Y (Fig. 5E). Subsequent development involves the differentiation of two more condensations of Element Y (Fig. 5 F-J) and their

fusion in a pyramidal cartilage or definitive Element Y. The origin of Distal Carpal 2 and the proximal prepollical element takes place before the fusion of Distal Carpal 3 and Distal Carpal 5-4. The sequence of digit formation is IV-V-III-II.

Microhylidae. The carpus of most adult microhylids is composed of the ulnare, the radiale, Element Y, Distal Carpal 5-4-3, Distal Carpal 2, and elements of the prepollex. In a few taxa, Distal Carpal 2 is fused with Element Y, and in others a large distal carpal is formed by the fusion of the four distal carpals and Element Y (Howes and Ridewood, 1888; Fabrezi, 1992). Adults of *Dermatonotus muelleri* and *Elachistocleis bicolor* have the first-mentioned morphology.

Early larval stages display well-defined condensations of the ulnare, intermedium, Distal Carpal 5, radiale, and Metacarpals V and IV. Metacarpal IV is the most developed (Fig. 6A). In some specimens, cartilaginous connections between the ulna and ulnare, and the ulnare and Distal Carpal 5, can be observed. Development progresses with the origin of distinct condensations of Distal Carpal 4 (Fig. 6B,C). Fusion of Distal Carpals 5 and 4 takes place before differentiation of a single cartilaginous primordium of Element Y (Fig. 6B). Differentiation of Distal Carpal 2 and fusion of Distal Carpal 3 to Distal Carpal 5-4 take place before the fusion of ulnare and intermedium (Fig. 6D). The sequence of digit formation is IV-V-III-II.

We obtained a picture of hand development in a larval stage of an unidentified species of *Dasypops* that clearly shows the branching end of the ulna segmenting to produce the ulnare and intermedium (Fig. 7).

Developmental features for each taxon are summarized in Table 1.

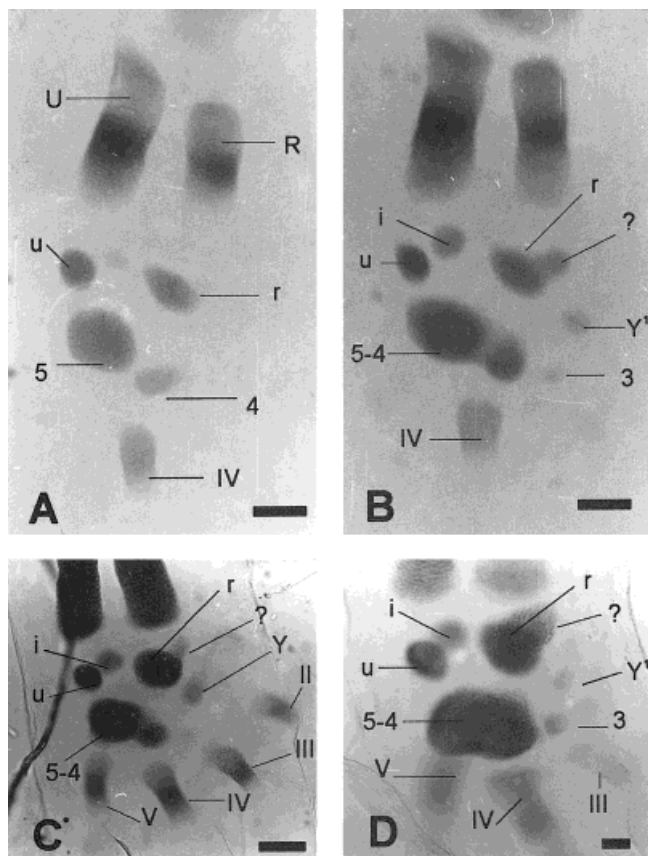


Fig. 4. Carpal development in selected leptodactylids. **A:** *Telmatobius cf. marmoratus*, larval Stage 32. Primary axis and radiale condensations are present. Distal Carpals 5 and 4 are separate. **B:** *Telmatobius cf. marmoratus*, larval Stage 34. A proximal process develops from the radiale and the first condensation of Element Y complex arises. Also, the incipient condensation of the intermedium is visible. Metacarpal IV is the only metacarpal differentiated at this stage. **C:** *Leptodactylus chaquensis*, larval Stage 35. The ulnare and intermedium are separate. A proximal process is developed from the radiale. Fusion of Distal Carpals 5 and 4 has occurred and Distal Carpal 3 and the single condensation forming Element Y have begun to appear. **D:** *Odontophrynus lavillai*, larval Stage 35. The ulnare, the intermedium, Distal Carpals 5-4, 3, and 2, two condensation that will form the radiale, and the first cartilage of Element Y, are differentiated. Scale Bar = 0.1 mm. 5, 4, 3, 2, distal carpals; i, intermedium; r, radiale; u, ulnare; U, ulna; V, IV, III, II, metacarpals; Y¹, Y¹¹, Y¹¹¹, cartilaginous condensations of Element Y; ?, unidentified condensation of the radiale.

