

Drivers and patterns of bite force evolution in liolaemid lizards

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Phenotypic variation is the result of selection on traits that are relevant in a given ecological context. Phylogenetic history, genetic drift, and any developmental or structural constraints may, however, limit variation in trait expression. It has been proposed that organismal performance traits take up a pivotal role in driving variation in morphology due to their central role in survival and reproductive success. However, how strong the links are between morphology and performance, and how the strength of this relationship impacts the rate of evolution of form and function need to be studied across a wider variety of systems to better understand the origin and evolution of biodiversity. Here we used data on the jaw system (muscle architecture and head dimensions) of liolaemid lizards to investigate the drivers of *in vivo* bite force variation and test for differences in evolutionary rates in morphology and performance. Our results show high rates of evolution for performance traits compared to morphological traits such as external head dimensions. Many-to-one mapping of morphology to performance, that is the possibility that different anatomical trait combinations lead to similar levels of performance, appears to be common in the jaw system of these lizards. Finally, traits showing greater mechanical sensitivity (muscle cross-sectional areas) showed higher rates of evolution compared to traits involved in other functions and that are probably subject to trade-offs (e.g. head width).

ADDITIONAL KEYWORDS: bite force – diet – evolutionary drivers – jaw system – liolaemid – many-to-one mapping.

INTRODUCTION

Phenotypic variation is the result of selection on traits relevant in a given ecological context, phylogenetic history, genetic drift, and any developmental or structural constraints that may limit variation in trait expression (Arnold, 1993; Wainwright, 2007). Selection is often considered to act at the level of the whole organism. As such, it has been proposed

that organismal performance traits take up a pivotal role in explaining the adaptive variation of phenotypic traits (Arnold, 1983). Variation in performance traits is known to influence fitness (Huey & Stevenson, 1979) as they impact survival and reproductive success. Given that selection acts directly on these ecologically relevant performance traits, the relationships between morphological and performance traits can be expected to be strong, especially for biomechanically relevant traits that are direct drivers of variation in performance (Muñoz *et al.*, 2017; Muñoz, 2019). How strong these links are between morphology and performance, and how

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the strength of this relationship impacts the rate of evolution of form and function across different systems remain relatively poorly known despite several recent studies addressing these questions (e.g. Muñoz *et al.*, 2017, 2018).

Many-to-one mapping, the process by which many different anatomical trait combinations can give rise to similar levels of performance, has been shown to be common in biomechanical systems (Alfaro *et al.*, 2004, 2005; Wainwright *et al.*, 2005; Wainwright, 2007). Based on this expectation, one can predict that rates of evolution should be lower for morphological traits compared to whole-organism performance traits which are dependent on the interactions of many morphological structures. Interestingly, rates for different morphological traits have been shown to vary considerably (Adams, 2012; Muñoz *et al.*, 2018), suggesting that selective pressures may drive higher rates specifically in functionally relevant traits. The few studies comparing evolutionary rates across different types of traits have suggested that sexually selected traits evolve faster than other morphological or life-history traits (see Pitchers *et al.*, 2014). Yet, whether whole-organism performance traits such as bite force, locomotor speed or manoeuvrability evolve at the same rate as morphological traits such as head size or limb length remains largely unknown. Most studies investigating how many-to-one mapping may impact the rate of evolution of higher level performance traits have used four-bar linkage systems (i.e. the simplest movable closed-chain linkage consisting of four bars or links connected in a loop by four joints). These linkage systems provide relatively simple and tractable arrangements with clearly and a priori defined relationships between morphology and the kinematic output of the system as the mechanical output of the system is predefined by its geometry (Alfaro *et al.*, 2004, 2005; Wainwright *et al.*, 2005; Muñoz *et al.*, 2017, 2018). Consequently, the mechanical output of the system is predefined by its geometry. However, most biomechanical systems underlying ecologically relevant performance traits are not well represented by four-bar linkage systems. Rather, many biomechanical systems related to locomotion or feeding are driven by more-or-less complex lever systems where muscles and bones interact to produce the biomechanical output of the system (McHenry, 2012). Consequently, the mechanical output of these lever systems cannot be predicted by their geometry. Whether the same patterns can be observed in these systems where many muscles and bones interact, compared to four-bar linkages, remains unknown. Despite their complexity, these biomechanical systems provide the advantage that the functional output of the system can be quantified *in vivo* rather easily by

measuring whole-organism performance traits such as sprint speed or bite force.

