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RESEARCH ARTICLE

Anuran Locomotion: Ontogeny and Morphological Variation of a Distinctive Set of Muscles

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Abstract Adult morphological variation of muscles originating on the iliac shaft (M. iliacus externus, M. internus, and tensor fasciae latae) and vertebrae (M. longissimus dorsi, M. coccygeosacralis, and M. coccygeoiliacus) that are involved in postmetamorphic anuran locomotion was recorded in 41 neobatrachians and coded in 13 more based on the literature, for a total of 54 anuran species. In addition, we explored the spatial and temporal sequences in the ontogeny of these set of muscles from larval series of 19 neobatrachians whose adults differ in locomotion and lifestyle. Our findings suggest that: (1) jumping, swimming, and/or walking are capabilities that could have been achieved from novelties of limbs and protractor muscles of the femur rather than from changes in the axial musculoskeletal system; (2) the initial ontogenetic phase of the locomotion comprises the capability to escape, when the tail is still present; (3) the secondary phase of locomotion comprises changes in the axial skeleton and muscles integrated to the pelvis and might develop simultaneously with the new feeding mechanism of the recently metamorphosed frog.

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Introduction

During their entire life cycle, anurans are characterized by the absence of discrete caudal vertebrae and a truncated axial skeleton (Handrigan and Wassersug 2007). Tadpoles have elongate tails that comprise all axial structures except for the skeleton, which terminates at the level of the anus in most anurans (Handrigan and Wassersug 2007). The tadpole tail, as a midline axial propeller, permits undulatory aquatic locomotion independent of appendicular locomotion (Wassersug and Hoff 1985; Wassersug 1989).

Girdles and paired appendages develop at larval stages and integrate with the axial skeleton simultaneously with tail regression (Rocková and Rocek 2005; Pugener and Maglia 2009; Manzano et al. 2013). They become part of the new mechanism of locomotion, which may involve jumping, swimming and/or walking (Emerson 1979). In fact, once the new mechanism exists it forms a device for collecting other specializations or variations (Gans and Parsons 1966). Furthermore, anuran variation comprises digging, climbing, and flying as additional behaviors for locomotion achieved by morphological specializations of the autopodia—e.g., the spade for digging; intercalary elements, muscles extensores breves, muscles tendo superficialis, and adhesive digit pads to climb; and full webbing on digits and accessory skin flaps on the limbs to fly (Burton 1998; Emerson and De Jongh 1980; Emerson and Diehl 1980; Emerson and Koehl 1990; Manzano et al. 2007).

Biomechanical, morphological and systematic studies have emphasized anuran postcranial specializations as

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essential to determining the typical postmetamorphic anuran mode of locomotion (Emerson and De Jongh 1980; Rage and Roček 1989; Jenkins and Shubin 1998; Prikryl et al. 2009; Reilly and Jorgensen 2011; Sigurdsen et al. 2012; Jorgensen and Reilly 2013; among others). However, although there is consensus that anuran locomotion initially evolved as an escape mechanism (Gans and Parsons 1966; Wassersug and Sperry 1977), earlier studies are not consistent in interpreting the precise functional context for its origin and evolution.

In anurans, jumping is the dominant locomotor mode on land (Emerson 1978, 1979, 1982); however, frogs are also able to swim or walk, depending on the habitat and the lifestyle. Emerson and De Jongh (1980) demonstrated that the basic musculoskeletal complex formed by hind limb, pelvis, and vertebral column allows anurans to use two or more locomotion mechanisms with different performances. Differences in muscle activity among jumping, swimming and walking revealed that the unique morphology represented by the pelvis, urostyle, and vertebrae of the adult frog represents a functional complex equally appropriate for swimming and jumping, whereas walking represents a different pattern in which right and left muscles of a pair fire asynchronously, unlike the pattern of jumping and swimming, in which right and left muscles fire synchronously (Emerson and De Jongh 1980).

Přikryl et al. (2009) compared the variation in the pelvic and thigh musculoskeletal system in various taxa representing the main anuran locomotor types (jumping, swimming, crawling and burrowing) and conducted a functional analysis of locomotion. They concluded that the muscles responsible for protractions of the femur (e.g., M. iliacus externus, M. iliacus internus, and M. tensor fasciae latae) and axial muscles that insert on the urostyle and the pelvis are derived with respect to the patterns in salamanders. These authors suggested these muscles are likely the ones determining locomotion in anurans, and they proposed jumping to be the primary type of locomotion in anurans, and swimming (in pipids) and walking to be derived (not transitional) locomotor types.

Reilly and Jorgensen (2011) sampled pelvic skeletal characters in a large sample of anuran species, and interpreted the relationships between pelvic systems and locomotor modes with respect to a phylogenetic hypothesis; they found that the generalized frog is a walker that can hop better than most vertebrates, with some proclivity toward burrowing. Many lineages of Neobatrachia adopted convergent patterns that increase jumping distance on land, or climbing and jumping among trees (Reilly and Jorgensen 2011). Further morphometric analyses demonstrated that the shape of the frog sacrum and lengths of the pelvis and hind limb could be associated with locomotor mode, whereas forelimb length and pelvic length were conserved across frogs (Jorgensen and Reilly 2013).

The fossil record documents instances of the evolution of some morphological traits associated with the anuran locomotion (Table 1). The Triassic *Triadobatrachus massinoti* had an axial skeleton consisting of up to 14 presacral vertebrae with ribs; tail with few caudal vertebrae; elongated iliac shaft exhibiting an unusual articulation with the sacral rib; pubes apparently cartilaginous as in the anurans; separated zeugopodia markedly shorter than stylopodia; and two (tibiale and fibulare) of the four tarsalia elongated (Rage and Roček 1989). In another study, Sigurdsen et al. (2012) focused on the anatomical features of the forelimbs of *Triadobatrachus massinoti* and described some anatomical features of the forelimbs that extant anurans use both in the take-off and landing phases of a jumping. These authors concluded that the structure of the humerus and the deltopectoral crest, the anteriorly directed ilium, and the reduced tail could also be associated with jumping or hopping as part of the locomotor repertoire of *Triadobatrachus massinoti*.

Many of the distinctive anatomical features of modern anurans were established by Early Jurassic time, as found in *Prosalirus bitis* (Shubin and Jenkins 1995): these comprise elongation of the hind limbs and especially the proximal tarsals, zeugopodial fusion, elongation of the ilium, presence of a urostyle, and a new localization and mobility of the ilio-sacral joint (Table 1). Jenkins and Shubin (1998) proposed the ilio-sacral articulation and the sacro-urostylic joint were critical to launching a jump and diverse locomotor activities, since the urostyle and caudopelvic muscles have largely involved in the function of force transmission between hind limb and axial skeleton.

Despite the significant literature about the morphological features that could be involved in typical anuran locomotion and interpretations about what evolutionary changes would have been engaged in the origin of this locomotor pattern, some questions remain. The aim of this study was to examine the interspecific and intraspecifc variation of muscles originating on the iliac shaft (M. iliacus externus, M. iliacus internus and M. tensor fasciae latae) and vertebrae (M. longissimus dorsi, M. coccygeosacralis and M. coccygeoiliacus) and inserting on the femur and the urostyle since these muscles are directly involved in the postmetamorphic anuran locomotion (Jenkins and Shubin 1998; Prikryl et al. 2009).

For that purpose, we recorded morphological variation of this set of muscles in adults of 41 neobatrachians and coded it in more than 50 anuran species. The evolution of this variation is interpreted on the most recent anuran phylogeny (Pyron and Wiens 2011) in order to look for those variations that could be linked with locomotor modes. Further, we

Table 1 Skeletal fossil data versus recent anuran morphology show that zeugopodial (fusion of long bones) and autopodial (elongation of proximal tarsalia) modifications evolved concomitant with caudopelvic (ilium and posterior vertebrae) changes. Most studies on the origin of anuran locomotion have focused on the sacro-iliac articulation (Emerson 1978, 1979; Emerson and De Jongh 1980; Reilly and Jorgensen 2011); thigh and caudopelvic musculature (Jenkins and Shubin 1998; Přikryl et al. 2009) to interpret the origin of the jumping capability and/or to address the ancient locomotor mode of the anuran (swimming or jumping)

Triadobatrachus massinoti (Rage and Roček 1989)	*Prosalirus bitis* (Jenkins and Shubin 1998)	Morphological change associated with the new locomotion
Tibiale and fibulare quite elongated and massive, free intermedium and a centrale	One proximal relatively elongate tarsal preserved in comparison with other Paleozoic amphibians	Long tibiale and fibulare, which resemble the lower leg bones (zeugopodium) by maintained expression of *Hoxa-11* (Blanco et al. 1998). Intermedium and central absent
Zeugopodial elements separated and shorter than the stylopodial bones	Tibiofibula and radioulna	Tibiofibula and radioulna
Atlas, 14 presacral, one sacral and six caudal vertebrae	Atlas, unknown number of presacral, one sacral vertebrae and a short urostyle	Atlas and nine or less (including the sacrum) vertebrae, accompanied by the urostyle
Elongation of the pelvis that corresponds to about the length of the last three presacral vertebrae. A distinct tuber superior	Pelvis elongated. The iliac shaft has the cranial terminus not ossified. Reduction of tuber superior	Elongation of the pelvis posteriorly relative to the sacrum. Reduction of tuber superior

explored the spatial and temporal sequences in the origin of these muscles during larval development of 19 neobatrachians whose adults differ in locomotor mode (jumping, hopping, swimming, walking) and habitat (terrestrial, arboreal, aquatic). Since anuran metamorphosis links aquatic and terrestrial locomotion, independently of the lifestyle of postmetamorphic individuals, our observations on the development of muscles and their integration with the axial and pelvic skeletons concomitant with the disappearance of the larval locomotor organ (i.e., the tail) may provide some evidence about how adult locomotion was achieved evolutionarily. Our findings suggest that muscles that act as protractors of the femur (M. iliacus externus, M. iliacus internus and M. tensor fasciae latae) have derived in jumpers and hoppers whereas the ontogeny reveals that anuran locomotor modes (jumping, swimming, and/or walking) are achieved before the pelvis + sacrum + urostyle, and hind limbs complex act as a unit.

