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Title: The influence of locomotion and habitat use on tendo-muscular units of an anuran clade (Anura, Diphyabatrachia)

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Keywords: tendon; locomotion; habitat use; neotropical frogs; Leptodactylidae; Centrolenidae

Abstract: Tendons have unique mechanical properties, contributing in the transmission, amplification and recycling of muscle energy. In this light, anuran tendons are especially interesting due to their highly mechanically demanding locomotor modes. Herein we aim to investigate the relation between tendons and muscles from two perspectives: (1) the tendinous area in relation to the potential force produced by associated muscles, and how this relation varies for different tendons of the anuran body; (2) the tendinous proportion of a tendo-muscle unit in relation to different locomotor modes and habitat uses in a monophyletic unranked anuran clade (Diphyabatrachia) which includes Leptodactylids and Centrolenids, two highly diverse frog family groups of the Neotropics. Our data suggests a significant and positive relation between tendon dimensions and potential muscle force for most of the analyzed elements. We also found that species of different locomotion and habitat uses show divergence of tendinous proportions for tendo-muscle units of the pectoral girdle and hindlimb. Notably, jumper-arboreal species differentiate from all others in all tendo-muscle units tested. Finally, optimization of tendinous proportions showed a great phylogenetic correspondence. These results are in agreement with the hypothesis that morphological variation is related to a combination of phylogenetic, functional and ecological factors.

The influence of locomotion and habitat use on tendo-muscular units of an anuran clade

(Anura, Diphryabatrachia)

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Abstract

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4 and recycling of muscle energy. In this light, anuran tendons are especially interesting due to
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6 their highly mechanically demanding locomotor modes. Herein we aim to investigate the
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10 relation to the potential force produced by associated muscles, and how this relation varies for
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12 different tendons of the anuran body; (2) the tendinous proportion of a tendo-muscle unit in
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14 relation to different locomotor modes and habitat uses in a monophyletic unranked anuran
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32 combination of phylogenetic, functional and ecological factors.
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43 Key words: tendon; locomotion; habitat use; neotropical frogs; Leptodactylidae;

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Abbreviations

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53 AT – Achilles tendon

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56 CS – *coccygeosacralis*
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1 FDC – *flexor digitorum communis* (forelimb)

2 FL – *fascia latae*

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4 FT-IV – flexor tendon of digit IV (hindlimb)

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7 LD – *longissimus dorsi*

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9 PIB – *puboischiofemoror* *alis internus B*

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11 SC – *supracoracoideus-coracoradialis*

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13 TB – *triceps brachii*

14
15 TEN – *tenuissimus*

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17 TM – tendo-muscle

23 24 **1 Introduction**

25
26 Tendons are viscoelastic elements with unique mechanical properties (Biewener 2003;
27 Lis et al. 2012; Hessel & Nishikawa 2017). Tightly integrated with muscles, tendons are part
28
29 of a functional unit that promotes movement and enables a diverse range of locomotor
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31 activities, contributing in the transmission, amplification and recycling of energy (Roberts
32
33 2002; Biewener 2003; Benjamin et al. 2008; Azizi & Roberts 2009; Konow & Roberts 2015).
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35 Specific tendinous morphologies related to structure-function specializations of the locomotor
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37 system have been found throughout Tetrapod groups (e.g. Manzano et al. 2008; Abdala et al.
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39 2009, 2018; Azizi & Roberts 2010; Tulli et al. 2012, 2016; Astley & Roberts 2014; Astley
40
41 2016). Anurans are an especially interesting study group due to their highly mechanically
42
43 demanding locomotor modes. While jumps are short burst events with high power generation,
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45 hopping and swimming implicate less explosive and regularly repeated movements (Navas et al.
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47 1999; Nauwelaerts et al. 2007). Habitat use also has an impact on locomotion, since more
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complex habitats such as the arboreal requires more precision in movement (Sustaita et al. 2013).

Tendinous contribution to locomotor performance of anuran species have been so far identified mainly on limb elements (Manzano et al. 2008; Astley & Roberts 2014). The Achilles tendon has been widely studied and participates on a mechanism of loading followed by a rapid release of energy which allows the ankle to extend using elastic recoil during the propulsive phase of the jump (Roberts & Azizi 2011; Roberts et al. 2011; Astley & Roberts 2014). On the forelimb, higher independence of hand flexor tendons is related to arboreality (Manzano et al. 2008; Sustaita et al. 2013). A more general overview provided by Fratani et al. (2018ab) described tendinous variation of the pelvis and limbs of leptodactylid species, and found a combination of allometry, phylogeny and function related to tendon morphology.

There are morphological and mechanical variations among tendons of different parts of an organism, and these differences are related to the intensity of inflicted tension (Birch 2007). Based on previous studies on tendon-muscle mechanics, the relation between tendon and the force produced by its associated muscle is expected to be direct and positive (Abdala & Ponssa 2012; Ponssa & Abdala 2016; Azizi & Roberts 2010; Astley & Roberts 2014). However, this hypothesis remains to be tested on a gross morphology level of anuran specimens. If confirmed, a tendo-muscle direct relation can be applicable to broader morphology studies based on collection specimens, as well as for refining inference of muscle size and movement in fossil material. Another example of an assumed direct relation is the case of muscle and bone insertion, since muscle force is known to promote skeletal growth (Chamay & Tschantz 1972). Nonetheless, Ponssa et al. (2018) revealed that the expected correlation between the potential force produced by a muscle and its insertion areas on bone crests is not always true for anurans. Therefore, the assumed direct relation between tendon

1 and muscle might also not be that certain, especially when taking into account the variety of
2 tendinous forms and functions.
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4 Another possible approach to investigating tendo-muscle (TM) relationship is looking at
5 their relative lengths, i.e. using tendinous proportion as a variable. High tendinous proportions
6 of a TM unit can be related to high energetic requirements and/or movement precision
7 (Roberts 2002; Manzano et al. 2017), and these functional demands will vary across different
8 locomotor modes. Variation is also expected regarding different tendons of the anuran body,
9 since they participate in different phases of the jump cycle. The pelvic girdle and hindlimb
10 elements are mainly related to the propulsive phase (Emerson 1988; Nauwelaerts & Aerts
11 2006; Fabrezi et al. 2014). While pectoral girdle and forelimb elements are responsible for
12 supporting and stabilizing the frog as it lands (Emerson 1983; Peters et al. 1996; Reilly et al.
13 2016). Herein, we analyze pectoral and pelvic girdle, as well as limbs tendo-muscle units of a
14 monophyletic anuran clade – Dyphiabatrachia – that includes two neotropical family groups,
15 Leptodactylidae and Centrolenidae (sensu Frost 2006). We selected Dyphiabatrachia as our
16 model group because of its great ecological diversity, encompassing over 350 species of
17 jumper and hopper species, ranging from arboreal to terrestrial and semi-aquatic habitats
18 (Guayasamin et al. 2009; De Sá et al. 2014; Frost, 2019).
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41 The goal of this study was to investigate the variation between tendons and their
42 associated muscles, under the hypothesis that morphological variation is related to a
43 combination of phylogenetic, functional and ecological factors. Our first question is how
44 tendon morphology relates to the force produced by their associated muscles, inferred by their
45 cross sectional area, and how this relation varies for different tendons of the anuran body. We
46 expect that both elements act as a unit, and this would be evidenced by a significant and
47 positive relation, in other words, bigger tendons would associate to muscles that potentially
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1 produce higher forces. Second, we ask if the tendinous relative proportion of tendo-muscle
2 units can be related to locomotion and habitat use in the Diphyabatrachia clade. Taking into
3
4 account the group's diversity, we expect to find differences in tendinous proportions in
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6 relation to the locomotor challenges of displacement in arboreal, terrestrial and semi-aquatic
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8 environments. As a complementary hypothesis, the phylogenetic relatedness may influence
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10 tendon morphology, resulting in tendon variability constrained to phylogenetic topology.
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13 **2 Material and Methods**

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15 To investigate the diversity of tendo-muscle units we analyzed 99 species and 188
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17 specimens of Diphyabatrachia, which represents approximately 30% of the group's diversity
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19 (Frost 2019). Three additional species were included as outgroup for the polarization of
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21 variables in the optimization: *Odontophrynus americanus* (Odontophrynidae), *Rhinella major*
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23 (Bufonidae) and *Scinax nasicus* (Hylidae). *Hydrolaetare schmidti* (Leptodactylidae) is the
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25 only case of a strictly aquatic species in the group (Rodríguez & Duellman 1994), which
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27 would cause a low representation of this locomotor category for statistical analysis, therefore
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29 this species was included only for the optimization of tendon proportions. Full details on
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31 specimens can be found in Supplementary Data S1. Specimens were dissected and
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33 photographed with a Leica M205A stereomicroscope. Measurements were taken in
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35 millimeters using a digital caliper (0.01 mm; Mitutoyo, Kawasaki, Japan) and using the
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37 software ImageJ (Schneider et al. 2012). The anatomical nomenclature used in this study
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39 follows that presented in Gaupp (1896) for trunk and pelvic structures, and Abdala & Diogo
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41 (2010), Diogo & Abdala (2010), Diogo & Ziermann (2014), Diogo & Molnar (2014) for
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43 pectoral and limb structures.
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53 To test the relation between tendon dimensions and the potential force produced by its
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55 associated muscle we selected the most conspicuous tendo-muscles units of the postcranium,
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1 described previously by Fratani et al. (2018). We selected the superficial tendon area as a
2 proxy of the collagen matrix of each element, and the maximum diameter cross-sectional
3 muscle area since it is directly related to force (Josephson, 1975; Biewener, 1998; Azizi &
4 Roberts, 2010). The following elements were included: *longissimus dorsi* (LD),
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coccygeosacralis (CS), *puboischiofemorobralis internus B* (PIB), *supracoracoideus-*
coracoradialis (SC), *triceps brachii* (TB), *flexor digitorum communis* – forelimb (FDC),
tenuissimus (TEN), and *flexor digitorum communis* – hindlimb (for clarity this element is
referred as Achilles tendon in the text, AT) (Fig. 1). For the remaining question, regarding the
relation between tendinous proportion of a tendo-muscle unit and locomotion/habitat use
categories, the *fascia latae* (FL) and the flexor tendon of digit IV – hindlimb (FT-IV) were
also included (Fig. 1). The mean species values for tendon area, muscle cross-sectional area
and tendinous proportions and the number of studied individuals/species are detailed in
Supplementary Data S2.