Bite force is an excellent example of an ecologically relevant whole-organism performance trait (Arnold *et al.*, 1983; McBrayer & White, 2002; Anderson *et al.*, 2008). Bite force has been suggested to be related to diet (Aguirre *et al.*, 2003; Herrel *et al.*, 2008a; Sagonas *et al.*, 2014; López-Darias *et al.*, 2015), intra- and interspecific competition (male–male interactions, aggression, etc.; Herrel *et al.*, 1999, 2009; Huyghe *et al.*, 2005; Lappin & Husak, 2005; Husak *et al.*, 2006; Vanhooydonck *et al.*, 2010; Donihue *et al.*, 2016; Dufour *et al.*, 2018), and defence (Herrel *et al.*, 2007). Previous studies have shown that bite force is correlated with variation in external morphological traits including head size and shape (Herrel *et al.*, 2001; López-Darias *et al.*, 2015; Zabolocki-Thomas *et al.*, 2018), as well as biomechanical traits such as lever arms (Meyers *et al.*, 2018). However, muscle size (Wittorski *et al.*, 2016) and its architecture (Herrel *et al.*, 2008b), and physiology (Herrel *et al.*, 2007) also play an important role in driving variation in bite force and may provide alternative pathways to increase bite force. Bite force is thus an ideal performance trait to investigate whether many-to-one mapping impacts the evolutionary rate of performance relative to morphological traits.

Liolaemid lizards are an ideal test case to investigate the proximate drivers of bite force variation as disparity in diet as well as sexual and head size dimorphism exist in these animals. Moreover, data on *in vivo* bite force are available (Vanhooydonck *et al.*, 2010), providing an *in vivo* estimator of performance and allowing us to test for differences in evolutionary rates in morphology and performance. We predict (1) differences between males and females in the traits (muscle architecture, lever arms, external head dimensions) that drive variation in bite force, given that bite force is driven more by sexual selection in males whereas this is not the case in females, where variation is in relation to diet (Vanhooydonck *et al.*, 2010). We also predict that (2) muscles, and more specifically their cross-sectional area, should be the strongest drivers of bite force variation given their direct impact on the input force of the system. Of all the jaw adductors important in generating bite force, we predict that the pseudotemporalis muscle will particularly strongly impact bite force (i.e. will have a high mechanical sensitivity) given its orientation perpendicular to the jaw and its position away from the jaw joint, providing it with the largest moment arm of all jaw adductors (Fig. 1). Variation in lever arms is also predicted to be a significant driver of variation in bite force together with differences in external head

