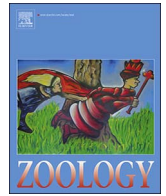




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# Tendinous framework of anurans reveals an all-purpose morphology

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## ABSTRACT

Tendons are directly associated with movement, amplifying power and reducing muscular work. Taking into account habitat and locomotor challenges faced by anurans, we identify the more conspicuous superficial tendons of a neotropical anuran group and investigate their relation to the former factors. We show that tendons can be visualized as an anatomical framework connected through muscles and/or fascia, and describe the most superficial tendinous layer of the postcranium of *Leptodactylus latinasus*. To analyze the relation between tendon morphology and ecological characters, we test the relative length ratio of 10 tendon–muscle (t-m) elements in 45 leptodactylid species while taking phylogeny into account. We identify the evolutionary model that best explains our variables. Additionally, we optimize t-m ratio values, and the shape of the longissimus dorsi insertion onto a selected phylogeny of the species. Our data show the existence of an all-purpose morphology that seems to have evolved independently of ecology and functional requirements. This is indicated by no significant relation between morphometric data of the analyzed tendons and habitat use or locomotion, a strong phylogenetic component to most of the analyzed variables, and a generalized pattern of intermediate values for ancestral states. Ornstein-Uhlenbeck is the model that best explains most t-m variables, indicating that stabilizing selection or selective optima might be driving shifts in tendon length within Leptodactylidae. Herein, we show the substantial influence that phylogeny has on tendon morphology, demonstrating that a generalized and stable morphological configuration of tendons is adequate to enable versatile locomotor mode and habitat use. This is an attempt to present the tendinous system as a framework to body support in vertebrates, and can be considered a starting point for further ecomorphological research of this anatomical system in anurans.

## 1. Introduction

Tendons are defined as a fibrous connective tissue that joins and transmits force from muscles to bones. They allow for dexterity of movement and the distribution of force from limbs to digits (Kardong, 2002). As most tendons are connected to muscle, they are usually regarded as part of the muscle mass, and have accordingly been treated in literature (Ecker, 1889; Gaupp, 1996; Dunlap, 1960; Duellman and Trueb, 1986). Tendons, however, have a unique structure, physiology and developmental origin that allow for their consideration as an independent system (Summers and Koob, 2002; Benjamin, 2008; Huang et al., 2015). Tendons are directly associated with movement, as the release of their elastic energy, muscle aponeuroses and ligaments amplifies power and reduces muscular work (Roberts, 2002; Biewener, 2003). Tendons enhance muscle performance for a wide range of locomotor activities (Roberts, 2002) and their modification in response to these challenges has been widely studied in sports medicine (Magnusson et al., 2003; Lidén et al., 2008; Gluck et al., 2010;

Kongsgaard et al., 2010, 2011; Malliaras et al., 2013; de Jonge et al., 2015 among many others). In comparison, fewer studies have been undertaken in the context of the comparative morphology of tetrapods (e.g., Azizi et al., 2002; Hutchinson, 2002; Shadwick et al., 2002; Summers and Koob, 2002; Abdala et al., 2006, 2008, 2009; Tulli et al., 2012a, 2012b, 2016; Sustaita et al., 2013; Carrizo et al., 2014).

The tendinous system of anurans has been taken into consideration as part of broad studies centered on muscle anatomy, biomechanics, and performance (Dunlap, 1969; Nauwelaerts et al., 2007; Přikryl et al., 2009; Astley, 2016). An exception can be found in Manzano et al. (2008), who detected a unique tendon pattern on the palmar surface of an arboreal anuran species (*Phyllomedusa bicolor*) that permits complex hand movements (grip and wrist rotation) essential to this species' particular life mode (Gray et al., 1997; Manzano et al., 2008). Until now, no study has focused on tendons in the context of anuran biodiversity studies, and despite their anatomical and functional uniqueness, there are no descriptions of the anuran tendinous system available.

Anurans are an excellent group for ecomorphological studies since

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**Table 1**Muscles and tendons mentioned in this work following the nomenclature of [Diogo and Ziermann \(2014\)](#), synonyms commonly used in the literature, and abbreviations.

	Diogo and Ziermann (2014)	Gaupp (1986), Dunlap (1960)	Abbreviations
Pectoral/ Forelimb	supracoracoideus-coracoradialis	sternoradialis	SC
	triceps brachii	triceps brachii	TB
	flexor digitorum communis (forelimb)	flexor digitorum communis	FDC <sub>f</sub>
Pelvic/ Hindlimb	longissimus dorsi	longissimus dorsi	LD
	coccygeosacralis	coccygeosacralis	CS
	puboischiofemoralis internus B	iliacus externus	PIB
	extensor iliotibialis A	tensor fascia latae	EIA
	fascia latae	fascia latae	FL
	–	knee aponeurosis	KA
	cruralis	cruralis	-
	tenuissimus	iliofibularis	TEN
	flexor digitorum communis (hindlimb)	gastrocnemius	FDC <sub>h</sub>
	Achilles tendon	Achilles tendon	AT
flexor tendon of digit IV	flexor tendon of digit IV	FT-IV <sub>f</sub>	

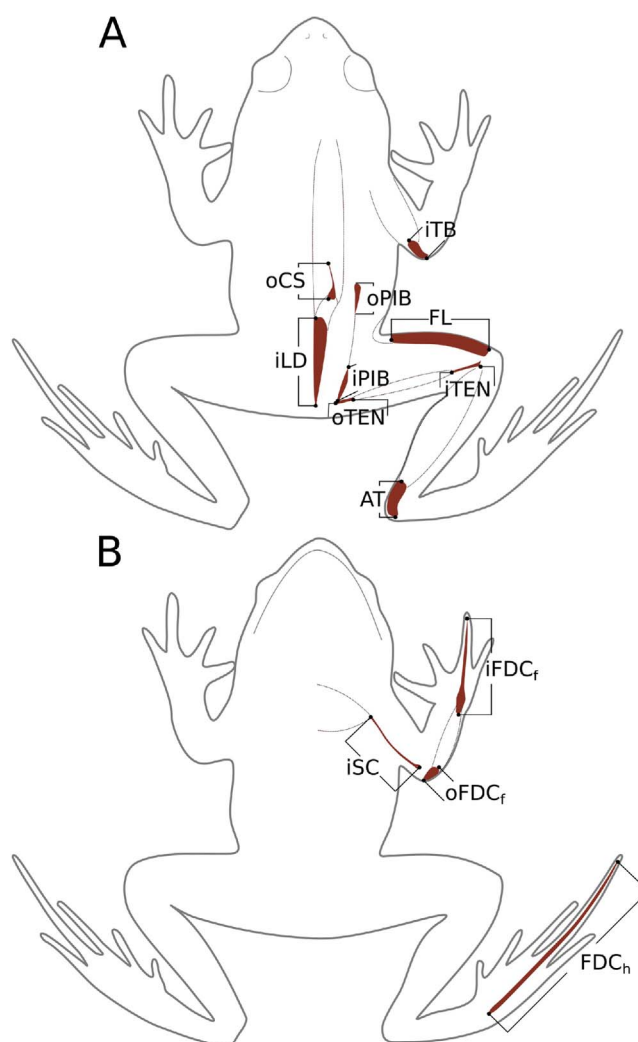
they present a large diversity of habitat use and locomotor modes that impose considerable mechanical challenges related to limb, girdle and vertebral column morphology ([Emerson, 1982](#); [Pügenger and Maglia, 2009](#); [Fabrezi et al., 2014](#)). Distinct habitats impose distinct locomotor challenges; for example, during terrestrial movement limbs function as support pillars for the body and provide stability and strength ([Wren et al., 1998](#); [Zani et al., 2005](#)) whereas in water the limbs are used principally to propel the body ([Hildebrand, 1985](#)). Leptodactylids are an ideal model for evaluating the relationship between tendon morphology and ecology, since they include species with a wide range of locomotor modes and use both aquatic and terrestrial habitats for reproduction and breeding ([Heyer, 1969](#)). Additionally, recent studies have shown that reproductive modes have not evolved linearly towards terrestriality as previously thought ([Heyer, 1969](#); [de Sá et al., 2014](#); [Pereira et al., 2015](#)), rendering more room for adaptive inquiry.

Herein, we offer a first approach to the general morphology of the anuran tendon system using comparative anatomy and identifying the more conspicuous tendons of the superficial layer of the postcranium in two representative species, *Leptodactylus latinasus* and *Physalaemus biligonigerus*. To investigate the relationship between morphometric data and ecological factors (habitat use and locomotor mode), we compared the relative tendon–muscle length of 45 species of leptodactylid frogs, while taking into account the phylogenetic relationship among species. We tested whether our variables adjust best to evolutionary patterns of random fluctuations through time, stabilizing selection or early modifications followed by deceleration. Finally, we optimized morphometric data and the shape of the insertion of the longissimus dorsi onto the phylogeny to identify evolutionary tendencies within the group.

