Phylogenetic patterns and correlation of key structures for jumping: bone crests and cross-sectional areas of muscles in *Leptodactylus* (Anura, Leptodactylidae)

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Abstract

Anurans are characterized by their saltatory mode of locomotion, which is associated with a specific morphology. The coordinated action of the muscles and bones of the pelvic girdle is key to the transmission of the force of the hindlimbs to the axial skeleton during jumping. Two features are critical for optimal locomotory performance: the cross-sectional area of muscle and the bone crest attachment sites. The first character is a proxy of the force exerted by the muscle, whereas the crests are muscle attachments sites related to muscle force. The provisory relationship between these features has previously been identified and bone crest size can be used to infer the magnitude and, therefore, muscle force in fossils records. In this work, we explore the correlation between the cross-sectional area of essential muscles to the jumping mechanism (longissimus dorsi, extensor iliotibialis B, tenuissimus, puboischiofemoralis internus B, coccygeo-sacralis and coccygeo-iliacus) and the bone crests where these muscles are inserted (dorsal tubercle, dorsal crest and urostylar crest) in species of the genus *Leptodactylus*. This genus, along with other leptodactylids, exhibits a diversity of locomotor modes, including jumping, hopping, swimming and burrowing. We therefore analyzed the morphometric variation in the two features, cross-sectional area and bone crest area, expecting a correlation with different locomotor types. Our results showed: (i) a correlation between the urostylar crest and the cross-sectional area of the related muscles; (ii) that the bone crest surface area of urostyle and ilium and the cross-sectional area of the corresponding muscles can be utilized to infer locomotor faculties in leptodactylid frogs; and (iii) that the evolution of both characters demonstrates a general tendency from lower values in leptodactylid ancestors to higher values in the *Leptodactylus* genus. The results attest to the importance of the comparison of current ecological and phylogenetic analogues as they allow us to infer functionality and behavior in fossil and extant groups based on skeletal evidence. Phylogenetic patterns in character evolution and their correlation with locomotory types could imply that functional restrictions are also inherited in leptodactylid.

Key words: frogs; functional anatomy; heritability traits; locomotion; skeleton-muscle architecture.

Introduction

Anurans are characterized by their saltatory mode of locomotion, which is associated with a specific morphology including shortened presacral vertebral series, the consolidation of caudal vertebral elements into a urostyle, elongated ilia and hindlimbs, and the development of mobile ilio-sacral and sacro-urostylar joints (Gans & Parsons, 1966; Emerson, 1979; Shubin & Jenkins, 1995). Precedents for this morphology have been found as far back as ca. 200 Ma before the crown-group anurans diversified and were already present in the earliest anuran fossils (Rage & Roček, 1989; Sanchiz & Roček, 1996; Jenkins & Shubin, 1998; Lires et al. 2016). The mobile sacro-urostylar and ilio-sacral joints are essential structures for saltatory locomotion (Gans & Parsons, 1966; Emerson & De Jongh, 1980). During the launch phase of a jump, the longissimus dorsi muscle extends the sacrum and presacral vertebral column at these joints aided by a thrust of the forelimbs. The coccygeo-iliacus muscles anchor the urostyle between the ilia and

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transmit the propulsive thrust to the sacrum and presacral column (Emerson & De Jongh, 1980; Jenkins & Shubin, 1998). Emerson & De Jongh (1980) found that three muscles, the coccygeo-iliacus, coccygeo-sacralis and longissimus dorsi, are maximally active during the initial phase of saltatory locomotion in frogs (data taken from *Bufo americanus*, *Kaloula pulchra* and *Rana esculenta*). The coordination of the muscles and skeleton of the pelvic girdle is, therefore, critical to force transmission between the hindlimbs and axial skeleton when launching a jump.

Two features of the pelvic girdle are particularly important for saltatory locomotion: muscle configuration and bone crests. A number of factors contribute to muscle performance, including the chemical composition of various muscle components and the structural organization of these components (Josephson, 1975; Astley, 2016). Muscle performance is also affected by its size; a larger muscle accumulates more tension and contracts faster than a small one (Josephson, 1975). The muscle cross-sectional area is particularly relevant in locomotion, since it is a measure of the maximal force that a striated muscle can exert (Josephson, 1975; Powell et al. 1984). For example, Nauwelaerts et al. (2007) found that the cross-sectional area of the m. gracilis major is critical for jumping and that that of the m. cruralis is critical for swimming performance. The first muscle is an extensor, with an abduction/adduction function, whose contraction rotates the femur internally. The second one is a hip flexor and knee extensor (Nauwelaerts et al. 2007; Přikryl et al. 2009).

Bone crests are muscle attachment sites and are therefore associated with muscle force (Zumwalt, 2005). The relationship between the cross-sectional areas of muscles and bone crest surface area has important connotations for morphology, as the magnitude of bone crests can be used to infer the muscle size in fossils (Zumwalt, 2005). The pelvic girdle contains two main crests: the dorsal and the urostylar. The dorsal crest, located in the superior edge of the ilium, serves as an attachment for the muscles, aponeurosis and fascia of the nearby structures. In frogs, the muscles attached to the dorsal crest are the coccygeo-iliacus, which originates on the surface of the crista of the iliac shaft (Přikryl et al. 2009); and the m. iliacus externus, whose pars interna emanates from the anterior part of the inner surface of the iliac shaft, whereas the pars externa originates from the antero-lateral end of the iliac shaft (Přikryl et al. 2009; Fratani et al. 2017). The dorsal tubercle, an area of muscular attachment is located on the anterior end of the dorsal crest, on whose dorsolateral surface m. extensor iliotibialis B and tenuissimus (Přikryl et al. 2009) insert, may also be relevant. The urostylar crest is a thin, extensive structure visible above the urostyle (Holman, 2003). The muscles attached to this crest are the m. coccygeo-iliacus and the m. longissimus dorsi, which start superficially at the midpoint of the urostyle length and extend to the posterior end (Fratani et al. 2017); and the m. coccygeo-sacralis, which originates on the sacral

diapophysis and inserts fleshly of the urostyle (Přikryl et al. 2009; Fratani et al. 2017).

Although jumping is the typical locomotor mode of anurans and its associated body plan is highly constrained (Fabrezi et al. 2017), anuran species display substantial diversity in their locomotion, with species ranging from those that perform powerful leaps to others that almost exclusively swim, hop or walk (Astley, 2016). The relationship between locomotor mode and morphological diversity in anurans has been little explored from a comparative perspective (Zug, 1972, 1978; Emerson, 1988) and only in the last years has an explicit phylogenetic framework been incorporated (Nauwelaerts et al. 2007; Reilly & Jorgensen, 2011; Jorgensen & Reilly, 2013; Astley, 2016). At a more exclusive level, research is even scarcer, e.g. in Hylids (Soliz et al. 2017), Nenirana clade (Engbrecht et al. 2011) and in Western Mediterranean anurans (Enriquez-Urzelai et al. 2015). In the genus *Leptodactylus*, no relationship has been found between locomotor modes and other morphological characters such as the morphometry of limbs (Ponssa & Medina, 2016) or the tendinous system (Fratani et al. 2017) and these same studies recommend that other structures involved in locomotion should be explored further (Ponssa & Medina, 2016). The genus *Leptodactylus* is an excellent subject for the investigation of muscle-skeletal specializations in the context of locomotion variability. The genus includes 74 species distributed in four species groups: *Leptodactylus latrans*, *Leptodactylus melanonotus*, *Leptodactylus pentadactylus* and *Leptodactylus fuscus* (Heyer, 1969), and belongs to the family Leptodactylidae that encompasses 203 species (Frost, 2017) that represent a large range of locomotor modes including hopper, jumper, walker, swimmer and burrower species (Fratani et al. 2017). Species in the genus also adhere to both aquatic and terrestrial breeding and reproduction modes (Heyer, 1969), including; (i) species that oviposit in foam nests on water (e.g. species of the *L. latrans* group); and (ii) species that exhibit terrestrial reproduction habits. These include foam nest deposition basins (e.g. some species of the *L. melanonotus* and *L. pentadactylus* groups) and the construction of terrestrial incubation chambers for foam nest placement and initial larval development (e.g. species of the *L. fuscus* group).