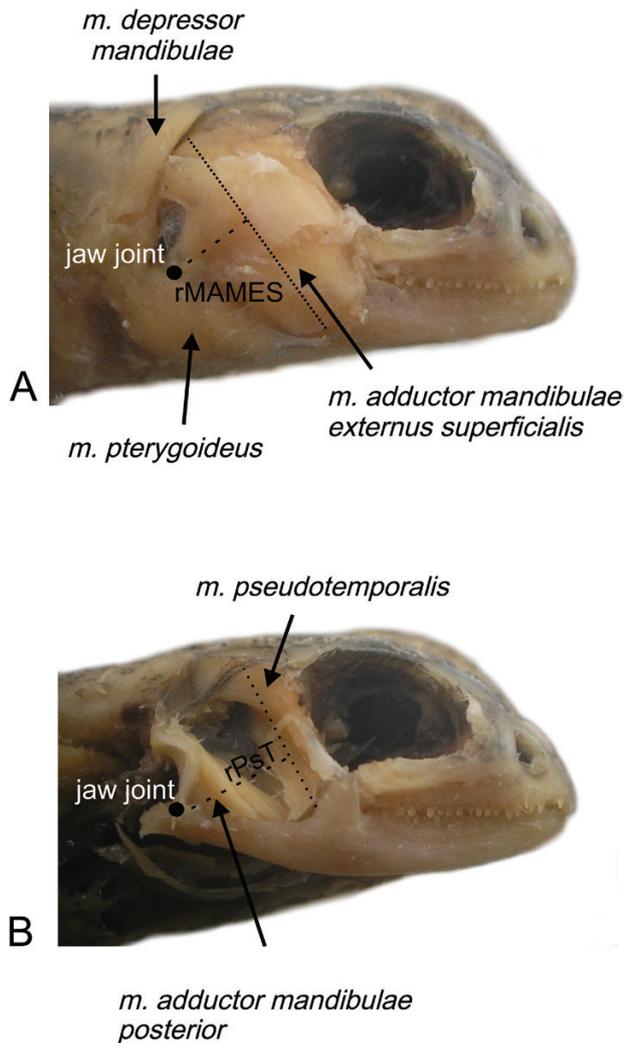


Figure 1. Lateral view of the head of *Liolaemus pseudoanomalus* illustrating the muscles dissected. A, superficial view after removal of the skin; B, deeper view after removal of the external adductor group. Indicated in A are the line of action of the *m. adductor mandibulae externus superficialis* (dotted line) and its moment arm (rMAMES; dashed line). In B the line of action of the *m. pseudotemporalis* (dotted line) and its moment arm (rPsT; dashed line) are indicated. Note how the moment arm of the *m. pseudotemporalis* is much greater than that of the *m. adductor mandibulae externus superficialis*.

dimensions. Finally, we predict that (3) the rates of evolution should be greater for performance traits (i.e. bite force) relative to morphological traits due to the many-to-one mapping of morphology to function (i.e. the same functional output can be achieved by many different combinations of underlying traits; Alfaro *et al.*, 2004, 2005; Wainwright *et al.*, 2005; Collar & Wainwright, 2006).

MATERIALS AND METHODS

SPECIMENS

Specimens from 26 species of Liolaemidae lizards were collected by hand or noose at different localities in Argentina, from Andean regions of northern Argentina (Salta and Catamarca provinces) to southern Patagonia of Argentina (Supporting Information, Table S1), at the end of February 2009, when lizards are post-reproductive (see Medina & Ibargüengoytía, 2010). We empirically observed that females were in good condition, even after egg-laying. The fact that experiments were conducted in the post-reproductive season implies that motivational differences between the sexes are reduced. Lizards were transferred to the lab where bite force was measured *in vivo*. While they remained in captivity, lizards were released into a terrarium (1.2 × 0.6 × 0.4 m, L × W × H) every 3 days, for 4 h. A 150-W infrared bulb on one side of the terrarium provided heat; illumination was provided by parabolic aluminized reflector (PAR) lights. While in the terrarium, lizards were fed crickets (*Achaeta domestica*) *ad libitum* and were sprayed with water at least twice a day. We added flowers in the case of herbivorous species. Once *in vivo* measurements were completed, lizards were killed, fixed in 4% formalin, and stored in 70% ethanol. The specimens were then deposited in the herpetological collection of the Instituto de Herpetología of the Fundación Miguel Lillo in Tucumán, Argentina (FML), and used for dissection. Species were collected with the approval of all Argentinean provinces (Fauna authorities: S. Montanelli, Chubut-; L. B. Ortega, Santa Cruz; M. Faillá, Rio Negro; F. Lonac, Neuquén; P. Barlanga, Mendoza; L. Fra, Catamarca; A. Ortin, Salta; A. Norino, La Rioja; W. López, Buenos Aires; and M. Jordán, San Juan) and National Parks of Argentina administration (L. Buria). This investigation was performed under the institutional animal care guidelines established by CONICET, and with the approval of ANPCyT (Argentinean National Agency for Promoting Research and Technology in Spanish) for the procedures and methodology.

DISSECTIONS

The muscle nomenclature of Haas (1973) is used throughout. All jaw muscles from the left side of the head were removed on each specimen (Fig. 1). Muscles were weighed using a digital scale (model ABT 5DM, Kern, Balingen, Germany). Note that all specimens were treated identically and data thus were comparable despite the known reduction in muscle mass due to fixation and preservation in ethanol (Vervust *et al.*, 2009). Next, fibre lengths were obtained by submerging the muscles in a 30% aqueous