Materials and Methods

Descriptions of the variation in the following muscles: M. iliacus externus, M. iliacus internus, tensor fasciae latae, M. longissimus dorsi, M. coccygeosacralis, and M. coccygeoiliacus were obtained from two different sources: (1) adult specimens of 41 neobatrachians representing Hyloides (Hylidae, Hylidae, Leptodactylidae, Ceratophryidae, Telmatobiidae, Odontophryinidae, Rhinodermatidae, and Bufonidae) and Ranoides (Microhylidae, Hyperoliidae, Phrynobatrachidae, Ptychadenidae, Ranidae, Mantellidae, and Pyxicephalidae), and (2) larval series of 19 anuran species belonging to five clades of Hyloides (Hylidae, Leptodactylidae, Ceratophryidae, Telmatobiidae, and Bufonidae) and one of Ranoides (Microhylidae). Larval series were staged following the standard table of Gosner (1960) from stages in which all five toes are separated (Stage 36) up to stages at which the tail is completely lost (Stage 46). Species, specimen numbers, and collection data are listed in "Appendix 1".

Anatomical descriptions of muscles were based on manual dissections of whole alcohol preserved specimens that were double stained with Alcian Blue and Alizarin Red S (Wassersug 1976) 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 muscles (Bock and Shear 1972).

Observations were made with a Nikon SMZ 800 stereomicroscope with attached digital camera. Terminology and criteria to identify the muscles follow those of Přikril et al. (2009).

A total of six morphological characters and its variation were scored for 41 anuran species belonging to our sample. Additional data from the literature (Limeses 1964; Přikril et al. 2009) allows us to get a data set for 13 additional taxa. Character change was traced along the hypothesis of the anuran relationships based on molecular data proposed by Pyron and Wiens (2011) using a parsimony software (TNT of Goloboff et al. 2003) and applying deltran optimization in those cases of ambiguous assignation for internal nodes.

Results

Intraordinal Variation

Table 2 defines characters and character states used to explore the phylogenetic occurrence of the variation.

Table 2 States of characters observed in the M. longissimus dorsi, M. coccygeoiliacus, M. tensor fascia latae, M. iliacus externus and M. iliacus internus in a sample of anurans from different lineages

Character	States
Character 0: length of the M. longissimus dorsi	State 0- long, covering totally the MM. coccygeosacralis (Figs. 1a, 2a)
	State 1—short, covering anteriorly the MM. coccygeosacralis (Fig. 2b, c)
Character 1: orientation of the fibers of the M. coccygeoiliacus	State 0—parallel to the orientation of the iliac shafts (Fig. 6a, b)
	State 1—oblique with respect to the iliac shafts (Fig. 7a–c)
Character 2: length of the M. tensor fasciae latae	State 0—long, formed by numerous parallel fibers originated on the anterior half of the iliac shaft (Fig. 3c).
	State 1- medium, formed by fibers originating on the inner surface on the posterior half of the iliac shaft (Fig. 3b, c–e, f, i–n).
	State 2: short, sometimes reduced or absent, few fibers originating on the posterior third of the iliac shaft (Figs. 1b, 3f, g, h, o)
Character 3: origin of the M. iliacus externus	State 0—at the anterior half of the iliac shaft (the muscle covers more than 70 % of the iliac shaft length (Fig. 2b, e, f).
	State 1—at the middle of the iliac shaft (the muscle covers between 40 and 70 % of the iliac shaft length (Fig. 2a, c)
	State 2—at the posterior half of the iliac shaft (Figs. 1d, e, g, 2d)
Character 4: pars interna of M. iliacus externus	State 0—absent
	State 1—Well differentiated (Fig. 1g)
Character 5: extension of the origin of the M. iliacus internus	State 0—wide (Figs. 1c, g, 2d, f)
	State 1—narrow (Fig. 2e)

The paired muscles M. longissimus dorsi, M. coccygeosacralis, M. iliolumbaris, and M. coccygeoiliacus originate from the axial skeleton (vertebrae and urostyle) and insert on the urostyle and the iliac shaft. The M. longissimus dorsi extends from sacral and presacral vertebrae to the urostyle (Figs. 1a, 2a–c). Its length varies and it may insert anywhere between the posterior end of the urostyle and the anterior half of the urostyle, immediately posterior to the articulation with the sacrum. Depending on the degree of extension, the M. longissimus dorsi covers the M. coccygeosacralis completely or partially.

The M. coccygeoiliacus originates on the urostyle and inserts on the iliac shaft (Figs. 1b, 2a–c). Depending on the location of the origin, their fibers form a compact muscular mass bordering and paralleling the iliac shaft to insert anteriorly, or the muscle is laminar and its fibers, originating along the urostyle, run obliquely to insert along the iliac shaft.

The M. coccygeosacralis exhibits little variation (Figs. 1b, 2b, c). This muscle is fan-shaped, laminar and its fibers run from the sacral vertebra to insert on the anterior half of the urostyle.

The M. iliolumbaris (Fig. 1a) originates on the presacral vertebrae and inserts on the epiphysis of the iliac shaft. It may be attached to the M. iliacus externus by ligaments.

The M. iliacus externus, M. iliacus internus, M. tensor fasciae latae run from the ilium to the femur or fascia lata and are protractors of the femur (Přikryl et al. 2009). The M. pyriformis has its origin on the end of the urostyle and inserts on the femur.

The M. tensor fasciae latae was reported as an accessory muscle that puts tension on the fascia lata without obvious movements of the appendicular skeleton (Přikryl et al. 2009). The muscle originates on the laterointernal surface of the iliac shaft and inserts on the fascia lata of the crus. The muscle may vary in length and strength, both qualities that seem to be correlated, since the shortest muscle is formed by few and weak fibers and the opposite is a long muscle with numerous fibers (Fig. 3).

For most species, the M. iliacus externus is a single and fusiform mass attached to the outer face of the iliac shaft (Figs. 1, 2). It may be observed in dorsal view, sometimes partially hidden by the M. coccygeoiliacus. This muscle inserts on the posterior face of the caput femoris via a short tendon. Interspecific variation is evident in its length since the origin of this muscle may be placed at different positions on the iliac shaft. Furthermore, the M. iliacus externus presents a fully differentiated pars interna in some species. The M. iliacus externus pars interna originates on the inner face of the iliac shaft and runs ventrally to the M. iliacus internus and inserts on the anterior face of the crista femoralis (Fig. 1g). The M. iliacus externus pars interna may be short or long, depending on whether the origin is located immediately contiguous to the origin of the M. iliacus internus or on the lateral surface of the anterior half of the iliac shaft.

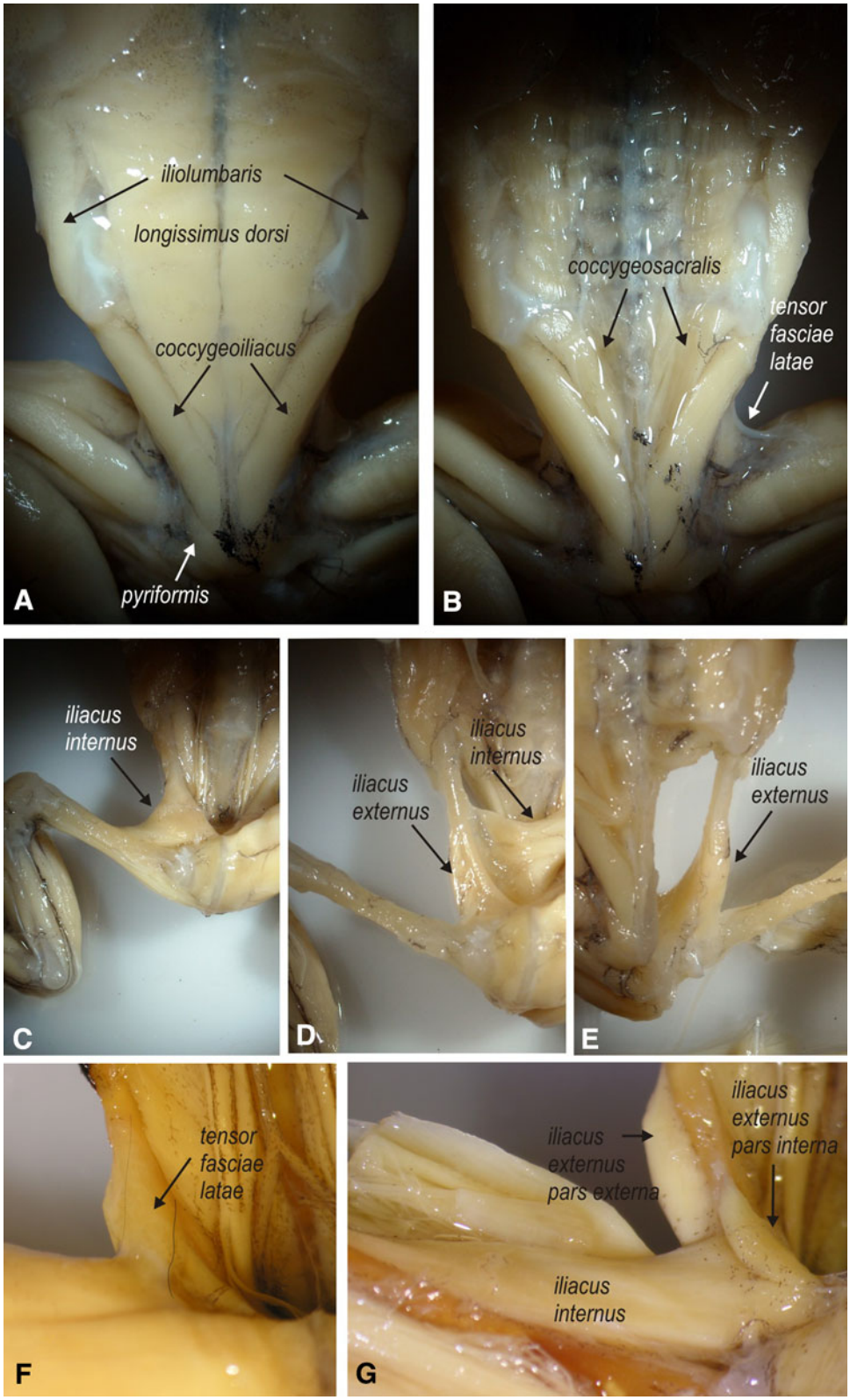

Fig. 1 **a**–**e** Adult specimen of *Lepidobatrachus laevis,* skin removed. **a** Dorsal view. **b** Same as in **a** but M. longissimus dorsi removed. **c** Ventral view. Muscles of thigh removed to show the M. iliacus internus. **d** Same as in **c** but M. iliacus internus removed. **e** Dorsal view of iliac shaft showing the M. iliacus externus. **f** and **g** Adult specimen of *Telmatobius oxycephalus*, skin removed. **f** Ventral view of pelvis showing the tensor fasciae latae originated on the inner face of the iliac shaft. **g** Same as in **g** but muscles of thigh removed to show the M. iliacus internus, and both parts of M. iliacus externus

The M. iliacus internus originates on the latero-internal surface of the ilium, opposite to the anterior part of the acetabulum, and runs laterally to insert on the anteroventral surface of the femur (Figs. 1, 2). Both insertion and origin are in the same plane. The muscle is laminar and visible upon dissection by removing the thigh musculature. The variation observed is that the origin may be wide or narrow.