DISCUSSION

Carpal morphology in anurans seems to be conserved in a basic plan in which adult variation is limited to distal carpal associations. However, different underlying ontogenetic pathways produce the uniform carpal pattern observed involving the ulnare, radiale, and Element Y (Fabrezi and Alberch, 1996). These elements develop from different axes: postaxial and preaxial. Differentiation of limb elements in postaxial axis is a focus of interest in the study of the tetrapod limb because it involves the origin of digits (i.e., Coates and Clack, 1990; Sordino et al., 1995), whereas differentiation in the preaxial

axis could be an important issue for discussing amphibian limb diversification.

Differentiation in the Postaxial Axis

Limb development proceeds in a proximodistal direction (Shubin and Alberch, 1986). The primary axis and digital arch form the postaxial axis of limb development that produces the differentiation of ulnare, intermedium, centrals, distal carpals, and metacarpals in tetrapod limbs (Shubin and Alberch, 1986). In reptiles, Burke and Alberch (1985) recognized the primary axis as a column of precociously developed elements formed by the ulnare, Distal Carpal 4, and Metacarpal IV. The primary axis is present during early limb development in anurans and amniotes (Shubin and Alberch, 1986). The ulnare gives rise to the digital arch. The digital arch is defined as the sequential bifurcation of distal carpals that yields a distal carpal proximally and a metacarpal distally, oriented along the anterior-posterior axis (Shubin and Alberch, 1986). Fabrezi and Alberch (1996) recognized the digit posterior to the primary axis as Digit V and proposed that this is evidence of the loss of Digit I in anurans. However, the participation of Digit V in the primary axis and digital arch has not been discussed and there are few references on the origin of Digit V in the literature. Shubin and Alberch (1986) only mentioned the differentiation of Digit V as a *de novo* condensation in turtles. We observed a primary axis, in full agreement with the definition in *Xenopus laevis*, where the sequence of digit formation was IV-V-III-II.

In some leptodactylids and microhylids, Distal Carpal 5 appears earlier than Distal Carpal 4 and they are distinct condensations, with Metacarpal IV always being the first to originate. In other leptodactylids, as well as bufonids, hylids, and pseudids, a postaxial condensation differentiates simultaneously with Metacarpal IV; this condensation grows anteriorly while Metacarpal V becomes evident. This postaxial condensation was interpreted as a primary fusion of Distal Carpals 5 and 4 that fails to separate (Shubin and Alberch, 1986; Fabrezi and Alberch, 1996).

We focus our attention on one developmental feature in neobatrachians—the early differentiation of Distal Carpal 5 and the inclusion of Digit V in the digital arch. The early differentiation of Distal Carpal 5 and its segmentation from the ulnare support its origin from the primary axis. For neobatrachian forelimbs, Distal Carpal 5 and Metacarpal V are included in the global morphogenetic events involved in postaxial autopodium development. The ulnare could branch, as most elements of postaxial series do, and produce Distal Carpal 5 and Distal Carpal 4. Thus, the early appearance of a distinct condensation of Distal Carpal 5 or a postaxial carpal complex formed by the primary fusion of Distal Carpals 5 and 4 could be a developmental divergence