Prior to the main analyses we explored for sexual dimorphism effects on a subset of
Leptodactylus bufonius specimens, both for tendon areas and TM units, by performing an
analysis of covariance (ANCOVA). It was significant for LD ($F_{2,96}=6.8$, $P<0.01$) and TB
($F_{2,17}=3.6$, $P=0.05$) tendon areas. In this case, subsequent analyses were performed using
exclusively male specimens, since they represent most part of the dataset. For all other
elements analyzed (CS, PIB, SC, FDC, TEN, TA, FL, and FT-IV) there was no significant
sexual dimorphism effect, therefore both male and female specimens were included in other
analyses.

All tendon and muscle morphometric variables were \log_{10} transformed to meet the
requirements of normality and homoscedasticity (Zar 1999). A linear regression was
performed to address the relation between tendon and muscle area variables, including size

1 (snout-vent length) as a covariable. For testing the relation between morphology and
2 locomotor mode (LM) we calculated the relative lengths of tendon and muscles to get a tendo-
3 muscle (TM) unit index. A TM bigger than 1 ($TM > 1$) corresponds to higher tendinous
4 proportions, while $TM < 1$ corresponds to lower tendinous proportion in a TM unit. We
5 adopted the same categorization of locomotor modes and habitats used in Fratani et al.
6 (2018ab) and Ponssa et al. (2018) for leptodactylids (Fig. 2). The authors subcategorized the
7 jumping species to best represent their locomotor diversity based on literature information on
8 species natural history, field observations and morphological characters (Fratani et al. 2018ab;
9 Ponssa et al. 2018). All centrolenid species were categorized as jumper-arboreal based on
10 documented natural history information (Lynch & Duellman 1973; McDiarmid & Adler 1974;
11 Greer & Wells 1980; Drake & Ranvestel 2005; Cisneros-Heredia & McDiarmid 2007;
12 Guayasamin et al. 2009; Delia et al. 2010; Cabanzo-Olarte et al. 2013; Guevara-Molina &
13 Vargas-Salinas 2014; Lehtinen et al. 2014; among others). See Supplementary Data S3 for
14 detailed information on literature references and locomotor modes/habitat uses of each
15 species.

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36 As data for species are not independent because of shared phylogenetic history
37 (Blomberg et al. 2003), a phylogenetic framework was included for all statistical analyses.
38 We built a composite tree based on the general topology of the latest large-scale amphibian
39 trees (Pyron & Wiens 2011; Jetz & Pyron 2018), and specific phylogenies for relationships
40 within each genera (Faivovich et al. 2012; Castroviejo-Fisher et al. 2014; Fouquet et al. 2014;
41 de Sá et al. 2014; Veiga-Menoncello et al. 2014; Lourenço et al. 2015) (Fig. 2). As branch
42 lengths were not available for all species included in this study, so we tested three different
43 types of arbitrary branch lengths: constant (all branch lengths equal to 1), Grafen's (1989),
44 and Pagel's (1992) methods. To test for the statistical adequacy of the branch lengths we
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1 performed an independent contrasts analysis (Garland et al. 1992) using the PDTREE module
2 of Mesquite 3.04 (Midford et al. 2005; Maddison & Maddison, 2015). Constant branch
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4 lengths gave the best results for the standardization of phylogenetically independent contrasts,
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6 as indicated by the absence of statistically significant trends in diagnostic plots for most traits
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9 (Garland et al. 1992).
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11 We applied the phylogenetic generalized least squares method (PGLS) to estimate the
12 relationship between each TM variable and locomotor modes while taking phylogeny into
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14 account (Grafen 1989; Garland & Ives 2000). We tested three different evolutionary models
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16 to investigate the processes that could best explain the evolution of these morphological traits:
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18 Brownian motion (BM), Ornstein-Uhlenbeck (OU), and early burst (EB). The BM model
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20 supposes that the evolution of a trait results from random fluctuations through time
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22 (Felsenstein 1988; Harmon et al. 2010). The OU model focuses on sections of lineages where
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24 a trait varies in relation to an optimum or stabilizing selection (Butler & King 2004; Harmon
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26 et al. 2010). Finally, the Early Burst (EB) model predicts variation in a trait's evolutionary
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28 rates (Harmon et al. 2010). We included an accelerated model (EB+) and a decelerated model
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30 (EB-) in our analyses. Akaike's information criterion (AIC) was used as a heuristic indicator
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32 for the fit of the different evolutionary models (Akaike 1974) together with the weights
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34 (wAIC) as a measure of strength for each model, and indicating the probability that a given
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36 model is the best among a series of candidate models (Burnham & Anderson 2002). These
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38 analyses were conducted using the APE (Paradis et al. 2004) and Geiger (Harmon et al. 2010)
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40 packages in R 3.2.3 (R Core Team 2018). TM indexes were also optimized onto the
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42 composite phylogenetic tree using Mesquite 3.04 (Maddison & Maddison 2015) and
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44 parsimony as optimization criteria.
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55 **3 Results**

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1 A significant and positive relation between tendon area and the potential force produced
2 by the associated muscle areas was detected for SC, TB, FDC, PIB, TEN, and AT (Fig. 3).

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4 While LD and CS tendon and muscle showed no significant association.
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7 Considering a global average for tendinous proportion of each tendo-muscle unit, FL,
8 FT-IV, FDC, and SC showed the highest values, which overpass muscle length (Table 1).
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10 While CS, LD and PIB had tendinous proportions of approximately half of muscle length and
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12 AT, TB, and TEN units had the lowest tendinous proportions (Table 1).
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17 The relation between tendinous proportions of tendo-muscle units and locomotion
18 revealed the differentiation of jumper-arboreal from other species under most of the models
19 analyzed (Table 2). All species of different locomotion/habitat use categories were
20 significantly differentiated for SC, TEN, FL, and FT-IV units under Ornstein-Uhlenbeck
21 model, and for the FL unit under accelerated Early Burst model as well (Table 2). At least two
22 of the locomotion/habitat use categories were differentiated for CS, PIB, and TB (Table 2).
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24 Ornstein-Uhlenbeck and accelerated early burst had the best fits among the evolutionary
25 models tested (Table 3).
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36 Jumper-arboreal species showed the highest values of TM for SC, FDC, LD, FL and
37 TEN, and the lowest values for CS, TB, AT y FT-IV (Fig. 4). Among other categories of
38 locomotion/habitat use, the jumper-burrower species had the highest TB and the lowest FL
39 tendinous proportions, the jumper-terrestrials had higher CS and lower FL, SC, PIB and TEN
40 values, and hopper-terrestrials had the higher values for PIB. Lastly, Jumper and hopper-
41 terrestrials share the higher values for FT-IV.
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51 Ancestral state reconstruction of tendo-muscle units reveals a pattern of low and
52 intermediate values for group nodes (Fig. 5). High tendinous proportion of tendo-muscle units
53 seem to have been independently acquired by non-related lineages throughout the phylogeny.
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1 A possible exception for this pattern is the FL reconstruction, in which high values are shared
2 by *Hyalinobatrachium* species (Fig. 5G). Shifts in tendo-muscle values mainly reflect a
3
4 phylogenetic pattern in which low and intermediate tendo-muscle ratio is plesiomorphic for
5
6 the main clades (Centrolenidae, Leptodactylinae and Leiuperinae), with some non-related
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8 shifts in distant related lineages (Fig. 5).
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11 **4 Discussion**