## 2. Materials and methods

### 2.1. Morphology

The superficial tendinous layer of the anuran postcranium was studied as a representative model of the tendinous framework as an independent support system, in the sense of an ectoskeleton as reported by [Wood-Jones \(1944\)](#) for the fascial system of the human hindlimb. Seven adult individuals of *L. latinasus* were examined in order to establish a general tendinous framework and three adult *P. biligonigerus* specimens were examined for comparison (Datasheet S1 in the supplementary online Appendix). Two adult *L. latinasus* (FML 29483 and 29484) and one *Ph. biligonigerus* (FML 29485) were partially cleared and stained for use as illustrations following an adapted protocol from [Wassersug \(1976\)](#). These species belong to two subfamilies within Leptodactylidae and were selected because of their availability for dissections.



**Fig. 1.** Measurements of origin and insertion tendons used for morphometric analysis. (A) Dorsal and (B) ventral views. Abbreviations as in [Table 1](#), with i for insertion or o for origin where necessary.

The muscle, tendon, and other connective tissue nomenclature used in this study follows that presented in [Gaupp \(1986\)](#) for trunk and pelvic structures, and [Abdala and Diogo \(2010\)](#), [Diogo and Abdala \(2010\)](#), [Diogo and Ziermann \(2014\)](#), [Diogo and Molnar \(2014\)](#) for

**Table 2**  
Mean morphometric measurements of tendon-muscle ratio length. See Table 1 for abbreviations.

Species	N	AT	CS	FL	FDC <sub>r</sub>	FT-IV <sub>h</sub>	LD	PIB	SC	TEN	TB
<i>Adenomera</i>											
<i>A. andreae</i>	1	0.280	0.545	1.333	1.192	1.287	0.681	0.651	1.075	0.561	0.386
<i>A. hylaedactyla</i>	3	0.359	0.377	1.963	1.697	1.350	0.508	0.664	1.012	0.471	0.421
<i>A. marmorata</i>	2	0.254	0.430	1.974	1.093	1.515	0.673	0.444	0.941	0.441	0.410
<i>Engystomops</i>											
<i>E. petersi</i>	1	0.405	0.576	1.702	1.128	1.330	1.006	1.219	1.173	0.661	0.458
<i>Leptodactylus</i>											
<i>L. albilabris</i>	1	0.369	0.647	1.267	1.148	1.361	0.674	0.747	1.102	0.494	0.577
<i>L. bolivianus</i>	1	0.452	0.800	1.765	1.097	1.229	0.929	0.863	0.952	0.660	0.434
<i>L. bufonius</i>	12	0.364	0.715	1.455	1.158	1.257	0.643	0.780	1.060	0.562	0.566
<i>L. chaquensis</i>	12	0.367	0.735	1.647	1.156	1.297	0.863	0.842	0.968	0.700	0.371
<i>L. colombienseis</i>	2	0.533	0.527	1.080	1.227	1.377	0.700	0.767	1.051	0.668	0.472
<i>L. diedrus</i>	1	0.393	0.699	1.374	1.027	1.318	0.616	0.758	1.001	0.789	0.473
<i>L. elenae</i>	2	0.267	0.684	1.580	1.383	1.077	0.833	0.673	0.910	0.685	0.571
<i>L. flavopictus</i>	1	0.494	0.892	1.304	1.099	1.252	0.762	0.963	1.032	0.841	0.414
<i>L. furnarius</i>	1	0.377	0.598	1.827	1.146	1.353	0.907	0.664	1.344	0.602	0.441
<i>L. fuscus</i>	2	0.382	0.600	0.836	1.137	1.330	0.601	0.728	0.958	0.652	0.481
<i>L. gracilis</i>	1	0.380	0.619	1.865	1.148	1.343	0.571	0.796	0.990	0.604	0.672
<i>L. griseularis</i>	1	0.576	0.782	1.336	1.138	1.337	0.568	0.879	1.157	0.508	0.603
<i>L. knudseni</i>	1	0.653	0.914	1.179	1.138	1.148	0.819	0.905	0.875	0.708	0.387
<i>L. labyrinthicus</i>	2	0.422	0.775	0.679	1.107	1.238	0.676	0.889	0.935	0.718	0.650
<i>L. laticeps</i>	1	0.402	0.849	1.281	1.044	1.260	0.825	0.902	0.839	0.706	0.493
<i>L. latinasus</i>	1	0.266	0.649	1.375	1.248	1.449	0.732	0.634	1.067	0.607	0.379
<i>L. latrans</i>	2	0.415	0.780	1.396	1.263	1.288	0.964	0.810	1.052	0.621	0.503
<i>L. leptodactyloides</i>	2	0.327	0.769	1.236	1.157	1.354	0.680	0.840	1.020	0.555	0.449
<i>L. macrosternum</i>	1	0.481	0.709	1.529	1.082	1.315	0.926	0.813	0.948	0.725	0.328
<i>L. melanonotus</i>	2	0.447	0.494	1.370	1.210	1.446	0.524	0.806	1.011	0.558	0.375
<i>L. myersi</i>	1	0.713	0.885	1.254	1.009	1.245	0.877	0.832	0.880	0.779	0.474
<i>L. mystaceus</i>	1	0.348	0.751	0.503	1.249	1.321	0.679	0.823	1.022	0.583	0.505
<i>L. mystacinus</i>	2	0.373	0.772	1.208	1.252	1.313	0.697	0.820	1.033	0.676	0.458
<i>L. natalensis</i>	1	0.498	0.679	1.312	1.187	1.434	0.584	0.737	1.089	0.530	0.554
<i>L. pentadactylus</i>	1	0.540	0.774	1.259	1.099	1.284	0.916	0.942	0.956	0.794	0.467
<i>L. petersii</i>	1	0.484	0.740	1.283	1.159	1.420	0.568	0.708	0.991	0.710	0.418
<i>L. podicipinus</i>	3	0.370	0.607	1.614	1.184	1.470	0.528	0.804	1.025	0.553	0.453
<i>L. pustulatus</i>	1	0.372	0.806	1.535	1.185	1.439	0.745	0.803	0.935	0.651	0.675
<i>L. rhodomystax</i>	1	0.517	0.875	1.289	1.164	1.423	0.911	0.986	1.031	0.714	0.616
<i>L. syphax</i>	1	0.488	0.941	1.302	1.088	1.316	0.803	0.877	0.899	0.594	0.422
<i>L. validus</i>	2	0.495	0.607	1.251	1.205	1.373	0.617	0.819	1.034	0.531	0.522
<i>L. ventrimaculatus</i>	1	0.417	0.775	1.340	1.095	1.384	0.364	0.725	1.006	0.562	0.476
<i>L. wagneri</i>	2	0.500	0.697	1.384	1.196	1.392	0.693	0.858	0.956	0.634	0.582
<i>Physalaemus</i>											
<i>Ph. nattereri</i>	1	0.458	0.948	1.107	1.412	1.421	0.557	0.815	0.985	0.535	0.360
<i>Ph. offersii</i>	1	0.583	0.692	2.076	1.201	1.389	0.820	1.177	1.230	0.703	0.299
<i>Ph. biligonigerus</i>	1	0.531	0.620	1.060	1.124	1.366	0.753	0.905	1.044	0.519	0.520
<i>Ph. centralis</i>	1	0.291	0.646	1.782	1.184	1.444	0.610	1.019	1.288	0.631	0.410
<i>Ph. cuvieri</i>	1	0.288	0.503	1.506	1.141	1.492	0.816	0.817	1.183	0.499	0.332
<i>Pleurodema</i>											
<i>Pl. borellii</i>	1	0.374	0.758	1.375	1.221	1.412	0.657	0.880	1.097	0.617	0.717
<i>Pl. tucumana</i>	1	0.380	0.545	1.192	1.252	1.481	0.621	0.753	1.203	0.628	0.384
<i>Pseudopaludicola</i>											
<i>Ps. falcipes</i>	1	0.326	0.578	1.488	1.339	1.508	0.801	0.548	1.058	0.414	0.274

pectoral and limb structures (see Table 1 for nomenclature synonyms).

## 2.2. Morphometric data

To investigate the diversity of tendinous patterns we analyzed 82 adult specimens from 45 leptodactylid species: *Adenomera* (3 spp.), *Engystomops petersi*, *Leptodactylus* (33 spp.), *Physalaemus* (5 spp.), *Pleurodema* (2 spp.), and *Pseudopaludicola falcipes* from various museum collections (for species and collection numbers see Datasheet S1 in the supplementary online Appendix).