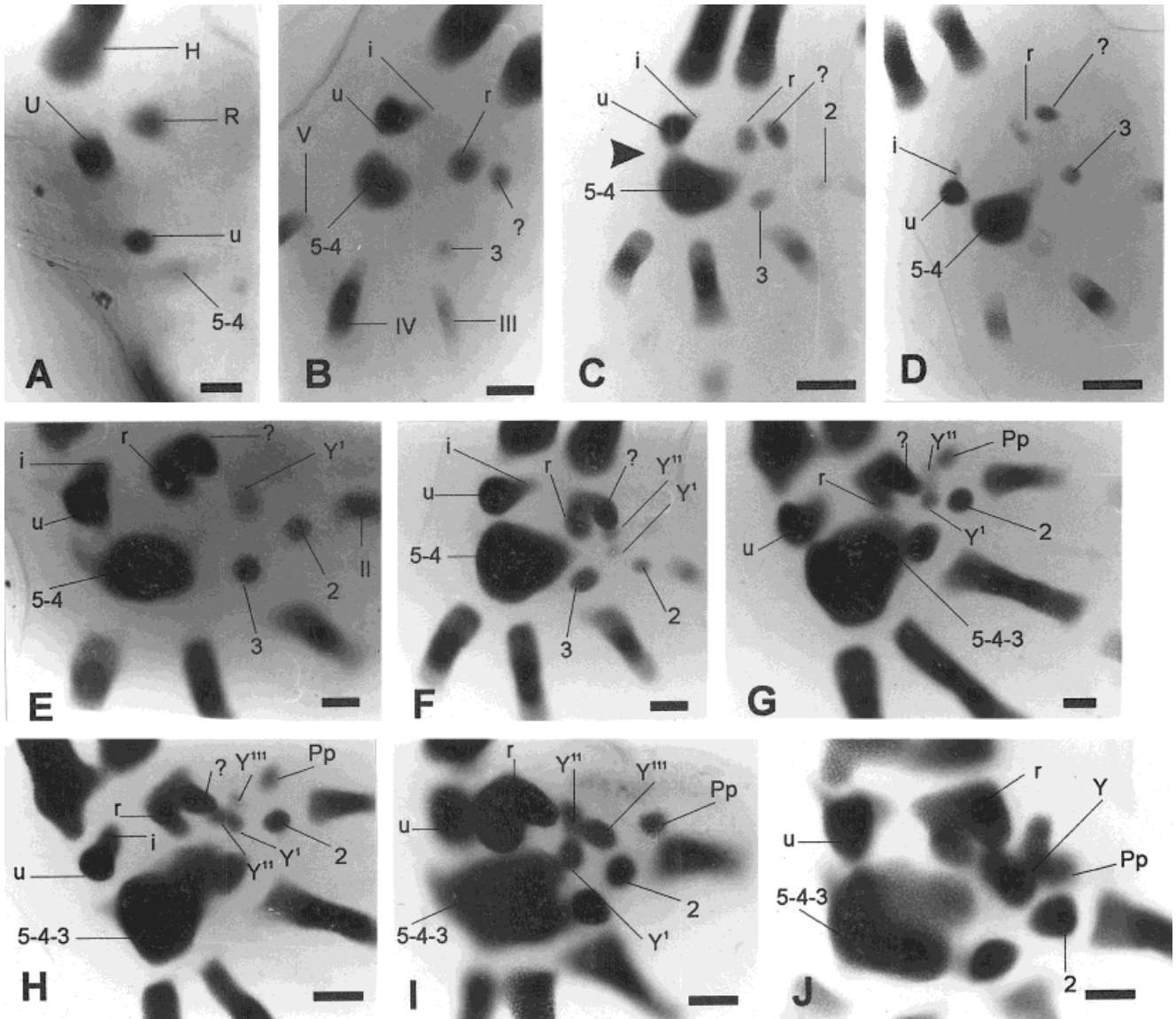


Fig. 5. Carpal development in selected pseudids and hyliids. **A:** *Pseudis paradoxa*, larval Stage 31. Proximal condensations of the primary axis are well defined. **B:** *Pseudis paradoxa*, larval Stage 33. Two condensations distal to the radius, the intermedium as an ulnare process, and digital arch condensations are differentiated. **C:** *Scinax fuscovarius*, larval Stage 35. The postaxial cartilages forming the primary axis and the digital arch are present. Distal Carpal 3 arises early, when the ulnare and Distal Carpal 5-4 are still connected. Two condensations are visible distal to the radius. The intermedium is present as an ulnare process. **D:** *Scinax acuminatus*, larval Stage 35. The same condensations as shown in *S. fuscovarius*. **E:** *Hyla andina*, larval Stage 34. The intermedium is developed from the ulnare. The fusion of two condensations distal to the radius is advanced and the first condensation of the Element Y complex is seen. **F:** *Hyla andina*, larval Stage 35. The second condensation of the Element Y is present. **G:** *Hyla andina*, larval Stage 36. Proximal prepollical element is well-defined and fusion between Distal Carpal 5-4 and Distal Carpal 3 is evident. **H:** *Phyllomedusa hypochondrialis*, larval Stage 34. The third condensation of Element Y has appeared. **I:** *Phyllomedusa hypochondrialis*, larval Stage 36. Three cartilages of Element Y complex are well-defined. **J:** *Phyllomedusa hypochondrialis*, larval Stage 37. The fusion of three condensations of the Element Y is advanced. Scale Bar = 0.1 mm. 5, 4, 3, 2, distal carpals; i, intermedium; Pp, proximal prepollical element; r, radiale; u, ulnare; V, IV, III, II, metacarpals; Y¹, Y², Y³, cartilaginous condensations of Element Y; ?, unidentified condensation of the radiale.

between the forelimbs of *Xenopus* and neobatrachians (Table 1).

Interpretation of the condensations that form the ulnare was discussed by Fabrezi and Alberch (1996). We confirm the identity of the intermedium condensation given by these authors. *Dasylops* sp. (Fig. 7) shows that the branched end of the ulna segments to

form the ulnare and, in agreement with the morphogenetic criterion of Shubin and Alberch (1986), the intermedium. In species in which the intermedium develops as an ulnare projection, it seems suitable to interpret this as heterochronic development in which the intermedium and ulnare are segmented from the ulna end before it branches (Fabrezi and