12 The goal of this study was to explore the relation of tendon dimension and the force
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14 produced by their associated muscles, as inferred by their cross-sectional area. Second, we
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16 aimed to test whether there is specialization of tendinous proportion of tendon-muscle units
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18 among anuran species with different locomotor modes and habitat uses, taking phylogeny into
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20 account. Our data suggests a significant and positive relation between tendon dimension and
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22 muscle cross-sectional area for most of the elements analyzed. We also found that four of the
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24 ten tendo-muscle units analyzed in this study could be differentiated among all locomotion
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26 and habitat use categories. Notably, jumper-arboreal species differentiate from all others in all
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28 tendo-muscle units tested. Complementing these results, optimization of tendinous
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30 proportions showed plesiomorphic low and intermediate tendo-muscle ratio and shifts for
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32 higher values in distant related lineages.
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41 Tendons and muscles have a deep functional and morphological relation which starts
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43 early on development (Kardon 1998; Huang 2017). Although these tissues have independent
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45 cell precursors, subsequent elongation and differentiation of tendon depends on attachment to
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47 muscle (Huang et al. 2015). Even with fully formed components, movement stimuli is still
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49 essential to the proper assembly and maintenance of functionality of the locomotor system of
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51 anuran juveniles (Abdala & Ponssa 2012; Ponssa & Abdala 2016). Our analysis focused on
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53 adult individuals also showed a significant and positive correlation of tendons and their
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1 associated muscles, in which broader tendons relate to stronger muscles (higher values for
2 cross-sectional area). This general pattern has two exceptions, the *longissimus dorsi* and the
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4 *coccygeosacralis* tendons, both inserting on the posterodorsal region of the body. The lack in
5
6 correspondence between the former tendons area and their respective associated muscles
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8 potential force reflects other possibly more complex power managing mechanisms. Although
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10 based on external gross morphology alone, we theorize that other subtle characteristics of the
11
12 two elements can be influencing the results. The *longissimus dorsi* has extra anchoring spots
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14 on the vertebral processes through its extension along the axial column (Fratani et al. 2018a),
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16 which might also contribute to elastic activity. Regarding the other case, the origin of
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18 *coccygeosacralis* is only partially tendinous, therefore the direct muscle insertion on bone also
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20 plays an important role on force transmission. These results highlight the risks of
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22 generalizations and the importance of taking into account the particularities of each element,
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24 as pointed out by Ponssa et al. (2018) in their study of muscles and bone crests.
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31 Tendinous proportions of tendo-muscle units from the pectoral girdle (SC) and from the
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33 hindlimb (TEN, FL and FT-IV) were significantly different for all locomotor/habitat use
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35 categories. Forelimbs and pectoral girdle play an important role on the stabilization of the
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37 body and energy dissipation during landing (Akella & Gillis 2011). Simultaneous contraction
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39 of both extensors and flexor elements protects the joints, and the resulting forces are directed
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41 medially through the pectoral girdle (Emerson 1983; Kardong 2009). The supracoracoideus-
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43 coracoradialis muscle extends from the epicoracoideus, the medial-most elements of the
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45 pectoral girdle, has its interface between muscle and tendon on the ventral shoulder region, a
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47 long tendinous extension throughout the humerus and inserts on the proximal radioulna
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49 (Fratani et al. 2018a). Akella & Gillis (2011) found a positive relation between jump distance
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51 and SC muscle activity, pointing to a central role of this unit on the dissipation of landing
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1 forces. Surprisingly, jumper species such as *Leptodactylus chaquensis* and *L. latinasus*
2 showed lower values for SC tendinous proportion when compared to hopper-terrestrials.
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4 However, previous data on a hand flexor tendon ultrastructure of the former *Leptodactylus*
5 species showed impressive high values of collagen fibril diameter associated to the absorption
6 of landing forces (Abdala et al. 2018). Thus, other properties of the SC tendon might be
7 contributing to its functionality on species with more demanding locomotor modes.
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14 Hindlimb elements, in turn, participate mostly on the take-off phase of jumping (Přikryl
15 et al. 2009). Among the tendo-muscle units with significant relation with locomotion/habitat
16 use, the tenuissimus is a leg extensor (Přikryl et al. 2009); the fascia latae does not have a
17 clear function but it is thought to promote optimal function of thigh elements by increasing
18 the overall tension (Fairclough et al. 2007); and the flexor tendon of digit IV (hindlimb) is
19 mostly related to the propulsion for take-off (Nauwelaerts & Aerts 2006). Our results indicate
20 that jumper-arboreal species have a higher tendinous proportion of FL and TEN, while
21 species of other locomotor/habitat use categories showed lower and similar values of
22 tendinous proportions. The opposite pattern was observed for FT-IV, in which all
23 locomotor/habitat use categories have similar tendinous proportions but the jumper arboreal
24 species showed a relatively lower value.
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41 Jumper-arboreal taxa distinguished themselves from all others species, with higher
42 tendinous proportions for half of the tendo-muscle units tested. Locomotion on arboreal
43 habitats requires precise movement in a complex three-dimensional stratus with the additional
44 challenge of gravity (Sustaita et al. 2013). In anurans, anatomical traits such as intercalary
45 skeletal elements, adhesive sub-digital pads, and forelimb tendon patterns have been related to
46 arboreality (Manzano et al. 2007, 2008, 2018; Sustaita et al. 2013). The higher tendinous
47 proportions on limb and girdle tendo-muscle units shown by jumper-arboreal species in our
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1 data can be related to more jump power and precision in movement while allowing the
2 possibility of a lighter body, favoring vertical habitat occupation. It should be also considered
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4 that power amplification improves jumping activity (Roberts & Azizi 2011). Power
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6 amplification is achieved through elastic elements such as tendons, which slowly stores
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8 energy from muscle work and releases it rapidly (Roberts & Azizi 2011; Roberts et al. 2011;
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10 Astley & Roberts 2012, 2014). This unique feature of tendons can explain their increased
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12 proportion in tendo-muscle units that requires energy storage to fulfill an efficient jump. This
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14 power amplification can be especially relevant in those tendons such as the Achilles tendon,
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16 which act as a catapult during take-off in jumping (Astley & Roberts 2012).
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22 Another interesting result is the differentiation between jumper-terrestrial and jumper-
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24 arboreal species from all others, with the highest and the lowest tendinous proportion of
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26 *coccygeosacralis*, respectively. This muscle-tendon unit originates mainly on the sacral
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28 diapophysis and stabilizes the urostyle on a horizontal plane during locomotion (Emerson &
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30 De Jongh 1980; Fratani et al. 2018a). Jumper-terrestrial species included in this study, such as
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32 some species of *Leptodactylus* and *Pseudopaludicola* have a very specific sacral morphology,
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34 characterized by rod-like diapophysis (IIB of Emerson 1979, 1982). While jumper-arboreal
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36 taxa, represented mainly by Centrolenidae species, have broader diapophyses enveloped by
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38 wide ligaments (IB of Emerson 1979, 1982). The two morphologies are related to different
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40 jump mechanisms, respectively vertical rotation and fore-after sliding of the pelvic girdle in
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42 relation to the main axis of the body (Emerson 1982, Reilly & Jorgensen 2011). Our results
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44 suggest a stronger tendinous participation of the CS tendon on the locomotion of terrestrial
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46 jumpers, and offers evidence that new tendinous traits could possibly be useful for the
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48 diagnosis of pelvic types.
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Tendinous proportions of the *triceps brachii* were significantly different for jumper-
arboreal and jumper-burrower species, with higher values for the latter. This tendo-muscle
unit is a forelimb extensor and, during locomotion, is related to energy dissipation of landing
forces (Duellman & Trueb 1986; Cox & Gillis 2015). Tendons can act as dampers, serving as
a power attenuation mechanism by absorbing mechanical energy and releasing it more slowly
(Azizi & Roberts 2010; Konow & Roberts 2015). A high tendinous proportion of TB in
jumper-burrowers is unexpected, since these species burrow using hindlimbs and snout
(Heyer 1978; Ponssa & Medina 2016). The jumper-burrower species included in this study
are mainly part of the *Leptodactylus fuscus* group, in which burrowing behavior is related to
reproduction and males build incubation chambers for clutch deposition (Heyer 1969, 1974).
Thus, we speculate that this result could be related to other ecological factors not included in
this study, such as reproductive behavior. Forearm muscles are directly involved in the
clasping mechanism during amplexus (Oka et al. 1984; Liao et al. 2012), and TB was one of
the few tendinous elements in which we have found significant differences between males and
females. This outcome is especially interesting and should be further investigated since sexual
dimorphism was not found in other morphological traits of the *L. fuscus* group by previous
studies (Ponssa & Medina 2016; Ponssa et al. 2018).

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Among the evolutionary models included in the analysis, Ornstein-Uhlenbeck had a
better fit for most models. It is important to highlight, however, that differences in wAIC
among the tested models are not strong enough to come to a definite conclusion. This low
resolution of the adaptive optimum model might be explained by recent microhabitat
transitions in Anura (Moen et al. 2016). Consequently, species may not have been in their
current microhabitat long enough to reach its estimated morphological optimum (Moen et al.
2016). Still, our results are consistent with previous studies which have also estimated a

1 phenotypic optimum for morphological traits in tetrapods (Soliz et al. 2017; Tulli et al. 2016;
2 Moen et al. 2016; Fratani et al. 2018ab; Ponssa et al. 2018). Additionally, optimization
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4 showed a distribution of tendo-muscle values highly correspondent to the greater clades
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6 (Leiuperinae, Leptodactylinae and Centrolenidae). It is possible that these results derive from
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8 the coincidence between locomotion/habitat use categories and monophyletic clades for most
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10 cases in our study group (e.g. all jumper-arboreal species are centrolenids).
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13 **4.1 Conclusions**

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16 In summary, our main findings support the hypothesis that tendon variables respond to
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18 mechanical demands and are also affected by the shared history among species. Overall, our
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20 results indicate a correlation between tendons and muscles, a divergence of tendinous
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22 morphology related to locomotion and habitat use, and congruence with phylogeny. It also
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24 provides a framework for testing other potential cases of tendinous phenotypic evolution in
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26 Anura. More generally, it adds evidence to a pattern of an interconnected effect between
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28 environment and shared phylogenetic history both influencing phenotypic diversity. This
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30 pattern has been consistently found in vertebrates (e.g. Muschick et al. 2012; Moen et al.
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32 2016).
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Figure captions

1
2 Fig. 1. Schematic representation of the studied tendons (colored) and muscles (dashed lines)
3
4 in dorsal (A) and ventral (B) view. Tendons highlighted in orange were used exclusively for
5
6 tendon x muscle force analysis, and all tendons (both orange and brown) were used in the TM
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8 x locomotion and habitat use analysis. Abbreviations: *coccygeosacralis* (CS); *flexor digitorum*
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10 *communis* (FDC); *fascia latae* (FL); *longissimus dorsi* (LD); *puboischiofemorialis internus B*
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12 (PIB); *supracoracoideus-coracoradialis* (SC); Achilles tendon (AT); *triceps brachii* (TB);
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14 flexor tendon of digit IV (TD-IV); *tenuissimus* (TEN).