nitric acid (HNO₃ 30%) solution for a minimum of 24 h to dissolve all connective tissue. Muscle fibres were then put in a 50% aqueous glycerol solution to stop further digestion. Fibres were teased apart and pictures with a scale were taken. Fibre lengths were quantified using ImageJ v.1.47 (W. Rasband, National Institutes of Health, Bethesda, MD, USA). Next, we calculated the average length of the fibres for each muscle. Finally, the physiological cross-sectional area (PCSA) of each muscle was calculated by dividing muscle mass by the product of muscle density and fibre length (see Table 1 for the species means). We then summed muscle cross-sectional areas for each muscle group: the *m. adductor mandibulae externus* (Mame), *m. adductor mandibulae posterior* (MAMP), *m. pseudotemporalis* (MPsT) and *m. pterygoideus* (MPt) areas were calculated. We also estimated the summed muscle cross-sectional area of all closer muscles (closers).

HEAD DIMENSIONS

Six morphological measurements were taken for all specimens (26 species) before dissection (see Table 1 for the species means). We used the same measurements as illustrated in Herrel & Holanova (2008) and Vanhooydonck *et al.* (2010). Snout–vent length (SVL) was measured from the tip of the snout to the posterior edge of the anal scale, head length (Headl) from the back of the parietal bone to the tip of the upper jaw, head width (Headw) at the widest part of the head (at the level of jugal bones), head height (Headh) at the highest part of the head (posterior to the orbits), lower jaw length (lower jaw) from the back of the retroarticular process to the tip of lower jaw, snout length (tip-coron) from the tip of the lower jaw to the posterior edge of the jugal (as an indicator of the position of the coronoid), and jaw outlever (tip-quadr) from the tip of the lower jaw to the anterior edge of the ear opening (corresponding to the posterior edge of the quadrate). Both lower jaw length and the distance from the tip of the jaw to the coronoid reflect the biomechanics of the jaw system. By subtracting ‘tip-coron’ from ‘tip-quadr’ we calculated the length of the jaw closing in-lever (close). All measurements (Table 2) were taken using digital callipers (CD-20DC, Mitutoyo, Kawasaki, Japan; precision: 0.01 mm), and were taken on the left side of the specimens whenever possible. If measurements could not be taken on the left side because the head was damaged, then measurements were taken on the right side.

BITE FORCE

In vivo bite forces for 23 liolaemid species (Table 1) were taken as follows, 18 from Vanhooydonck *et al.* (2010)

and five additional species (*Liolaemus lineomaculatus*, *L. magellanicus*, *L. poecilochromus*, *L. scrochii* and *Phymaturus tenebrosus*). Only data for adults were used in this study. Bite forces were measured using an isometric Kistler force transducer (type 9203, range 7500 N; Kistler, Zurich, Switzerland) mounted on a purpose-built holder and connected to a Kistler charge amplifier (type 5995A, Kistler; for more detailed descriptions of the setup and data collection see Herrel *et al.*, 1999; Vanhooydonck *et al.*, 2010). Gape angle (30°) and bite position were standardized across all trials. Gape was adjusted to lizard size by moving the bite plates and the angle was chosen based on studies of lizard feeding kinematics showing that lizards regularly crush hard prey at gape angles of around 30° (e.g. Herrel *et al.*, 1996); bite position was standardized such that lizards bit at the front of the tooth row, and all bites were bilateral. Bite plates were not covered with leather or rubber as this decreases maximal bite forces by dissipating part of the force in the deformation of the material. Bite force were higher for all species tested (over 50) when using metal bars compared to bars covered with rubber or leather, at least in lizards (A. Herrel, unpubl. data). This is not the case for birds and mammals where a significantly lower force is observed when biting on metal (A. Herrel, unpubl. data). *In vivo* bite forces were not available for three of the species in our anatomical data set (*Liolaemus cayanus*, *L. multimaculatus*, *L. pseudoanomalus*). All lizards were post-reproductive when measured and the repeatability of the measurements was high, suggesting that the motivational state was similar across individuals and species.