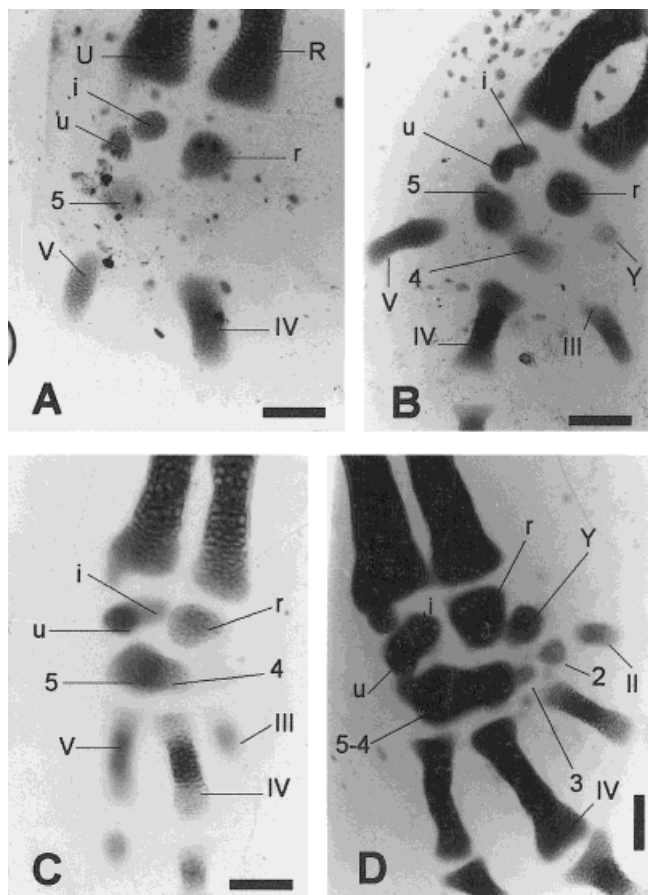


Fig. 6. Carpal development in selected microhylids. **A:** *Dermatonotus muelleri*, larval Stage 31. The radiale, intermedium, ulnare, Distal Carpal 5, and Metacarpals IV and V are differentiated. The primary axis is still incomplete because Distal Carpal 4 has not developed. **B:** *Dermatonotus muelleri*, larval Stage 32. Distal Carpal 4 and Metacarpal III are evident. The condensation of Element Y is incipient. **C:** *Elachistocleis bicolor*, larval Stage 31. Well-differentiated condensations of the ulnare, intermedium, radiale, Distal Carpal 5, Distal Carpal 4, and Metacarpals IV and V are present. **D:** *Elachistocleis bicolor*, larval Stage 36. Element Y, Distal Carpal 5-4-3, and Distal Carpal 2 are present, whereas the intermedium and ulnare are not yet fused. Scale Bar = 0.1 mm. 5, 4, 3, 2, distal carpals; i, intermedium; Pp, proximal prepollical element; r, radiale; u, ulnare; U, ulna; V, IV, III, II, metacarpals; Y, Element Y.

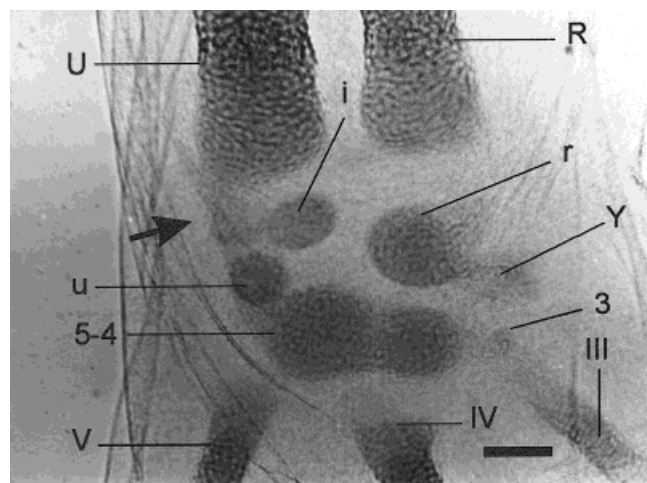


Fig. 7. *Dasylops* sp., larval Stage 35. The origin of the ulnare and intermedium from the end of the ulna via branching and segmentation is shown. Scale Bar = 0.1 mm. 5-4, 3, distal carpals; i, intermedium; r, radiale; u, ulnare; U, ulna; V, IV, III, metacarpals; Y, Element Y.

Alberch, 1996). Our current information suggests that the intermedium primordium is always present in anurans. There is no subsequent independent development of the intermedium and the embryonic condensation is incorporated into the adult ulnare in late development.

Progressive differentiation of Digits III and II is similar among different taxa, and the formation of Distal Carpal 5-4-3 could involve primary or secondary fusion of Distal Carpal 3 with Distal Carpal 5-4 (Fabrezi and Alberch, 1996).

Differentiation in the Preaxial Axis

The radiale, Element Y, and prepollical elements are differentiated in the preaxial axis of the autopodium (Shubin and Alberch, 1986). In anurans, the formation of the adult radiale and Element Y involving different cartilaginous primordia was mentioned by Fabrezi and Alberch (1996). The adult radiale develops from one embryonic condensation in *Xeno-*