In this study we explore the locomotor systems of *Leptodactylus* by first evaluating the cross-sectional area of the muscles and the corresponding pelvic bone crests, features that have a previously established correlation, and subsequently analyzing the variation in these specific muscle-skeletal features from an ecomorphologycal perspective. The mobile link sacro-urostylar and ilio-sacral articulation were specifically selected because of their particular implication in anuran locomotion (Gans & Parsons, 1966; Emerson & De Jongh, 1980; Jenkins & Shubin, 1998). The mechanical stress (e.g. muscle force) suffered by a surface area is proportional to the strength applied to that surface (Biewener, 1992) and the hypertrophy of bone attachments for larger

muscles is considered to be a valuable mechanism to reduce or maintain acceptable levels of mechanical stress (Zumwalt, 2006). Based on these assumptions and that distinct loco-motor modes require conflicting morphological designs, we expect (i) a close relationship between pelvic girdle cross-sectional muscle and bone crest area and (ii) a correlation between the studied variables and locomotor mode. We also optimized the gathered morphometric data onto the phylogeny to identify evolutionary tendencies within the group.

Materials and methods

Morphometric data

The cross-sectional area of six muscles related to the sacro-urostylar and ilio-sacral articulation were analyzed: m. longissimus dorsi, extensor m. iliotibialis B, m. tenuissimus, m. puboischiofemoralis internus B, m. coccygeo-sacralis and m. coccygeo-iliacus (Fig. 1). The bone crest surface areas correspondent to the muscle insertions were also measured, including: the dorsal tubercle (=tuber superius=tuber superior=processus superior; Gómez & Turazzini, 2015), the dorsal crest (=Crista dorsalis) and urostylar crests (Fig. 2). The terminology for pelvic and hindlimbs muscles was taken from Diogo & Ziermann (2014) and Diogo & Molnar (2014). The terminology used for postcranial osteology follows Trueb (1973). Gómez & Turazzini (2015) were referenced for the ilial terminology. To collect osteological data, the specimens were cleared and stained following the protocol from Wassersug (1976). The snout-vent length (SVL) of each specimen and the total area of ilium and urostyle were also taken to remove the size effect in the muscle and bone crests areas. We worked with an approximation that responds to an anatomical concept of muscle cross-sectional area, under the premise that the force produced is greater when the cross-sectional area is bigger (Biewener, 1998; Carrizo et al. 2014), which reflects a larger number of parallel myofilaments (Josephson, 1975). The specimens were dissected, muscles were removed, and the cross-sectional area of their middle region – corresponding to the most voluminous area in a perpendicular plan in relation to the fibers – and the lateral view of the ilium and urostyle were examined and photographed with a Leica M205 stereomicroscope. Measurements were taken using the software IMAGEJ (Schneider et al. 2012). The mean species values for muscle cross-sectional and bone crest areas and the number of studied individuals/species are detailed in Supporting Information Data S1.

Specimens and ecological settings

Fifty species of *Leptodactylus* were sampled, in conjunction with 18 species of other Leptodactylidae genera, to ensure that the analyzed locomotor modes were not limited to only one taxonomic group and to adequately to polarize evolutionary changes in the phylogeny. The sampled species appropriately represents the taxonomic, morphological and locomotory diversity of leptodactylid frogs. The additional genera included were *Adenomera* (three spp.), *Engystomops* (two spp.), *Physalaemus* (eight spp.), *Pleurodema* (four spp.) and *Pseudopaludicola* (one spp). Specimen collection numbers are detailed in Supporting Information Data S2. Osteological and muscular data were examined from 39 species of Leptodactylidae to calculate the relationship between bone crest and muscle cross-
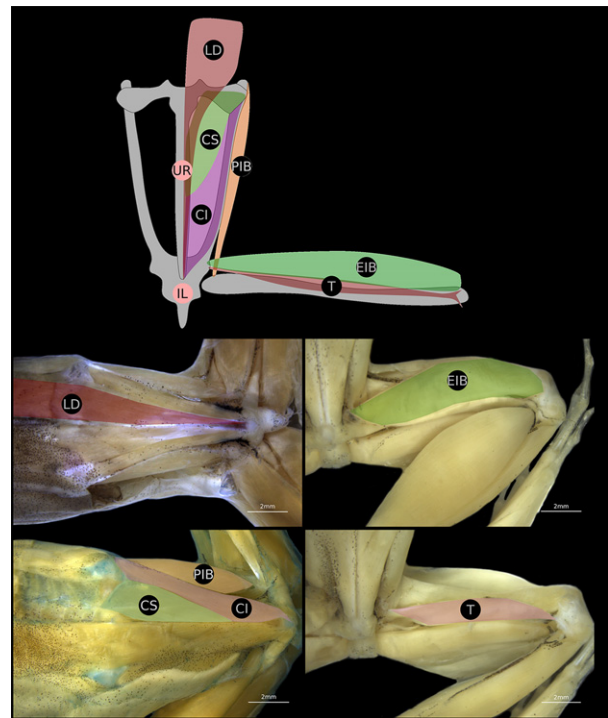

Fig. 1 Muscles analyzed for their relationship with the sacro-urostylar and ilio-sacral joints. Above, schematic representation of relationship between the muscles and bone crest analyzed. Below, muscles whose cross-sectional area was measured. Specimens of *Leptodactylus latinasus* (FML 29482, FML 29481, FML 29478). CI, coccygeo-iliacus; CS, coccygeo-sacralis; EIB, extensor iliotibialis B; IL, ilium; LD, longissimus dorsi; PIB, puboischiofemoralis internus B; T, tenuissimus; UR, urostyle.
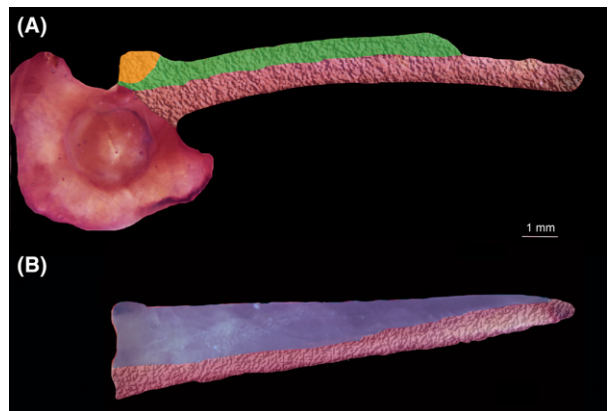

Fig. 2 Hemipelvic girdle (A) and urostyle (B) of *Leptodactylus elenae* (FML11955) showing the measured bone areas. Textured area: total area of ilium (A) and urostyle (B); green: dorsal crest area; yellow: dorsal tubercle area; light blue: urostylar crest area.

sections (Supporting Information Data S1–S4). We compared the anatomy of species with different locomotor modes to validate the existence of morphometric variances in muscle and bone features associated with locomotion. Although species in the Leptodactylinae subfamily have previously been categorized as jumpers in various studies (Zug, 1978; Emerson, 1978, 1979, 1982; Jorgensen & Reilly, 2013; Fabrezi et al. 2014), the extensive scope of those

investigations overlook subtle differences in locomotor modes within the group. For this study, we adhere to the categorization presented by Fratani et al. (2017), who further subcategorized the jumper species to best represent their locomotory diversity. The subcategories defined by these authors are jumper-swimmers (species known for the ability to displace in water, e.g. *Leptodactylus chaquensis*), jumper-burrowers (species known to build depressions or chambers as reproduction sites, e.g. *L. fuscus*), jumpers and hoppers (including species that have previously been categorized in the literature: Zug, 1978; Emerson, 1979, 1982; Jorgensen & Reilly, 2013; Fabrezi et al. 2014). For the species not included in that previous work, we followed the same criteria as Fratani et al. (2017) to assign the locomotor mode. The assigning of subcategories was based on observations documented in the literature (Heyer, 1978; Ponssa & Heyer, 2007; Ponssa, 2008; Ponssa et al. 2011; Ponssa & Barrionuevo, 2012; de Sá et al. 2014) , personal observations on field expeditions and morphological evidence conspicuously associated with swimming (presence of toe fringes: Heyer, 1969; Goldberg & Fabrezi, 2008; de Sá et al. 2014), jumper or hopper (pelvic type: Reilly & Jorgensen, 2011; Jorgensen & Reilly, 2013). Although burrowing has generally been considered a locomotor mode (Emerson, 1978, 1979, 1988; Jorgensen & Reilly, 2013), some authors regard it as an activity pattern related to substrate use (Lires et al. 2016). For this study, we considered the burrowing habit as an ability particular to the species' locomotor mode. While using the snout to dig is characteristic of burrowing species of this genus, there is evidence that some species also use their hindlimbs (Pisanó et al. 1993; Reading & Jofré, 2003; Giaretta & Kokubum, 2004; Silva et al. 2005). The locomotor mode of each species is presented in Table 1.