For the morphometric analysis we used the relative lengths of 10 tendon–muscle elements that correspond to 13 origin and insertion tendons (Fig. 1). All analyses were performed considering the ratio between the length of each selected tendon and its associated muscle (t-m ratio), allowing access to the functional importance of the tendon–muscle unit (Biewener and Gillis, 1999; Biewener and Roberts, 2000;

Benjamin et al., 2008). We selected the most conspicuous tendons of the superficial layer which exhibited interspecific variability. Five of the studied tendons are associated with muscles of the pectoral and fore-limb region: insertion tendon of m. supracoracoideus–coracoradialis, insertion tendon of m. triceps brachii, origin and insertion tendons of m. flexor digitorum communis, and flexor tendon of digit IV of the hand; and nine with the pelvic and hindlimb region: insertion of m. longissimus dorsi, origin of m. coccygeosacralis, origin and insertion of m. puboischiofemoralis internus B, origin and insertion of m. tenuissimus, fascia latae, insertion of m. flexor digitorum communis (henceforth denoted as Achilles tendon to avoid confusion), and flexor tendon of digit IV of the foot. Specimens were dissected and photographed with a Leica M205 stereomicroscope. Measurements were taken in millimeters using a digital caliper (0.01 mm; Mitutoyo, Kawasaki, Japan) and using the software ImageJ (Schneider et al., 2012). The mean species values for tendon length and the number of

**Table 3**  
Habitat use character descriptions.

Characters/Coding	(0) association to aquatic habitat	(1) association to terrestrial habitat
Toe fringes	Webbed toes	Weak basal fringes and/or webbing, toes with fringes extending through the length of toes except for the tips, no web or fringe
Clutch size	≥ 1000	< 1000
Egg placement	Water	Natural and/or built depressions and chambers
Tadpole development	Water	Built chamber

**Table 4**  
Habitat use character scores (see coding in Table 3). Species with missing entries were not included.

Species	Toe fringes	Clutch size	Egg placement	Tadpole development	Habitat use
<i>Adenomera</i>					
<i>A. andreae</i>	1	1	1	1	4
<i>A. hylaedactyla</i>	1	1	1	1	4
<i>A. marmorata</i>	1	1	1	1	4
<i>Engystomops</i>					
<i>E. petersi</i>	1	1	0	0	2
<i>Leptodactylus</i>					
<i>L. bufonius</i>	1	1	1	0	3
<i>L. chaquensis</i>	0	0	0	0	0
<i>L. elenae</i>	1	1	1	0	3
<i>L. furnarius</i>	1	1	1	0	3
<i>L. fuscus</i>	1	1	1	0	3
<i>L. knudseni</i>	1	0	1	0	2
<i>L. labyrinthicus</i>	1	0	1	0	2
<i>L. latinasus</i>	1	1	1	0	3
<i>L. latrans</i>	0	0	0	0	0
<i>L. leptodactyloides</i>	0	0	0	0	0
<i>L. macrosternum</i>	0	0	0	0	0
<i>L. melanonotus</i>	0	0	0	0	0
<i>L. mystaceus</i>	1	1	1	0	3
<i>L. mystacinus</i>	1	0	1	0	2
<i>L. petersii</i>	0	0	0	0	0
<i>L. podicipinus</i>	0	0	1	0	1
<i>L. rhodomystax</i>	1	1	0	0	2
<i>L. syphax</i>	1	1	1	0	3
<i>L. wagneri</i>	0	0	0	0	0
<i>Physalaemus</i>					
<i>Ph. nattereri</i>	0	0	0	0	0
<i>Ph. biligonigerus</i>	1	0	0	0	1
<i>Ph. centralis</i>	1	0	0	0	1
<i>Ph. cuvieri</i>	1	1	0	0	2
<i>Ph. olfersii</i>	1	1	0	0	2
<i>Pleurodema</i>					
<i>Pl. tucumana</i>	0	0	0	0	0
<i>Pseudopaludicola</i>					
<i>Ps. falcipes</i>	0	1	0	0	1

individuals/species are detailed in Table 2.

### 2.3. Ecological settings

The ecological characters analyzed included habitat use and locomotor mode. Habitat use categories were assigned using morphological characters adapted from the literature which are known to be associated with habitat use: presence of toe fringes (Goldberg and Fabrezi, 2008; de Sá et al., 2014), clutch size (Duellman and Trueb, 1986; Pereira et al., 2015), egg placement (Duellman, 1989; Haddad and Prado, 2005), and tadpole development site (Haddad and Prado, 2005; Zamudio et al., 2016). Characters were coded as in Table 3; state (0)

denotes aquatic habitat, and state (1), terrestrial habitat. The habitat category assigned to a given species corresponded to the sum of its character states, ranging from 0 (strongly associated with water) to 4 (strongly associated with land) (Table 4). Since ecological information was not available for all *Leptodactylus* species, 15 species with missing entries were discarded from this analysis. The compiled information and accompanying literature references are listed in Datasheet S2.

Leptodactylinae species have been consistently categorized as jumpers or hoppers in previous studies (Zug, 1978; Emerson, 1979, 1982; Fabrezi et al., 2014). Considering, however, that burrowing and swimming are notable locomotory abilities also observed in *Leptodactylus*, we subcategorized the jumper species in order to best represent the diversity of this group (Table 5). We are aware that leptodactylids are capable of a range of locomotory modes, so we estimate the following categories as an approximation based on what the species most frequently do. Subcategories were defined as: swimmer-jumpers (species known for the ability to displace in water – e.g., *Leptodactylus chaquensis*); burrower-jumpers (species known to build depressions or chambers for reproduction sites – e.g., *Leptodactylus fuscus*); jumpers and hoppers (including species that have been previously categorized in the literature (Zug, 1978; Emerson, 1979, 1982; Jorgensen and Reilly, 2013; Fabrezi et al., 2014). All the above-mentioned scores were assigned based on observations documented in the literature (Heyer, 1978; Ponssa and Heyer, 2007; Ponssa, 2008; Ponssa and Barrionuevo, 2008; Ponssa et al., 2011; de Sá et al., 2014) and personal observations during field expeditions. We also used morphological evidence conspicuously associated with swimming (presence of toe fringes – Heyer, 1969; Goldberg and Fabrezi, 2008; de Sá et al., 2014) or burrowing (presence of spatulate snout and ossification of the nasal cartilages – Heyer, 1978; Ponssa and Barrionuevo, 2010) for scoring locomotory categories.

### 2.4. Statistical analyses

Prior to statistical tests all morphometric variables were  $\text{LOG}_{10}$  transformed to meet the requirements of normality and homoscedascity (Zar, 1999). To explore the effect of sexual dimorphism we performed an analysis of covariance (ANCOVA) using 20 individuals of known sex of *Leptodactylus bufonius* as a proxy of the genus. This analysis was conducted using R 3.2.3 (R Core Team, 2015) for each tendon–muscle ratio measure.

#### 2.4.1. Phylogenetic context

For statistical tests and optimization we used the topology of the most sampled and most inclusive hypothesis for *Leptodactylus* (de Sá et al., 2014). A few changes were added for taxa not included in this study, using better sampled phylogenies for species within *Physalaemus* (Lourenço et al., 2015) and *Adenomera* (Fouquet et al., 2014). Branch lengths were not available for all the species included in this study, so we tested three different types of arbitrary branch lengths: constant (all branch lengths equal to 1), Grafen's (1989), and Pagel's (1992) methods. To test for the statistical adequacy of the branch lengths we performed an independent contrasts analysis (Garland et al., 1992) using the PDTREE module of Mesquite 3.04 (Midford et al., 2005; Maddison and Maddison, 2015). Constant branch lengths gave the best results for the standardization of phylogenetically independent contrasts, as indicated by the absence of statistically significant trends in diagnostic plots for most traits (Garland et al., 1992).

#### 2.4.2. Phylogenetic generalized least squares (PGLS)

As data for species may be non-independent because of shared phylogenetic history (Blomberg et al., 2003; Revell, 2008, 2009), we applied the phylogenetic comparative method to estimate the relationship between tendon length and ecological factors while taking phylogeny into account. To reduce the number of variables a phylogenetically-based principal component analysis (Phylo PCA – Revell,

**Table 5**

Locomotor mode character scores. Toe fringes: (0) webbed toes, (1) weak basal fringes and/or webbing, fringes extending through the length of the toes except for the tips, no web or fringe. Spatulate snout: (A) absent, (P) present.