TABLE 1. Summarized observations of hand development among selected anuran taxa

| Developmental feature | <i>Xenopus laevis</i> | Bufoinae | Leptodactylidae | Hylidae and Pseudidae | Microhylidae |
|---|-----------------------|-------------------|---------------------------------------|--------------------------|--------------------|
| First distal carpals to originate | Distal Carpal 4 | Distal Carpal 5-4 | Distal Carpal 5 or 5-4 | Distal Carpal 5-4 | Distal Carpal 5 |
| Condition of intermedium | Ulnare process | Ulnare process | Distinct cartilage | Ulnare process | Distinct cartilage |
| Number of condensations to form the radiale | One cartilage | One cartilage | Two separated or connected cartilages | Two separated cartilages | One cartilage |
| Number of condensations to form Element Y | One cartilage | Three cartilages | One or three cartilages | Three cartilages | One cartilage |
| Digital sequence | IV-V-III-II | IV-V-III-II | IV-V-III-II | IV-V-III-II | IV-V-III-II |

Different ontogenetic features involved in the development of distal carpals, intermedium, radiale, and Element Y are integrated in patterns that appear to contain potentially useful phylogenetic information.

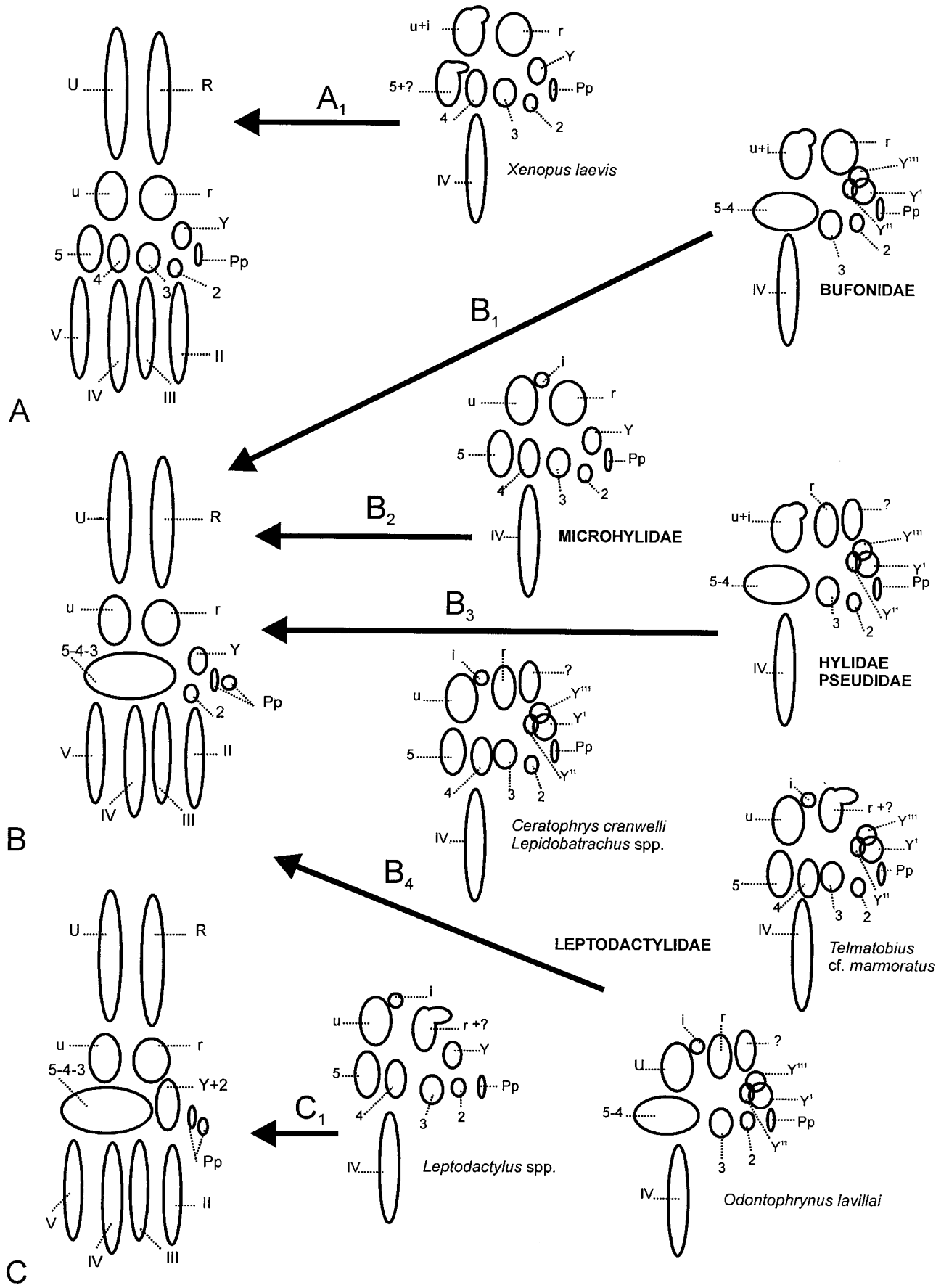


Figure 8

pus, bufonids, and microhylids, or from two embryonic primordia that are separate in hylids, pseudids, and some leptodactylids, but connected in other leptodactylids (Table 1). The ontogenetic pathway for Element Y also differs. In *Xenopus*, *Leptodactylus* spp., and microhylids a single condensation grows into Element Y, whereas in bufonids, hylids, pseudids, and most leptodactylids, three embryonic primordia fuse to form Element Y (Table 1). It is not yet possible to propose the identity of these extra-condensations in the radiale and Element Y.