Statistical analyses

Prior to statistical tests, all morphometric variables were logarithmized to meet the requirements of normality and homoscedascity (Zar, 1999).

Correlation between bone crest area and muscle cross-sectional area

To test the relationship between each bone crest area and the associated muscles we performed a linear regression between size-corrected residual data, obtained from each $\log_{10}$-transformed variable. These analyses were performed in R 3.3.1 (R Core Team, 2016). This analysis was carried out only with the species for which both skeletal and muscle data were available (Data S1) (musculature dissections of some species were not accessible).

Correlation between bone crest area, muscle cross-sectional area and locomotor mode

Phylogenetic context

We utilized the most inclusive and well sampled phylogenetic hypothesis of *Leptodactylus* for our statistical analysis and optimizations (de Sá et al. 2014). Additions were made for taxa not covered in de Sá et al. (2014), including species within *Adenomera* (Fouquet et al. 2013) and Leiuperinae (Faivovich et al. 2012; Lourenço et al. 2015). Branch lengths were not available for all the species included in this study, so we tested three different types of arbitrary branch lengths using the PDTREE module of MᴇꜱQᴜɪᴛᴇ 3.04 (Midford et al. 2005; Maddison & Maddison, 2015): constant (all branch lengths equal to 1), Grafen's (1989) and Pagel's (1992) methods. The

absence of statistically significant trends in diagnostic plots of independent contrasts analysis for most traits was considered an indicator of the best fit method (Garland et al. 1992). Constant branch lengths gave the best results for muscle cross-sectional area and bone crest variables.

Phylogenetic generalized least squares

In this study, we address the evolutionary processes that affect bone crest surface areas and cross-sectional area of muscles in leptodactilids. To determine the best evolutionary explanation for these morphological traits, we applied three different possible evolutionary models: Brownian motion (BM), Ornstein-Uhlenbeck (OU) and Early Burst (EB). The BM model considers that the evolution of a trait results from random fluctuations over time (Felsenstein, 1988; Harmon et al. 2008). The OU model focuses on portions of lineages where a feature differs in relation to an optimum or stabilizing selection (Butler & King, 2004; Harmon et al. 2008). The third hypothesis, EB, predicts that trait modifications intensify early in the evolutionary tree followed by a gradual deceleration of the evolutionary rate (Harmon et al. 2008). Akaike's information criterion (AIC) was used as a heuristic indicator for the fit of the different evolutionary models (Akaike, 1974), and the weights (wAIC) were used as a measure of strength for each model. These two factors together indicated the probability that a given model was the best among a series of candidate models (Burnham & Anderson, 2002).

We applied the phylogenetic comparative method (Grafen, 1989; Garland & Ives, 2000), recognized as a robust comparative methodology (Revell, 2009; Barr & Scott, 2013; Symonds & Blomberg, 2014), to analyze the relationship between locomotor modes and bone crest area and cross-sectional muscle areas in species with shared phylogenetic history. The advantage of comparative methods is that the absence of significant results suggests that trait correlation could be due to common ancestry (Barr & Scott, 2013).

To optimize our statistical models and accommodate differences in species samples for muscular and skeletal features, the selected traits were incorporated as dependent variables in two separate phylogenetic generalized least squares (PGLS) analyses. To prevent the size effect, SVL and total bone surface area were also included as variables in the analyses. PGLS analyses were conducted using the packages APE (Paradis et al. 2004), Phytools (Revell, 2012), and nlme (Pinheiro et al. 2016) in R 3.3.1 (R Core Team, 2016).

Ancestral state reconstruction

The ancestral state reconstruction of nine continuous characters: longissimus dorsi/SVL (character 1), extensor iliotibialis B/SVL (character 2), tenuissimus/SVL (character 3), puboischiofemoralis externus B/SVL (character 4), coccygeo-sacralis/SVL (character 5), coccygeo-iliacus/SVL (character 6), dorsal tubercle/ilium (character 7), dorsal crest/ilium (character 8) and urostylar crest/urostyle (character 9) were estimated using MᴇꜱQᴜɪᴛᴇ version 3.04 (Maddison & Maddison, 2015) with parsimony and BM as models for evolutionary change (Felsenstein, 1985).

Results

Correlation between bone crest area and muscle cross-sectional area

The regression analyses found significant relationships ($P = 0.0223$) for only the urostylar crest area and cross-

Table 1 Locomotor mode character scores.

Species	Toe fringes	Spatulate snout	Tectum and solum nasi	Pelvis type	Burrowing/no burrowing	Locomotor mode
Leptodactylus						
L. albilabris	1	P	Ossified	SG	b	Jumper-burrower
L. bolivianus	0	A	Cartilaginous	SG	–	Jumper-swimmer
L. bufonius	1	P	Ossified	SG	b	Jumper-burrower
L. camaquara	1	P	Ossified	SG	–	Jumper-burrower
L. chaquensis	0	A	Cartilaginous	SG	–	Jumper-swimmer
L. colombiensis	0	A	Cartilaginous	SG	–	Jumper-swimmer
L. cunicularis	1	P	Ossified	SG	b	Jumper-burrower
L. didymus	1	P	Ossified	SG	–	Jumper-burrower
L. discodactylus	0	A	Cartilaginous	SG	–	Jumper-swimmer
L. diedrus	0	A	Cartilaginous	SG	–	Jumper-swimmer
L. elenae	1	A/P	Ossified	SG	–	Jumper-burrower
L. flavopictus	1	A	Cartilaginous	SG	–	Jumper
L. fragilis	1	P	Cart/Ossif	SG	–	Jumper-burrower
L. furnarius	1	P	Cart/Ossif	SG	b	Jumper-burrower
L. fuscus	1	P	Ossified	SG	b	Jumper-burrower
L. gracilis	1	P	Ossified	SG	b	Jumper-burrower
L. griseigularis	0	A	Cartilaginous	SG	–	Jumper-swimmer
L. insularum	0	A	Cartilaginous	SG	–	Jumper-swimmer
L. jolyi	1	P	Ossified	SG	–	Jumper-burrower
L. knudseni	1	A	Cartilaginous	SG	–	Jumper
L. labrosus	1	P	Cartilaginous	SG	–	Jumper-burrower
L. labyrinthicus	1	A	Cartilaginous		b	Jumper-burrower*
L. laticeps	1	A	Cartilaginous	SG	–	Jumper
L. latinasus	1	P	Cartil/Ossif	SG	b	Jumper-burrower
L. latrans	0	A	Cartilaginous	SG	–	Jumper-swimmer
L. leptodactyloides	0	A	Cartilaginous	SG	–	Jumper-swimmer
L. lineatus	1	A	Cart/Ossif	SG	–	Jumper
L. longirostris	1	P	Ossified	SG	–	Jumper-burrower
L. macrosternum	0	A	Cartilaginous	SG	–	Jumper-swimmer
L. marambaie	1	P	Cartilaginous	SG	–	Jumper-burrower
L. melanonotus	0	A	Cartilaginous	SG	–	Jumper-swimmer
L. myersi	1	A	Cartilaginous	SG	–	Jumper
L. mystaceus	1	P	Ossified	SG	b	Jumper-burrower
L. mystacinus	1	P	Ossified	SG	b	Jumper-burrower
L. natalensis	0	A	Cartilaginous	SG	–	Jumper-swimmer
L. notoaktites	1	P	Ossified	SG	b	Jumper-burrower
L. pentadactylus	1	A	Cartilaginous	SG	–	Jumper
L. plaumanni	1	P	Ossified	SG	–	Jumper-burrower
L. petersii	0	A	Cartilaginous	SG	–	Jumper-swimmer
L. podicipinus	0	A	Cartilaginous	SG	–	Jumper-swimmer**
L. poecilochilus	1	P	Ossified	SG	–	Jumper-burrower
L. pustulatus	0	A	Cartilaginous	SG	–	Jumper-swimmer
L. rhodomystax	1	A	Cartilaginous	SG	–	Jumper
L. rhodonotus	1	A	Cartilaginous	SG	–	Jumper
L. riveroi	0	A	Cart/Ossif	SG	–	Jumper-swimmer
L. syphax	1	A	Cartilaginous	SG	–	Jumper
L. troglodytes	1	P	Ossified	SG	b	Jumper-burrower
L. validus	0	A	Cartilaginous	SG	–	Jumper-swimmer
L. ventrimaculatus	1	A	Cartilaginous	SG	–	Jumper
L. wagneri	0	A	Cartilaginous	SG	–	Jumper-swimmer
Adenomera						
A. andreae	1	P	Carti/Ossif	SG	–	Jumper-burrower
A. hylaedactyla	1	P	Ossified	SG	b	Jumper-burrower
A. marmorata	1	P	Carti/Ossif	SG	–	Jumper-burrower