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19 Fig. 2. Composite tree representing the phylogenetic relationship of the analyzed taxa.
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21 Categories of locomotor mode and habitat use: jumper-arboreal (JA); hopper-terrestrial (HT);
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23 jumper-burrower (JB); jumper-swimmer (JS); jumper-terrestrial (JT); swimmer (SW).
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27 Fig. 3. Relationships of (log-transformed) tendons and muscle areas with p-values,
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29 highlighted with asterisks (*) when significant.
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32 Fig. 4. Boxplots comparing tendinous proportions among different categories of locomotion
33
34 and habitat use. Boxes represent the 25%/75% quartiles, and the median is shown with a
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36 horizontal line. Significantly different groups are highlighted with an asterisk (*).
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38
39 Abbreviations: jumper-arboreal (JA); hopper-terrestrials (HT); jumper-burrowers (JB);
40
41 jumper-swimmers (JS); jumper-terrestrial (JT).
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44 Fig. 5. Optimization of tendinous proportion of each tendon-muscle unit. Low values are
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46 represented in blue, intermediate values in green, and high values in yellow.
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Table 1. Summary of tendinous proportions for each tendo-muscle (TM) unit.

Abbreviations: coccygeosacralis (CS); flexor digitorum communis (FDC); fascia latae (FL); longissimus dorsi (LD); puboischiofemoralis internus B (PIB); supracoracoideus-coracoradialis (SC); Achilles tendon (AT); triceps brachii (TB); tenuissimus (TEN); flexor tendon of digit IV (FT-IV).

	CS	FDC	FL	LD	PIB	SC	AT	TB	TEN	FT-IV
Mean	0.41	1.53	3.14	0.48	0.72	1.21	0.15	0.23	0.34	2.34
Standard deviation	0.12	0.20	1.43	0.15	0.21	0.26	0.04	0.06	0.08	0.49
Maximum value	0.89	2.70	9.97	1.21	1.54	2.82	0.33	0.47	0.69	4.21
Minimum value	0.08	0.89	0.23	0.09	0.24	0.58	0.07	0.09	0.18	1.01

Table 2. Locomotion/habitat use categories with significant differences in tendo-muscle (TM) under different evolutionary models (evol models). Abbreviations: TM units: coccygeosacralis (CS); flexor digitorum communis (FDC); fascia latae (FL); longissimus dorsi (LD); puboischiofemoralis internus B (PIB); supracoracoideus-coracoradialis (SC); Achilles tendon (AT); triceps brachii (TB); tenuissimus (TEN); flexor tendon of digit IV (FT-IV). Evolutionary models: BM: brownian motion; OU: Ornstein-Uhlenbeck; EB: accelerated (+) and deaccelerated (-) early burst. Locomotion/habitat use: jumper-arboreal (JA); hopper-terrestrial (HT); jumper-burrowers (JB); jumper-swimmers (JS); jumper-terrestrial (JT).

TM/ Evol models	BM	OU	EB+	EB-
CS	JA	JA - JT	JA	-
FDC	JA	JA	JA	JA
FL	JA	JA - HT JB - JS JT	JA - HT JB - JS JT	JA
LD	JA	JA	JA	-
PIB	JA	JA - HT JB - JT	JA	JA
SC	JA	JA - HT JB - JS JT	JA - JB JS - JT	JA
AT	JA	JA	JA	-
TB	JA	JA - JB	JA	-
TEN	JA	JA - HT JB - JS JT	JA	JA
FT-IV	JA	JA - HT JB - JS JT	JA - JB JS	-

Table 3. Akaike (AIC) and weighted Akaike (wAIC) values for each evolutionary model included in the analysis. Abbreviations: coccygeosacralis (CS); flexor digitorum communis (FDC); fascia latae (FL); longissimus dorsi (LD); puboischiofemoralis internus B (PIB); supracoracoideus-coracoradialis (SC); Achilles tendon (AT); triceps brachii (TB); tenuissimus (TEN); flexor tendon of digit IV (FT-IV). The best model for each tendo-muscle unit are highlighted in bold.

	BM		OU		EB+		EB-	
	AIC	wAIC	AIC	wAIC	AIC	wAIC	AIC	wAIC
CS	-90	0.08	-93	0.33	-94	0.57	-18	<0.01
FDC	64	<0.01	47	0.99	58	<0.01	66	<0.01
FL	327	0.16	328	0.09	324	0.65	328	0.08
LD	-20	0.01	-28	0.84	-25	0.143	-18	<0.01
PIB	21	<0.01	7	0.97	14	0.019	23	<0.01
SC	60	<0.01	43	0.99	54	0.004	62	<0.01
AT	-227	<0.01	-261	0.99	-234	<0.01	-224	<0.01
TB	-169	<0.01	-200	0.99	-176	<0.01	-166	<0.01
TEN	-127	<0.01	-149	0.99	-133	<0.01	-125	<0.01
FT-IV	180	<0.01	153	0.99	173	<0.01	183	<0.01