In nonanuran tetrapods, the radiale is a single condensation that may fuse to the intermedium (Shubin and Alberch, 1986; Müller, 1992). Element Y is developed from one embryonic primordium (Blanco and Alberch, 1992; Vorobyeva and Hinchliffe, 1996) and in anurans it always supports the prepollical elements (Fabrezi, 2000). The radiale, Element Y, and prepollex are fully developed preaxial structures that share structural identity among anurans, but the developmental features observed have not been described in other taxa yet and could represent novelties of anuran limbs. However, more detailed anatomical studies on preaxial limb elements in other amphibians and early tetrapods will be required to evaluate the evolutionary significance of these elements and understand the early tetrapod limb evolution.

Developmental Variation

Developmental variation in the ulnare, radiale, and Element Y among neobatrachians converges to the same adult carpal morphology (Fig. 8). This kind of developmental variation is compatible with the structural identity that is conserved during the anu-

ran phylogeny and can be explained in terms of the biological homology concept (Wagner, 1994).

Bufonids, hylids, pseudids, and microhylids could have stereotypic patterns, whereas leptodactylids are more variable because they are an artificial group (Ford and Cannatella, 1993) and taxa considered in our analysis belong to different subfamilies (Ceratothryinae: *Ceratothryus*, *Lepidobatrachus*; Leptodactylinae: *Leptodactylus*; Telmatobiinae: *Telmatobius*, *Odontophrynus*) (Duellman and Trueb, 1986).

Most of the results presented in this article are in agreement with the observations presented by Fabrezi and Alberch (1996) and Schmalhausen (1907). Some literature that mention developmental features of the anuran hand were not considered (Holmgren, 1933; de Saint-Aubain, 1981; Fabrezi, 1992) because they were carried out on incomplete ontogenetic series or were biased by theoretical expectations based on the concept of the chyropterygium (Gregory et al., 1923). With regard to *Xenopus laevis*, there are discrepancies in the literature on carpal development in this species. Holmgren (1933), Jarosova (1973), and de Saint-Aubain (1981) mentioned different condensations incorporated with the ulnare, the radiale, Element Y, and Distal Carpal 5, while Shubin and Alberch (1986) reported the origin of every adult carpal element from a single embryonic condensation. Therefore, more detailed studies of carpal development in species of *Xenopus* and other pipids are needed to define patterns and explain carpal morphologies in *Hymenochirus* and *Pipa* (Howes and Ridewood, 1888; Fabrezi, 1992; Trueb et al., 2000).

Finally, individual/intraspecific variation in developing anuran limbs has not been observed. We

Fig. 8. Different patterns of carpal development in anurans. **A:** *Xenopus laevis*, adult carpal morphology consists of eight elements: ulnare, radiale, Element Y, Distal Carpal 5, 4, 3, and 2 and one prepollical element. **A₁:** Embryonic cartilages in *Xenopus laevis*. The intermedium develops as an ulnare process. The radiale and Element Y are single condensations. Distal Carpals 5, 4, 3, and 2 are distinct condensations. A well-defined lobe developed from Distal Carpal 5 suggests a complex origin of this distal carpal. The ulnare, Distal Carpal 4, and Metacarpal IV are the first cartilages to appear and form the primary axis. **B:** The adult carpal morphology found in most neobatrachian species, with the ulnare, radiale, Element Y, a postaxial distal carpal (formed by the fusion of Distal Carpals 5, 4, and 3), Distal Carpal 2, and two or more prepollical elements present. **B₁:** Embryonic cartilages in selected bufonids. The intermedium develops as an ulnare process. The radiale is a single condensation. Three cartilages form the Element Y. Distal Carpal 5 and 4 originate fused. The ulnare, Distal Carpal 5-4, and Metacarpal IV form the primary axis. **B₂:** Embryonic cartilages in selected microhylids. The intermedium and ulnare are separate condensations. The radiale and Element Y are single condensations. Distal Carpal 5 and 4 appear as different primordia. The ulnare, Distal Carpals 5 and 4, and Metacarpal IV form the primary axis. **B₃:** Embryonic cartilages in selected hylids and pseudids. The intermedium develops as an ulnare process. Two distinct cartilages form the radiale. Three cartilages make up the Element Y. Distal Carpal 5 and 4 originate fused. The ulnare, Distal Carpal 5-4, and Metacarpal IV form the primary axis. **B₄:** Embryonic cartilages in selected leptodactylids. The intermedium and ulnare are distinct condensations. The ulnare, Distal carpals 5 and 4 or Distal carpal 5-4, and Metacarpal IV form the primary axis. The radiale will be formed by two separated condensations in *Ceratothryus cranwelli*, *Lepidobatrachus* spp. and *Odontophrynus lavillai*, and two connected primordia in *Telmatobius* cf. *marmoratus* and *Leptodactylus* spp. Element Y arises from one condensation in *Leptodactylus* spp. and three condensations in *Ceratothryus cranwelli*, *Lepidobatrachus* spp., *Odontophrynus lavillai*, and *Telmatobius* cf. *marmoratus*. Distal Carpals 5 and 4 are distinct cartilages in *Ceratothryus cranwelli*, *Lepidobatrachus* spp., *Leptodactylus* spp., and *Telmatobius* cf. *marmoratus*, and are fused in *Odontophrynus lavillai*. **C:** Adult carpal morphology found in some neobatrachian species characterized by the presence of the ulnare, radiale, Element Y fused to Distal Carpal 2, a postaxial distal carpal (formed by the fusion of Distal Carpals 5, 4, and 3), and two or more prepollical elements. **C₁:** Embryonic cartilages in some species of *Leptodactylus*. The fusion between Element Y and Distal Carpal 2 is added to the same pattern described for other *Leptodactylus* species. 5, 4, 3, 2, distal carpals; 5+?, Distal Carpal 5 with a proximal unidentified process; i, intermedium; Pp, prepollex; r, radiale; R, radius; u, ulnare; U, ulna; V, IV, III, II, metacarpals; Y, Element Y, Y¹, Y¹¹, Y¹¹¹, cartilaginous condensations of Element Y; ?, unidentified condensation connected or separate that will form the radiale.