(continued)

Table 1. (continued)

Species	Toe fringes	Spatulate snout	Tectum and solum nasi	Pelvis type	Burrowing/no burrowing	Locomotor mode
Engystomops						
E. petersi	1	A	–	NSG	–	Hopper
E. pustulosus	1	A	Cartilaginous	NSG	–	Hopper
Physalaemus						
P. biligonigerus	1	A	Cart/Mineralized	NSG	-	Hopper
P. centralis	1	A	–	NSG	–	Hopper
P. cuqui	1	A	Cartilaginous	NSG	–	Hopper
P. cuvieri	1	A	Cart/Mineralized	NSG	–	Hopper
P. nattereri	0	A	Cart/Ossif	NSG	–	Hopper
P. olfersii	1	A	–	NSG	–	Hopper
P. riograndensis	1	A	Catilaginous	NSG	–	Hopper
P. santafecinus	1/0	A	Catilaginous	NSG	–	Hopper
Pleurodema						
P. borellii	0	A	Catilaginous	NSG	–	Hopper
P. bufoninum	1	A	–	NSG		Hopper
P. thaul	1	A	Catilaginous	NSG	–	Hopper
P. tucumanum	0	A	Catilaginous	NSG	–	Hopper
Pseudopaludicola						
P. boliviana	0	A	Cartilaginous	NSG	–	Hopper

Toe fringes: (0) webbed toes or with fringes extending through the length of toe except for the tip, (1) weak basal fringes and/or webbing or toes with no web or fringe. Spatulate snout: (A) absent, (P) present. Pelvis type: (SG) sagittal-hinge pelvis, (NSG) non sagittal-hinge pelvis. Published data about burrowing behavior (b) (see Data S3) in species of Leptodactylinae.

*According to the morphological characters there is no evidence of *L. labyrinthicus* being an excavator; however, Silva et al. (2005) described that 'the male excavates soil with his hindlimbs…"

**Prado et al. (2002) described a slightly different reproductive mode in *Leptodactylus podicipinus* from the Pantanal, Mato Grosso do Sul, in southwestern Brazil, which deposit eggs in foam nests in water-filled basins that are either natural depressions or constructed by males with their snouts (Martins, 1996). This reproductive mode was not observed in populations from central and southern Paraguay or northern Argentina (Cei, 1980; Brusquetti, personal observations; Ponssa et al., 2011). In the present study we analyzed data from several localities, including specimens from Pantanal (Data S2). We consider the species to be jumper-swimmer since the more parsimonious criteria would be assigned as the more frequent or common mode for the species.

sectional area of the longissimus dorsi, coccygeo-sacralis and coccygeo-iliacus muscles; even though the percentage of variation explained is low: R^2 of 14.05% (Table 2, Fig. 3). The dorsal crest and dorsal tubercle superficial area did not show a significant relationship with their corresponding muscles ($P = 0.5$ and 0.4, respectively) (Table 2, Fig. 3).

Relationship between muscle cross-sectional area, bone crest area and locomotor mode

We found that the OU model was the best fit for all the analyses performed (Table 3). The PGLS showed a significant relationship between locomotion mode and the muscle cross-sectional areas LD + CS + CI (muscles related to the urostyle) and CI + PIB (muscles related to the dorsal crest) (Table 4). The PGLS demonstrated significant differentiation between hopper and jumper species, with low and high values, respectively (Fig. 4). We also found a significant relationship between all tested crest areas and the sampled locomotor modes (Table 4). These results would imply that

character correlations are not simply due to a common evolutionary history, as all leptodactlid locomotor groups could be significantly differentiated by the bone crest area surface (Fig. 5). For the three bone crest areas variables, jumper species presented the highest values and hoppers the lowest, with jumper-burrowers and jumper-swimmers demonstrating intermediary mean values (Fig. 5).

Ancestral state reconstruction

The ancestral state reconstruction of the surface area of the bone crests and cross-section of muscles showed clear evolutionary trends within *Leptodactylus* and the related genera (Figs 6 and 7). The hypothetical ancestor of the leptodactlids would have lower values for the cross-sectional pelvic and hindlimb muscles (e.g. long. dorsi/SVL = 0.04; extensor iliotibialis B/SVL = 0.07; tenuissimus/SVL = 0.04; puboischiofemoralis internus B/SVL = 0.01; coccygeo-sacralis/SVL = 0.04; coccygeo-iliacus/SVL = 0.07) and for the bone crest area of the ilium (e.g. dorsal tubercle/SVL = 0.04;

Table 2 Regression analysis showing the relationship between the cross-sectional area of pelvic and hindlimb muscles and the bone crest area where these muscles insert.

Relationship	SE	t-value	Probability
Urostylar crest vs. long dorsi + coccsac + cocciliac	0.02	2.39	0.02
Dorsal crest vs. cocciliac + puboischiofem int B	0.07	0.68	0.50
Dorsal tubercle vs. ext iliotibialis B + tenuissimus	0.083	0.88	0.39

Cocciliac, coccygeo-iliacus; coccsac, coccygeo-sacralis; ext iliotibialis, extensor iliotibialis B; long dorsi, longissimus dorsi; puboischiofem int B, puboischiofemoralis internus B.
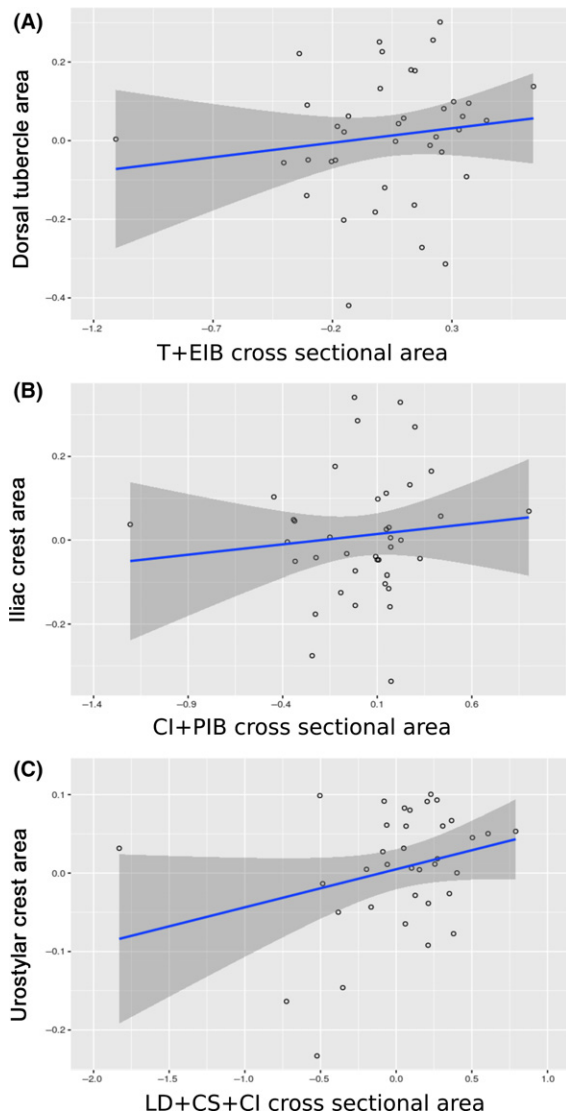