Figure 1

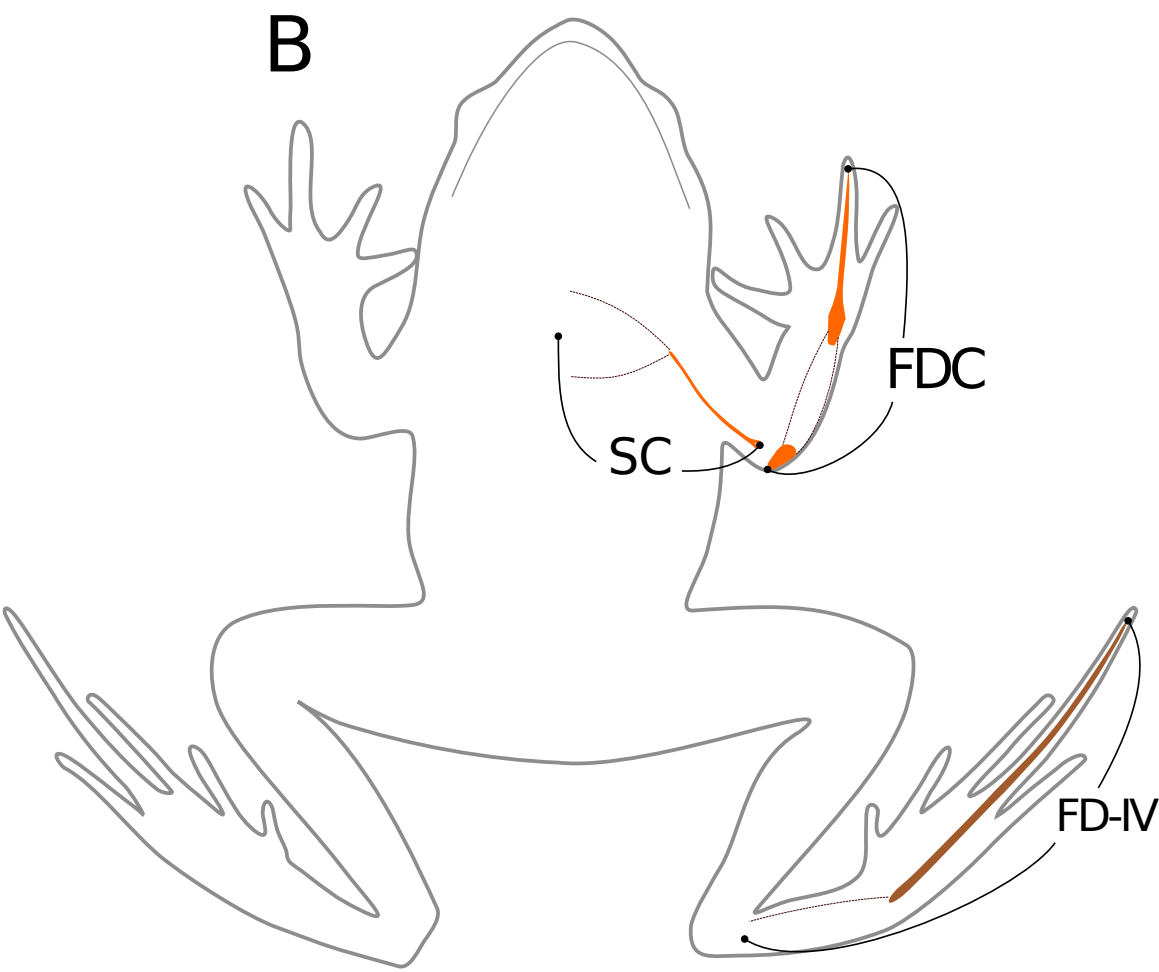
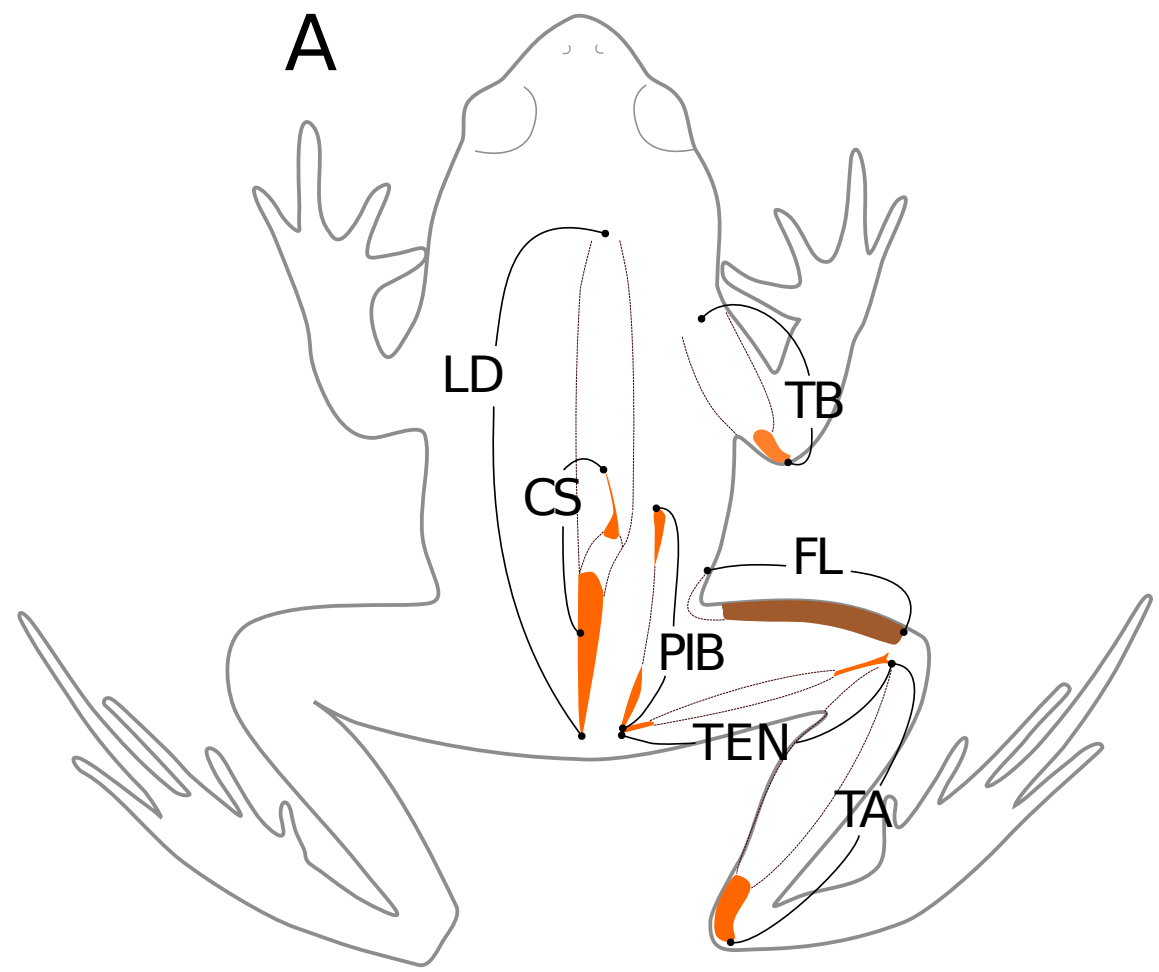


Figure 2

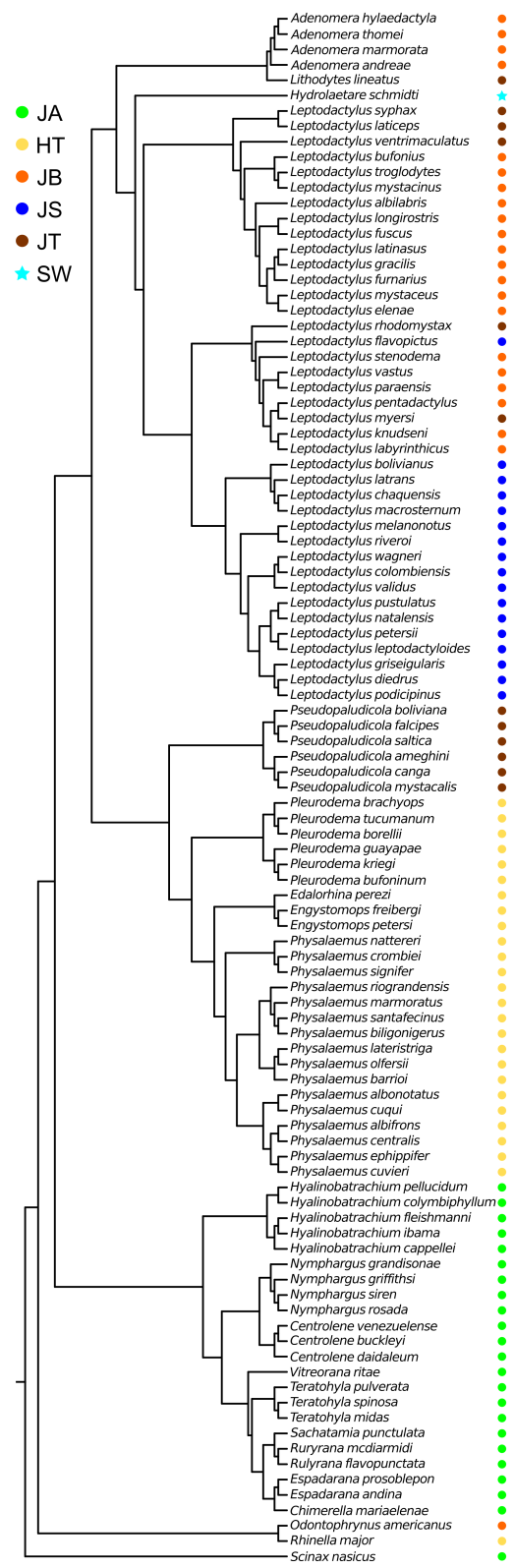
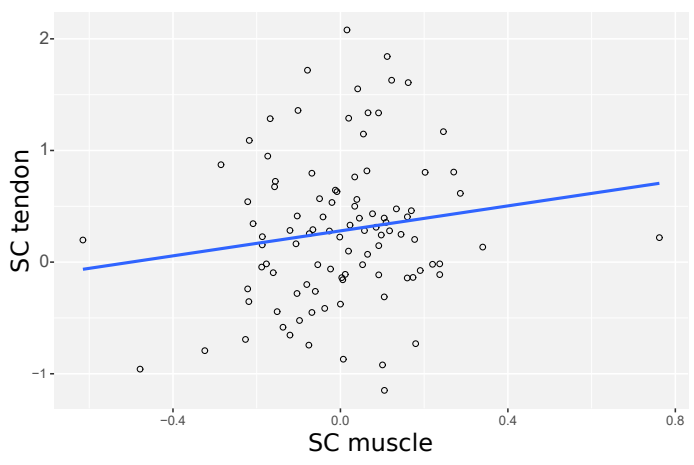
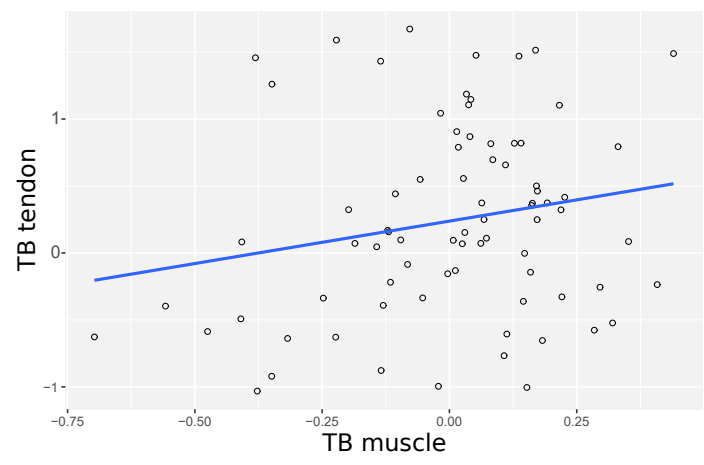


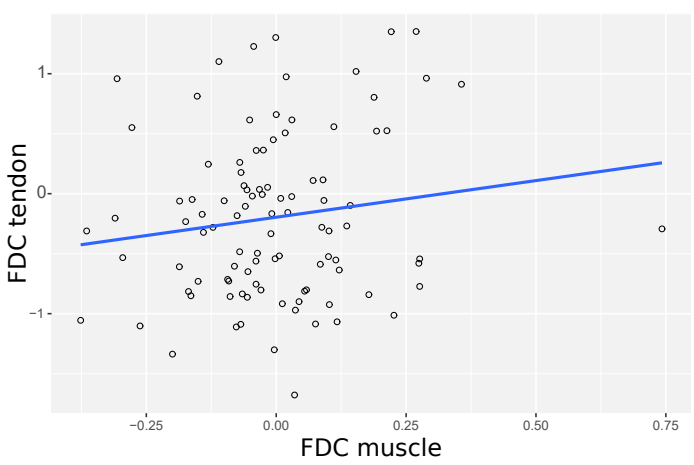
Figure 3 A) supracoracoideus-coracoradialis $P < .01^*$



