# Forelimb musculoskeletal-tendinous growth in frogs

Mónica Carina Soliz[1]*, María José Tulli[2]*, Abdala Virginia[3]

[1] Universidad Nacional de Salta, Facultad de Ciencias Naturales, Cátedra Vertebrados, Salta, Argentina

[2] UEL-CONICET- Instituto de Herpetología- Fundación Miguel Lillo. Cátedra de Biología Animal, Facultad de Ciencias Naturales, UNT. Tucumán, Argentina ORCID: 0000-0003-4763-6973

[3] Instituto de Biodiversidad Neotropical (IBN), UNT-CONICET. Cátedra de Biología General, Facultad de Ciencias Naturales, UNT. Tucumán, Argentina ORCID: 0000-0002-4615-5011

Corresponding Author:

[1] Mónica Carina Soliz Universidad Nacional de Salta (UNSa), Facultad de Ciencias Naturales, Cátedra Vertebrados, Salta, Argentina (4200). monica.c.soliz@gmail.com

[2] María José Tulli UEL-CONICET- Instituto de Herpetología- Fundación Miguel Lillo. Cátedra de Biología Animal, Facultad de Ciencias Naturales, UNT. San Miguel de Tucumán, Tucumán, Argentina (4000). majotulli@gmail.com.

## Abstract

The tendons unite and transmit the strength of the muscles to the bones, allowing movement

dexterity, the distribution of the strength of the limbs to the digits, and an improved muscle

31    performance for a wide range of locomotor activities. Tissue differentiation and maturation of

32    the structures involved in locomotion are completed during the juvenile stage; however, few

33    studies have investigated the ontogenetic variation of the musculoskeletal-tendinous system. We

34    ask whether all those integrated tissues and limb structures growth synchronically between them

35    and along with body length. We examined the ontogenetic variation in selected muscles, tendons

36    and bones of the forelimbs in seventy-seven specimens belonging to seven anuran species of

37    different clades and of three age categories, and investigate the relative growth of the forelimb

38    musculoskeletal-tendinous structures throughout ontogeny. Ten muscles and nine tendons and

39    their respective large bones (humerus and radioulna) were removed intact, and their length was

40    measured and analysed through a multivariate approach of allometry. We obtained an allometry

41    coefficient, which indicates how the coefficient departures from isometry as well as allometric

42    trends. Our data suggest that along with the post-metamorphic ontogeny, muscles tend to

43    elongate proportionally to bone length, with a positive allometric trend. On the contrary, tendons

44    show a negative allometric growth trend. Only two species show different patterns: *Rhinella*

45    *granulosa* and *Physalaemus biligonigerus*, with an isometric and positive growth of muscles and

46    bones, and most tendons being isometric.

47

48    **Keywords**: muscles, bones, tendons, allometry, ontogeny, anuran

## Introduction

49
50
51    The musculotendinous system is particularly active in the general limb movements. The tendons

52    unite and transmit the strength of the muscles to the bones, allowing movement dexterity, and the

53    distribution of the strength of the limbs to the digits (Kardong, 2002). The release of the elastic

54    energy of muscular aponeuroses and ligaments amplifies the power and reduces muscle work

55  (Roberts, 2002; Biewener, 2003). The capacity for the differential jump between frog species is

56  related to the relative amount of musculature of the hindlimb and the use of the energy stored in

57  tendons and ligaments (Emerson, 1978). Further, tendons improve muscle performance for a

58  wide range of locomotor activities (Roberts, 2002).

59  During ontogeny, the characteristics of muscle architecture and connective tissue vary

60  according to the body length increase, and to the increase of daily activity functional demands.

61  Thus, many of the evolutionary and developmental transformations in structures related to the

62  locomotor function in anurans occur during larval stages and metamorphosis (Ročkova & Roček,

63  2005; Púgener & Maglia, 2009; Manzano et al., 2013; Fabrezi et al., 2014). Studies on anuran

64  ontogeny reveal that locomotor modes (walking, jumping, and swimming) are achieved before

65  the acquisition of the pelvis-sacral-urostil complex coordination, and the hindlimbs are acting as

66  a unit (Fabrezi et al., 2014). Although metamorphosis has been considered as the period in which

67  the most critical anatomical characteristics for adult locomotion are developed (for example,

68  those related to girdles and limbs; Fabrezi et al., 2014), it is known that tissue differentiation and

69  maturation of the structures involved in locomotion are completed during the juvenile stage

70  (Vera, Ponssa & Abdala, 2015).

71  There are numerous studies on anuran ontogeny (Hanken & Hall, 1984; De Sá, 1988; Hall &

72  Larsen, 1988; Banbury & Maglia, 2006; Handrigan & Wassersug, 2007; Handrigan, Haas &

73  Wassersug, 2007; Manzano et al., 2013; among many others); and on the relationship between

74  different ontogenetic trajectories and modes of life (Haas, 1999; Haas & Richards, 1998;

75  Ročková & Roček, 2005; Púgener & Maglia, 2009; Manzano et al., 2013; Fabrezi et al., 2014;

76  Soliz & Ponssa, 2016). However, considerably less attention has been given to the changes

77  occurring through juveniles and metamorphic stages (Ponssa & Vera Candioti, 2012). Juveniles

78    of most species must maneuver in the same environment and avoid the same predators as adults,

79    but they undergo ontogenetic changes that allow them to sprint and jump almost as fast and far

80    as adults. The allometric changes in juveniles include relatively longer limbs, muscular forces,

81    and relatively greater contractile speeds and higher muscular mechanical advantage (Carrier,

82    1995; Ponssa & Vera Candioti, 2012).