Fig. 3 Relationship of (A) (log-transformed) dorsal tubercle area with tenuissimus and extensor iliotibialis B; (B) (log-transformed) dorsal crest area with coccygeo-iliacus and puboischiofemoralis internus B; (C) (log-transformed) urostylar crest area with longissimus dorsi, coccygeo-sacralis and coccygeo-iliacus. The blue lines show the best fit model regression.

dorsal crest/SVL = 0.4) and the urostyle (e.g. urostylar crest/SVL = 0.4). The Leiuperinae clade (hopper species) preserves this tendency in both cases. For Leptodactylinae, the *Adenomera* clade maintains a low ancestral value for the cross-sectional area of muscles and the clade *Leptodactylus* shows a tendency to increase in value (Fig. 6). The ancestor of the jumper clade *Leptodactylus* has a bone crest with a magnitude of 0.05 to dorsal tubercle/SVL, 0.54 to the dorsal crest/SVL and 0.5 to the urostylar crest; and a cross-sectional area of muscles with dimensions of 0.09 to the long. dorsi/SVL, 0.12 to the extensor iliotibialis B/SVL, 0.07 to the tenuissimus/SVL, 0.07 to the puboischiofemoralis internus B/SVL, 0.07 to the coccygeo-sacralis/SVL and 0.14 to the coccygeo-iliacus/SVL. Reacquisition of low values is shown in *L. melanonotus* (character 1), *Leptodactylus gracilis* + *Leptodactylus jolyi* + *Leptodactylus sertanejo* (character 2); *Leptodactylus elenae*, *Leptodactylus riveroi* + *L. melanonotus*, *Leptodactylus validus* (character 3); *L. melanonotus* (character 4); *Leptodactylus elenae*, *Leptodactylus latinasus*, *Leptodactylus ventrimaculatus*, *L. riveroi* + *L. melanonotus*, *Leptodactylus diedrus* + *Leptodactylus podicipinus*, *Leptodactylus natalensis* (character 5); *L. melanonotus* and *L. natalensis* (character 6). The *L. pentadactylus* group, which includes the largest species in the genus, shows a clear tendency to increase the cross-sectional area of muscles, with the highest values for the jumper-burrower *Leptodactylus labyrinthicus* (character 1). Independent acquisitions of high values are present in the jumper-swimmer species *Leptodactylus insularum* (character 9), *Leptodactylus bolivianus* (character 6) and *L. latrans* (character 5), in the jumper species *Leptodactylus laticeps* (character 3) and *Leptodactylus syphax* (character 4), and in the jumper-burrower species *Leptodactylus furnarius* (character 5). The bone crests show a clear tendency to increase their surface area in the Leptodactylinae clade, where low values reappear for the dorsal tubercle area of *Leptodactylus flavopictus* (jumper), *L. riveroi* (jumper-swimmer), *L. laticeps* (jumper) and *Leptodactylus didymus* (jumper-burrower). The highest values appear in the dorsal tubercle and dorsal crest of *Leptodactylus longirostris* (jumper-burrower), dorsal crest of *L. validus* (jumper-swimmer) and *L. chaquensis* (jumper-swimmer) (Fig. 7).

Discussion

This study demonstrates that (i) there is a correlation between the urostylar crest bone attachment surface and the cross-sectional area of related muscles in Leptodactylus; (ii) the bone crest surface area of the urostyle and ilium in

Table 3 Values of log likelihood (LogL), AIC_c and wAIC corresponding to the evolutionary models tested [Brownian motion (BM), Ornstein-Uhlenbeck (OU) and Early Burst (EB).

Model	BM			OU			EB		
	LogL	AIC_c	wAIC	LogL	AIC_c	wAIC	LogL	AIC_c	wAIC
LD + CS + CI ~ Locomotion + Size	37.85	87.69	0.31	36.80	87.61	0.33	37.97	87.95	0.30
CI + PIB ~ Locomotion + Size	17.10	46.20	0.28	15.95	45.91	0.32	17.30	46.61	0.27
EIB + T ~ Locomotion + Size	15.29	42.58	0.13	**12.54**	**39.08**	**0.77**	15.58	43.17	0.10
Dorsal tubercle ~ Locomotion + Size	26.64	40.58	0.02	**31.01**	**48.03**	**0.96**	25.62	39.25	0.01
Dorsal crest ~ Locomotion + Size	27.61	43.23	0.03	**31.95**	**49.91**	**0.95**	26.89	41.79	0.02
Urostyle ~ Locomotion + Size	30.10	48.20	0.01	**35.48**	**56.96**	**0.98**	29.52	47.04	0.01

Values in bold indicate the evolutionary model that best fit the data based on the wAIC values.
AIC, Akaike's information criterion; CI, coccygeo-iliacus; CS, coccygeo-sacralis; EIB, extensor iliotibialis B; LD, longissimus dorsi; PIB, puboischiofemoralis internus B; T, tenuissimus; wAIC, weighted Akaike's information criterion.

Table 4 Best fitting PGLS model of each area (cross-sectional muscle and bone crests) against locomotion mode. See Supporting Information (Data S4) for all models.

Model	Locomotion type	Value	SE	*t*-value	*P*-value
LD + CS + CI ~ Locomotion + Size	Hopper	−6.79	0.86	−7.88	< 0.05*
OU	Jumper	0.92	0.41	2.22	0.03*
	Jumper-Burrower	0.61	0.38	1.61	0.11
	Jumper-Swimmer	0.59	0.40	1.48	0.15
CI + PIB ~ Locomotion + Size	Hopper	−5.12	0.44	−11.61	< 0.05*
OU	Jumper	0.41	0.20	2.07	0.04*
	Jumper-Burrower	0.21	0.18	1.24	0.22
	Jumper-Swimmer	0.20	0.27	0.07	0.29
Dorsal tubercle ~ Locomotion + Size	Hopper	−1.40	0.07	−19.88	< 0.05*
OU	Jumper	0.35	0.08	4.43	< 0.05*
	Jumper-Burrower	0.33	0.07	4.45	< 0.05*
	Jumper-Swimmer	0.38	0.08	4.72	< 0.05*
Dorsal crest ~ Locomotion + Size	Hopper	−0.63	0.06	−9.73	< 0.05*
OU	Jumper	0.34	0.07	4.60	< 0.05*
	Jumper-Burrower	0.32	0.07	4.72	< 0.05*
	Jumper-Swimmer	0.39	0.08	5.26	< 0.05*
Urostyle crest ~ Locomotion + Size	Hopper	−47.99	0.04	−10.78	< 0.05*
OU	Jumper	0.11	0.05	2.01	0.04*
	Jumper-Burrower	0.17	0.04	3.94	< 0.05*
	Jumper-Swimmer	0.19	0.05	3.45	< 0.05*

CI, coccygeo-iliacus; CS, coccygeo-sacralis; EIB, extensor iliotibialis B; LD, longissimus dorsi; PGLS, phylogenetic generalized least squares; PIB, puboischiofemoralis internus B; T, tenuissimus. Significant values are marked with an asterisk (*)

conjunction with the cross-sectional area of the associated muscles are a reliable proxy to infer some locomotor faculties in leptodactylids frogs; (iii) the evolution of both characters, bone crest surface and cross-sectional area of muscles, shows a general tendency from lower values for the ancestor of the leptodactylids to higher values in the *Leptodactylus* genus.