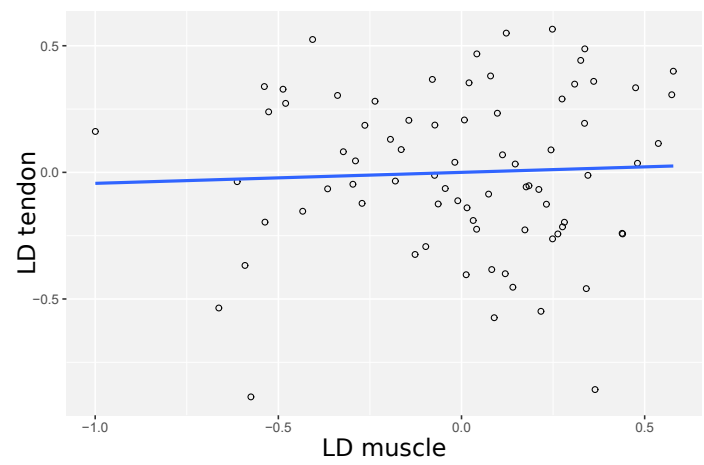
B) triceps brachii $P < .01^*$



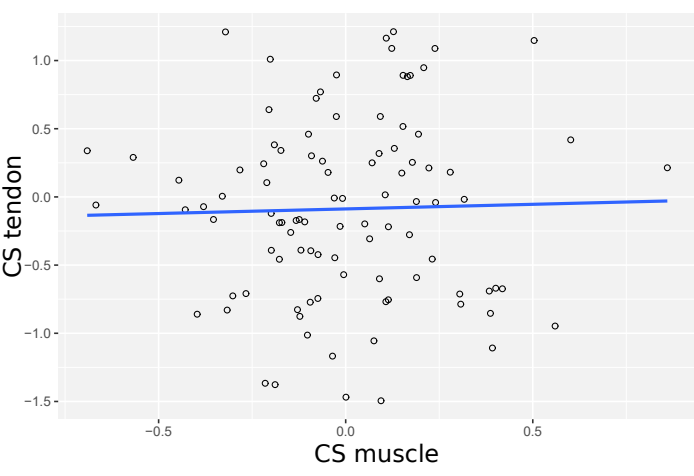
C) flexor digitorum communis $P = .02^*$