83    Several researchers found that subtle structural differences in the proportion of hindlimbs

84    would facilitate functional diversity, allowing for wide-ranging exploitation of environments

85    (Duellman & Trueb, 1994; Emerson, 1978; 1979; 1983; 1985; Emerson & De Jongh, 1980;

86    Nauwelaerts, Ramsay & Aerts, 2007). Ontogenetic processes have been considered responsible

87    of the morphological variability among species and could have a profound impact on the shape

88    of morphological structures (Thompson, 1942; Gould, 1977; Alberch, Gould & Wake, 1979;

89    Calder, 1984; Schmidt-Nielsen, 1984; Emerson & Bramble, 1993; Reilly, Wiley & Meinhardt,

90    1997; Vukov et al., 2018). However, few studies have investigated the ontogenetic variation of

91    the musculotendinous system, which could be responsible for generating and transmitting force

92    to produce and control body movements. This active force is produced by the muscle fibers and

93    is transmitted to the bones through aponeuroses and tendons (Biewener, 1998; Böl, Leichsenring

94    & Siebert, 2017).

95    Here, we examined the ontogenetic variation in selected muscles, tendons, and bones of the

96    forelimbs in seven anuran species. Our main goal was to investigate the relative growth of the

97    forelimb musculoskeletal-tendinous structures throughout ontogeny. All the ontogenetic stages

98    considered here belong to the fully functional category (Muntz, 1976), implying that all tissues

99    and limb structures are integrated. We ask whether all those integrated tissues and limb

100    structures growth synchronically between them and along with body length, and hypothesize that

101   our data will show a general allometric pattern of increased growth rate with larger body length.

102   The study of the effect of the ontogeny onto the longitudinal growth of the musculoskeletal-

103   tendinous structures of frogs represents a starting point to a more global analysis considering

104   other variables such as volume or cross-sectional areas.

105

106   **Materials and Methods**

107   The right forelimb of seventy-seven specimens belonging to six species encompassing different

108   clades of the anuran phylogeny (Duellman et al. 2016 and Pyron and Wiens, 2011) were

109   dissected (Table 1). Then, 10 muscles and 9 tendons, and their respective large bones (humerus

110   and radioulna) (Table 2) were removed intact, and their length was measured (Fig. 1). The

111   specimens were staged in three estimated categories according to Gosner (1960): metamorphic

112   46 (2); juvenil (3); and adult (4). Dissections of the anatomical traits were performed between the

113   origin and insertion points under a binocular microscope (Nikon SMZ645), and were measured

114   in mm with digital callipers (±0.01 mm; Mitutoyo CD-15B, Mitutoyo Corp., Kure, Japan). When

115   referring to muscles, abbreviation "m." before muscle names was added; otherwise names refer

116   to tendons associated with those muscles (Table 1). The terminology used follows Přikryl et al.

117   (2009), Abdala & Diogo (2010), Diogo & Ziermann (2014). Data of muscles measurements,

118   body length and number of individuals used per species are detailed as Table S1. All the

119   examined specimens are deposited in systematic collections, and listed in Table 1.

120

121   **Statistical Analysis**

122   To estimate the scaling of muscles and tendons throughout postnatal ontogeny of the forelimb we

123   performed a multivariate allometric tests based on the generalized allometric equation proposed

124    by Jolicoeur (1963). We performed a principal component analysis (PCA) to obtain the 1st PC

125    eigenvector that expresses the scaling relationships among all variables with the latent size

126    regarded as a latent variable affecting all measured variables simultaneously (Giannini, Abdala

127    & Flores, 2004; Giannini et al., 2010; Segura & Prevosti, 2012). This eigenvector is extracted

128    from a variance-covariance matrix of $\log_{10}$-transformed variables and scaled to unity (Jolicoeur,

129    1963). The significance of multivariate allometry coefficients was tested using a resampling

130    strategy based on jackknife. Each specimen was removed from the sample at a time, generating n

131    pseudovalues to calculate confidence intervals (CIs) for the original coefficients (Giannini et al.,

132    2004; Flores, Giannini & Abdala., 2006). If the interval excluded an expected value of isometry,

133    the variable was considered positively or negatively allometric. For all multivariate coefficients

134    of allometry, the expected value of isometry, which depends only on the number of variables (p),

135    is calculated as $1/p^{0.5}$ (0.21) for our set of 22 variables). Trimming the largest and smallest m

136    pseudovalues (with m=1) for each variable may significantly decrease the standard deviations

137    calculated under jack-knife, and allow for more accurate allometric estimations (Manly, 1997;

138    Giannini et al., 2004). Here, untrimmed and trimmed calculations are reported, but the chosen

139    results are those with either lower average standard deviation, or lower bias (with the latter

140    defined as the difference between observed and jackknifed allometry coefficient; Giannini et al.,

141    2004).

142

143    **Results**

144       Individual values of the analysed morphological variables are shown in Table S1. Scaling

145    analyses describing ontogenetic growth in length and width of forelimb muscles and tendons in

146    seven frog species are shown in Table 3. Results of allometry multivariate analyses are given in

147    Table 3. We report untrimmed (m=0) as well as trimmed (m=1) calculations of confidence

148    intervals, opting for the results with lower average standard deviation or bias, trimmed with CI

149    95% which combines the conservative safety of interval and estimated bias in these analyses .