Species	Toe fringes	Spatulate snout	Tectum and solum nasi	Sagittal-hinge articulation	Locomotor mode
<i>Adenomera</i>					
<i>A. andreae</i>	1	P	ossified	Jumper	Jumper-burrower
<i>A. hylaedactyla</i>	1	P	ossified	Jumper	Jumper-burrower
<i>A. marmorata</i>	1	P	ossified	Jumper	Jumper-burrower
<i>Engystomops</i>					
<i>E. petersi</i>	1	A	–	Hopper	Hopper
<i>Leptodactylus</i>					
<i>L. albilabris</i>	1	P	ossified	Jumper	Jumper-burrower
<i>L. bolivianus</i>	0	A	cartilaginous	Jumper	Jumper-swimmer
<i>L. bufonius</i>	1	P	ossified	Jumper	Jumper-burrower
<i>L. chaquensis</i>	0	A	cartilaginous	Jumper	Jumper-swimmer
<i>L. colombiensis</i>	0	A	cartilaginous	Jumper	Jumper-swimmer
<i>L. diedrus</i>	0	A	cartilaginous	Jumper	Jumper-swimmer
<i>L. elenae</i>	1	A/P	ossified	Jumper	Jumper-burrower
<i>L. flavopictus</i>	1	A	cartilaginous	Jumper	Jumper
<i>L. furnarius</i>	1	P	cart./ossified	Jumper	Jumper-burrower
<i>L. fuscus</i>	1	P	ossified	Jumper	Jumper-burrower
<i>L. gracilis</i>	1	P	ossified	Jumper	Jumper-burrower
<i>L. grisegularis</i>	0	A	cartilaginous	Jumper	Jumper-swimmer
<i>L. knudseni</i>	1	A	cartilaginous	Jumper	Jumper
<i>L. labyrinthicus</i>	1	A	cartilaginous	Jumper	Jumper-burrower
<i>L. laticeps</i>	1	A	cartilaginous	Jumper	Jumper
<i>L. latinasus</i>	1	P	cart./ossified	Jumper	Jumper-burrower
<i>L. latrans</i>	0	A	cartilaginous	Jumper	Jumper-swimmer
<i>L.leptodactyloides</i>	0	A	cartilaginous	Jumper	Jumper-swimmer
<i>L. macrosternum</i>	0	A	cartilaginous	Jumper	Jumper-swimmer
<i>L. melanonotus</i>	0	A	cartilaginous	Jumper	Jumper-swimmer
<i>L. myersi</i>	1	A	cartilaginous	Jumper	Jumper
<i>L. mystaceus</i>	1	P	ossified	Jumper	Jumper-burrower
<i>L. mystacinus</i>	1	P	ossified	Jumper	Jumper-burrower
<i>L. natalensis</i>	0	A	cartilaginous	Jumper	Jumper-swimmer
<i>L. pentadactylus</i>	1	A	cartilaginous	Jumper	Jumper
<i>L. petersii</i>	0	A	cartilaginous	Jumper	Jumper-swimmer
<i>L. podicipinus</i>	0	A	cartilaginous	Jumper	Jumper-swimmer
<i>L. pustulatus</i>	0	A	cartilaginous	Jumper	Jumper-swimmer
<i>L. rhodomystax</i>	1	A	cartilaginous	Jumper	Jumper
<i>L. syphax</i>	1	A	cartilaginous	Jumper	Jumper
<i>L. validus</i>	0	A	cartilaginous	Jumper	Jumper-swimmer
<i>L.ventrimaculatus</i>	1	A	cartilaginous	Jumper	Jumper
<i>L. wagneri</i>	0	A	cartilaginous	Jumper	Jumper-swimmer
<i>Physalaemus</i>					
<i>Ph. biligonigerus</i>	1	A	–	Hopper	Hopper
<i>Ph. centralis</i>	1	A	–	Hopper	Hopper
<i>Ph. cuvieri</i>	1	A	–	Hopper	Hopper
<i>Ph. nattereri</i>	0	A	cart./ossified	Hopper	Hopper
<i>Ph. olfersii</i>	1	A	–	Hopper	Hopper
<i>Pleurodema</i>					
<i>Pl. borellii</i>	0	A	–	Hopper	Hopper
<i>Pl. tucumana</i>	0	A	–	Hopper	Hopper
<i>Pseudopaludicola</i>					
<i>Ps. falcipes</i>	0	A	cartilaginous	Hopper	Hopper

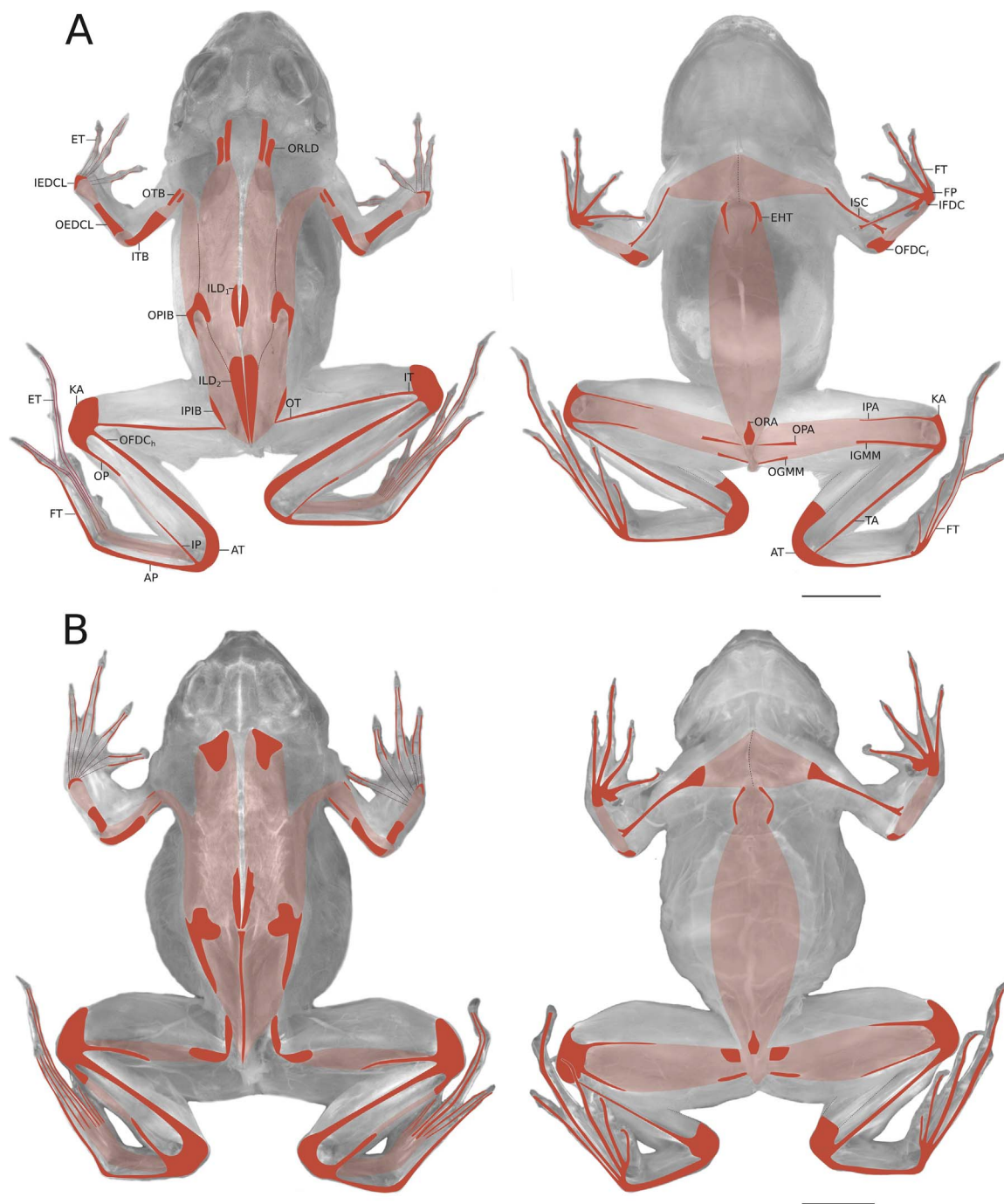
**Table 6**

Loading values of each trait for the first three principal components (PCs). Percentages in parentheses indicate the proportion of variance of each axis. Higher absolute values of morphological loadings are highlighted in boldface.

Morphometric variables	PC1 (52%)	PC2 (11%)	PC3 (9%)
Achilles tendon	–0.033	–0.201	<b>–0.249</b>
coccygeosacralis	0.088	<b>–0.514</b>	–0.412
flexor digitorum communis	–0.029	0.438	<b>–0.589</b>
fascia latae	<b>–0.997</b>	0.058	–0.020
flexor tendon of digit IV (foot)	0.227	<b>0.442</b>	0.271
puboischiofemoralis internus B	–0.270	<b>–0.667</b>	–0.336
tenuissimus	–0.216	<b>–0.512</b>	–0.013
longissimus dorsii	–0.326	–0.530	<b>0.581</b>
supratoracoideus–coracoradialis	–0.192	0.064	<b>0.214</b>
triceps brachii	0.027	<b>–0.401</b>	–0.420

2009) was conducted, and the principal components (PCs) with greatest contributions of morphometric variables were selected based on higher absolute values of morphological loadings (bold values in Table 6).

For subsequent analyses we used the phylogenetic generalized least squares method (PGLS) (Grafen, 1989; Garland and Ives, 2000), which incorporates a matrix of variance and covariance into the calculation of model parameters based on the pattern of relatedness among species (Schweizer et al., 2014). We used the scores of PC 1, 2 and 3 as dependent variables, representing the morphological information. Habitat use and locomotor modes were incorporated as independent variables in two separate analyses: PC1 + PC + PC3 ~ habitat use and PC1 + PC + PC3 ~ locomotor mode. These analyses were conducted using R 3.2.3 (R Core Team, 2015), packages APE (Paradis et al., 2004), Phytools (Revell, 2012), and nlme (Pinheiro et al., 2016).



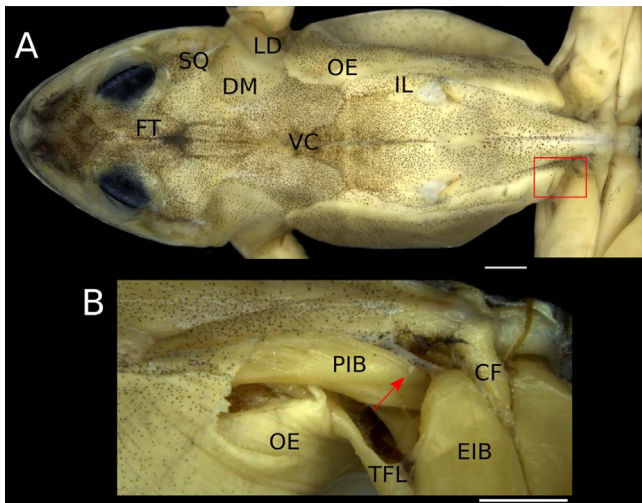
**Fig. 2.** Postcranial superficial tendinous framework of (A) *Leptodactylus latinasus* and (B) *Physalaemus biligonigerus*. Abbreviations as in Table 1, with i for insertion or o for origin where necessary. AP, aponeurosis plantaris; FP, flexor plate; FT, flexor tendons. Scale bars = 5 mm.