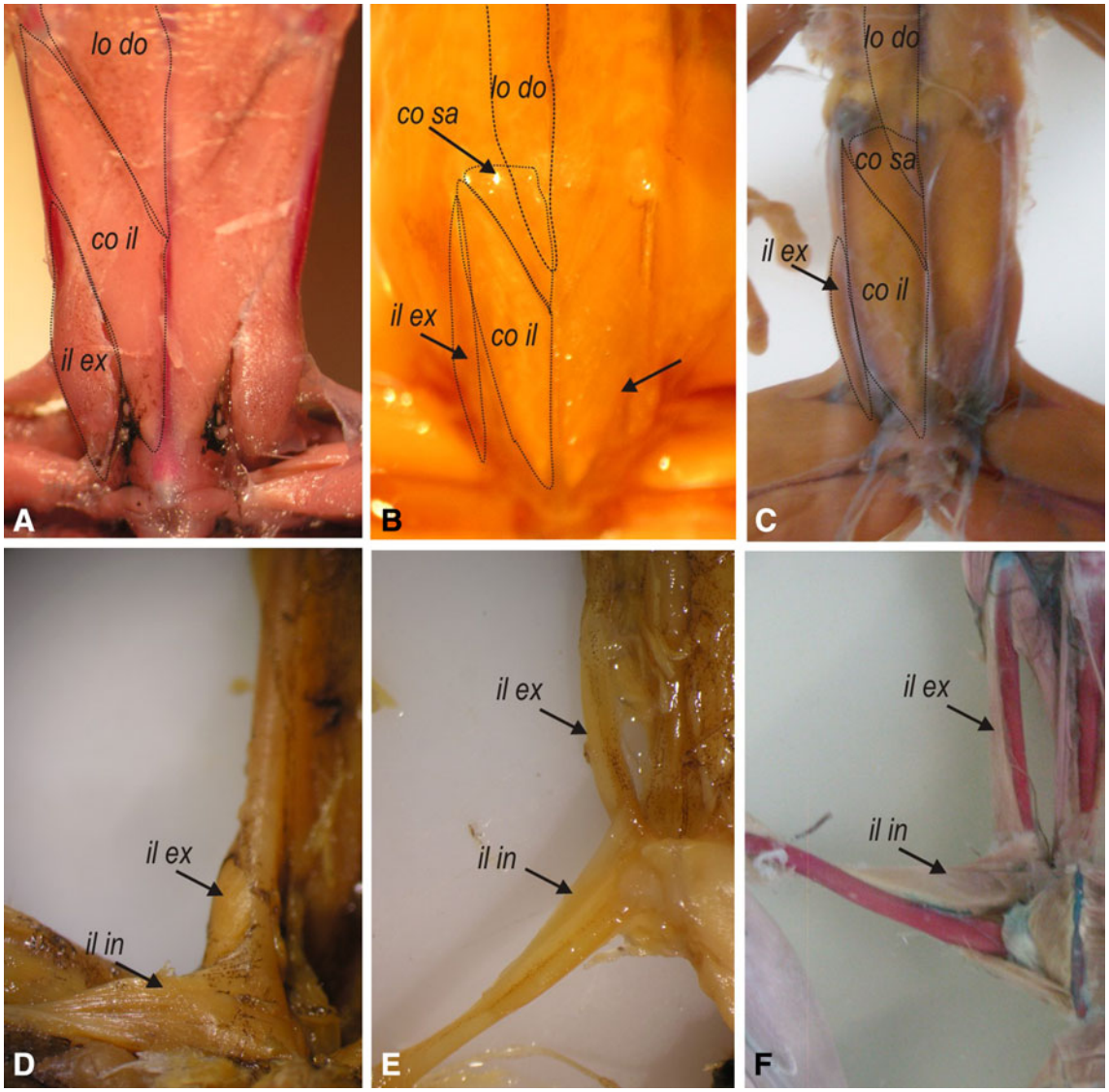

Fig. 2 **a** Dorsal view of M. longissimus dorsi, M. iliacus externus, and M. coccygeolumbaris in *Pleurodema borellii*. **b** Dorsal view of M. longissimus dorsi, M. iliacus externus, M. coccygeoiliacus and M. coccygeosacralis in *Arthroleptis lameerei*. **c** Dorsal view of M. longissimus dorsi, M. iliacus externus, M. coccygeoiliacus and M. coccygeosacralis in *Hylarana albolabris*. **d** Ventral view of M. iliacus externus, and M. iliacus internus in *Odontophrynus americanus*. Muscles of thigh removed. **e** Ventral view of M. iliacus externus, and M. iliacus internus in *Plectrohyla charadricola*. Muscles of thigh removed. **f** Ventral view of M. iliacus externus, and M. iliacus internus in *Leptodactylus chaquensis*. Muscles of thigh removed. *co il* M. coccygeoiliacus, *co sa* M. coccygeosacralis, *il ex* M. iliacus externus, *il in* M. iliacus internus, *lo do* M. longissimus dorsi

The variation was coded as detailed in Table 2, recorded in anuran species as is listed in Table 3, and was used to interpret the morphological changes in the anuran phylogeny (Fig. 4).

Ontogenetic Variation

The axial, pelvic and hind limb skeletons and their joints are completed in the general sequence of events depicted in Fig. 5a. The latest developmental event defining the juvenile anuran stage is the fusion of the hypochord and the coccyx to form the urostyle simultaneously with the complete loss of the tail.

During larval development, when the cartilages of the femur and pelvic girdle are already differentiated, the organization of the thigh musculature begins, as well as development of the muscles with origin in the ischium and ilium (e.g. the M. iliacus internus) which will insert along the femur, knee aponeurosis, tibia, and fibula (Figs. 5b, 6a). The M. iliacus internus is developed early.

At larval Stage 36, the iliac shaft has differentiated and the M. iliacus externus appears on its external surface. The

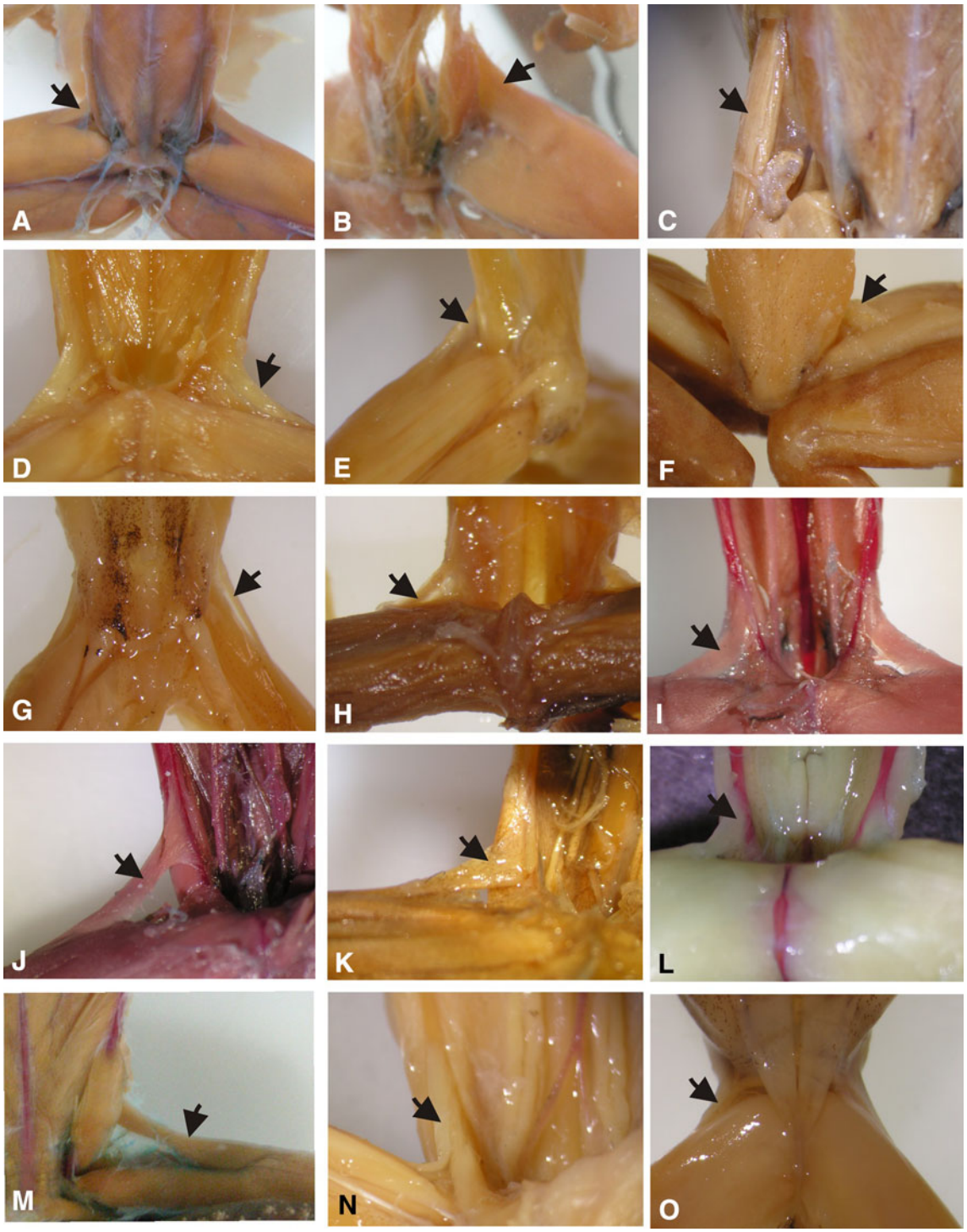

Fig. 3 Morphological variation of the tensor fasciae latae in different species. The *arrow* indicates the muscle. **a** *Hylarana albolabris*, dorsal view. **b** *Amietia angolensis*, dorsal view. **c** *Ptychadena oxyrhinchus*, dorsal view. **d** *Hyperolius phantasticus*, ventral view. **e** *Arthroleptis lameerei*, dorsal view. **f** *Phrynobatrachus asper,* dorsal view. **g** *Plectrohyla siopela*, dorsal view. **h** *Rhinoderma darwinii*, ventral view. **i** *Pleurodema borellii,* ventral view. **j** *Physalaemus biligonigerus,* ventral view. **k** *Nanophryne variegata*, ventral view. **l** *Leptodactylus latinasus*, ventral view. **m** *Rhinella schneideri*, dorsal view. **n** *Phyllomedusa sauvagii*, ventral view. **o** *Plectrohyla charadricola,* ventral view

M. iliacus externus may be short or long, depending on the species, and it grows with the iliac shaft during subsequent stages. In some species (*Phyllomedusa* spp. and *Telmatobius atacamensis*), the M. iliacus externus is divided into two distinct parts identified as pars externa and pars interna (Figs. 6, 7d, j, k).