150

151    **Musculoskeletal-tendinous allometry in *Rhinella arenarum***

152        Trends obtained with untrimmed and trimmed values differed only in relation to five

153    variables: radioulna length (RUL), extensor carpi radialis tendon length (EcrT), humeralis

154    medialis tendon (HmedT) and m. coracoradialis (C). The greatest departure was observed in two

155    variables, m. flexor digitorum communis (Fdc) and m. flexor carpi ulnaris (Fcul) lengths (-0.213

156    and -0.208, respectively). Two variables showed the smallest observed departure from isometry:

157    m. flexor carpi radialis (FcrT) and flexor carpi ulnaris tendon lengths (FculT) (0.061 and -0.068,

158    respectively). Based on this result, twelve variables significantly departed from isometry:

159    radioulna length (RUL), m. humeralis lateralis (Hlat), m. extensor digitorum (Edig), m. extensor

160    carpi ulnaris (Ecul), m. flexor digitorum communis (Fdc) and extensor carpi radialis tendon

161    length (EcrT), and body length (BL), all positively allometric. M. coracoradialis (C), humeralis

162    medialis tendon length (HmedT), flexor digitorum communis tendon length (FdcT), flexor carpi

163    ulnaris tendon length (FculT) and flexor carpi radialis tendon length (FcrT) were all negatively

164    allometric (Table S2).

165

166    **Musculoskeletal-tendinous allometry in *Rhinella granulosa***

167        Trends obtained with untrimmed and trimmed values differed only in relation to five

168    variables: scapularis medialis tendon length (SMT), extensor carpi radialis tendon length (EcrT),

169    m. coracoradialis (C), m. flexor carpi ulnaris (Fcul) and m. flexor carpi radialis (Fcr). The

170 greatest departure was observed in two variables, m. extensor carpi ulnaris (Ecul) and extensor

171 carpi radialis tendon length (EcrT) (0.320 and 0.308, respectively). The smallest departure

172 observed from isometry was found in scapularis medialis tendon length (SMT) and humeralis

173 lateralis tendon length (HlatTL) (0.108 and 0.082, respectively). Based on this result, six

174 variables significantly departed from isometry: humeralis lateralis tendon length (HlatT),

175 scapularis medialis tendon length (SMT), and m. coracoradialis (C; all negatively allometric);

176 and extensor carpi radialis tendon length (EcrT), m. flexor carpi ulnaris (Fcu) and m. flexor carpi

177 radialis (Fcr; all positively allometric) (Table S3).

178

179 **Musculoskeletal-tendinous allometry in *Trachycephalus typhonius***

180 Trends obtained with untrimmed and trimmed values differed only in relation to seven

181 variables: humerus length (HL), humeralis lateralis (Hlat), m. extensor carpi radialis (Ecr) and

182 tendon length (EcrT), m. extensor carpi ulnaris (Ecu) and tendon length (EcuT) and flexor

183 digitorum communis tendon length (FdcT) (Table 3). The greatest departure was observed in

184 three variables, m. scapularis medialis (SM) (0.272), extensor carpi radialis (Ecr) (0.259) and

185 extensor digitorum communis (Edc) (0.265) (Table 3). The smallest observed departure from

186 isometry was in extensor carpi ulnaris tendon length (EculT) and humeralis medialis tendon

187 length (HmedT) (0.07 and 0.107, respectively). Based on this result, ten variables significantly

188 departed from isometry: humeralis medialis tendon length (HmedT), extensor carpi radialis

189 tendon length (EcrT), extensor carpi ulnaris tendon length (EcuT) and flexor digitorum

190 communis tendon length (FdcT; all negatively allometric); and humerus length (HL), extensor

191 carpi radialis tendon length (EcrT), m. flexor carpi ulnaris (Fcu) and m. flexor carpi radialis (Fcr;

192 all positively allometric) (Table S4).

193

**194**   **Musculoskeletal-tendinous allometry in *Physalaemus biligonigerus***

195     Trends obtained with untrimmed and trimmed values differed only in relation to seven

196   variables: body length (BL), scapularis medialis (SM), humeralis medialis (Hlat), m. extensor

197   digitorum (Edig), m. extensor carpi radialis (Ecr), m. coracoradialis (C) and flexor carpi radialis

198   tendon length (FcrT) (Table 3). The greatest observed departure from isometry was observed

199   radioulna length (RUL) and coracoradialis tendon length (CT) (0.278 and 0.277, respectively).

200   The smallest departure was observed in three variables: extensor carpi ulnaris tendon length

201   (EcuT) (0.069), extensor carpi radialis tendon length (EcrT) (0.082) and humeralis medialis

202   tendon length (HmedT) (0.109). Based on this result, six variables significantly departed from

203   isometry: humeralis medialis tendon length (HmedT), extensor carpi radialis tendon length

204   (EcrT), extensor carpi ulnaris tendon length (EcuT; all negatively allometric), and humerus

205   length (HL), radioulna length (RUL), coracoradialis tendon length (CT; all positively allometric)

206   (Table S5).

207

**208**   **Musculoskeletal-tendinous allometry in *Leptodactylus chaquensis***

209     Trends obtained with untrimmed and trimmed values differed only in relation to eight

210   variables: radioluna (RUL), m. scapularis medialis (SM), scapularis medialis tendon length

211   (SMT), humeralis medialis tendon length (HlatT), humeralis lateralis tendon length (HmedT), m.

212   coracoradialis (C), extensor carpi radialis tendon length (EcrT), and flexor carpi ulnaris tendon

213   length (FcuT) (Table 3e). The greatest observed departure from isometry was found in scapularis

214   medialis tendon length (0.29), m. extensor carpi ulnaris (0.295) and extensor carpi radialis

215   tendon length (0.315). The smallest departure was observed in four variables: humeralis medialis

216 tendon length (0.09), flexor digitorum communis tendon length (0.02), flexor carpi ulnaris

217 tendon length (EcuT) (0.068) and flexor carpi radialis tendon length (EcrT) (0.061). Based on

218 this result, ten variables significantly departed from isometry: body length (BL), m. humeralis

219 lateralis (Hlat), humeralis medialis (Hmed), m. extensor digitorum (Edig), m. extensor carpi

220 ulnaris (Ecul), m. flexor digitorum communis (Fdc), m. flexor carpi radialis (Fcr; all positively

221 allometric); and flexor carpi radialis tendon length (FcrT), flexor carpi ulnaris tendon length

222 (FcuT), and flexor digitorum communis tendon length (FdT; all negatively allometric) (Table

223 S6).

224

225 **Musculoskeletal-tendinous allometry in *Pleurodema borellii***

226    Trends obtained with untrimmed and trimmed values differ only in relation to five variables:

227 scapularis medialis tendon length (SMT), humeralis lateralis tendon length (HmedT), m.