analyzed large sample sizes from different populations of some species and variation of developmental features for single species was not noticeable.

General Features of Anuran Hand Development

Our analysis of anuran hand development reveals the following. 1) The sequence of digit formation (IV-V-III-II) is invariant. 2) In the neobatrachians examined, Digit V is included in the primary axis and the condensation of Distal Carpal 5 appears earlier than Distal Carpal 4. 3) The adult ulnare contains an embryonic condensation of the intermedium. 4) The ulnare, radiale, and Element Y arise from different ontogenetic pathways depending on the taxon. Bufonids, microhylids, and hylidspseudids show stereotypic patterns. Leptodactylids possess an embryonic free intermedium and have more variation in the differentiation of the other elements.

Postaxial limb development in anuran resembles, in many aspects, that of amniotes. In contrast, limb development in caudates is characterized by unique features distinguishing them from other tetrapods (Blanco and Alberch, 1992; Vorobyeva and Hinchliffe, 1996; Hinchliffe and Vorobyeva, 1999). These differences can be explained by molecular data on the *Hoxa-11* expression pattern that is unique among tetrapods (Wagner et al., 1999). Developmental data of the lissamphibian limb may provide valuable insight about the evolutionary history of the limb skeleton. However, other studies that focus on morphological diversity of preaxial limb elements are necessary to understand these anatomically specialized groups and discuss origins.

ACKNOWLEDGMENTS

We thank Richard J. Wassersug for extended corrections and comments on the manuscript. We appreciate the suggestions of the early draft of the manuscript from Linda Trueb. We also thank Raymond F. Laurent and Cristina Busch for encouragement and advice.

LITERATURE CITED

- Blanco MJ, Alberch P. 1992. Caenogenesis, developmental variability, and evolution in the carpus and tarsus of the marbled newt *Triturus marmoratus*. *Evolution* 46:677–687.
- Burke AC, Alberch P. 1985. The development and homologies of the chelonian carpus and tarsus. *J Morphol* 186:119–131.
- Coates MI, Clack JA. 1990. Polydactyly in the earliest known tetrapod limbs. *Nature* 347:66–69.
- de Saint-Aubin ML. 1981. Amphibian limb ontogeny and its bearing on the phylogeny of the group. *Zool Syst Evolut Forsch* 19:175–194.
- Duellman WE, Trueb L. 1986. *Biology of amphibians*. New York: McGraw Hill.
- Fabrezi M. 1992. El carpo de los anuros. *Alytes* 10:1–29.
- Fabrezi M. 2001. A survey on prepollex and prehallux variation in anuran limbs. *Zool J Linn Soc.* 131:22.