Table 3 Variation of six characters of muscles longisimus dorsi, coccygeosacralis, tensor fascia latae, iliacus externus, and iliacus internus as was recorded in this study (see Table 2) in a sample of 54 anurans

Family	Species	Source	Character					
			0	1	2	3	4	5
Allophrynidae	*Allophryne ruthveni*	This study	1	1	2	0	0	1
Alytidae	*Discoglossus pictus*	Prĭkryl et al. (2009)	0	0	2	0	1	1
Arthroleptidae	*Arthroleptis lameerei*	This study	1	1	2	0	0	0
Ascaphidae	*Ascaphus truei*	Prĭkryl et al. (2009)	0	0	1	1	0	0
Bombinatoridae	*Barbourula busuangensis*	Prĭkryl et al. (2009)	0	0	2	0	1	1
	Bombina orientalis	Prĭkryl et al. (2009)	0	0	2	0	0	1
Bufonidae	*Amietophrynus camerunensis*	This study	1	0	1	1	0	0
	Amietophrynus kisoloensis	This study	1	0	1	1	0	0
	Melanophryniscus rubriventris	This study	0	0	1	1	0	0
	Nannophryne variegata	This study	0	0	1	1	0	0
	Rhaebo guttatus	Prĭkryl et al. (2009)	0	0	1	1	0	0
	Rhinella arenarum	This study	0	0	1	1	0	0
Centrolenidae	*Nymphargus griffithsi*	This study	1	1	2	0	0	?
Ceratophryidae	*Ceratophrys cranwelli*	This study	0	0	2	2	0	0
	Chacophrys pierottii	This study	0	0	2	2	0	0
	Lepidobatrachus laevis	This study	0	0	2	2	0	0
	Lepidobatrachus llanensis	This study	0	0	2	2	0	0
Craugastoridae	*Oreobates discoidalis*	This study	1	0	2	1	0	0
Hemiphractidae	*Flectonotus fitzgeraldi*	This study	1	1	2	0	0	1
Hylidae	*Agalychnis lemur*	This study	1	1	0	0	1	1
	Dendropsophus nanus	This study	1	1	2	0	0	1
	Dendropsophus subocularis	This study	1	1	2	0	0	1
	Hypsiboas riojanus	This study	0	0	2	0	0	1
	Phyllomedusa sauvagii	This study	0	1	2	0	1	1
	Plectrohyla charadricola	This study	1	0	2	0	0	1
	Plectrohyla siopela	This study	?	?	2	0	0	1
	Pseudis paradoxa	This study	0	0	2	0	0	0
	Scinax fuscovarius	This study	1	0	2	0	0	1
	Smilisca puma	This study	1	1	2	0	0	1
	Trachycephalus typhonius	This study	0	0	2	0	0	0
Hyperoliidae	*Hyperolius phantasticus*	This study	1	1	2	0	0	1
Leptodactylidae	*Leptodactylus bufonius*	This study	0	0	1	0	0	1
	Leptodactylus chaquensis	This study	0	0	1	0	0	1
	Leptodactylus latinasus	This study	0	0	1	0	0	1
	Physalaemus biligonigerus	This study	1	0	1	1	0	0
	Pleurodema borellii	This study	1	0	1	1	0	0
Mantellidae	*Boophis madagascariensis*	This study	1	1	1	0	0	¿
Microhylidae	*Dermatonotus muelleri*	This study	0	0	2	0	0	0
Odontophrynidae	*Macrogenioglottus alipioi*	Limeses (1964)	?	?	1	2	0	0
	Odontophrynus americanus	This study	1	0	2	2	0	0
	Odontophrynus cultripes	Limeses (1964)	?	?	2	2	0	0
	Odontophrynus occidentalis	Limeses (1964)	?	?	2	2	0	0
	Proceratophrys boiei	Limeses (1964)	?	?	1	1	0	1
Pelobatidae	*Pelobates fuscus*	Prĭkryl et al. (2009)	0	0	2	1	1	0

Table 3 continued

Family	Species	Source	Character					
			0	1	2	3	4	5
Pipidae	*Pipa pipa*	Přikryl et al. (2009)	0	0	2	0	1	1
	Xenopus laevis	Přikryl et al. (2009)	0	1	1	1	2	1
Phrynobatrachidae	*Phrynobatrachus asper*	This study	1	0	1	1	0	1
Ptychadenidadae	*Ptychadena aequiplicata*	This study	1	0	0	0	0	1
	Ptychadena oxyrhynchus	This study	1	0	0	0	0	1
Pyxicephalidae	*Amietia angolensis*	This study	1	1	1	1	0	1
Ranidae	*Hylarana albolabris*	This study	1	1	1	1	0	0
	Pelophylax lessonae	Přikryl et al. (2009)	1	1	1	1	0	1
Rhinodermatidae	*Rhinoderma darwinii*	This study	0	0	2	1	0	1
Telmatobiidae	*Telmatobius oxycephalus*	This study	1	0	2	2	1	0

The tensor fasciae latae may be already present at larval Stage 36 or differentiates later (Figs. 6, 7) especially in those species in which it is weak.

At the beginning of metamorphosis (larval stage 42), forelimbs emerge and the tadpole becomes a tetrapod. From this stage onwards, tadpoles can use the limbs to move under water, jump over vegetation and climb vertical surfaces (Fig. 8). Furthermore, additional behaviors involving autopodial specializations, such as climbing, grasping, and digging are now functional (Fig. 8). At this stage, the M. pyriformis is present. It originates on the end of the hypochord (axial skeleton), inserts on the femur, and will produce rotation of the urostyle (which is not formed yet) towards the ilium. Hind limb movements only result from actions of the pelvic and limb muscles.

During the metamorphic stages, no muscles running between the axial skeleton and pelvis are differentiated and/or functional (Fig. 9). The larval trunk musculature is retained; only the caudal muscles (posterior to the obturator muscle) are reduced and lost at the end of metamorphosis. After the formation of the ilio-sacral articulation and the fusion of the coccyx and the hypochord into the urostyle, the superficial layer of trunk musculature loses the transverse myosepta and forms a paired continuous band along the back, the M. longissimus dorsi (Fig. 9). The M. iliolumbaris gradually differentiates anteriorly to the sacral diapophyses, quite simultaneously with the differentiation of the M. longissimus dorsi, both being derived from the larval epaxial musculature. The M. coccygeosacralis and M. coccygeoiliacus derive from hypaxial musculature; they lose their segmentation after the M. longissimus dorsi is already well differentiated and their fibers will become reoriented.

The general sequence of events of differentiation of the muscles that link the axial skeleton, pelvis and hind limb in a morpho-functional complex is depicted in Fig. 5.

Discussion

Morphological Variation, Locomotion and Lifestyles

Numerous studies have analyzed the relationships between musculoskeletal morphology and locomotion with the aim of exploring if there are morphological patterns associated with a particular locomotor mode, and/or any morphological traits influencing the evolution of locomotion (Emerson 1978, 1979; Emerson and De Jongh 1980; Přikryl et al. 2009; Reilly and Jorgensen 2011, Jorgensen and Reilly 2013; among others). Emerson (1979) proposed some differences in ilio-sacral articulation that were revisited by Reilly and Jorgensen (2011) to conclude that the skeletal features of pelvis, urostyle, vertebrae and their articulations may be correlated to locomotor mode (e.g. burrower, walker, hopper, terrestrial jumper, arboreal jumper, and aquatic). These authors found that the frog bauplan is preadapted for increasing performance: generalized frogs are walkers/hoppers; and among neobatrachians, some lineages convergently increase jumping distance on land and others to climb and jump within the trees.

However, it is convenient to distinguish locomotor modes (e.g. hopping, jumping, swimming, walking) from habitat/lifestyle (e.g. aquatic, arboreal, terrestrial) and additional behaviors implying morphological specializations in the hindlimbs or even in the autopodia (e.g. burrowing, grasping, flying).