228 coracoradialis (C), flexor digitorum communis tendon length (FdcT) and m. flexor carpi ulnaris

229 (Fcu) (Table 3). The greatest observed departure from isometry was observed in m. humeralis

230 lateralis and m. extensor digitorum (0.262 and 0.301, respectively). The smallest departure was

231 observed in two variables: extensor carpi ulnaris tendon length (EcuT) and flexor digitorum

232 communis tendon length (FdcT) (0.05 and 0.11, respectively). Based on this result, five variables

233 significantly departed from isometry: radioulna length, m. humeralis lateralis (Hlat), m. extensor

234 digitorum (Edig), m. extensor carpi ulnaris (Ecul; all positively allometric), and m. extensor

235 carpi ulnaris (Ecu; negatively allometric) (Table S7).

236

237 **Discussion**

238    The main goal of the present study was to investigate the relative growth patterns of the forelimb

239    musculoskeletal-tendinous system in seven anuran species of three age categories. The general

240    allometric growth patterns inferred from our data indicate that along with the post-metamorphic

241    ontogeny of most studied anuran species, muscles tend to elongate proportionally to bone length,

242    with a positive allometric trend. On the contrary, tendons show a negative allometric growth

243    trend. Only two species show different trends: *Rhinella granulosa* and *Physalaemus*

244    *biligonigerus*, with an isometric and positive growth of muscles and bones, and most tendons

245    being isometric. This trend represents a synchronic growth of all structures, which is an

246    interesting pattern. Overall, the couple antagonist-agonist muscles do not present the same

247    tendency in the surveyed species. For example, the triceps generally grows with positive

248    allometry, and the coracoradialis (biceps) with negative allometry. On the contrary, in the

249    forearm of *Rhinella* and *Physalaemus*, agonist and antagonist muscles presented the same trend.

250    Taken together, these data might indicate that the differential growth of the musculoskeletal-

251    tendinous system might be related to the intrinsic nature of each tissue e.g., tendons increase

252    their lengths by apposition of collagen fibril segments in a complex and hierarchical process

253    (Zhang et al. 2005), meanwhile bone growth in length is mainly achieved through the action of

254    chondrocytes in the proliferative and hypertrophic zones (Rauch, 2005).

255    Manzano et al. (2013) stressed that the tendon is the last tissue to form during limb ontogeny

256    in anurans and that it needs a fully functional limb to reach complete maturity. Our data shows

257    that this delay in tendon appearance and growth occurs after reaching the fully functional stage,

258    as evidenced by its negative allometric growth. On the contrary, muscles and bones show the

259    same positive allometric trend. This coordinated growth trend contrasts with the previous

260    morphogenetic processes in which muscular differentiation seems to be extremely fast compared

261  to the differentiation of the limb skeletal element (Manzano et al., 2013). Our results on postnatal

262  growth are concordant with the observations made by Huang et al. (2015) in mouse mutants

263  [Splotch delayed (Spd) mice (Vogan et al., 1993)]. These authors found that the first stage of

264  tendon development – in which muscles span the zeugopodium anchor to autopodium induced

265  tendons – might be better described through positive allometric muscle growth and a negative

266  allometric tendon elongation, and a subsequent reversal of this trend. Thus, the correct assembly

267  of the musculoskeletal-tendinous complex of a limb segment as unity is regulated by differential

268  growth, in a similar way to that proposed by Eilam (1997). In that study, a heterochronic process

269  was suggested as the critical factor to explain body morphology divergence in several rodent

270  taxa.

271  Heterochrony is a central process driving morphological diversity in mammals (Ravosa,

272  Meyers & Glander, 1993; Maunz & German, 1997; Richardson et al., 2009), which also seems to

273  modulate musculoskeletal-tendinous growth in anurans. The synchronic muscle-bone growth

274  combined with a negative allometric growth of tendon length results in a segment highly

275  occupied by muscle fibers. On the contrary, a positive allometric growth of the tendons length

276  combined with a synchronic and negative muscle-bone growth would result in a segment highly

277  occupied by tendons. This process could explain, for example, the differences between tendon

278  length of the forearm of a bat or a horse and that of a rat, and would provide a simple mechanism

279  to account for their highly specialized locomotor types.

280  When the relative growth of the arm and forearm structures of the analyzed anuran species are

281  compared, interesting trends emerge. In the forearm, there is a general trend of a positive bone

282  and muscle allometry, including extensors and flexors, and the already reported delayed tendon

283  growth. In the arm, there is a trend to a positive humerus and triceps allometry, combined with a

284   negative coracoradialis allometry. Strikingly, the coracoradialis tendon presents isometric

285   growth. The described growth of the coracoradialis tendon, combined with negative allometric

286   muscle growth, indicates the presence of a forearm flexor layer with long tendons. Interestingly,

287   the pattern highlighted by Bobbert (2001) as an intriguing design aspect of the human

288   musculoskeletal system (distal muscle-tendon complexes spanning the distance between origin

289   and insertion, with long tendons and very short muscle fibers) was only recorded for the

290   coracoradialis. The longer tendon compared with the muscle length indicates a segment with less

291   force but faster reaction, which could compensate for the great force with slower reaction

292   indicated by the relative growth of the triceps.