STATISTICAL ANALYSES

All muscular and morphological variables were logarithmically transformed (\log_{10}) before analyses to fulfil assumptions of normality and homoscedasticity. Given the known dimorphism in head dimensions and bite force (see Vanhooydonck *et al.*, 2010) we ran all subsequent analyses for males and females separately where possible. Species are not independent data points and, as such, phylogeny needs to be taken into account (Felsenstein, 1985). Thus, our analyses considered phylogeny; for this we ran a phylogenetic analysis by using DNA extractions from tissues of the studied lizard species. The phylogenetic tree of 26 liolaemid species studied was reconstructed using DNA (1726 bp) sequences spanning the mitochondrial DNA gene regions *ND1* to *COI* and includes complete and partial sequences representing three protein-coding genes and eight tRNA genes. Sequences were aligned using the molecular protocols and alignment structure described by Schulte *et al.* (2000). Mitochondrial DNA sequences representing the species reported here have

Table 1. List of species studied by sex

Species	N	SVL (±SE)	Mass (±SE)	Headl (±SE)	Headw (±SE)	Headh (±SE)	Close (±SE)	Maxbite (±SE)
<i>Liolaemus baguali</i>	6	90.76 (±0.024)	20.49 (±0.076)	19.49 (±0.024)	13.34 (±0.032)	8.25 (±0.032)	4.59 (±0.009)	22.93 (±0.029)
<i>L. baguali</i>	2	81.79 (±0.023)	12.97 (±0.121)	15.9 (±0.022)	10.98 (±0.037)	7.13 (±0.009)	3.96 (±0.022)	16.5 (±0.053)
<i>L. bibroni</i>	4	53.87 (±0.009)	4.35 (±0.033)	11.69 (±0.004)	7.64 (±0.016)	5.45 (±0.015)	2.47 (±0.031)	5.19 (±0.018)
<i>L. bibroni</i>	4	55.98 (±0.011)	4.99 (±0.011)	11.38 (±0.018)	7.81 (±0.005)	5.43 (±0.003)	1.93 (±0.058)	3.9 (±0.029)
<i>L. canqueli</i>	7	94.81 (±0.013)	22.78 (±0.022)	18.63 (±0.022)	12.77 (±0.015)	9.50 (±0.009)	4.22 (±0.010)	22.44 (±0.012)
<i>L. canqueli</i>	4	88.7 (±0.001)	15.97 (±0.020)	17.04 (±0.004)	11.27 (±0.003)	8.02 (±0.011)	3.39 (±0.012)	17.38 (±0.025)
<i>L. ceii</i>	3	76.86 (±0.026)	12.48 (±0.029)	16.48 (±0.004)	11.91 (±0.015)	7.65 (±0.004)	3.35 (±0.018)	11.39 (±0.026)
<i>L. ceii</i>	5	77.4 (±0.029)	13.81 (±0.107)	16.02 (±0.009)	11.64 (±0.011)	7.22 (±0.011)	3.31 (±0.056)	8.5 (±0.019)
<i>L. coeruleus</i>	13	61.5 (±0.004)	6.80 (±0.035)	12.90 (±0.000)	9.33 (±0.007)	5.79 (±0.010)	2.47 (±0.009)	8.07 (±0.010)
<i>L. coeruleus</i>	5	63.27 (±0.003)	6.63 (±0.033)	12.41 (±0.006)	9.09 (±0.006)	5.6 (±0.018)	2.27 (±0.008)	5.78 (±0.025)
<i>L. cuyanus</i>	3	80.52 (±0.005)	15.03 (±0.021)	17.03 (±0.012)	13.96 (±0.006)	10.67 (±0.008)	5.58 (±0.015)	NA
<i>L. cuyanus</i>	1	75.37 (NA)	13.04 (NA)	15.42 (NA)	12.63 (NA)	9.56 (NA)	3.86 (NA)	NA
<i>L. elongatus</i>	7	72.76 (±0.012)	10.26 (±0.072)	15.83 (±0.002)	10.17 (±0.021)	6.60 (±0.036)	3.34 (±0.013)	12.90 (±0.013)
<i>L. elongatus</i>	9	68.83 (±0.008)	8.5 (±0.003)	14.98 (±0.002)	9.64 (±0.008)	6.27 (±0.017)	3.06 (±0.021)	10.88 (±0.013)