For example, adult pipids are aquatic, they have webbed feet and a lateral line system, live in tropical lentic ponds, feed underwater and swimming is their locomotor mode. Adults of *Pseudis* spp. live in tropical ponds; have limbs with intercalary elements and webbed feet (Manzano et al. 2007; Goldberg and Fabrezi 2008), but lack a lateral line system, use floating vegetation as substrate for feeding, and present effective escape mechanisms: fast swimming by a jumping-style movements or long jumps on floating vegetation (Manzano

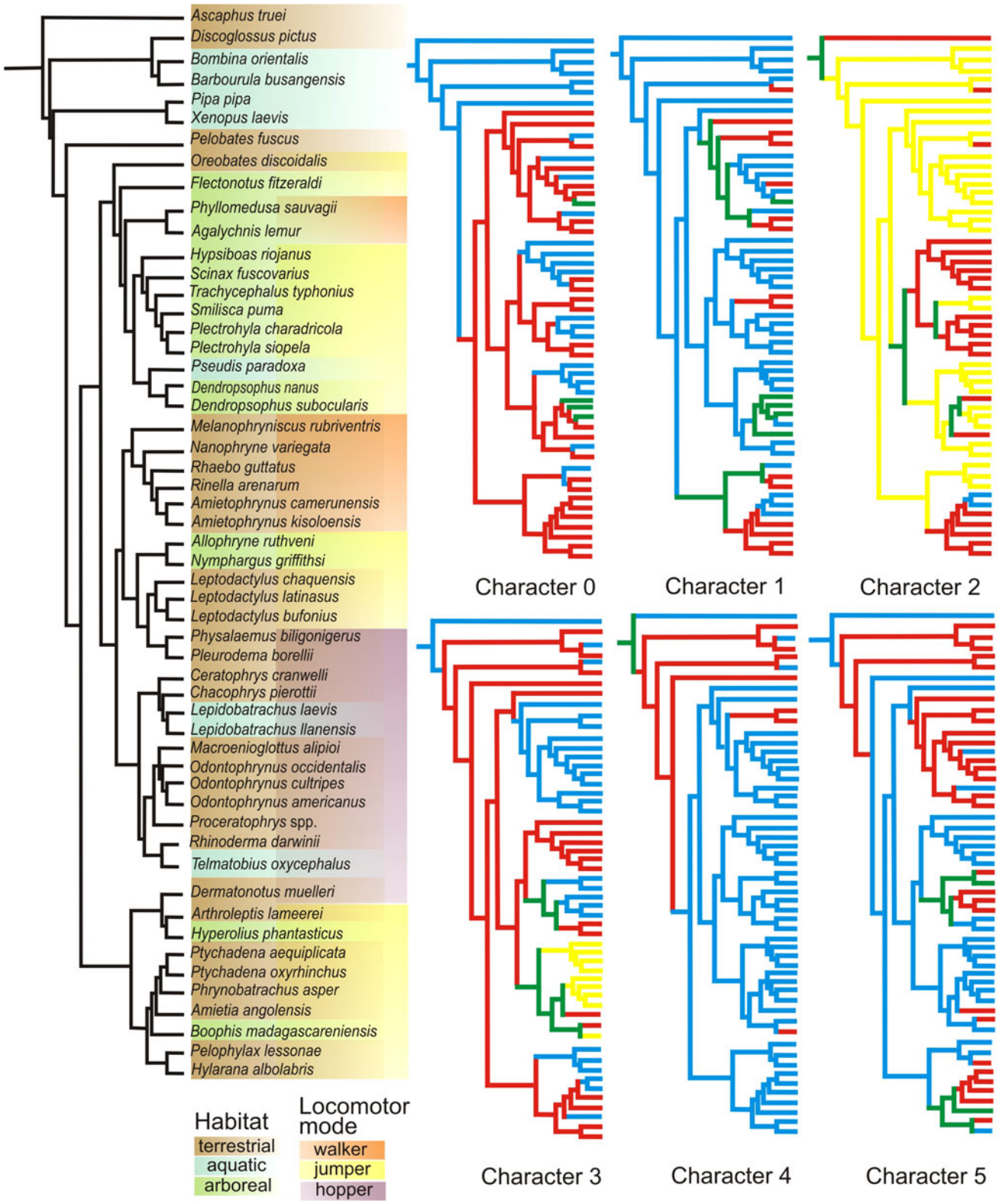

Fig. 4 Simplified anuran phylogeny by Pyron and Wiens (2011) in which the relationships of the analyzed taxa are depicted. *Shadows colors* represent habitat and the main locomotor modes. The six trees show change of characters (Color figure online)

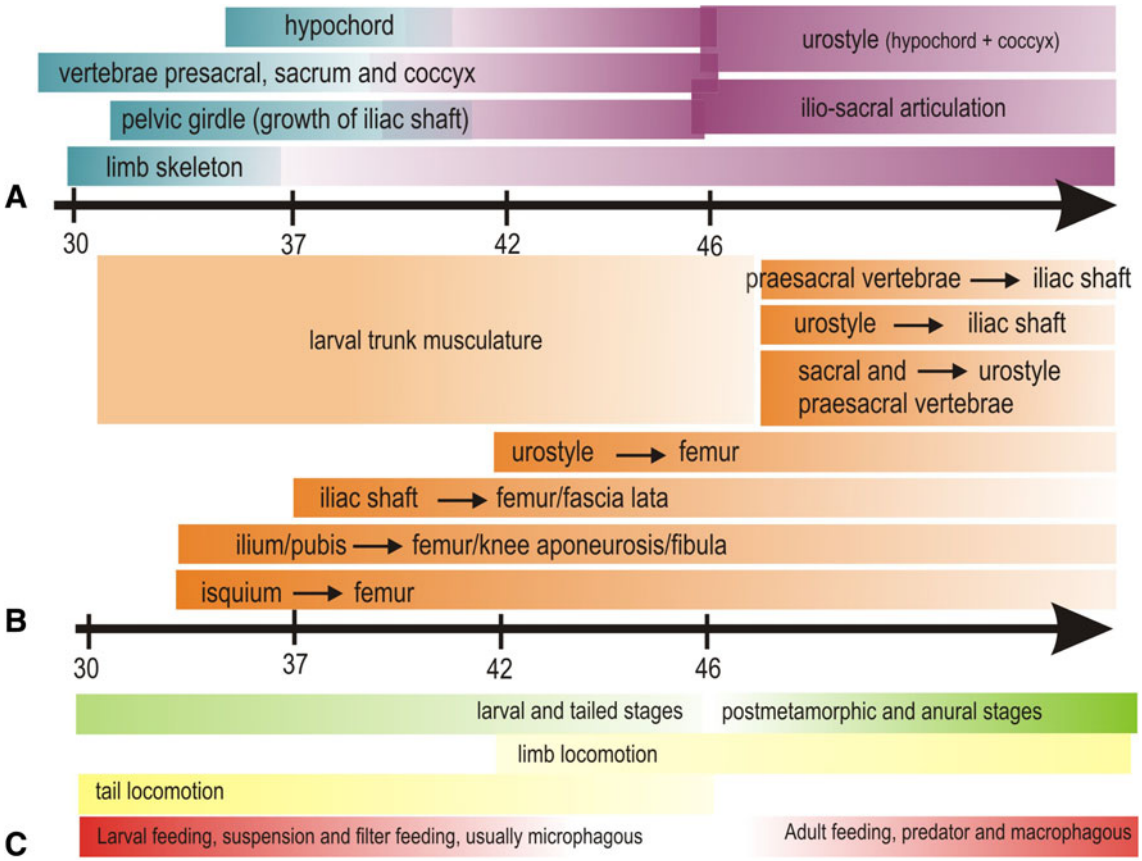

Fig. 5 **a** Sequence of development of the postaxial skeleton in most anurans according to Gosner's stages (Rocková and Rocek 2005; Pugener and Maglia 2009; Fabrezi and Goldberg 2009; Manzano et al. 2013). Differentiation of vertebrae takes place in cephalo-caudal direction; the coccyx is the last vertebral element to appear. In the limb skeleton, the primary axis differentiates in proximo-distal sequence and then, the autopodial elements develop in postaxial to preaxial direction. The three elements of the pelvic girdle (ilium, pubis, and ischium) become cartilaginous after femur differentiation, whereas the elongation of the ilium occurs when the autopodial elements are already differentiated. The cartilaginous hypochord appears after differentiation of vertebrae. The cartilaginous templates (marked in *blue*) start to ossify (marked in *violet*) at different stages. The ilio-sacral articulation and the formation of the urostyle are the last developmental events to define the adult postcranial skeleton. **b** Developmental sequence of pelvic and thigh muscles in anurans according to Gosner's stages (Manzano et al. 2013); the *arrow* indicates insertion of the muscle. Thigh muscles with origin on the ischium (M. sartorius, M. gracilis, M. semitendinosus, M. gemellus, M. adductor magnus, M. quadratus, M. obturator, M. pectineus) and the ilium (M. semimembranosus, M. cruralis; M. adductor longus, M. iliofemoralis, M. iliacus internus), and insertion on the femur are differentiated immediately once the cartilaginous femur and pelvic girdle are present. The M. iliacus externus and tensor fasciae latae appear simultaneously with iliac shaft elongation. The muscle pyriformis is well differentiated when the hypochord turns from horizontal to oblique position at the beginning of metamorphosis. Muscles that will insert on the pelvis or the urostyle start to differentiate after tail musculature is completely lost. **c** Morphological changes during anuran development: Gosner Stage 46 denotes the end of the metamorphosis, and separates tailed stages from juvenile stages. During larval development, larval and adult locomotion are functional simultaneously during the short period of tailed tetrapod stages that starts with forelimb emergence and finishes with the complete absence of a tail (Gosner Stages 42–46). Locomotion and feeding seem to be parallel: anuran larvae have a distinctive mechanism to feed from primary planktonic production, and tadpoles are usually suspension or filter feeders and microphagous (Wassersug 1975). On the other hand, the tailless stages are predators and use tongue protrusion as the mechanism for prey capture (with the exception of the aquatic pipids that lack a tongue). The hyoglossal apparatus of the postmetamorphic frog derives from the skeleton and muscles of the larval hyobranchial apparatus, whereas the tongue and its muscles are added at the end of the transformations (Fabrezi and Lobo 2009). Differentiation of the hyoglossal apparatus starts at larval Stage 42 and is finished a short time after metamorphosis. During this period the metamorphosing individual stops feeding (with the exception of *Lepidobatrachus* spp.). The development of the adult feeding mechanism and the integration of hind limb, pelvis and axial structures in a single unit are performed at the same time and suggest both could have evolved together (Color figure online)

and Barg 2005). By contrast, adults of *Lepidobatrachus laevis* have webbed feet with strong prehallical skeleton used for digging (Fabrezi 2001), a retained lateral line system, feed underwater, remain submerged in ephemeral ponds, and are slow swimmers and poor jumpers on land (Fabrezi 2011). These examples denote changes in the habitat that do not necessarily affect locomotor modes but add features useful for other specializations such as digging.