293      In conclusion, our data indicate that the musculoskeletal-tendinous growth is different than

294   posed in our initial hypothesis: limb bones and muscles tend to develop synchronically, with

295   tendons exhibiting a delayed growth.

296

297   **Data Availability**

298   **Supplementary File**

299   Tables S1: The raw of muscles, bones and body measurements and a number of individuals used

300   per species.

301   Tables S2-S7: Summary of results of multivariate allometry.

302   **Funding**

303   This work was funded by the PIUNT 26/G625 and PIP CONICET 389 and PICT 2016-2772.

304   The funders had no role in study design, data collection and analysis, decision to publish, or

305   preparation of the manuscript.

306

307   **Acknowledgments**

308   We are very thankful to Marta Cánepa and Sonia Kretzschmar from Herpetology Collection of

309   the Fundación Miguel Lillo and Museo de Ciencias Naturales de la Universidad Nacional de

310   Salta for allowing access to herpetological collections. For helpful for improving the English

311   language, we thank to Sofía Nanni for providing suggestions that greatly improved the

312   manuscript.

313   **References**
314

315   Abdala V, Diogo R. 2010. Comparative anatomy, homologies and evolution of the pectoral

316      and forelimb musculature of tetrapods with special attention to extant limbed amphibians and

317      reptiles. *Journal of Anatomy* 217:536-573.

318   Alberch P, Gould SJ, Wake DB. 1979. Size and Shape in Ontogeny and Phylogeny.

319      *Paleobiology* 5:296-317.

320   Banbury B, Maglia AM. 2006. Skeletal development of the mexican spadefoot, *Spea*

321      *multiplicata* (Anura: Pelobatidae). *Journal of Morphology* 267:803-821.

322   Biewener AA. 1998. Muscle–tendon stresses and elastic energy storage during locomotion in the

323      horse. *Comparative Biochemistry Physiology Part B* 120:73-87.

324   Biewener AA. 2003. *Animal locomotion*. Oxford: Oxford University Press.

325   Bobbert, MF. 2001. Dependence of human squat jump performance on the Series elastic

326      compliance of the triceps surae: A simulation study. *Journal of Experimental Biology*

327      204:533-542.

328   Böl M, Leichsenring J, Siebert T. 2017. Effects of growth on muscle, tendon, and aponeurosis

329      tissues in rabbit shank musculature. *The Anatomical Records* 300:1123-1136.

330   Calder WA III. 1984. *Size, Function, and Life History*. Harvard University Press.

331   Carrier DR. 1995. Ontogeny of jumping performance in the black–tailed jackrabbit (*Lepus*

332      *californicus*). *Zoology* 98: 309-313.

333   Cei JM. 1950. *Leptodactylus chaquensis* n. sp. y el valor sistemático real de la especie Linneana

334      *Leptodactylus ocellatus* en la Argentina. *Acta Zoologica Lilloana* 9:395-423.

335   Chaparro JC, Pramuk JB, Gluesenkamp AG. 2007. A new species of arboreal *Rhinella* (Anura:

336      Bufonidae) from cloud forest of southeastern Peru. *Herpetologica* 63:203-212

337    De Sá, R., 1988. Chondrocranium of ossification sequence of *Hyla lanciformis*. *Journal of*
338        *Morphology* 195:345-355.
339    Diogo R, Ziermann JM. 2014. Development of fore- and hindlimb muscles in frogs:
340        Morphogenesis, homeotic transformations, digit reduction, and the forelimb-hindlimb enigma.
341        *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* 322:86-05.
342    Duellman WE, Trueb L. 1994. *Biology of Amphibians*. The Johns Hopkins University Press.
343    Eilam D. 1997. Postnatal development of body architecture and gait in several rodent species.
344        *Journal of Experimental Biology* 200:339-1350.
345    Emerson S. 1978. Allometry and Jumping in frogs: helping the twain to meet. *Evolution* 32:551-
346        564.
347    Emerson SB. 1979. The ilio-sacral articulation in frogs: form and function. *Biological Journal of*
348        *the Linnean Society* 11:153-168.
349    Emerson SB. 1982. Frog Postcranial morphology: identification of a functional complex. *Copeia*
350        3:603–613.
351    Emerson SB. 1983. Functional analysis of frog pectoral girdles. The epicoracoid cartilages.
352        *Journal of Zoology* 201:293-308.
353    Emerson SB. 1985. Heterochrony and frogs: the relationship of life history trait to
354        morphological form. *American Naturalist* 127:167-183.
355    Emerson SB, De Jongh HD. 1980. Muscle activity at the iliosacral articulation of frogs. *Journal*
356        *of Morphology* 166:129-144.
357    Emerson SB, Bramble DM. 1993. Scaling, allometry, and skull design. In: Hanken J & Hall BK,
358        eds. *The Skull, Vol. 3, Functional and Evolutionary Mechanism*s. Chicago, IL: University of
359        Chicago Press, 384–421.
360    Fabrezi M, Manzano A, Lobo F, Abdala V. 2014. Anuran locomotion: ontogeny and
361        morphological variation of a distinctive set of muscles. *Evolutionary Biology* 41:308-326
362    Flores DA, Giannini N, Abdala F. 2006. Comparative postnatal ontogeny of the skull in the
363        australidelphian metatherian *Dasyurus albopunctatus* (Marsupialia: Dasyuromorpha:
364        Dasyuridae). *Journal of Morphology* 267:426-440.
365    Giannini NP, Abdala F, Flores DA. 2004. Comparative postnatal ontogeny of the skull in
366        Dromiciops gliroides (Marsupialia: Microbiotheriidae). *American Museum Novitates* 3460:1-
367        17.