<i>L. escachardosi</i>	4	79.64 (±0.007)	12.92 (±0.017)	17.27 (±0.006)	13.72 (±0.013)	8.95 (±0.003)	3.66 (±0.037)	16.53 (±0.023)
<i>L. escachardosi</i>	3	68.29 (NA)	6.32 (NA)	14.34 (NA)	8.81 (NA)	6 (NA)	2.89 (NA)	6.01 (±0.091)
<i>L. fitzingerii</i>	4	93.55 (±0.001)	22.71 (±0.033)	18.66 (±0.007)	12.76 (±0.002)	9.18 (±0.002)	3.90 (±0.012)	22.65 (±0.036)
<i>L. fitzingerii</i>	3	89.92 (NA)	19.12 (NA)	17.78 (NA)	11.46 (NA)	8.15 (NA)	3.39 (NA)	16.99 (±0.009)
<i>L. hatcheri</i>	7	59.94 (±0.011)	5.55 (±0.022)	13.05 (±0.023)	9.30 (±0.008)	5.77 (±0.009)	2.95 (±0.014)	6.45 (±0.013)
<i>L. hatcheri</i>	5	59.07 (±0.005)	4.82 (±0.033)	11.52 (±0.000)	8.48 (±0.022)	5.27 (±0.011)	2.63 (±0.033)	4.92 (±0.031)
<i>L. kingii</i>	3	73.78 (±0.017)	11.12 (±0.086)	15.71 (±0.034)	10.57 (±0.012)	7.64 (±0.053)	3.76 (±0.002)	14.21 (±0.026)
<i>L. kingii</i>	7	77.18 (±0.004)	10.89 (±0.024)	15.34 (±0.008)	10.18 (±0.009)	7.06 (±0.006)	3.44 (±0.011)	13.98 (±0.010)
<i>L. kolongh</i>	7	56.27 (±0.002)	4.89 (±0.005)	12.23 (±0.012)	8.96 (±0.013)	5.45 (±0.006)	2.78 (±0.013)	4.18 (±0.016)
<i>L. kolongh</i>	4	56.66 (±0.019)	5.03 (±0.046)	11.7 (±0.004)	8.45 (±0.001)	5.16 (±0.005)	2.51 (±0.041)	3.73 (±0.015)
<i>L. lineomaculatus</i>	2	57.77 (±0.005)	4.83 (±0.002)	11.52 (±0.007)	7.90 (±0.000)	5.93 (±0.009)	2.96 (±0.006)	5.40 (±0.070)
<i>L. lineomaculatus</i>	2	55.49 (±0.005)	4.23 (±0.005)	11.23 (±0.007)	8.05 (±0.010)	5.72 (±0.011)	2.61 (±0.017)	7 (±0.024)
<i>L. magellanicus</i>	2	56.82 (±0.001)	4.25 (±0.031)	11.47 (±0.000)	8.43 (±0.007)	6.01 (±0.017)	2.76 (±0.009)	2.57 (±0.003)
<i>L. magellanicus</i>	2	53.56 (±0.002)	3.28 (±0.023)	10.6 (±0.006)	7.41 (±0.014)	5.18 (±0.015)	2.41 (±0.053)	2.83 (±0.041)
<i>L. mapuche</i>	11	76.82 (±0.024)	13.23 (±0.067)	16.12 (±0.008)	11.33 (±0.028)	8.24 (±0.030)	3.51 (±0.014)	16.28 (±0.005)
<i>L. mapuche</i>	5	70.9 (±0.006)	7.97 (±0.006)	14.36 (±0.002)	10 (±0.004)	7.25 (±0.005)	3.06 (±0.016)	12.85 (±0.009)
<i>L. multimaculatus</i>	1	50.89 (NA)	3.78 (NA)	12.58 (NA)	10.38 (NA)	6.76 (NA)	2.86 (NA)	NA
<i>L. multimaculatus</i>	2	54.64 (±0.004)	5.47 (±0.005)	12.94 (±0.002)	10.69 (±0.005)	7.06 (±0.000)	2.9 (±0.024)	NA
<i>L. petrophilus</i>	2	84.29 (±0.011)	14.70 (±0.009)	18.77 (±0.021)	12.19 (±0.019)	7.13 (±0.018)	4.28 (±0.029)	13.58 (±0.010)
<i>L. petrophilus</i>	7	79.6 (±0.010)	13.29 (±0.014)	18.11 (±0.006)	11.43 (±0.003)	7.01 (±0.011)	3.5 (±0.002)	11.1 (±0.022)
<i>L. poeclichromus</i>	4	63.76 (±0.005)	7.20 (±0.021)	14.23 (±0.011)	10.23 (±0.005)	6.62 (±0.003)	3.34 (±0.027)	6.97 (±0.011)
<i>L. poeclichromus</i>	3	63.56 (NA)	7.14 (NA)	13.22 (NA)	8.62 (NA)	5.87 (NA)	2.72 (NA)	5.08 (±0.007)
<i>L. pseudoanomalous</i>	2	62.92 (±0.018)	8.54 (±0.093)	15.76 (±0.033)	14.23 (±0.017)	9.68 (±0.018)	5.32 (±0.044)	NA
<i>L. pseudoanomalous</i>	1	57.11 (NA)	7.51 (NA)	12.68 (NA)	11.89 (NA)	8.24 (NA)	4.14 (NA)	NA
<i>L. rothi</i>	3	79.35 (±0.005)	15.13 (±0.046)	16.55 (±0.002)	12.32 (±0.005)	8.48 (±0.009)	3.23 (±0.013)	9.14 (±0.030)