The morphological variation in pelvic musculature shows two facts: (1) change in one character is generally

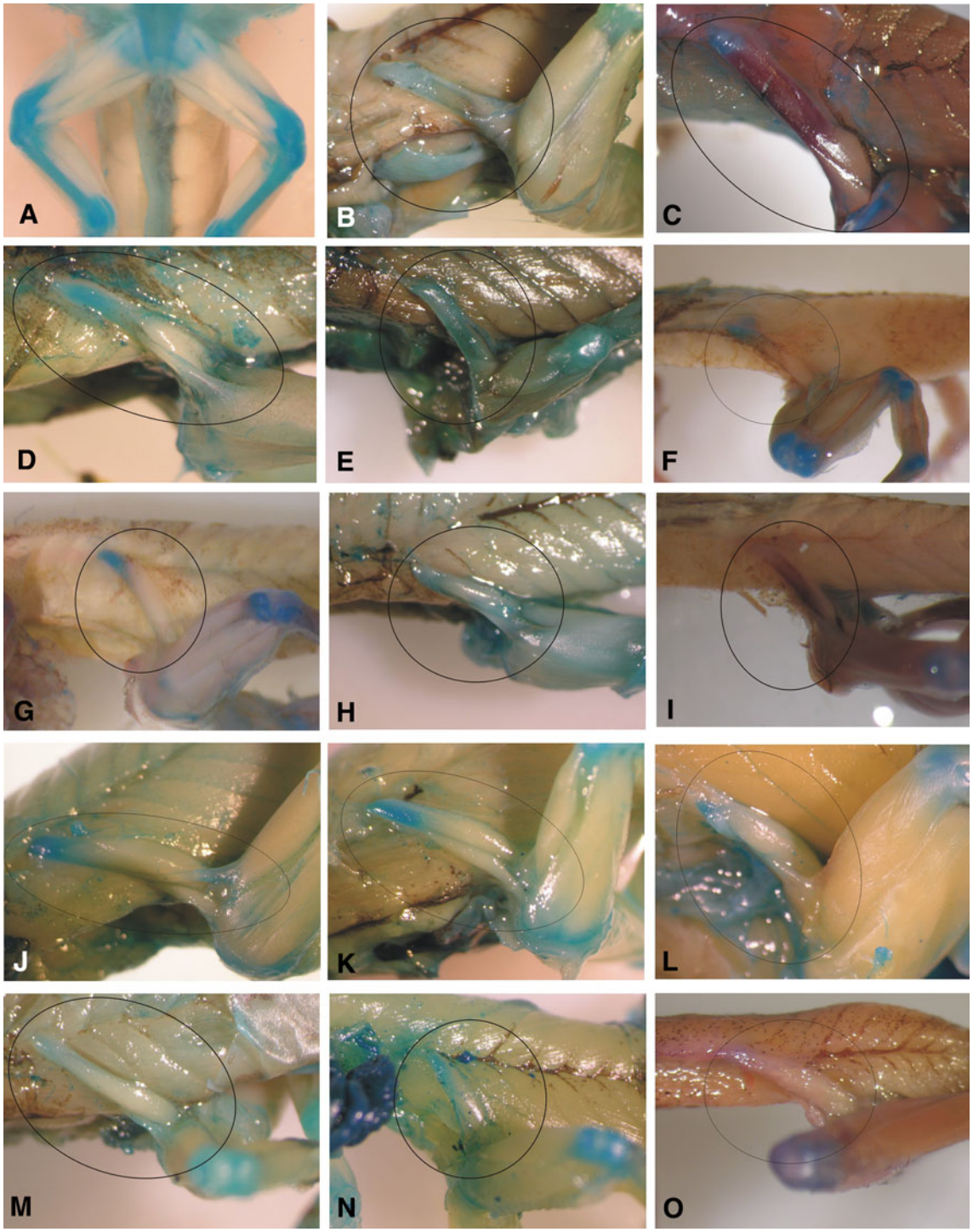

Fig. 6 **a** *Lepidobatrachus laevis*, ventral view of developing thigh musculature at larval stage 35. **b–o** Lateral view of pelvic and femoral muscles at larval stages in selected species. **b** *Ceratophrys cranwelli*, larval stage 37. **c** *Lepidobatrachus laevis,* larval stage 39. **e** *Telmatobius atacamensis*, larval stage 37. **f** *Odontophrynus lavillai*, larval stage 36. **g** *Rhinella schenideri*, larval stage 39. **h** *Dermatonotus muelleri*, larval stage 40. **i** *Leptodactylus chaquensis*, larval stage 37. **j** *Leptodactylus latinasus*, larval stage 39. **j** *Phyllomedusa sauvagii*, larval stage 37. **k** *Phyllomedusa azurea*, larval stage 37. **l** *Pseudis paradoxa*, larval stage 37. **m** *Trachycephalus thyphonius*, larval stage 37. **n** *Scinax acuminatus*, larval stage 36. **o** *Scinax fuscovarius*, larval stage 38

independent of change in another, and (2) there is not a unique combination of morphological characters related to any locomotor mode.

A long M. longissimus dorsi and a M. coccygeoiliacus with fibers parallel to the pelvis are conditions common among basal anurans, with reversions within neobatrachians. Their

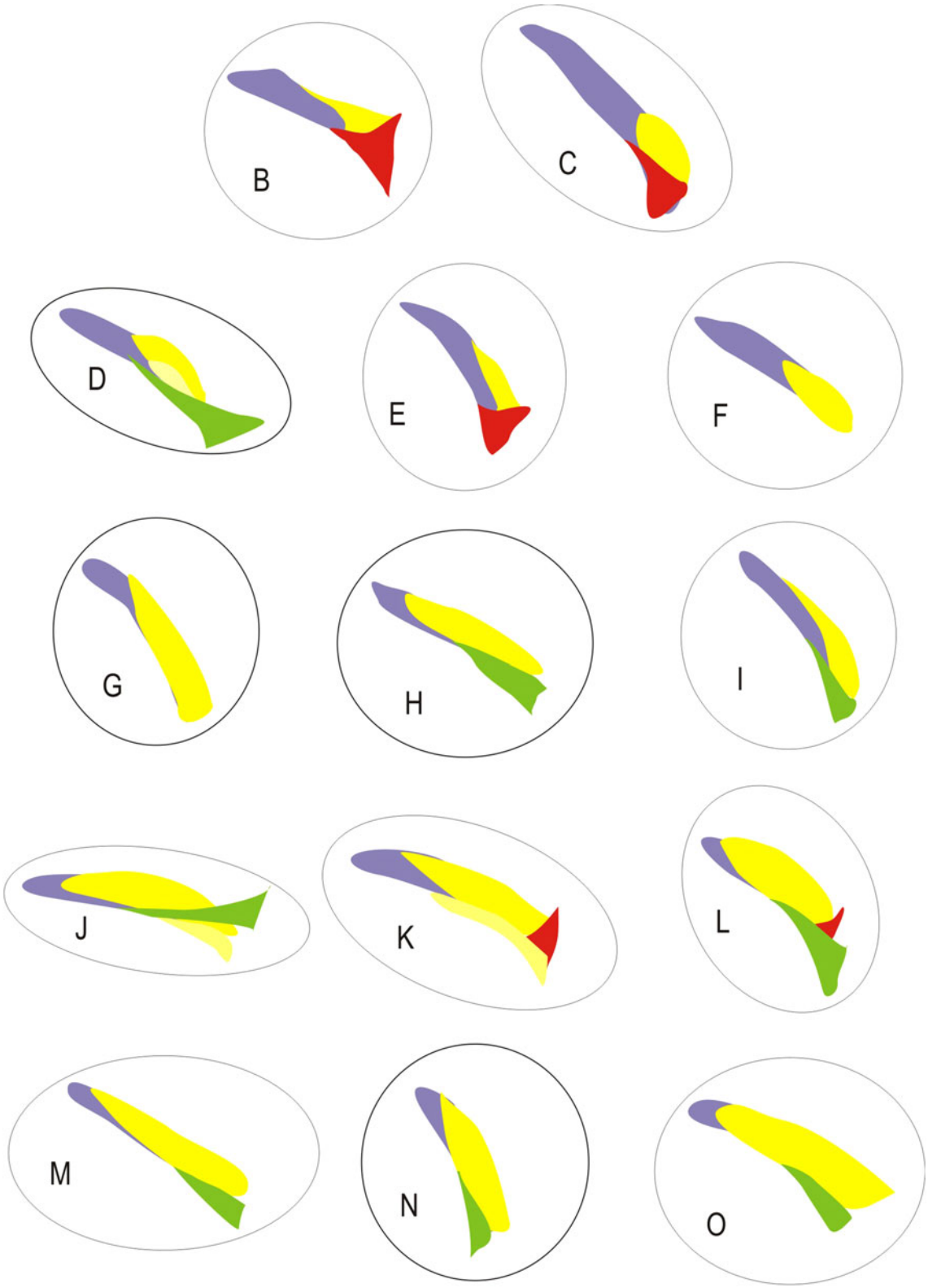

Fig. 7 Schematic representation of pelvic muscles in larvae of Fig. 2(**b–o**). The iliac shaft is *violet*. The tensor fasciae latae is *green*, the M. iliacus internus is red, the M. iliacus externus is *dark yellow*, and the M. iliacus externus pars interna is *light yellow* (Color figure online)

Fig. 8 Specimens during metamorphosis. **a–c** *Phyllomedusa azurea* at larval stage 44. The tail has lost the caudal fins and atrophy of caudal musculature is evident. Movements of limbs (fore and hind limbs) help the individual to displace under the water. **d–c** *Phyllomedusa azurea* at larval stage 45. Tail is atrophied. The individual may stay out of the water. Fingers and toes are able to oppose to help locomotion on different surfaces. **g–j** *Phyllomedusa azurea* at larval stage 45. Asynchronous movements of limbs (fore and hind limbs), prehensile capability of digits, and digital adhesive pads allow climbing on vertical surfaces. **k** *Scinax acuminatus* at larval stage 46. The specimen stays immobile on the vertical and plastic surface of the box and is able to jump quickly when a shake, noise, and/or contact occurs. **l–n** *Lepidobatrachus laevis*, larval stages 43, 44 and postmetamorphic individual. Before and after metamorphosis, individuals stay immobile at the bottom of the pond. In the presence of a threat or a prey, they move by stamping both feet repeatedly and swinging the arms. On land, tadpoles and postmetamorphic individuals are able to move by short and heavy jumps

derived conditions have evolved 13 and 8 times respectively (Fig. 4, Characters 0 and 1). The patterns of variation of these axial muscles do not seem to be related with locomotion.