368 Giannini NP, Segura V, Giannini MI, Flores D. 2010. A quantitative approach to the cranial
369      ontogeny of the puma. *Mammalian Biology* 75:547-554.

370 Gosner KL. 1960. A simplified table for staging anuran embryos and larvae with notes on
371      identification. *Herpetologica* 16:183-190.

372 Gould SJ. 1977. *Ontogeny and Phylogeny*. Cambridge MA: Harvard Univ. Press.

373 Haas A, Richards JS. 1998. Correlations of cranial morphology, ecology, and evolution in
374      australian suctorial tadpoles of the genera *Litoria* and *Nyctimystes* (Amphibia: Anura:
375      Hylidae: Pelodryadinae). *Journal of Morphology* 238:109-141.

376 Haas A. 1999. Larval and metamorphic skeletal development in the fast–developing frog
377      *Pyxicephalus adspersus* (Anura, Ranidae). *Zoomorphology* 119:23-35.

378 Hall JA, Larsen JH Jr. 1998. Postembryonic ontogeny of the spadefoot toad, *Scaphiopus*
379      *intermontanus* (Anura: Pelobatidae): Skeletal morphology. *Journal of Morphology* 238:179-
380      244.

381 Handrigan GR, Wassersug RJ. 2007. The metamorphic fate of supernumerary caudal vertebrae
382      in South Asian litter frogs (Anura: Megophryidae). *Journal of Anatomy* 211:271-279.

383 Handrigan GR, Haas A, Wassersug RJ. 2007. Bony–tailed tadpoles: the development of
384      supernumerary caudal vertebrae in larval megophryids (Anura). *Evolution & Development*
385      9:190-202.

386 Hanken J, Hall BK. 1984. Variation and timing of the cranial ossification sequence of the
387      oriental fire–bellied toad, *Bombina orientalis* (Amphibia, Discoglossidae). *Journal of*
388      *Morphology* 182:245-255.

389 Huang AH, Riordan TJ, Pryce B, Weibel JL, Watson SS, Long F, Tufa SF. 2015.
390      Musculoskeletal integration at the wrist underlies the modular development of limb
391      tendons. *Development* 142:2431-2441.

392 Jolicoeur P. 1963. The multivariate generalization of the allometry equation. *Biometrics* 19:497-
393      499.

394 Kardong KV. 2002. *Vertebrates: Comparative Anatomy, Function, Evolution* (3rd Ed.). New
395      York: McGraw-Hill.

396 Lavilla EO, Langone JA, Padial JM, de Sá RO. 2010. The identity of the crackling, luminescent
397      frog of Suriname (*Rana typhonia* Linnaeus, 1758) (Amphibia, Anura). *Zootaxa* 2671:17-30.

398    Manzano A, Abdala V, Ponssa ML, Soliz M. 2013. Ontogeny and tissue differentiation of the
399        pelvic girdle and hind limbs of anurans. *Acta Zoologica Stockholm* 94:420-436.

400    Maunz M, German RZ. 1997. Ontogeny and limb bone scaling in two New World marsupials,
401        *Monodelphis domestica* and *Didelphis virginiana*. *Journal of Morphology* 231:117-130

402    Muntz L. 1976. Myogenesis in the trunk and leg during development of the tadpole of *Xenopus*
403        *laevis* (Daudin 1802). *Journal of Embryology and Experimental Morphology* 33:757-77.

404    Nauwelaerts S, Ramsay J, Aerts P. 2007. Morphological correlates of aquatic and terrestrial
405        locomotion in a semi–aquatic frog, *Rana esculenta*: No evidence for a design conflict. *Journal*
406        *of Anatomy* 210:304-317.

407    Nieden F. 1923. Anura I. Subordo Aglossa und Phaneroglossa, Sectio 1. Arcifera. Das Tierreich
408        46, xxxii + 584.

409    Parker HW. 1927, A revision of the frogs of the genera *Pseudopaludicola*, *Physalaemus*, and
410        *Pleurodema*. *The Annals and Magazine of Natural History, Serie 9* 20:458.

411    Pramuk JB, Robertson T, Sites JW Jr, Noonan BP. 2008. Around the world in 10 million years:
412        biogeography of the nearly cosmopolitan true toads (Anura: Bufonidae). *Global Ecology and*
413        *Biogeography* 17:72-83.

414    Ponssa ML, Vera Candioti MF. 2012. Patterns of skull development in anurans: size and shape
415        relationship during postmetamorphic cranial ontogeny in five species of the Leptodactylus
416        fuscus Group (Anura: Leptodactylidae). *Zoomorphology* 131:349-362.

417    Přikryl T, Aerts P, Havelková P, Herrel A, Roček Z. 2009. Pelvic and thigh musculature in frogs
418        (Anura) and origin of anuran jumping locomotion. *Journal of Anatomy* 214:100-139

419    Púgener LA, Maglia AM. 2009. Developmental evolution of the anuran sacro–urostylic
420        complex. *South American Journal of Herpetology* 4:193-20.

421    Ravosa MJ, Meyers DM, Glander KE. 1993. Relative growth of the limbs and trunk in sifakas:
422        heterochronic, ecological, and functional considerations. *American Journal of Physical*
423        *Anthropology* 92:499-520.

424    Reilly SM, Wiley EO, Meinhardt DJ. 1997. An integrative approach to heterochrony: the
425        distinction between interspecific and intraspecific phenomena. *Biological Journal of the*
426        *Linnean Society* 60:119-143.