Table 1. Continued

<i>L. rothi</i>	3	87.38 (±0.003)	22.76 (±0.024)	18.36 (±0.008)	13.51 (±0.010)	8.92 (±0.003)	3.91 (±0.012)	10.08 (±0.060)
<i>L. scapularis</i>	3	58.15 (NA)	5.52 (NA)	13.72 (NA)	9.63 (NA)	6.34 (NA)	2.83 (NA)	9.21 (±0.058)
<i>L. scapularis</i>	2	55.5 (±0.007)	4.64 (±0.020)	12.54 (±0.006)	8.6 (±0.010)	5.64 (±0.002)	2.48 (±0.033)	3.83 (±0.069)
<i>L. scroccii</i>	1	90.44 (NA)	22.14 (NA)	20.31 (NA)	15.83 (NA)	9.00 (NA)	4.80 (NA)	15.30 (±NA)
<i>L. scroccii</i>	3	86.98 (±0.007)	19.38 (±0.015)	17.64 (±0.008)	14.01 (±0.003)	7.94 (±0.002)	4.15 (±0.016)	10.65 (±0.063)
<i>L. tenuis</i>	5	53.03 (±0.001)	3.94 (±0.008)	12.98 (±0.010)	8.62 (±0.014)	5.30 (±0.008)	2.64 (±0.001)	8.29 (±0.014)
<i>L. tenuis</i>	2	51.91 (±0.003)	2.66 (±0.004)	12.15 (±0.001)	7.34 (±0.003)	4.79 (±0.000)	2.38 (±0.033)	5.4 (±0.028)
<i>L. xanthoviridis</i>	5	82.39 (±0.002)	14.99 (±0.030)	16.81 (±0.007)	11.39 (±0.001)	7.99 (±0.014)	3.65 (±0.022)	18.77 (±0.019)
<i>L. xanthoviridis</i>	3	84.17 (±0.012)	15.3 (±0.040)	16.84 (±0.010)	10.7 (±0.008)	7.73 (±0.032)	3.68 (±0.028)	16.3 (±0.019)
<i>L. zulyae</i>	2	71.43 (±0.018)	9.28 (±0.017)	15.98 (±0.031)	10.60 (±0.025)	7.83 (±0.054)	3.75 (±0.032)	12.43 (±0.020)
<i>L. zulyae</i>	2	63.48 (±0.004)	6.29 (±0.070)	13.25 (±0.009)	8.98 (±0.022)	6.63 (±0.002)	2.84 (±0.008)	6.78 (±0.030)
<i>Phymaturus tenebrosus</i>	1	93.57 (NA)	24.95 (NA)	17.90 (NA)	14.77 (NA)	7.90 (NA)	3.51 (NA)	19.43 (NA)
<i>P. tenebrosus</i>	2	92.3 (±0.017)	22.7 (±0.027)	16.55 (±0.007)	13.81 (±0.004)	7.41 (±0.012)	2.58 (±0.007)	18.55 (±0.011)