Correlations between bone crest and muscle cross-sectional areas

As direct observations of behavior or measurement of physiological variables is impossible for extinct forms, three basic approaches can be used to infer function and behavior from fossil records: empirical evidence, comparison with modern analogs and biomechanical modeling (Benton, 2010; Vizcaíno et al. 2015).

The main source of empirical data in vertebrate paleontology comes from the analysis of skeletal material to infer habitual activities of past populations (Rabey et al. 2015). Muscle attachment sites in particular, such as crests and ridges, have been used to deduce the type, and even performance, of locomotion in historic populations or extinct species (e.g. Robb, 1998; Eliot & Jungers, 2000; Wang et al. 2004; Zumwalt, 2006; Rhodes & Steven, 2008; Holliday, 2009; Benton, 2010). The inference that the size of the bone
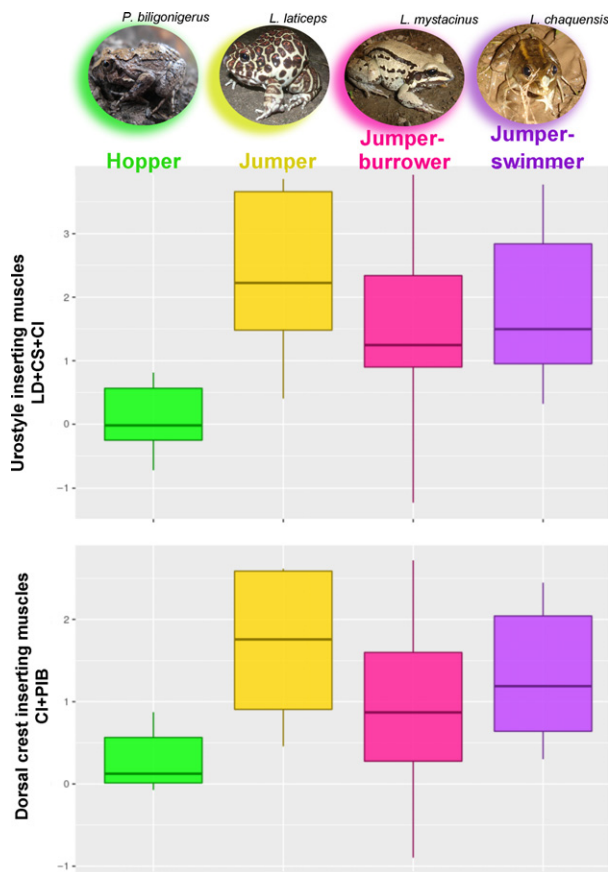
Fig. 4 Box plot comparing the differences among locomotor mode groups according the cross-sectional muscle variables. Boxes represent the 25%/75% quartiles, and the median is shown with a horizontal line. The minimal and maximal values are in the outermost point of the vertical lines.

crest reflects the magnitude of the muscle and consequently locomotor capabilities, derives from the fact that the blood flow to periosteal bone increases under the effect of the force of a contracting muscle, which would stimulate bone growth and increase the size of the attachment site to strengthen it (Chamay & Tschantz, 1972). In other words, since the mechanical stress experienced on a surface area is proportional to the applied force in each unit area of that surface (Biewener, 1992), greater bone crests for larger and/or more active muscles would be an optimum system to maintain adequate stress magnitudes (Zumwalt, 2006). Although the correlation between morphological muscle constraints that influence function (e.g. fiber length, cross-sectional area) and the morphology of associated muscle attachment sites has frequently been assumed (Zumwalt, 2006), it is poorly understood (Zumwalt, 2006; Rabey et al. 2015). Our results confirm that the cross-sectional area of the pelvic muscles correlates with the magnitude of the urostylar crest and, therefore, this feature could be used as a proxy for the configuration and force of the muscle attached to this bone. This is important as it enables certain anatomical and behavioral inferences to be
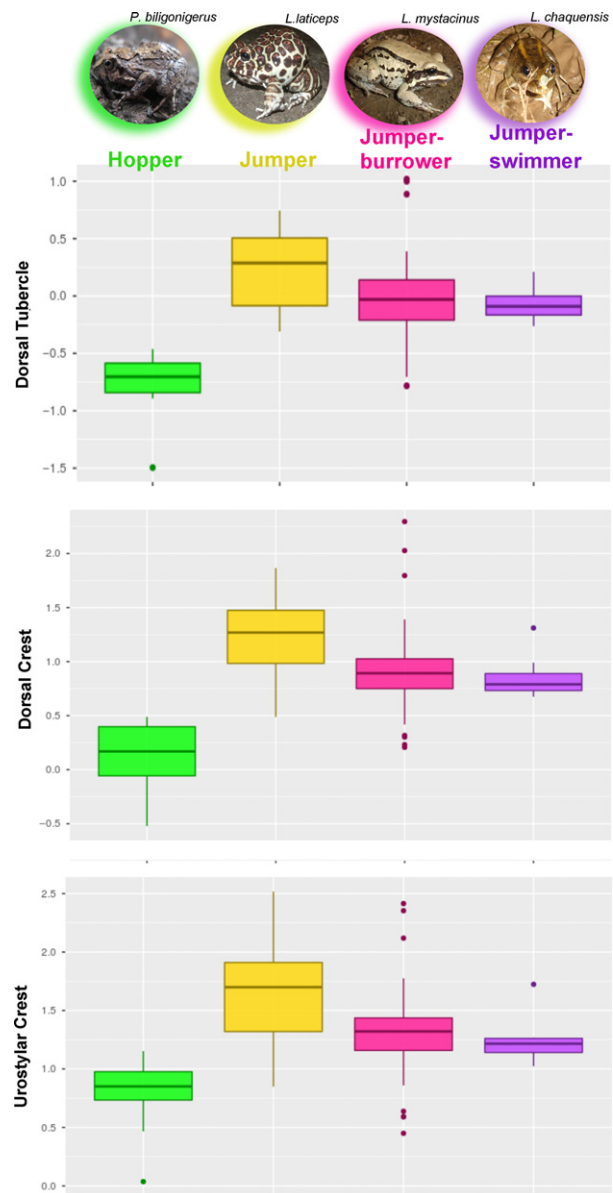

Fig. 5 Box plot comparing the differences among locomotor mode groups according to the bone crest area variables. Boxes represent the 25%/75% quartiles, and the median is shown with a horizontal line. The minimal and maximal values are in the outermost point of the vertical lines.