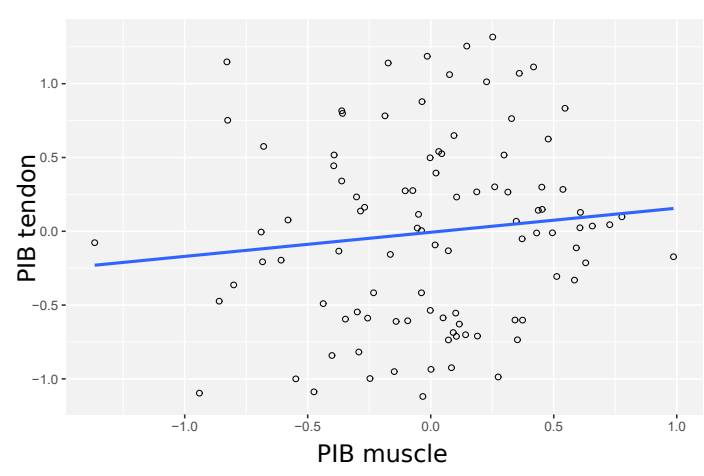
D) longissimus dorsi $P = 0.7$



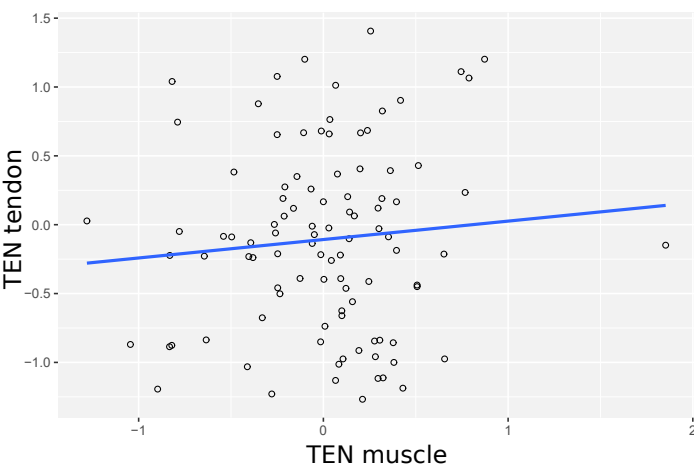
E) coccygeosacralis $P = .16$



F) puboischiofemoralis internus B $P < .01^*$



G) tenuissimus $P = .03^*$



H) Achilles tendon $P < .01^*$

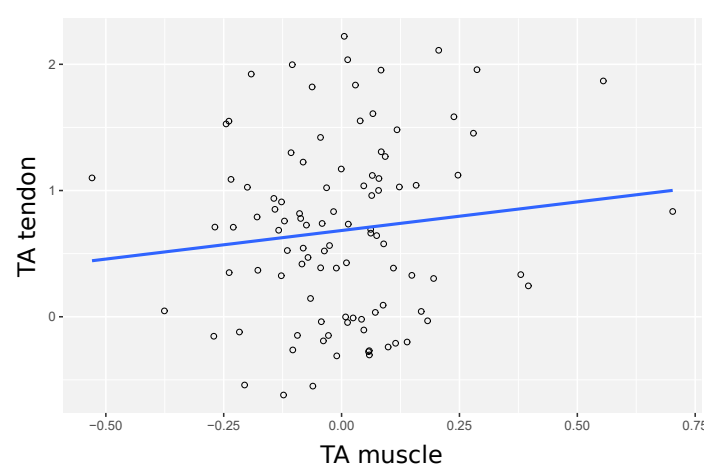


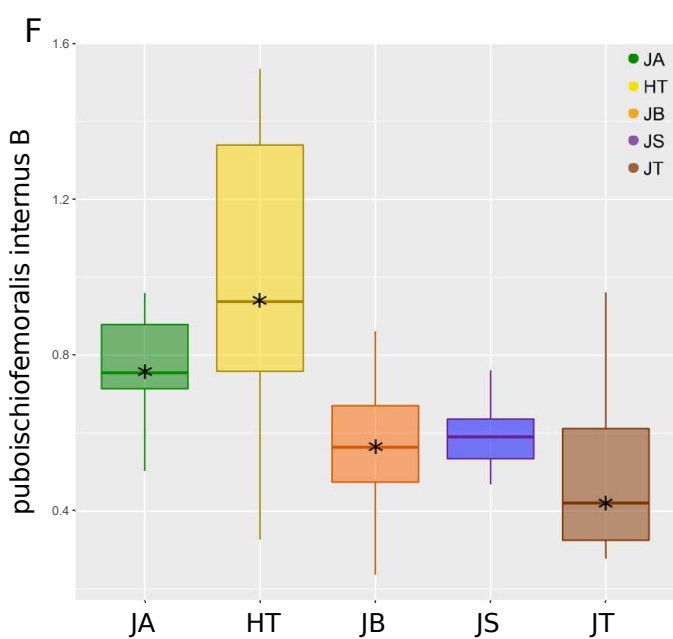
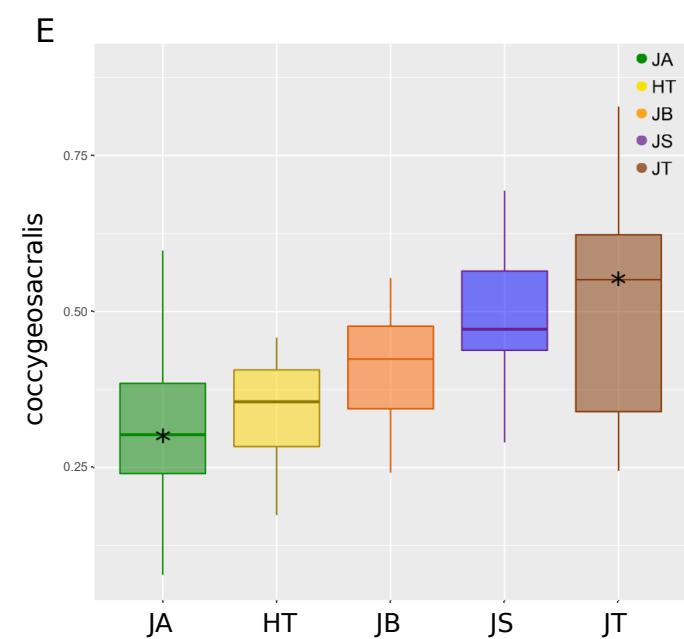
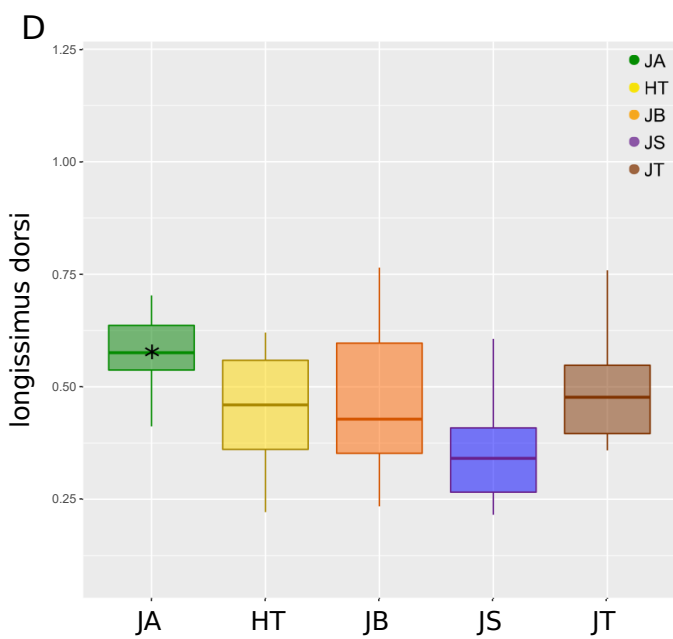
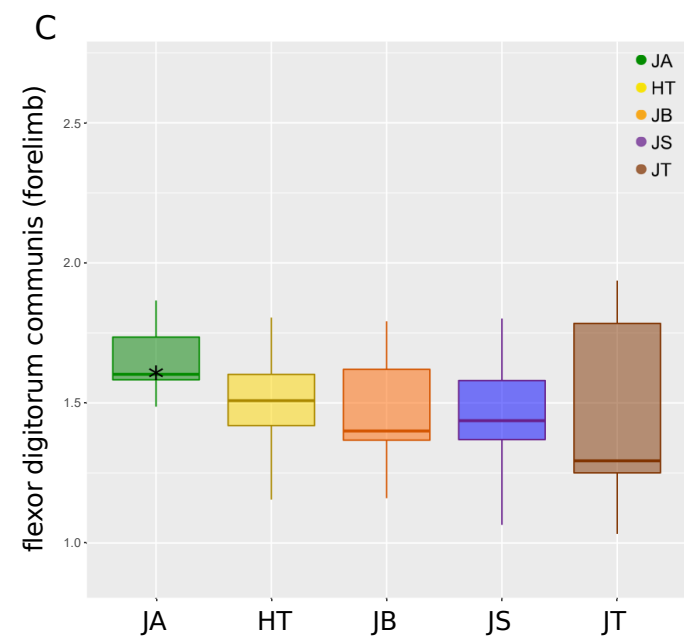
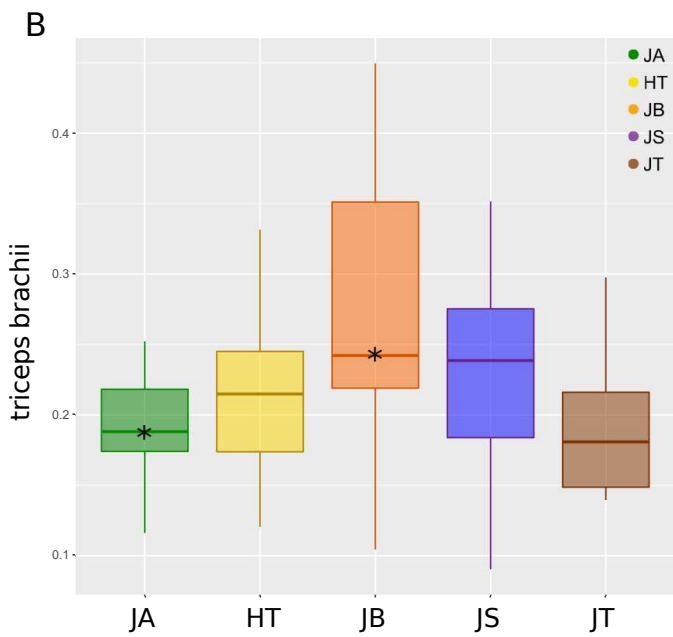
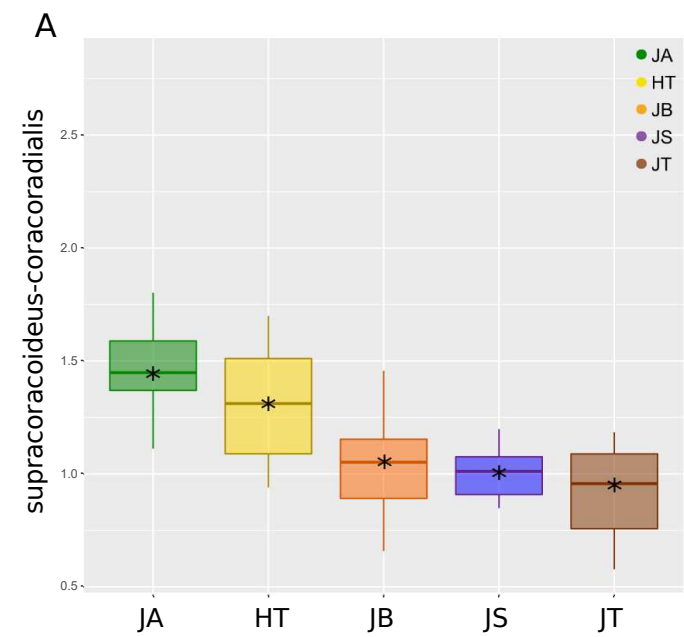
Figure 4