#### 2.4.3. Phylogenetic signal and evolutionary model testing

To calculate the phylogenetic signal of t-m ratio data we used Pagel's lambda (Pagel, 1999). Significant values ( $P < 0.05$ ) denote the presence of phylogenetic signal, and the estimated lambda value for each trait ranges from 0 (trait similarity independent of phylogeny) to 1 (trait similarity directly correlated with evolutionary history).

Next we investigated the evolutionary processes through which t-m ratio traits may have evolved in leptodactylids. We tested three different evolutionary models to investigate the processes that could best explain the evolution of these morphological traits: Brownian motion (BM), Ornstein–Uhlenbeck (OU), and early burst (EB). The BM model supposes that the evolution of a trait results from random fluctuations through time (Felsenstein, 1988; Harmon et al., 2010). The OU model

focuses on sections of lineages where a trait varies in relation to an optimum or stabilizing selection (Butler and King, 2004; Harmon et al., 2010). Finally, the third hypothesis predicts intensified trait modifications early in the evolutionary tree followed by a gradual deceleration of the evolutionary rate (EB; Harmon et al., 2010). These analyses were conducted using the 'fitContinuous' function in the Geiger (Harmon et al. 2010) and APE (Paradis et al., 2004) packages for R 3.2.3 (R Core Team, 2015). Akaike's information criterion (AIC) was used as a heuristic indicator for the fit of the different evolutionary models (Akaike, 1974) together with the weights (wAIC) as a measure of strength for each model, and indicating the probability that a given model is the best among a series of candidate models (Burnham and Anderson, 2002).



**Fig. 3.** *Leptodactylus latinasus* in dorsal view. (A) General overview of muscles that originate in the fascia dorsalis. (B) Detailed view of highlighted area in (A); m. obliquus externus was partially removed to show a flat tendinous insertion of the fascia dorsalis on the ilial protuberance (arrow). Abbreviations: CF, caudofemoralis; DM, depressor mandibulae; EIB, extensor iliotibialis B; FT, frontoparietal; IL, ileolumbaris; LD, latissimus dorsi; OE, obliquus externus; PIB, puboischiofemoralis internus B; SQ, squamosal; EIA, extensor iliotibialis A; NS, neural spine. Scale bars = 2 mm.

## 2.5. Ancestral state reconstruction

Tendon–muscle ratio data were optimized onto the composite phylogeny and categorized into three groups of t-m ranges (low, medium and high) to facilitate data visualization.

Shape variation of the tendons was small in comparison to length, except for the insertion tendon of m. longissimus dorsi. For this element, the shape was discriminated into three different categories described in Section 3.3 and optimized onto the selected phylogeny. T-m ratio continuous data and longissimus dorsi shape were mapped onto the tree using Mesquite 3.04 (Maddison and Maddison, 2015) and parsimony as optimization criterium.

## 3. Results

### 3.1. Morphology

The postcranial superficial tendinous framework of *L. latinasus* presented itself as a suitable anuran reference system (Fig. 2A). The most prominent tendons are related to the body parts associated with mobility: (a) head–trunk; trunk–pelvic girdle; (b) girdles–limbs; (c) the main limb joints: elbow; knee; tibia–fibula/tibiale–fibulare; ankle; and (d) palmar and plantar surfaces. These tendons can be visualized as an independent anatomical system connected through muscles and/or fascias.

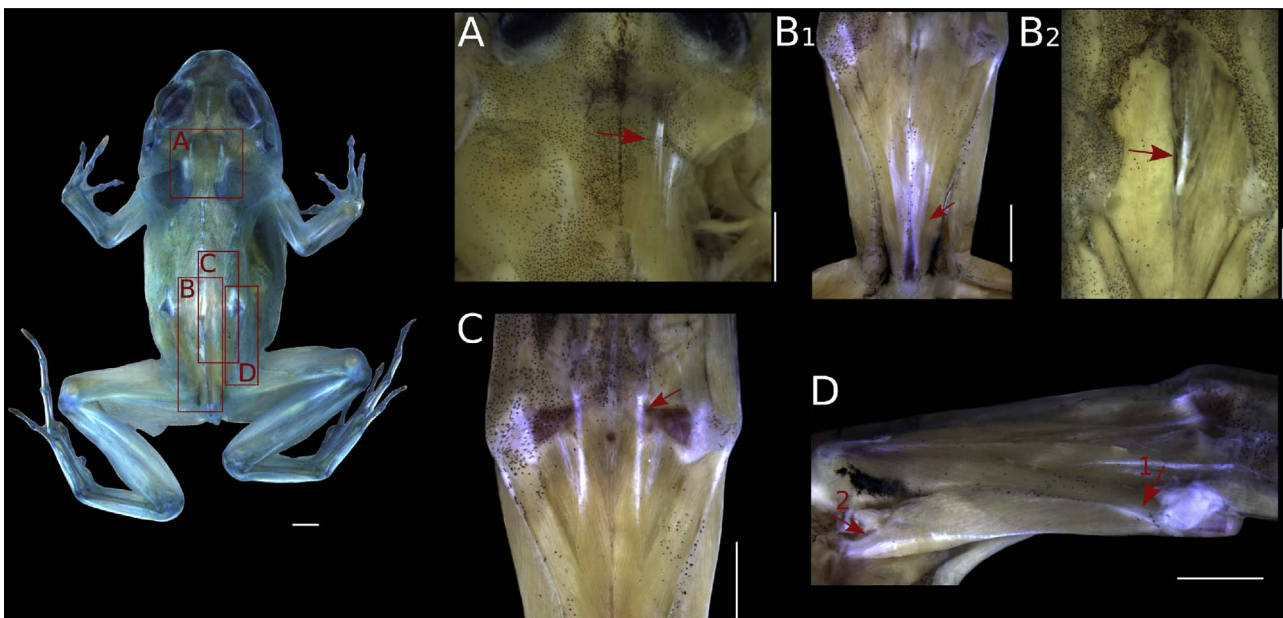
The tendinous system of *Ph. biliginigerus* showed a similar composition (Fig. 2B), with variation in relative size and shape between the frameworks of the two species, particularly in: (a) the origin and insertion tendon of m. longissimus dorsi; (b) the origin tendon of m. puboischiofemoralis internus B; (c) the insertion tendon of m. supra-coracoideus–coracoradialis; (d) the tendon of origin of m. flexor digitorum communis.

The tendinous structures of *L. latinasus* are described below.

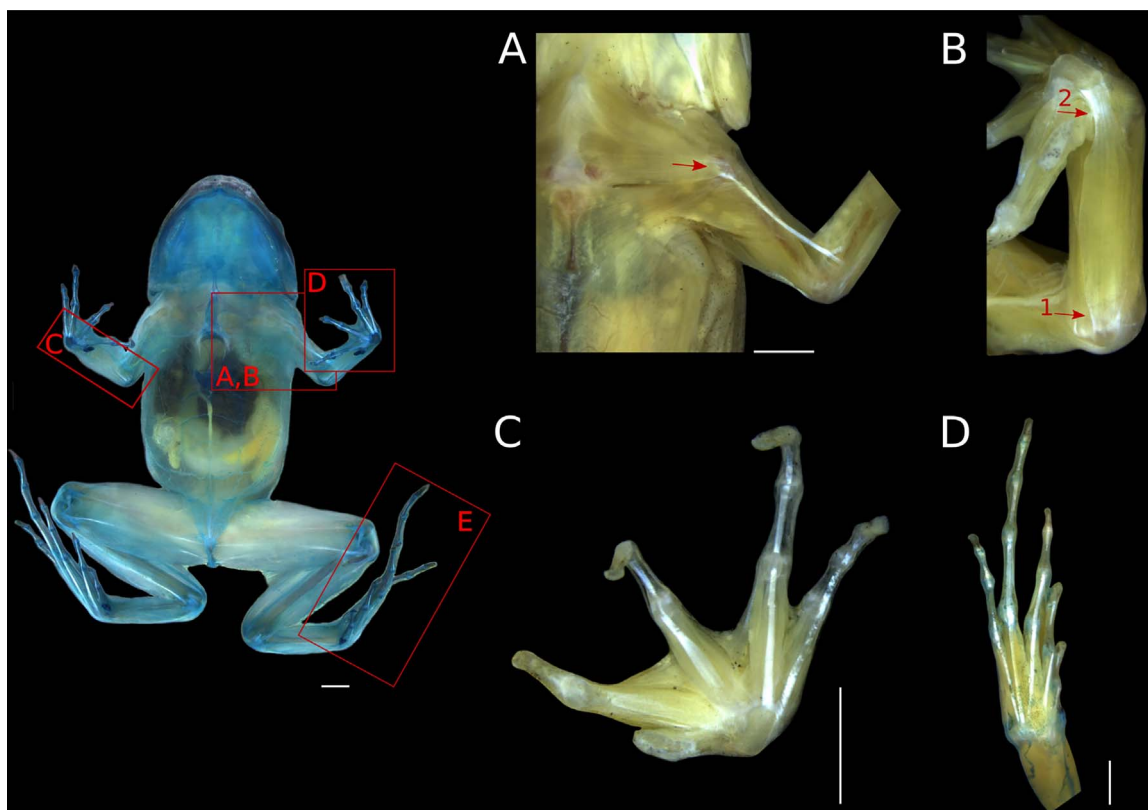
#### 3.1.1. Superficial tendons of the trunk and girdles

The fascia dorsalis overlies the muscles of the dorsal part of the trunk (Fig. 3A). Anteriorly, it is connected to the frontoparietals, the fibrous ring that surrounds the eye, and the squamosals. Medially it is attached to the vertebral centra from the third centrum to the sacral vertebrae. The dorsal fascia is the point of origin for the muscles depressor mandibulae, latissimus dorsi, and puboischiofemoralis internus B, and laterally it is attached to the fascia of the medial portion of the m. ileolumbaris. Posterolaterally, the fascia attaches to the fibrous sheath of the distal end of the sacral diapophyses, and to the iliac ridges, and is contiguous with the fascia of the puboischiofemoralis internus B. Posteriorly, it overlays the muscles of the sacral region (longissimus dorsi, coccygeosacralis and coccygeoiliacus), attaches to the posterior end of the urostyle, and has a flat tendinous connection to the ilial protuberance (Fig. 3B).