made from biomechanical and phylogenetic contexts, i.e. muscular configuration in jumping species, and allows us to draw reliable conclusions regarding fossil and extant anurans. Our data, however, do not allow for generalized conclusions, as no correlation was found between all the crests and muscles analyzed; it is very probable that other constraints outside the scope of this study act on the ilium crests. The muscles that we analyzed related to the ilium crests are also not intrinsically involved in the movement of this bone. The studied urostylar crest muscles, on the other hand, directly impact the movement of the urostyle. During locomotion, the longissimus dorsi and the coccygeo-sacralis
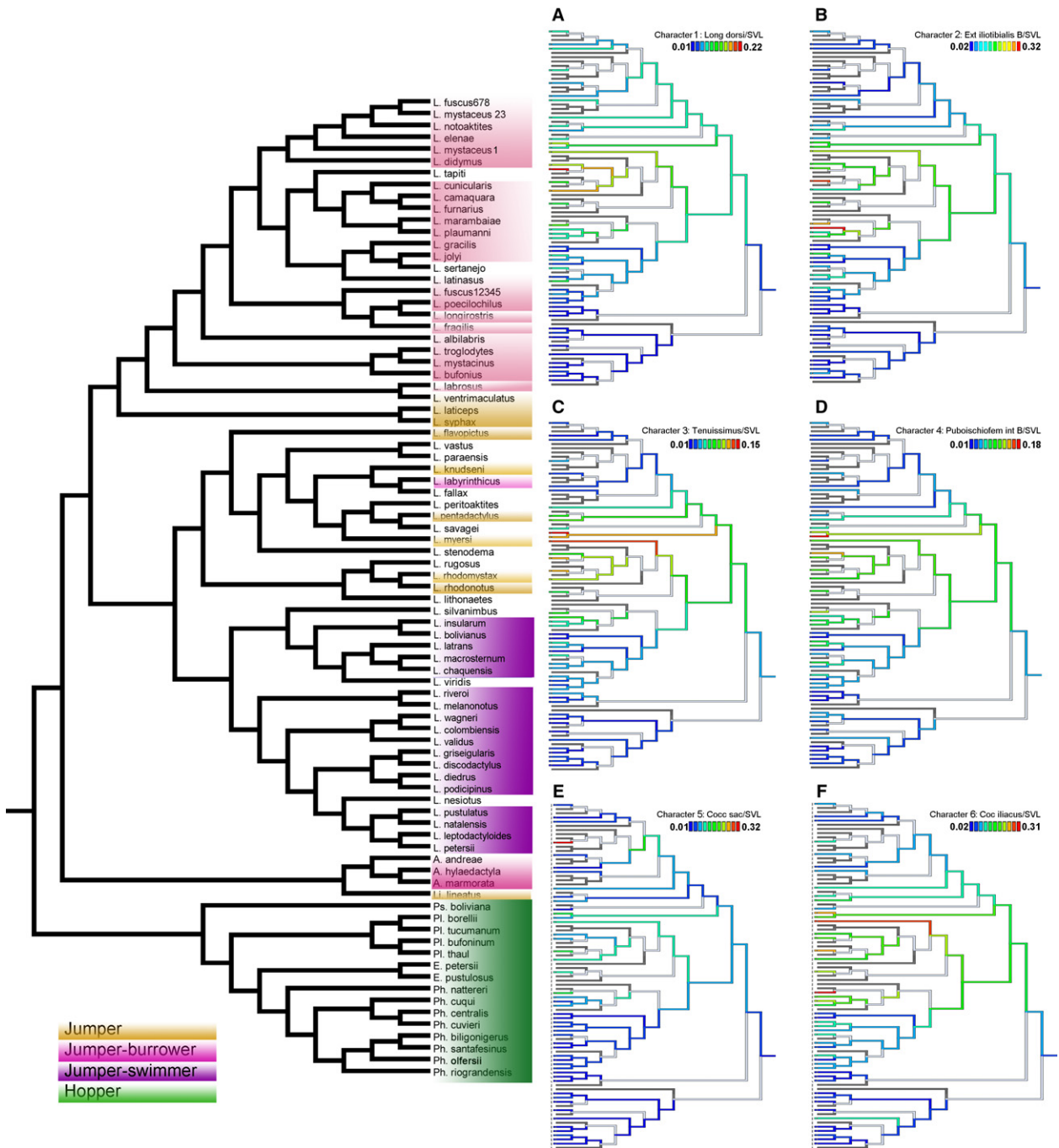
Fig. 6 Ancestral state reconstruction of cross-sectional muscle characters. Composite *Leptodactylus* tree on the left representing phylogenetic relationship of the analyzed taxa. The trees on the right represent ancestral state reconstruction of characters 1–6. (A) Longissimus dorsi/SVL (character 1). (B) Extensor iliotibialis B/SVL (character 2). (C) Tenuissimus/SVL (character 3). (D) Puboischiofemoralis externus B/SVL (character 4). (E) Coccygeo-sacralis/SVL (character 5). (F) Coccygeo-iliacus/SVL (character 6).

rotate the urostyle dorsally, whereas the coccygeo-iliacus rotates the urostyle ventrally or shifts the pelvis posteriorly (Přikryl et al. 2009). The extensor iliotibialis B acts in knee extension, the tenuissimus in the retraction of the femur and flexion at the knee, and the puboischiofemoralis internus B in the protraction of the femur. A more significant morpho-functional correlation could probably be found between the cross-sectional area of these muscles and their attachment sites in knee and femur. We did consider the relationship of the origins and insertions of the coccygeo-iliacus with the dorsal and urostylar crests, as it is the only muscle dedicated to the anterior gliding of the ilium along the sacral diapophysis (Přikryl et al. 2009). This action, along with the movement that it produces in the urostyle, would

Fig. 7 Ancestral state reconstruction of bone crest area characters. Composite *Leptodactylus* tree on the left representing phylogenetic relationship of the analyzed taxa. The trees on the right represent ancestral state reconstruction of characters 7–9. (A) Dorsal tubercle/ilium (character 7). (B) Dorsal crest/ilium (character 8). (C) Urostylar crest/urostyle (character 9).

be key in the initial phases of jumping in all species (Emerson & De Jongh, 1980).

Physiologically and interspecifically, these inferences should be drawn with caution. It has been found that, in mammals, exercise produces relatively large muscular cross-sections but does not influence the bone attachment sites (Zumwalt, 2006; Rabey et al. 2015). It would be interesting to compare the experimental data on the effects of muscle activity on bone crest morphology during locomotion in frogs with that from the evolutionary perspective. This study also raises questions about the pattern of genetic

expression that induces the hypertrophy of the attachment sites. Previous studies describe alterations at a molecular level that produce an increased periosteal cell proliferation as consequence of mechanical loading (Raab-Cullen et al. 1994).

Correlation between muscle cross-sectional area, bone crest area and locomotor mode

As predicted, the structure of the cross-sectional areas of the pelvic girdle muscles and their corresponding bone crest

surface areas allow us to make inferences about the loco-motor modes in leptodactylid frogs. Differences in muscle configuration can distinguish primarily between jumpers and hoppers, as the jumping ability requires higher muscle surface values. Jumping in Leptodactylinae and hopping in Leiuperinae are also reflected in their different pelvic morphology: the first having a sagittal-hinge and the second a non-sagittal-hinge (Jorgensen & Reilly, 2013). Morphological differences between the subcategories of jumper-swimmers and jumper-burrowers are minimal in Leptodactylinae, as the primary locomotor mode for the genus is jumping. Burrowing and swimming are secondary in Leptodactylinae and do not seem to require modification or a specialized phenotype in the cross-sectional area of the studied muscle group. The burrowing behavior found in this genus, and in the related genus *Adenomera*, has not had a significant adverse effect on their ability to jump, as some species are able to cover nine body lengths per jump (Emerson, 1979, 1988). Burrowing in these genera is exclu-sively associated with the biological role of reproduction. The burrower species dig depressions or build underground incubation chambers for the foam nest where the eggs are deposited (Heyer, 1978). In *Leptodactylus* and *Adenomera,* this behavior exclusively involves the use of the hindlimbs and/or head (Philibosian et al. 1974; Pisanó et al. 1993; Prado et al. 2002; Marahão Dos Santos & Oliveira Amorim, 2005; Silva et al. 2005) and, in this case, the only associated morphological modifications are the development of a rigid chisel-like snout and the ossification of the nasal cartilage region (Heyer, 1978; Ponssa, 2008; Ponssa et al. 2011; Ponssa & Barrionuevo, 2012; Ponssa & Medina, 2016). Other more typical burrower anurans demonstrate muscle features par-ticular to more persistent digging behavior: *Hemisus,* for example, shows powerful muscles used for head-first bur-rowing that involves flexing the head and retracting the front legs (Emerson, 1976; Wells, 2008); hindlimb burrowers (e.g. *Myobatrachus*) exhibit modifications of the hindlimb muscles that serve to place the digging tubercle appropri-ately for soil displacement and to increase the force of the legs (Emerson, 1976; Kley & Kearney, 2007). The ability to swim is linked to aquatic habitats, although many species that can move in water are not considered strictly aquatic. Only a few anuran species can actually be considered strictly aquatic (e.g. Pipidae, *Barbourula kalimantanensis, Lanke-nectes corrugatus* and several species of *Telmatobius*). These species spend the greater part of their life in water and typ-ically show morphological traits related to aquatic life, such as a lateral line, reduced tongue, reduced lungs and skin bagginess (Barrionuevo, 2015). Species in the *L. melanono-tus* group utilize aquatic habitats for reproduction, building foam nests on the top of the water (Heyer, 1978). This reproductive behavior categorizes these species as jumper-swimmers and the presence of an inter-digital web is suffi-cient for optimal performance in water. The absence of a correlation between the cross-sectional area of muscles and certain locomotor modes raises the question of whether other muscle characteristics might reflect modifications associated with the locomotor subcategories of lepto-dactylids. Several other factors can affect muscle perfor-mance, including the proportion of the cross-sectional area of a muscle occupied by myofibrils, the amount of overlap between the thick and thin filaments, myofilament length and resting sarcomere length (Josephson, 1975). Variations in traits in the tendinous system may also allow for distinc-tion among locomotor subcategories. Roberts et al. (2011) have shown that muscle properties of frogs are not enough to determine their locomotor performance. When jumping, several species use a catapult-like mechanism to store and rapidly to release elastic energy and, in this case, the tendon would act as a site of elastic energy storage during jumping (Astley & Roberts, 2012).