- Fabrezi M, Alberch P. 1996. The carpal elements of anurans. *Herpetologica* 52:188–204.
- Ford LS, Cannatella DC. 1993. The major clades of frogs. *Herpetol Monogr* 7:94–117.
- Gosner KL. 1960. A simplified table for staging anuran embryos and larvae, with notes on identification. *Herpetologica* 16:183–190.
- Gregory WK, Minner R, Noble GK. 1923. The carpus of *Eryops* and the structure of the primitive chiropterygium. *Bull Am Mus Nat Hist* 48:279–288.
- Hinchliffe JR, Vorobyeva EI. 1999. Developmental basis of limb homology in urodeles: heterochronic evidence from the primitive hynobiid family. In: Hall BK, editor. *Homology*. Novartis Foundation Symposium 222. Chichester, UK: John Wiley. p 95–109.
- Holmgren N. 1933. On the origin of the tetrapod limb. *Acta Zool (Stockholm)* 14:185–295.
- Howes GB, Ridewood R. 1888. On the carpus and tarsus of the Anura. *Proc Zool Soc Lond* 1888:141–180.
- Jarosova J. 1973. The components of the carpus in *Paleobatrachus* and their development in two related recent species. *Caenopsis Narodniho Muzea* 142:82–106.
- Müller G. 1992. Evolutionary transformation of limb pattern: heterochrony and secondary fusion. In: Hinchliffe JR, Hurlle JM, Summerbell D, editors. *Developmental patterning of the vertebrates limbs*. New York: Plenum Press. p 395–405.
- Nieuwkoop PD, Faber J. 1956. Normal table of *Xenopus laevis* (Daudin). A systematical and chronological survey of the development from the fertilized egg till the end of metamorphosis. Amsterdam: North Holland. p 252.
- Schmalhausen JJ. 1907. Die Entwicklung des skelettes der vorderen extremität der Anuren Amphibien. *Anat Anz* 31:177–187.
- Shubin N, Alberch P. 1986. A morphogenetic approach to the origin and basic organization of the tetrapod limb. *Evol Biol* 20:319–387.
- Sordino P, van der Hoeven P, Duboule D. 1995. *Hox* gene expression in teleost fins and the origin of vertebrate digits. *Nature* 375:678–681.
- Trueb L, Pügener LA, Maglia AM. 2000. Ontogeny of the bizarre: an osteological description of *Pipa pipa* (Anura: Pipidae), with an account of skeletal development in the species. *J Morphol* 243:75–104.
- Vorobyeva EI, Hinchliffe JR. 1996. Developmental pattern and morphology of *Salamandrella keyserlingii* limbs (Amphibia, Hynobiidae) including some evolutionary aspects. *Russ J Herpetol* 3:68–81.
- Wagner GP. 1994. Homology and the mechanisms of development. In: Hall BK, editor. *Homology*. The hierarchical basis of comparative biology. New York: Academic Press. p 274–299.
- Wagner GP, Khan PA, Blanco MJ, Misof B, Liversage RA. 1999. Evolution of *Hoxa-11* expression in amphibians: is the urodele autopodium an innovation? *Am Zool* 39:686–694.
- Wassersug R. 1976. A procedure for differential staining of cartilage and bone in whole formalin-fixed vertebrates. *Stain Technol* 51:131–134.

APPENDIX

Specimens Examined

Bufonidae. *Bufo fernandezae*, MCN 492, 38 specimens of larval Stages 32–46. *B. paracnemis*, MCN 395, 35 specimens of larval Stages 32–42. *B. spinulosus*, MCN 604, 32 specimens of larval Stages 32–46; *Melanophryniscus stelzneri*, MCN 493, 47 specimens of larval Stages 31–46.

Hylidae. *Hyla andina*, MCN 024, 30 specimens of larval Stages 32–44. *Phrynohyas venulosa*, MCN 238, six specimens of larval Stages 32–35, and 13 specimens of larval Stages 37–44. *Phyllomedusa boliviana*, MCN 596, 14 specimens of larval Stages 35–43. *P. hypochondrialis*, MCN 598, eight speci-

mens of larval Stages 32–38. *P. sauvagii*, MCN 061, 34 specimens of larval Stages 32–43; MCN 599, 12 specimens of larval Stages 33–39. *Scinax acuminatus*, MCN 601, seven specimens of larval Stages 33–37. *S. nasicus*, MCN 646, 12 specimens of larval Stages 32–38. *S. fuscovarius*, MCN 239, 18 specimens of larval Stages 33–42; MCN 600, 12 specimens of larval Stages 33–39.

Leptodactylidae. *Ceratophrys cranwelli*, MCN 021, 13 specimens of larval Stages 32–35; MCN 425, 18 specimens of larval Stages 33–46. *Lepidobatrachus laevis*, MCN 647, 15 specimens of larval Stages 33–42. *L. llanensis*, MCN 567, 12 specimens of larval Stages 34–40. *Leptodactylus bufonius*, MCN 064, 12 specimens of larval Stages 31–37; MCN 200, 25 specimens of larval Stages 33–43. *L. chaquensis*, MCN 048, 22 specimens of larval Stages 32–37; MCN 070, 19 spec-

imens of larval Stages 35–39; MCN 240, 13 specimens of larval Stages 36–43. *L. latinasus*, MCN 243, 14 specimens of larval Stages 33–41. *Odontophrynus lavillai*, MCN 230, 23 specimens of larval Stages 33–42. *Telmatobius cf. marmoratus*, MCN 181, 6 specimens of larval Stages 33–36.

Microhylidae. *Dermatonotus muelleri*, MCN 234, 12 specimens of larval Stages 31–36; MCN 236, 14 specimens of larval Stages 35–42; MCN 603, 12 specimens of larval Stages 31–38. *Elachistocleis bicolor*, MCN 602, seven specimens of larval Stages 32–37. *Dasypops* sp. No catalogued larval specimen in Stage 35.

Pipidae. *Xenopus laevis*, MCN 490, 14 specimens of larval Stages 50–56 and 58.

Pseudidae. *Pseudis paradoxa*, MCN 597, six specimens of larval Stages 31 (two), 33 (two), 35, 37.