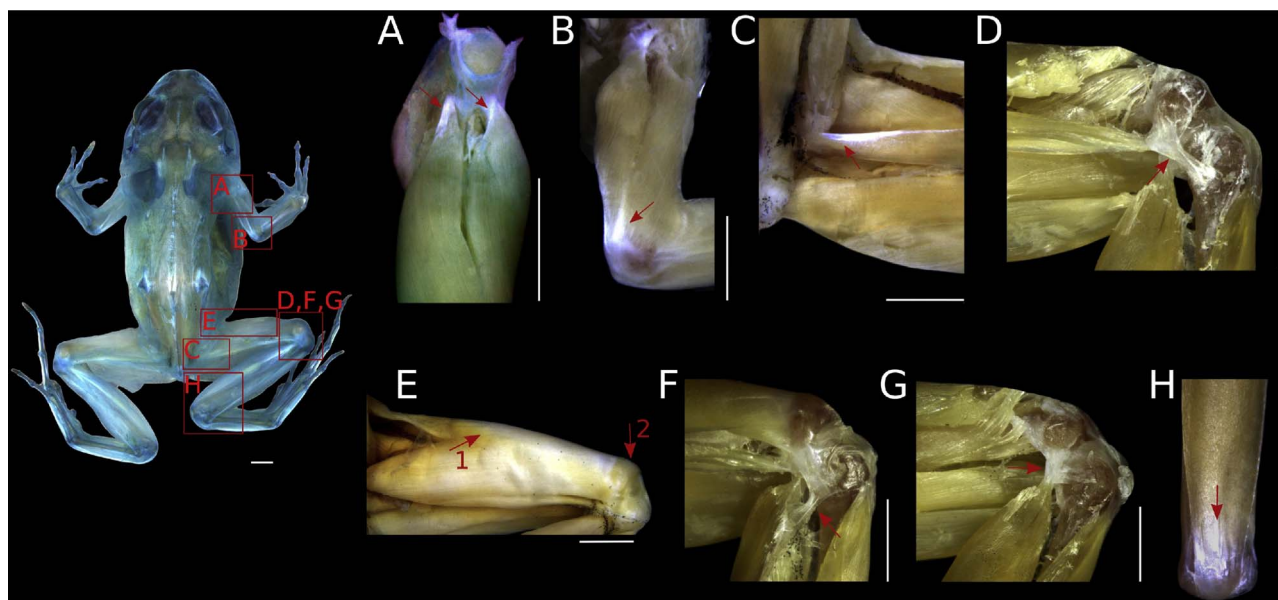
The origin tendons of m. longissimus dorsi are a pair of superficial tendons that originate from the otic crest and the dorsal region of the



**Fig. 4.** Tendons of the trunk dorsal region of *Leptodactylus latinasus*. (A) Origin tendons of longissimus dorsi (notice the part tendinous, part fleshy origin of this muscle). (B<sub>1</sub>) Tendinous insertion of longissimus dorsi to the anterior end of the urostyle (m. longissimus dorsi partially removed); (B<sub>2</sub>) tendinous insertion of m. longissimus dorsi to the posterior half of the urostyle shaft. (C) Insertion tendon of coccygeosacralis arising from the lateral end of the transverse process of vertebra XVIII. (D) Origin (arrow 1) and insertion (arrow 2) tendons of puboischiofemoralis internus B. Scale bars = 2 mm.



**Fig. 5.** Tendons of the ventral surface of *Leptodactylus latinasus*. (A) Insertion tendon of supracoracoideus-coracoradialis. (B) Origin (arrow 1) and insertion (arrow 2) tendons of the flexor digitorum communis. (C) Tendons of the flexor surface of the hand. (D) Tendons of the flexor surface of the foot. Scale bars = 2 mm.



**Fig. 6.** Tendons of the fore and hind limbs of *Leptodactylus latinasus*. (A) Origin tendons of m. triceps brachii from the ventral and dorsal region of the proximal humerus epiphysis. (B) Insertion tendon of m. triceps brachii to the elbow joint. (C) Origin tendon of m. tenuissimus attached to the ilial protuberance. (D) Bifurcated insertion tendon of m. tenuissimus (extensor iliobtibialis B and knee aponeurosis partially removed). (E) Detail of the fascia latae (whitish color indicated by arrow 1) and insertion of m. cruralis (arrow 2). (F) One origin tendon of m. flexor digitorum communis attached to the knee capsule. (G) More internal origin tendons attached to the inner region of the knee joint, arising from the epiphysis of the femur and the tibiofibular. (H) Achilles tendon. Scale bars = 2 mm.

exoccipital (base of the frontoparietals); the longissimus dorsi also exhibits a fleshy origin (Fig. 4A). Various thin tendinous slips, comprised of small fibrous bundles fused to the main part of the muscle, arise from the transverse process of the IV–VI vertebrae and constitute a second origin point. A series of transversal wavy tendinous partitions subdivide the longissimus dorsi. Posteriorly, a strong intramuscular tendon inserts

on the most anterior region of the urostyle dorsal crest (Fig. 4B<sub>1</sub>), and a second insertion tendon is attached to the posterior half of the urostyle (Fig. 4B<sub>2</sub>). The longissimus dorsi inserts directly, without tendon, into the first half of the urostyle.

The tendon of m. coccygeosacralis is a thin slip that originates on the lateral end of the transverse process of the XVIII vertebra and gives



**Table 7**  
Values for phylogenetic signal (Pagel's lambda) of tendon–muscle (t-m) ratio lengths and *P*-values. Significant values are marked with an asterisk (\*).

PC variables	$\lambda$	<i>P</i>
PC1	0.075	0.76
PC2	0.999	< 0.05*
PC3	< 0.01	1

rise to a portion of the *m. coccygeosacralis*; most of this muscle originates directly from the sacral diapophyses (Fig. 4C). The *m. coccygeosacralis* is completely covered by the *m. longissimus dorsi* and inserts directly into the anterior two thirds of the urostyle.

The insertion tendon of *m. puboischiofemoralis internus B* is flat, shared by both parts of this muscle, and is attached to the proximal epiphysis of the femur (Fig. 4D). The *puboischiofemoralis internus B* has two points of origin that are attached directly to bone in *L. latinasus*. The main muscular mass (*pars externa*) originates from the anterolateral end of the iliac shaft and a small bundle of fibers (*pars interna*) originates from the lateral aspect of the iliac shaft and runs posteriorly adjacent to the *pars externa*.

The insertion tendon of *m. supracoracoideus-coracoradialis* is shared by the *supracoracoideus* and *coracoradialis* (Fig. 5A). It runs distally along the length of the humerus and attaches to the ventral surface of the radioulna, adjacent to its proximal epiphysis. The insertion is Y-shaped, allowing one branch of tendon to be inserted onto the radial region and another onto the ulnar region. The *supracoracoideus-coracoradialis* insertion tendon is also attached to the base of the *crista ventralis* by a sheath formed by the insertion tendons of the *pectoralis* and by the *procoracohumeralis* fibers.

### 3.1.2. Superficial tendons of the forelimb

A broad and thin tendon attached to the ventral region of the elbow joint gives rise to the *flexor digitorum communis* muscle (Fig. 5B). Distally, the insertion tendon adjoins the tendinous flexor plate of the hand.

The tendons of the flexor surface of the hand are in accordance with the general pattern previously described by Burton (1998) for lepto-dactylids. In *L. latinasus* the most superficial layer of flexor tendons arises either from the flexor plate or from flexor muscles attached to the carpals (Fig. 5C). The tendon of digit II is attached to *m. flexor indicis superficialis proprius* which arises from carpals 3–4–5. The flexor tendon of digit III has a double origin; it arises both from the flexor plate and from *m. caput profundum*. The flexor tendons of digits III and IV arise from the flexor plate.

The two short origin tendons that give rise to the *m. triceps brachii* are attached to the ventral and dorsal regions of the proximal humerus epiphysis (Fig. 6A); this muscle is also firmly attached to the glenoid cavity capsule. The short, broad insertion tendon is attached to the elbow joint (Fig. 6B).