The bone crest surface area of the ilium and urostyle effectively allowed us to distinguish among the four defined locomotor types. Abundant research shows that skeletal tissue is susceptible to mechanical stress (Quinn et al. 1998; Huang et al. 2010; Kelly & Jacobs, 2010; Nowlan et al. 2010; among others), since mechanical signals regu-late the skeletogenesis of mesenchymal stem cells (Kelly & Jacobs, 2010). Our data support this premise, as the bone crests corresponded to the proposed locomotor types. Soft tissues are thought to be more susceptible to changes in the mechanical stress implied in different locomotor modes, whereas muscles are not as sensitive and do not allow for the subtle distinction of subcategories (i.e. jumper-bur-rowers, jumper-swimmers). Morphometric traits in the ten-dons of leptodactlids also showed no relation with the locomotor modes and appear to be restricted by phylogeny (Fratani et al. 2017).

According to the OU model, the variation in our data would be explained by a stabilizing selection or selective optima. It is important to highlight, however, that there were minimal differences in the AIC_c and wAIC between the tested models and, therefore, some consideration should be given to whether these models really do have a substantially better or worse fit than other models. Coinci-dently, this hierarchical pattern has also been observed in tendon variations in the same leptodactylidae group (Fra-tani et al. 2017), in pelvic girdle characters in hylids (Soliz et al. 2017) and in tendinous muscle variation in lizards in general (Tulli et al. 2016). These new results support our previous hypothesis that a stabilizing selection would best explain many of the morphological traits related to the evo-lution of the locomotor system in tetrapods (Fratani et al. 2017).

The mobile ilium-sacral and sacrum-urostyle articulations are keys to locomotor function in anurans. Other characters related to these functional dispositions also show variability associated with locomotor types, e.g. the magnitude of the sacrum diapophyseal expansion, the pelvic models (e.g. sagittal-hinge, lateral-bender), urostylar fusion or bicondyly,

and the ilium length that is key to the pelvic glide (Emerson, 1979, 1982; Reilly & Jorgensen, 2011; Jorgensen & Reilly, 2013; Herrel et al. 2014). All these characters contribute to the development and functionality of the hip module (Dos Santos et al. 2017), whose integrated skeletal, muscular and tendinous elements are closely tied to functional variations.

Ancestral state reconstruction

The ancestral reconstruction showed that bone crests and muscle cross-sectional area would have had a relatively lower magnitude in the ancestor of Leptodactylid. The powerful muscles linked to the sacrum-urostyle and ilium-sacral joints are related to the capacity to jump in *Leptodactylus*, whereas an increase in the ilium and urostyle bone crest is linked to this ability in Leptodactylinae.

The optimization of the tree resulted in a pattern consistent with that of the comparative method in which the hopper group, Leiuperinae, have smaller crests and the jumper group, Leptodactylinae, shows a tendency for increased crest areas. Larger ridges on the ilia and the urostyle provide larger muscle attachment surfaces for control of the movements of the pelvis relative to the body and the limbs relative to the pelvis (Emerson & De Jongh, 1980; Duellman & Trueb, 1986; Přikryl et al. 2009). A smooth iliac shaft and urostyle are basal for Anura and, within the genus Neobatrachia, the iliac ridge appears in Eleutherodactylidae, Natatanura and Leptodactylidae, whereas the urostylic ridge is widespread in Hyloides (Reilly & Jorgensen, 2011). Reilly & Jorgensen (2011) also showed well developed iliac and urostylic ridges in Leptodactylinae (Leptodactylidae in their phylogenetic framework) that are characterized as jumper terrestrial. For the walker-hopper genus Leiuperinae (Leiuperidae in their phylogenetic framework), these authors characterized a dorsal ridge for the ilium and a half ridge for the urostyle. Similar to our findings, these authors note a clear pattern of presence of ridges in the ilium and urostyle and terrestrial jumping. In a widespread phylogenetic framework it has been found that long-distance jumping and jumping traits do not appear until well into the evolution of Neobatrachia (Reilly & Jorgensen, 2011), contrary to the hypothesis that jumping is primitive (Shubin & Jenkins, 1995). Terrestrial jumpers seem to have acquired the same configurations independently in distant lineages (Reilly & Jorgensen, 2011), signifying strong functional constraints on the evolution of terrestrial jumping in frogs that tend to have larger bodies and hindlimb muscle masses (Marsh, 1994; Choi & Park, 1996) and, according to our data, the biggest muscle attachment sites and muscle cross-sectional area linked to the sacrum-urostyle and ilium-sacral joints.

The characteristics studied here do not demonstrate a clear phylogenetic pattern for either the jumper-burrowers or jumper-swimmer clades, proving that these characteristics are not related to the acquisitions of these faculties in Leptodactylinae. The consistent pattern between phylogenetic and ecological groups detected in the hopper Leiuperinae and jumper Leptodactylinae, was also found in hylids. These monophyletic groups have locomotor modes or habitats in common (Soliz & Ponssa, 2016). This implies that, as morphological traits respond to shared phylogenetic history, the functional constraints that restricted the heritability of these features are also inherited. The current morphological make-up of an animal is a conglomeration of its present adaptations and its evolutionary history (Vizcaíno et al. 2015).

Author contributions

Contributions to concept/design: M.L.P.; acquisition of data: M.L.P., J.F.; data analysis/interpretation: M.L.P., J.F., V.A.; drafting of the manuscript: M.L.P., J.F.; critical revision of the manuscript and approval of the article: M.L.P., J.F., V.A.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Number of studied individuals/species and the mean species values for (a) bone crest areas, (b) SVL and muscle cross-sectional area.

Data S2. List of species examined for morphological data collection. m Specimens used for muscular characters, s specimens used for skeletal characters. Acronyms of collections are: Fundación Miguel Lillo (FML), Museu Nacional-UFRJ (MNRJ), Museu de Zoologia da Universidade de São Paulo (MZUSP); Museo de la Plata (MLP); Smithsonian Institution National Museum of Natural History (UNSM), Pontificia Universidad Católica del Ecuador (QCAZ), Universidade Estadual Paulista –Rio Claro (CFBH), and personal collect of María Laura Ponssa (L).

Data S3. Locomotor mode matrix with literature references for toe fringes: (0) webbed toes or toes with fringes extending through the length of toes except for the tips, (1) weak basal fringes and/or webbing, no web or fringe; spatulare snout, tectum and solum nasi and pelvis type (SG: sagittal-hinge pelvis/ NSG: non sagittal-hinge pelvis).

Data S4. Summary of PGLS models.