Figure 4

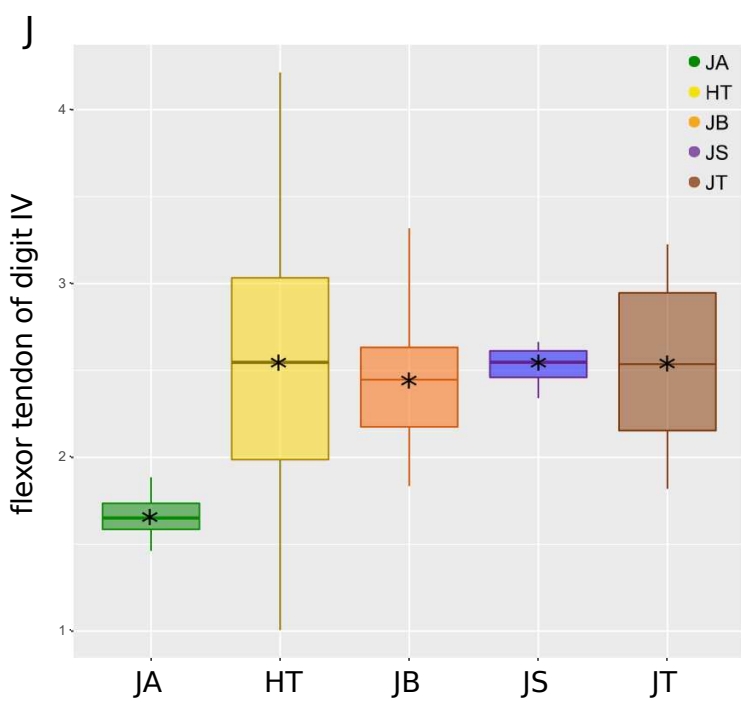
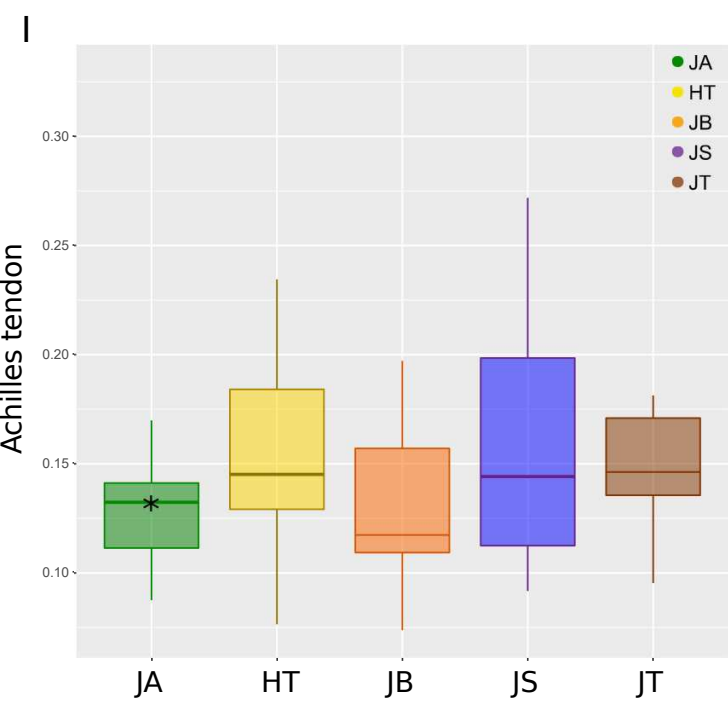
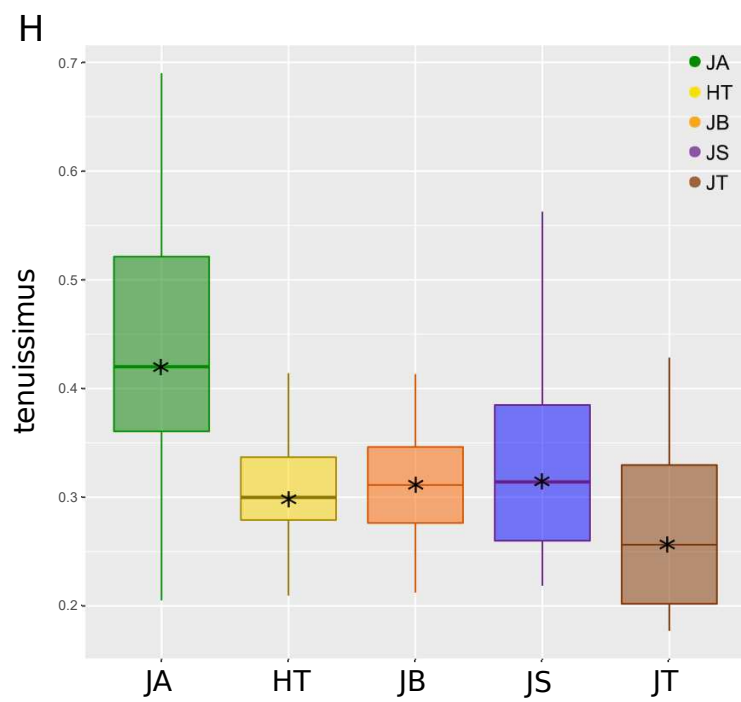
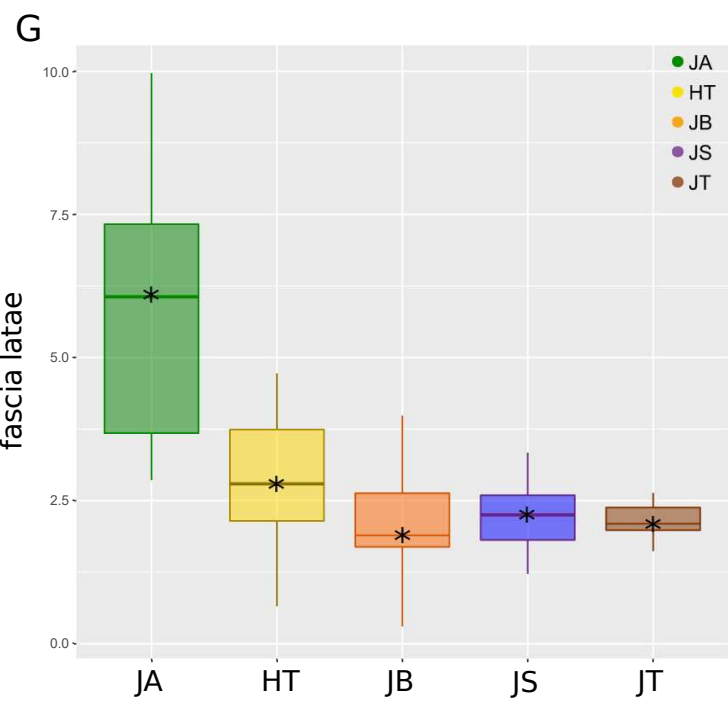
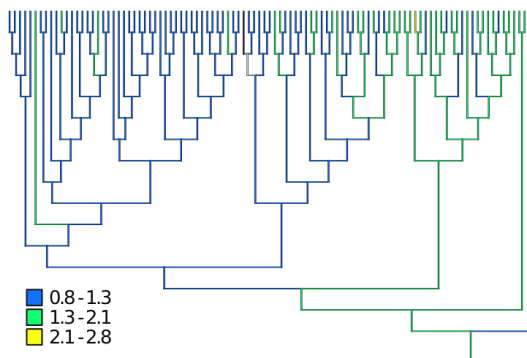
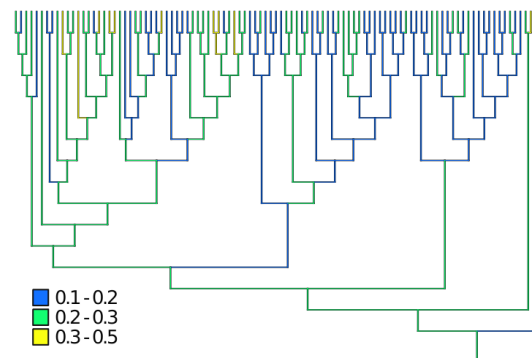


Figure 5

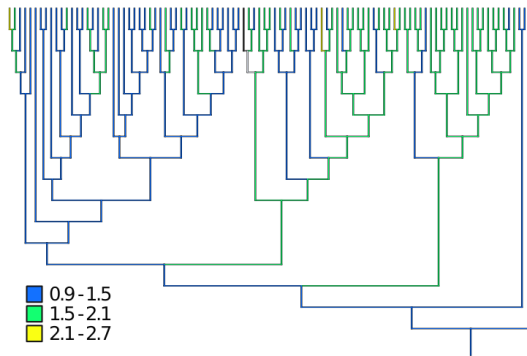
A) supracoracoideus-coracoradialis



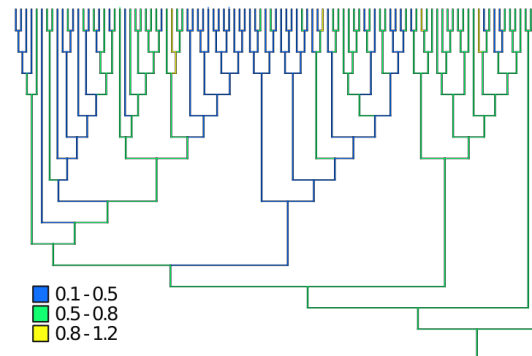
B) triceps brachii



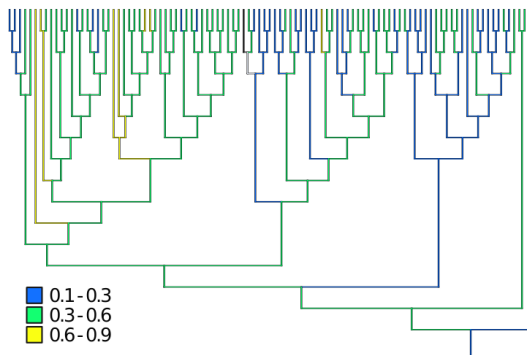
C) flexor digitorum communis



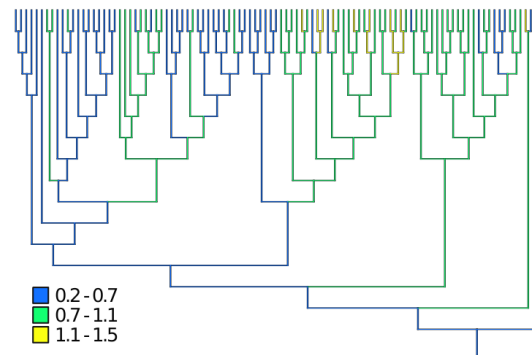
D) longissimus dorsi



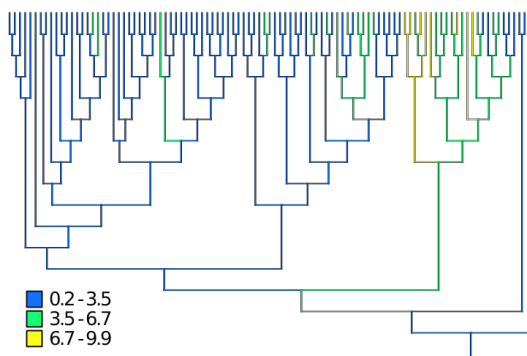
E) coccygeosacralis



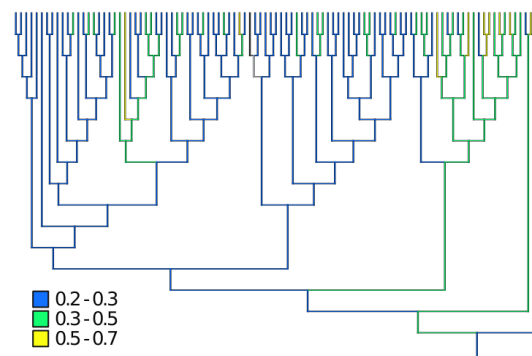
F) puboischiofemorales internus B



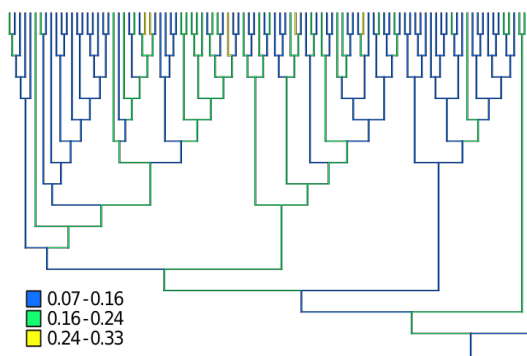
G) fascia latae



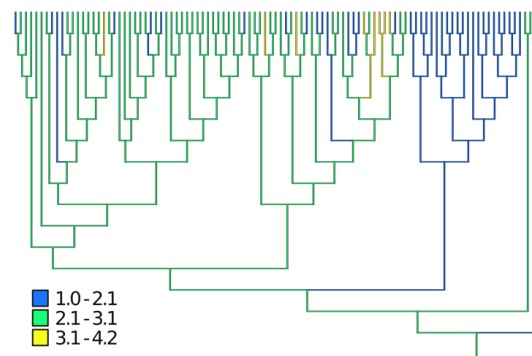
H) tenuissimus



I) Achilles tendon



J) flexor tendon of digit IV



Supplementary Material

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