### 3.1.3. Superficial tendons of the hindlimb

The origin tendon of the *tenuissimus* muscle is flat and attaches to the ilial protuberance (Fig. 6C). The insertion tendon is bifurcated, flat,

**Table 8**

Values log likelihood (LogL), AICc and wAICc correspondent to the evolutionary models tested (BM, Brownian motion; OU, Ornstein–Uhlenbeck; EB, early burst) for all the morphological variables. Values for best models are highlighted in boldface.

PC var	BM			OU			EB		
	LogL	AICc	wAICc	LogL	AICc	wAICc	LogL	AICc	wAICc
PC1	−19.13	42.55	< 0.01	−11.09	28.78	<b>0.99</b>	−19.13	44.85	< 0.01
PC2	15.71	−27.13	0.04	15.76	−24.93	0.01	19.91	−33.23	<b>0.94</b>
PC3	21.34	−38.39	< 0.01	33.07	−59.56	<b>0.99</b>	21.98	−37.37	< 0.01

and attaches to the epiphysis of the femur and tibiofibula (Fig. 6D).

The fascia latae is the insertion tendon of *m. extensor iliobtibialis A*; it is a broad tendinous sheet that covers the *cruralis* muscle and is conjoined with the knee aponeurosis (Fig. 6E).

The insertion tendon of *m. cruralis* is broad, short, and contiguous with the knee aponeurosis (Fig. 6E). The *cruralis* originates directly from the acetabular region of the ilium.

One origin tendon of the *flexor digitorum communis* muscle is attached to the knee capsule, encompassing the distal femoral head and the proximal tibiofibular head (Fig. 6F). A second internal origin tendon is flat and attaches to both the popliteal space (inner region) of the knee joint and to the epiphysis of the tibiofibula (Fig. 6G). The insertion tendon of the *flexor digitorum communis* is a flat aponeurosis that passes into the Achilles tendon (Fig. 6H).

The flexor tendons of the feet arise from the aponeurosis plantaris and from the *m. flexores breves superficiales*, run distally and are firmly attached to each metacarpal joint by a tendinous sheath inserted into the distal end of the distal phalanges (Fig. 5D). The flexor tendons of toes I, II and part of III arise from the aponeurosis, while those of toes III–V originate together from the *m. flexores breves superficiales*. The *prehallux* has a tendinous connection to the aponeurosis plantaris.

### 3.2. Statistical analyses

Differences between male and female specimens of *L. bufonius* were not significant ( $P > 0.05$ ); therefore, sexual dimorphism does not seem to have an effect on tendinous variability.

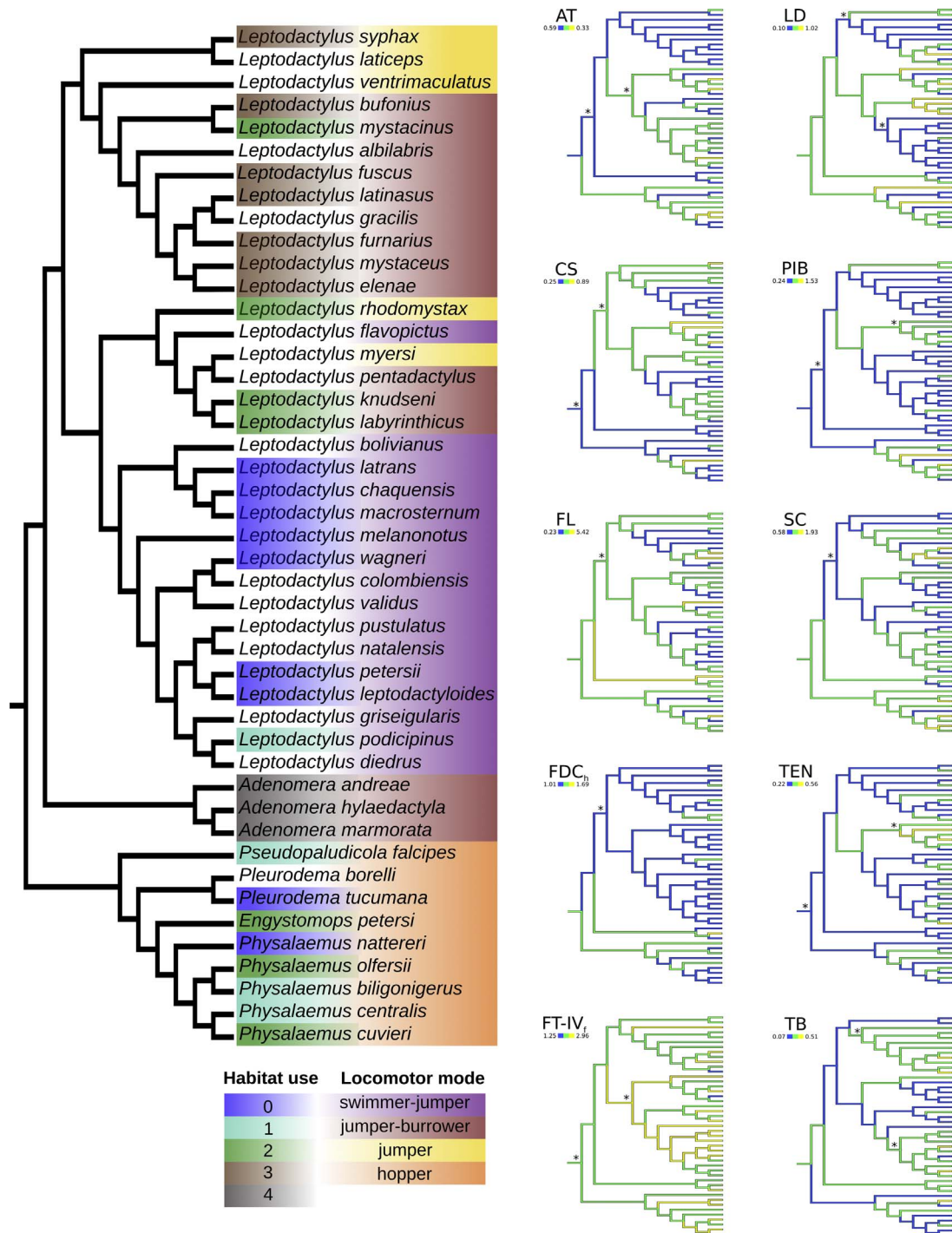
The first three resulting PC scores represented most of the variation (72%). Fascia latae t-m ratio contributed most importantly to PC1 (Table 6). In the case of PC2, *coccygeosacralis*, flexor tendon flexor of digit IV, *puboischiofemoralis internus B*, *tenuissimus*, and *triceps brachii* t-m ratio traits contributed most (Table 6). Finally, for PC3, the t-m traits which contributed most were the Achilles tendon, flexor digitorum communis, *longissimus dorsi*, and *supracoracoideus-coracoradialis* (Table 6). There was no significant relation between the PCs for the morphological variables and ecological factors (for a summary of the PGLS models see Datasheet S3).

Phylogenetic signal was significant ( $P < 0.05$ ) for PC2 (Table 7). Tendon–muscle (t-m) ratio evolution in Lepto-dactylidae seems to have followed different evolutionary processes, as a single model did not explain all variables (Table 8). The OU model showed a better fit for PC1 and PC3, whereas the EB model better explained the evolution of PC2 variables (Table 8). None of the variables showed a best fit under the BM model.

### 3.3. Ancestral state reconstruction

The ancestral state reconstruction of the t-m length ratio shows a pattern of intermediate and low values for the group ancestor (Fig. 7). High values of the t-m ratio are derived and scattered across the tree, except for the flexor tendon of digit IV, where it emerges as a shared state for the *L. pentadactylus*, *L. latrans* and *L. melanonotus* clades. Shifts of the t-m ratio are not associated with habitat use and locomotor modes, being more notably related to phylogeny (highlighted in Fig. 7).

The broad shape of the insertion tendon of *m. longissimus dorsi*



**Fig. 7.** Ancestral state reconstruction of tendon lengths. Composite *Leptodactylus* tree, on the left representing the phylogenetic relationships of the analyzed taxa. Species are colored according to habitat use (left) ranging from 0 (strongly associated with water) to 4 (strongly associated with land), and locomotor mode (right); species with missing ecological information were left uncolored. The trees on the right represent ancestral state reconstructions of tendon–muscle ratio length, and asterisks highlight t–m shifts in major clades. Abbreviations as in Table 1.

optimizes as a putative synapomorphy for *Leptodactylus* (Fig. 8, black box). A thinner tendon architecture was recovered as a putative synapomorphy for the *L. melanotus* species group (Fig. 8, white box), with independent acquisition of an intermediate tendon shape in *L. bufonius* and *L. ventrimaculatus* (Fig. 8, gray box).

#### 4. Discussion

In the present work we addressed the study of tendons as a singular and independent system directly related to movement. Our main result

is that leptodactylids present an all-purpose morphology that seems to have evolved independently of ecology and functional requirements tested herein. This is indicated by no significant relation between morphometric data of the analyzed tendons and habitat use or locomotion, a strong phylogenetic component in the analyzed variables, and a generalized pattern of intermediate values for ancestral states.