427    Richardson MK, Gobes SM, van Leeuwen AC, Polman JA, Pieau C, Sánchez-Villagra MR.
428        2009. Heterochrony in limb evolution: developmental mechanisms and natural selection.

*Journal of Experimental Zoology Part B Molecular and Developmental Evolution* 312:639-664.

Roberts TJ. 2002. The integrated function of muscles and tendons during locomotion. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology* 33:1087-99.

Ročková H, Roček Z. 2005. Development of the pelvis and posterior part of the vertebral column in the Anura. *Journal of Anatomy* 206:17-35.

Schmidt-Nielsen K. 1984. *Scaling: Why is animal size so important?* Cambridge: Cambridge University Press.

Soliz MC, Ponssa ML. 2016. Development and Morphological Variation of the Axial and Appendicular Skeleton in Hylidae (Lissamphibia, Anura). *Journal of Morphology* 277:786-813.

Steindachner F. 1867. Reise der österreichischen Fregatte Novara um die Erde in den Jahren 1857, 1858, 1859 unter den Bafehlen des Commodore B. von Wüllerstorf-Urbair. Zologischer Theil. 1. Amphibien. Wien: K. K. Hof- und Staatsdruckerei.

Thompson DW. 1942. *Growth and form*. New York: Macmillan.

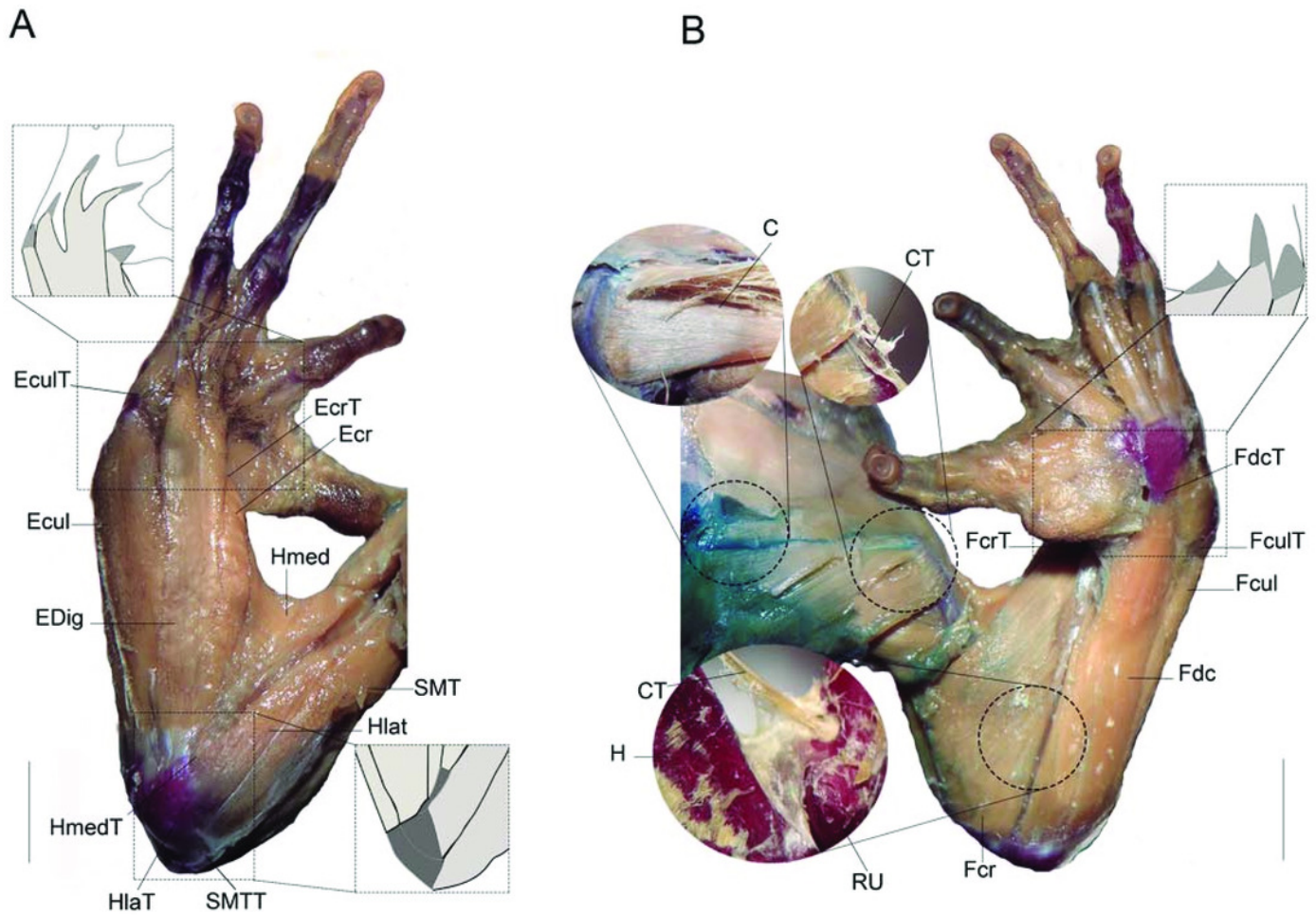
Vera MC, Ponssa ML, Abdala V. 2015. Further Data On Sesamoid Identity from Two Anuran Species. *Anatomical Records* 298:1376-1394.

Vogan KJ, Epstein DJ, Trasler DG, Gros P. 1993. The splotch delayed (Spd) mouse mutant carries a point mutation within the paired box of the Pax-3 gene. *Genomics* 17:364-369.

Vukov TD, Kuručki M, Anđelković M, Kolarov NT. 2018. Post–metamorphic ontogenetic changes in head size and shape of the pool frog (*Pelophylax lessonae*, Ranidae). *Archives of Biological Science* 70:307-312.