The conditions of the tensor fasciae latae vary among anurans. The muscle formed by numerous fibers (Fig. 4, State 1) appears seven times in *Ascaphus truei, Xenopus*

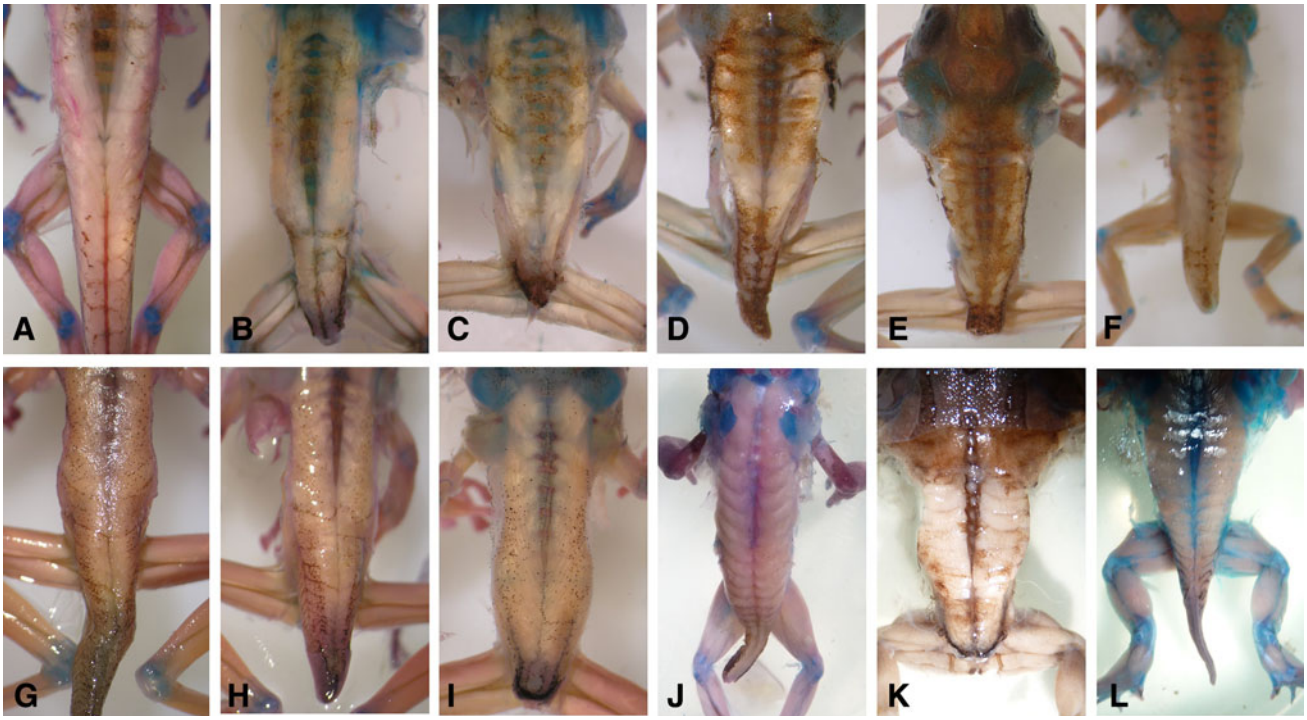

Fig. 9 Dorsal view of epaxial trunk musculature in metamorphic larvae of selected species. **a–c** *Dermatonotus muelleri,* larval stages 42, 45, 46, progressive loss of segmentation, differentiation of the M. longissimus dorsi, and reduction of caudal musculature. **d–e** *Leptodactylus latinasus*, larval stages 45, and 46, segmentation of the trunk musculature is retained at the end of metamorphosis. **f** *Rhinella schneideri*, larval stage 45, the trunk retains its segmentation and the caudal musculature is reduced but not atrophied. **g–i** *Scinax fuscovarius*, larval stages 43, 44, and 45. Progressive atrophy of caudal musculature and beginning of differentiation of the M. longissimus dorsi. **j** *Phyllomedusa sauvagii,* larval stage 45. Atrophied caudal musculature. **k** *Chacophrys pierottii,* larval stage 46, the caudal musculature has disappeared and the epaxial somites that will give origin to the longissimus dorsi are already attached to the urostyle. **l** *Lepidobatrachus laevis,* larval stage 44. Atrophied caudal musculature

laevis and neobatrachians. Reduction of fibers and loss of the M. tensor fasciae latae was described among terrestrial hyloides by Limeses (1964). Among the neobatrachians it is curious the muscle formed by numerous fibers originating on the inner surface on the posterior half of the iliac shaft is present in terrestrial good jumpers (e.g. *Leptodactylus* spp., Bufonidae, and some ranoids). The longest M. tensor fasciae latae seems to have only evolved in *Ptychadena* spp. (Fig. 4, Character 2, State 1 and 2).

A short M. iliacus externus is a derived condition for this muscle. This condition is would have evolved within the hyloids three times; once in the clade formed by *Ceratophrys* spp., *Chacophrys pierottii*, *Lepidobatrachus* spp., in *Odontophrynus* spp., and in *Telmatobius* spp. (Fig. 4, Character 3). Most of these taxa are terrestrial (or secondarily aquatic) and hoppers. Limeses (1963, 1964) studied the length of the M. iliacus externus among taxa that were considered relatives (ceratophryids, *Odontophrynus* spp., *Macrogenioglottus* and *Proceratoprhys* spp.) and she proposed relationships between the ceratophryids and *Odontophrynus*. Further, she described the shortening of the M. iliacus internus within *Lepidobatrachus* that increases in the sequence *L. llanensis, L. asper* and *L. laevis* (Limeses 1963). In the phylogenetic hypothesis used here (Pyron and Wiens 2011), the derived condition could be a synapomorphy for the large clade formed by Ceratophryidae + {Odontophryidae + [(Cyclorhamphidae + (Alsodidae + Hylodidae)) + (Rhinodermatidae + (Batrachylidae + Telmatobiidae))]} with reversions at least in *Proceratophrys* (Limeses 1964) and *Rhinoderma darwinii*. Other anuran phylogenies have proposed a monophyletic Ceratophryidae formed by Telmatobinae + (Batrachylini + Ceratophrynini) (Frost et al. 2006) or Batrachylini + (Telmatobiinae + Ceratophryinae) (Grant et al. 2006) with *Odontophrynus* forming another monophyletic group. In both cases the shortness of the M. iliacus externus would represent a synapomorphy for *Ceratophrys, Chacophrys* and *Lepidobatrachus.* Other terrestrial clades, such as bufonids and microhylids, exhibit a longer M. iliacus externus, which is very long in good jumpers (terrestrial, aquatic or arboreal, Fig. 4).

Division of the M. iliacus externus in pars externa and interna is observed in the phylogeny as reversals within hyloids; once in the walking tree frogs *Phyllomedusa sauvagii* and *Agalichnys lemur* (phyllomedusines), and the

other in the aquatic *Telmatobius* spp. (Fig. 4 Character 4). Přikryl et al. (2009) described variations in the presence of an independent pars interna by three different combinations relative to the length of pars interna and externa: pars interna long/pars externa long (*Discoglossus pictus),* pars interna short/pars externa intermediate (*Barbourula busuangensis)*; pars interna short/pars externa long (*Pipa pipa).* Here we report two additional combinations: pars interna intermediate/pars externa long for *Phyllomedusa* spp. and *Agalychnis lemur*; and pars interna short/part externa short for *Telmatobius*. Both parts of the iliacus externus produce large joint excursion of the femur-pelvic girdle joint since the moment arm is short (Oatis 2009) and when they act together gives it diverse functional capacities, such in explosive escapes or jumps that strong forces and large extensions of the legs need. Differences between the lengths of the parts could involve differences in the forces that elevate or depress the femur (Bell 1998), for example during swimming producing more forces the shorter parts.

The muscle iliacus internus with narrow origin could be the derived condition and would have evolved at least nine times within neobatrachians (Fig. 4).

Ontogeny of Locomotion

The axial skeleton (vertebrae + urostyle), pelvic girdle, hind limb and the associated musculature come together to form a unique morpho-functional system associated with anuran locomotion (Emerson and De Jongh 1980; Rage and Roček 1989; Jenkins and Shubin 1998; Přikryl et al. 2009; Reilly and Jorgensen 2011; Sigurdsen et al. 2012; Jorgensen and Reilly 2013; among others). This system develops during the larval stages while the tadpole is a free-living organism (Rocková and Rocek 2005; Pugener and Maglia 2009; Fabrezi 2011; Manzano et al. 2013). The locomotor system differentiates from two independent modules (limbs/girdles, and trunk) which are integrated after the tail has been completely lost (Fig. 5), although they are already functional before metamorphosis is finished.

The limb skeletons and their muscles are completely differentiated at larval stage 42, when ossifications in the long bones are in progress (Fig. 5a). At this stage, the forelimbs emerge and the tail undulatory movement is complemented by limb extension-flexion movements that permit rapid escape in a rectilinear plane of motion (Fig. 8) and/or rotation of the direction of this escape. When the forelimbs emerge, the pelvis and sacro-urostyle complexes are incompletely developed and still retain a larval position.

The ilio-sacral articulation and pelvic morphology have long been considered key to the origin of special features in anuran locomotion (Emerson 1978, 1979; Emerson and De Jongh 1980; Jenkins and Shubin 1998; Kargo and Rome 2002; Nauwelaerts et al. 2005, 2007; Přikryl et al. 2009). However, anurans achieve tetrapod locomotion at the beginning of metamorphosis, before the adult pelvis is completely formed (Pugener and Maglia 2009). Simultaneously with tail loss, the skeletal/joint system such as the urostyle and ilio-sacral articulation acquires their final form but the muscles integrating the axial skeleton and pelvis have still not differentiated (Fig. 5b).

It is generally accepted that the origin of anuran locomotion involved changes in the pelvis/axial skeleton, and those muscles that have a derived condition with respect to the ancestors would be associated with new locomotor modes (Jenkins and Shubin 1998; Přikril et al. 2009). Herein, we found the iliac shaft muscles (Mm. iliacus externus and internus, tensor fasciae latae) and thigh musculature seem to be enough for the occurrence of characteristic anuran locomotion before tail loss. On the other hand, differentiation of trunk muscles (e.g. M. longissimus dorsii, M. coccyegeoiliacus, M. iliolumbaris, and M. coccygeosacralis) occurs postmetamorphically (Figs. 5b, 9). The functions of these muscles correspond to movements such as lateral bending and gliding (Prikryl et al. 2009) and could add capabilities to the locomotion (e.g. trunk rotation, pelvis upload) for prey capture, or even during swimming or floating (Manzano and Barg 2005).

Metamorphosis is the period in which feeding and locomotion become distinctive among anurans (Fig. 5c). Most anurans stop feeding during metamorphosis and may undergo a short postmetamorphic period (days or weeks) without feeding [the exception is *Lepidobatrachus* spp., which feed during metamorphosis, probably because these frogs have adult-like tadpoles (Fabrezi 2011)]. Interesting, there is also a period after metamorphosis in which the axial muscles retain the larval morphology, which must then be transformed and integrated with the developing pelvic girdle (Fig. 5c).