Direct tendon-to-tendon interactions are not easily observed, as tendons and muscles are strongly associated from a functional perspective. However, there is evidence of the independent modular development of tendons (Huang et al., 2015) and there are studies that

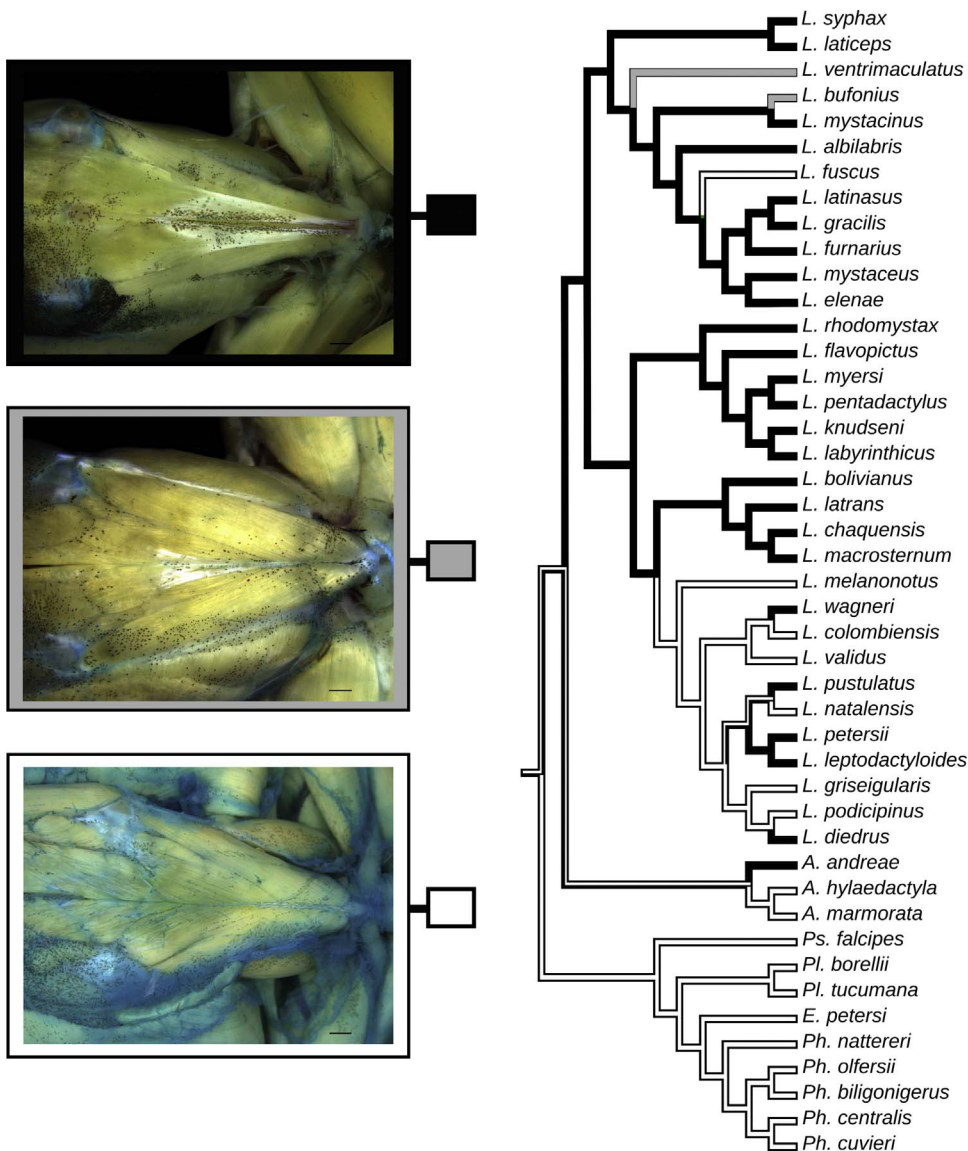


Fig. 8. Shape optimization of the insertion tendon of *m. longissimus dorsii*. Cone-shaped configuration (black) – *Leptodactylus elenae*; funnel-shaped configuration (gray) – *Leptodactylus bufonius*; thin configuration (white) – *Pleurodema borellii*.

approach connective tissue as a body-wide organ (Wood-Jones, 1944; Langevin, 2006; Benjamin, 2008). Herein, we propose a tendinous system conjoined by muscles and/or fascia (Fig. 2). Along with its association to movement, this continuous tendon framework also presents evidence of mechanisms that allow cells to perceive and interpret mechanical forces (Langevin et al., 2002, 2004). The load detection mechanism includes extracellular matrix components linked by a cascade of mechanical signals – cell secretion, modification of extracellular matrix, amplification and propagation of the signal along connective tissue planes, and modulation of sensory input via changes in the connective tissue (Langevin et al., 2002). Evidence points to an extensively interconnected and body-wide signaling system that responds to mechanical forces and influences other physiological systems, with local and downstream effects (Langevin et al., 2001, 2002, 2004).

Contrary to our prediction, t-m ratio and shape variation did not relate significantly to the ecological or functional aspects addressed in this work, reinforcing the premise that species are adequately, rather than perfectly, matched to their environments (Gans, 1993). Our results allow us to infer that a conservative morphological configuration is retained in Leptodactylidae, one that is versatile enough to allow for various different locomotor modes and the exploitation of distinct environments. This outcome fits well with the ecological versatility of leptodactylid species, which occupy and shift through a variety of

habitats to facilitate foraging and reproduction or to escape predators, and are capable of a wide range of locomotor modes including jumping, walking, hopping and burrowing (Heyer, 1969; Ponssa and Heyer, 2007; de Sá et al., 2014). *Leptodactylus* species' most striking ecological trait is their digging ability associated with clutch deposition (Heyer, 1969). Recent studies of *Leptodactylus* regarding the correlation between morphological traits and locomotion suggest that the morphology associated with saltatorial locomotion would scarcely require modification for effective use in hind feet digging behavior (Ponssa and Medina, 2016). This conservatism is also apparent in the strong phylogenetic signal exhibited for the t-m variables related to PC2.

Our results on evolutionary model analysis showed that more than one evolutionary process might be driving tendon–muscle morphology. OU is the model that best explains most variables, indicating that stabilizing selection or selective optima might be driving shifts in tendon length within Leptodactylidae. This result coincides with previous ecomorphological studies which have also identified OU as the best-fitting model, even though they were performed considering different groups and structures (frog postcranial skeleton: Soliz et al., 2017; lizards' myotendinous traits: Tulli et al., 2016). Other studies, although not designed for evolutionary model testing, also found hierarchical patterns (Reilly and Jorgensen, 2011; Jorgensen and Reilly, 2013); which would indicate that a stabilizing selection would best explain

many of the morphological traits related to the evolution of the locomotor system in tetrapods. However, it is important to highlight that there are minimal differences in AICc and wAIC between the tested models. Consequently, some caution is warranted when deciding whether these models really do have a substantially better or worse fit than the others.

The most conspicuous tendinous elements of the pelvic girdle and hindlimbs in leptodactylids are notably composed of elements directly related to locomotion in Anura. Axial muscles which insert on the urostyle (longissimus dorsi, coccygeosacralis), protractor of the femur (puboischiofemoralis internus B), and leg extensors (Achilles tendon, flexor tendon of digit IV, extensor ilioltibialis A, tenuissimus) are fundamental to jumping (Nauwelaerts et al., 2007; Přikryl et al., 2009). Herein, the t-m ratios of these elements show a constraint to intermediate and low values, the only exception being the feet tendon flexor. In this case there seems to be a release of the restrictions imposed on the other studied t-m elements, which could be a reflection of a more general tendency of feet lengthening in Anura. Regarding tendon shape optimization, in the phylogenetic hypothesis used here (de Sá et al., 2014), a broad morphology for the insertion of the longissimus dorsi optimizes as a synapomorphy for *Leptodactylus*. This finding emphasizes the importance of studies on tendon morphology and variability, since character coding generally focusses on muscle mass (e.g., Fabrezi et al., 2014; Blotto et al., 2017), whereas it can easily be observed that species having a similar longissimus dorsi length can present two different tendon morphologies (Fig. 8, black and gray boxes).

Our results seem to point to a high level of tendon morphological conservatism. It might be inferred that once the jumping body plan, which is strongly associated with particular properties of certain musculotendinous traits, was reached (Astley, 2016), variability within the anuran clade became much more subtle. However, to confidently infer that conserved intermediate tendon morphology allows for generalized and versatile locomotion mode and habitat use, more evidence from other, more specialist anuran taxa having more extreme tendon lengths than those found in Leptodactylidae, are necessary. Our results can also be an indication of the difficulty of assessing, understanding, and classifying ecological traits. Previous studies have shown that a refinement of ecological character categories can improve analysis (Engbrecht et al., 2011); unfortunately, reports of direct ecological and behavioral observations are scarce in relation to *Leptodactylus*' species diversity (Ponssa and Medina, 2016).

## 5. Conclusions

Our work on Leptodactylidae can be considered a starting point for further ecomorphological research with a comparative perspective that approaches tendons as an independent system. Herein, we found no relation between tendon–muscle length variables and habitat use or locomotor modes, and a historic maintenance of intermediate and low values of t-m ratio. This conservatism is also apparent in the high phylogenetic signal values and a hierarchical pattern of character evolution for the studied variables. Even the insertion of the longissimus dorsi shape showed a pattern of variation adjusted to phylogeny. This set of evidence indicates the preponderance of an “off-road” morphology in the studied group, which affords the ability to versatilely adjust to ecological requirements, without any obligatory corresponding shift in biological roles.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.zool.2017.08.007>.

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