# Figure 1

Figure 1. Muscles and tendons of the right forelimb of anuran species, and their respective large bones

A: dorsal view. B: ventral view. Light grey muscles and dark gray tendons. C: Coracoradialis. CT: Coracoradialis tendon. Ecr: Extensor carpi radialis. EcrT: Extensor carpi radialis tendon. Ecul: Extensor carpi ulnaris. EculT: Extensor carpi ulnaris tendon. EDig: Extensor digitorum. Fcr: Flexor carpi radialis. FcrT: Flexor carpi radialis tendon. Fcul: Flexor carpi ulnaris. FculT: Flexor carpi ulnaris tendón. Fdc: Flexor digitorum comunis. FdcT: Flexor digitorum comunis tendon. H: humerus. Hlat: Triceps humeralis lateralis. HlatT: Triceps humeralis lateralis tendon. Hmed: Triceps humeralis medialis. HmedT: Triceps humeralis medialis tendon. RU: radioulna. SMT: Triceps scapularis medialis. SMTT: Triceps scapularis medialis tendon. Scale bars: 5mm

List of anuran specimens examined. Acronyms for Argentinian institutions where specimens are deposited and personal catalogs: MCN: Museo de Ciencias Naturales de Salta, FML: Fundación Miguel Lillo. MS: field number of Mónica Soliz to be deposited at FML.

| Species | N | Specimen number |
|---|---|---|
| *Rhinella arenarum* | 18 | MCN594, MCN849, MCN858, MCN579, MCN776 |
| *Rhinella granulosa* | 9 | MCN775, MCN994, MCN779, MS0130, MS0131, MS0132 |
| *Pleurodema borellii* | 29 | MCN707, MCN592, MCN844, MS0133, MS0134, MS0135 |
| *Physalaemus biligonigerus* | 11 | MCN731, MS0137, MS136 |
| *Leptodactylus chaquensis* | 12 | MCN327, MCN166, MCN327, MCN583, MCN789 |
| *Trachycephalus typhonius* | 11 | FML29062, FML29133, FML29167, FML29168, MS0129 |

List of abbreviations of forelimb muscle, tendons, bones and body length of de
measured specimens

| Muscles | Abbreviations | Tendon | Abbreviations | Group |
|---|---|---|---|---|
| ***Extensor*** | | | | |
| *Triceps scapularis medialis* | SMT | x | SMTT | Triceps |
| *Triceps humeralis lateralis* | Hlat | x | HlatT | Triceps |
| *Triceps humeralis medialis* | Hmed | x | HmedT | Triceps |
| *Extensor carpi radialis* | Ecr | x | EcrT | Extensor of the forearm |
| *Extensor carpi ulnaris* | Ecul | x | EculT | Extensor of the forearm |
| *Extensor digitorum* | EDig | | | Extensor of the forearm |
| ***Flexor*** | | | | |
| Coracoradialis | C | x | CT | Flexor of the arm |
| *Flexor carpi radialis* | Fcr | x | FcrT | Flexor of the forearm |
| *Flexor carpi ulnaris* | Fcul | x | FculT | Flexor of the forearm |
| *Flexor digitorum comunis* | Fdc | x | FdcT | Flexor of the forearm |
| ***Bones*** | | | | |
| *Humerus length* | HL | | | Arm |
| *Radioulna length* | RUL | | | Forearm |
| ***Body length*** | BL | | | |

Summary of allometric trends in the seven species of anuran for 20 variables investigated. The used symbols are: ''+" (accelerated with respect to overall size or positive allometric), ''-" (respect to overall size or negative allometric), ''=" (isometric

| Species | Rhinella arenarum | | Rhinella granulosa | | Trachycephalus typhonius | | Physalaemus biligonigerus | | Leptodactylus chaquensis | | Pleurodema borelli | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variables | Untrimmed | Trimmed | Untrimmed | Trimmed | Untrimmed | Trimmed | Untrimmed | Trimmed | Untrimmed | Trimmed | Untrimmed | Trimmed |
| BL | + | + | = | = | = | = | + | = | + | + | = | = |
| HL | = | = | = | = | + | = | + | + | = | = | = | = |
| RUL | = | + | = | = | = | = | + | + | + | = | + | + |
| SM | = | + | = | = | + | + | + | = | + | = | = | = |
| SMTL | = | + | - | = | = | = | = | = | + | = | - | = |
| Hlat | + | + | = | = | + | = | = | = | + | + | + | + |
| HlatTL | = | - | - | - | = | = | = | = | - | = | - | = |
| Hmed | + | + | = | = | = | = | - | = | + | + | = | = |
| HmedTL | = | - | = | = | - | - | - | - | - | = | = | = |
| Edig | + | + | = | = | + | + | + | = | + | + | + | + |
| Ecul | + | + | = | = | + | = | = | = | + | + | + | + |
| EculT | = | = | = | = | - | = | - | - | = | = | - | - |
| Ecr | = | = | = | = | + | = | - | = | = | = | = | = |
| EcrT | = | + | + | = | - | = | - | - | + | = | = | = |
| C | = | - | - | = | = | = | + | = | - | = | - | = |
| CTL | = | = | = | = | = | = | + | + | = | = | = | = |
| Fdc | + | + | = | = | = | = | = | = | + | + | = | = |
| FdcT | - | - | = | = | - | = | = | = | - | - | - | = |
| Fcul | = | + | + | = | = | = | = | = | + | = | + | = |
| FculT | - | - | = | = | = | = | = | = | - | - | = | = |
| Fcr | + | + | + | = | = | = | = | = | + | + | = | = |
| FcrT | - | - | = | = | = | = | + | = | - | - | = | = |