Wassersug and Sperry (1977) demonstrated that the timecourse of acquisition of the new locomotion is a response to the high risk of predation during the metamorphosis; the larval tail is functional and essential to tadpole swimming, and is a limiting factor to delay the adult locomotion, whereas exposed forelimbs could diminish tadpole swimming ability during metamorphosis. However, some authors consider that the tail would not significantly disturb jumping locomotion, but instead shift the centre of mass, changing its stability (Gans and Parsons 1966). The combination of larval and adult morphological traits defines a short period in which tailed-tetrapod individuals are able to use the paired locomotor appendages to move on land, vegetation, and vertical surfaces (Fig. 8). This period implies that most important structures and features for adult locomotion are already developed in the metamorphosing body plan (e.g. girdles/limbs). These structures and features are: fusion of the elements of

zeugopodia, elongation of the proximal tarsalia, and differentiation of muscles of the thigh, and mm. iliacus externus and internus, and tensor fasciae latae.

After metamorphosis, there is a period that might occur simultaneously with the acquisition of a new feeding mechanism and comprises the establishment of the ilio-sacral articulation and the formation of the urostyle, and involves differentiation of the muscles longissimus dorsii, coccygeoiliacus, coccygeosacralis, and iliolumbaris (Fig. 5c).

Although we cannot determine how long after the end of metamorphosis the froglets have functional pelvic and axial muscles, we propose that this set of muscles would provide new movements which could have evolved for prey capture.

Handrigan and Wassersug (2007) defined the anuran bauplan by the following features: (1) absence of discrete caudal vertebrae, (2) a truncated axial skeleton, and (3) elongated tarsal elements. Despite the scarce fossil record of anurans, these three major features seem to have evolved during the 250 MYA of anuran history in a sequence in which limb features would have appeared prior to those in the axial skeleton (Table 1).

To conclude, morphological variation in skeleton and muscles show there is not a unique combination of morphological characters for each locomotor mode. The ontogeny of the systems participating in the anuran post-metamorphic locomotion reveals a sequence in which the development of paired limbs and girdles are the prerequisite to perform the tetrapod anuran locomotion independently of the larval organization of the axial musculoskeletal system. Tail loss, urostyle formation, formation of the ilio-sacral articulation and transformations of axial muscles add improvements to locomotion for other requirements (e.g. prey capture). Like the ontogeny, the fossil record suggests anuran postmetamorphic locomotion originated first by changes in paired appendages and girdles, with subsequent integration with axial structures.

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Appendix 1: Abbreviations of Museum

MCN, Museo de Ciencias Naturales, Universidad Nacional de Salta, Argentina.

MNHN, Museo Nacional de Historia Natural, Montevideo, Uruguay.

DIAM, Centro de Investigación y Transferencia Técnica a la Producción, Diamante, Argentina.

Larval Series

Bufonidae (Hyloidea): *Rhinella schneideri* (Werner, 1894): MCN 1316 (14 specimens, larval stages 38–46)

Ceratophryidae (Hyloidea): *Chacophrys pierottii* (Vellard, 1948): MCN 1029 (12 specimens, larval stages 35–46, and three froglets). *Ceratophrys cranwelli* Barrio, 1980: MCN 413 (7 specimens, larval stages 37–46), MCN 425 (7 specimens, larval stages 42–46), MCN 1318 (8 specimens, larval stages 34–37). *Lepidobatrachus laevis* Budgett, 1899: MCN 647 (12 specimens, larval stages 33–40), MCN 695/696 (three juvenile specimens), MCN 1320/1322 (8 specimens, stages 39–46). *Lepidobatrachus llanensis* Reig and Cei, 1963: MCN 1122 (8 specimens, larval stages 39–46), MCN 1178 (6 specimens, larval stages 32–38).

Hylidae (Hyloidea): *Phyllomedusa azurea* Cope, 1862: MCN 1054 (12 specimens, larval stages 34–40); MCN 1346 (12 specimens, larval stages 37–46). *Phyllomedusa sauvagii* Boulenger, 1882: MCN 1159 (12 specimens, larval stages 31–39); MCN 1330 (9 specimens, larval stages 38–46). *Pseudis paradoxa* (Linnaeus, 1758): MCN 1171 (8 specimens, larval stages 33–42); MCN 1197 (12 specimens, larval stages 33–46). *Scinax acuminatus* (Cope, 1862): MCN 1145 (12 specimens, larval stages 38–42). *Scinax fuscovarius* (Lutz, 1925): MCN1134 (7 specimens, larval stages 34–38), MCN 1144 (10 specimens, larval stages 38–44), MCN 1315 (7 specimens, larval stages 38–46). *Scinax nasicus* (Cope, 1862): MCN 1350 (13 specimens, larval stages, 36–43). *Trachycephalus typhonius* (Linnaeus, 1758): MCN1127 (9 specimens, larval stages 37–43); MCN 1333 (9 specimens, larval stages 38–46).

Leptodactylidae (Hyloidea): *Leptodactylus chaquensis* Cei, 1950: MCN 1343 (7 specimens larval stages 35–37); MCN 1335 (6 specimens, larval stages 39–42; 45). *Leptodactylus fuscus* (Schneider, 1799): MCN 1344 (6 specimens, larval stages 39–43). *Leptodactylus latinasus* Jiménez de la Espada, 1875: MCN 1352 (12 specimens, larval stages 38–46). *Physalaemus biligonigerus* (Cope, 1861): MCN 1097 (13 specimens, larval stages 37–46).

Microhylidae (Ranoidea): *Dermatonotus muelleri* (Boettger, 1885): MCN 1332 (12 specimens, larval stages 37–46)

Odontophrynidae (Hyloidea): *Odontophrynus lavillai* Cei, 1985: MCN 230 (8 specimens, larval stages 36–42).

Telmatobiidae (Hyloidea): Telmatobius atacamensis Gallardo, 1962: MCN 1325 (5 specimens, larval stages 37, 39–42).

Adults

Allophrynidae: MNHN 0539 *Allophryne ruthveni* Gaige, 1926 (1 specimen)

Arthroleptidae: MCN 941 *Arthroleptis lameerei* De Witte, 1921 (3 specimens)

Bufonidae: MCN s/n, col. Personal R.F. Laurent *Amietophrynus camerunensis* (Parker, 1936) (2 specimens); MCN s/n, col. Personal R.F. Laurent *Amietophrynus kisoloensis* (Loverdige, 1932) (2 specimens); MCN s/n Col. Marcos Vaira 88/89 *Melanophryniscus rubriventris* (Vellard, 1947) (2 specimens); MCN 018 *Nannophryne variegata* Günther, 1870, (2 specimens); MCN 266 *Rhinella arenarum* (Hensel, 1867) (2 specimens).

Centrolenidae: DIAM 325 *Nymphargus griffithsi* (Goin, 1961) (1 specimen)

Ceratophryidae: MCN 819 *Ceratophrys cranwelli* Barrio, 1980 (2males); MCN 987 *Chacophrys pierottii* (Vellard, 1948): (2 males); MCN 931 *Lepidobatrachus laevis* Budgett, 1899: (2 males); MCN 986 *Lepidobatrachus llanensis* Reig and Cei, 1963 (2 specimen, male and female);

Craugastoridae: MCN 466 *Oreobates discoidalis* (Peracca, 1895) (2 specimens)

Hemiphractidae: MCN 017 *Flectonotus fitzgeraldi* (Parker, 1934) (2 specimens)

Hylidae: MCN 012 *Agalychnis lemur* (Boulenger, 1882) (2 specimens); MCN 724 *Dendropsophus nanus* (Boulenger, 1889) (1 specimen); MCN 217 *Dendropsophus subocularis* (Dunn, 1934) (2 specimens); MCN 092 *Hypsiboas riojanus* (Koslowsky, 1895) (1 specimen); MCN 795 *Phyllomedusa sauvagii* Boulenger, 1882 (2 specimens); MCN 014 *Plectrohyla charadricola* (Duellman, 1964) (2 specimens); MCN 216 *Plectrohyla siopela* (Duellman, 1968) (2 specimens); MCN 1110 *Pseudis paradoxa* (Linnaeus, 1758) (2 specimens); MCN 799 *Scinax fuscovarius* (Lutz, 1925) (2 specimens); MCN 011 *Smilisca puma* (Cope, 1885) (2 specimens); MCN 794 *Trachycephalus typhonius* (Linnaeus, 1758) (1 specimen).

Hyperoliidae: MCN s/n, col. Personal R.F. Laurent 235 *Hyperolius phantasticus* (Boulenger, 1899) (2 specimens)

Leptodactylidae: MCN 520 *Leptodactylus chaquensis* Cei, 1950 (3 specimens); MCN 551 *Leptodactylus bufonius* Boulenger, 1894 (2 specimens); MCN 593 *Leptodactylus latinasus* Jiménez de la Espada, 1875 (2 specimens); MCN 725 *Physalaemus biligonigerus* (Cope, 1861) (3 specimens): MCN 545 *Pleurodema borellii* (Peracca, 1895) (2 specimens)

Mantellidae: DIAM 319 *Boophis madagascariensis* (Peters, 1874) (1 specimen)

Microhylidae: MCN 997 *Dermatonotus muelleri* (Boettger, 1885) (2 specimens)

Odontophrynidae: MCN 105 *Odontophrynus americanus* (Duméril and Bibron, 1841)(2 specimens)

Phrynobatrachidae: MCN s/n, col. Personal R.F. Laurent *Phrynobatrachus asper* Laurent, 1951 (2 specimens)

Ptychadenidadae: MCN s/n, col. Personal R.F. Laurent *Ptychadena aequiplicata* (Werner, 1898) (2 specimens); MCN s/n, col. Personal R.F. Laurent *Ptychadena oxyrhynchus* (Smith, 1849) (2 specimens)

Pyxicephalidae: MCN s/n, col. Personal R.F. Laurent *Amietia angolensis* (Bocage, 1866) (2 specimens)

Ranidae: MCN s/n, col. Personal R.F. Laurent *Hylarana albolabris* (Hallowell, 1856) (2 specimens)

Rhinodermatidae: MCN 020 *Rhinoderma darwinii* Duméril and Bibron, 1841 (2 specimens)

Telmatobiidae: MCN 440 *Telmatobius oxycephalus* Vellard, 1946 (2 males)

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