N is the number of specimens used for bite force measurements (Maxbite) and external morphology (for some species we did not measure bite force – NA). SVL is mean snout–vent length, mass is mean body mass; HeadL, Headw and Headh, are mean (±SE; NA when data correspond to a single specimen) head length, width and height, respectively; Close is the closing in-lever. External measurements are in millimetres, body mass in grams and bite force in newtons. Data are presented for males (white rows) and females (grey rows).

been deposited in GenBank and the alignment used for phylogenetic tree reconstruction is available in TreeBASE (<https://www.treebase.org/treebase-web/search/study/summary.html?id=14692>). GenBank accession numbers for all species are available from Schulte (2013). The phylogenetic tree was estimated using a priori partitioned mixed model maximum likelihood (ML) analysis of species for which bite force and muscle cross-sectional area were obtained (Fig. 2). For the analysis, model testing was performed in MEGA 5.05 (Tamura *et al.*, 2011). The models with the highest likelihood value for model testing were GTR+ Γ +I, and the default model in RAxML 7.2.8 on XSEDE (Stamatakis *et al.*, 2008) on the CIPRES Science Gateway (Miller *et al.*, 2010) is GTR+ Γ (Tavaré, 1986). Due to this limitation, GTR+ Γ was used for all analyses with model parameter values estimated from the data. For partitioned analyses, we assumed a priori partitions based on codon position with three partitions (codon positions 1, 2, 3) for all three protein-coding genes (*ND1*, *ND2*, *COI*) combined, and one partition for combined tRNA and non-coding positions for a total of four partitions. There are other possible partitioning schemes but after the results of Schulte & de Queiroz (2008) and studies cited therein, partitioning by codon position most often explains the data better than other partitioning schemes using likelihood ratio tests or Bayes factor estimates. Branch lengths were estimated using the evolutionary model above and the tree was converted to be ultrametric using r8s (Sanderson, 2003) and setting the root node representing the common ancestor of *Liolaemus* and *Phymaturus* to have diverged 100 Mya. This age is within the time frame estimated by Schulte (2013).

We tested for phylogenetic signal (the tendency of related species to resemble each other more than expected than species drawn at random from the same tree) in all variables (muscles, head dimensions, body size and bite force) by calculating Blomberg's *K* (Blomberg *et al.*, 2003) using a randomization test implemented in R ('phytools', Revell, 2012). Analyses of phylogenetic signal were carried out for the overall sample and for each sex separately (Supporting Information, Table S2).

To analyse drivers of variation in bite force we ran phylogenetic generalized least squares (PGLS) model analyses. Analyses were done in the 'caper' (Orme *et al.*, 2012) and 'ape' (Paradis *et al.*, 2004) libraries in R (R Development Core Team, 2014). This procedure is preferred as it has been shown to outperform or be equivalent to phylogenetic or non-phylogenetic procedures depending on the λ value obtained (Revell, 2010). Because of the large number of predictor variables, we divided the analyses into two sets, one for muscle-related traits and the other for the external head variables. PGLS were run for the overall sample, and for males and females separately.

Table 2. List of species studied by sex

Species	<i>N</i>	Mame (\pm SE)	MAMP (\pm SE)	MPsT (\pm SE)	MPt (\pm SE)	Closers (\pm SE)
<i>Liolaemus baguali</i>	2	0.0165 (\pm 0.084)	0.0045 (\pm 0.058)	0.0032 (\pm 0.138)	0.0241 (\pm 0.110)	0.0483 (\pm 0.098)
<i>L. baguali</i>	2	0.0121 (\pm 0.043)	0.003 (\pm 0.140)	0.0027 (\pm 0.057)	0.0124 (\pm 0.089)	0.0301 (\pm 0.072)
<i>L. bibroni</i>	2	0.0051 (\pm 0.014)	0.0012 (\pm 0.054)	0.0015 (\pm 0.053)	0.0068 (\pm 0.050)	0.0146 (\pm 0.038)
<i>L. bibroni</i>	2	0.0023 (\pm 0.020)	0.0005 (\pm 0.076)	0.0005 (\pm 0.088)	0.0056 (\pm 0.147)	0.009 (\pm 0.093)
<i>L. canqueli</i>	2	0.0285 (\pm 0.005)	0.0027 (\pm 0.013)	0.0058 (\pm 0.109)	0.0306 (\pm 0.033)	0.0676 (\pm 0.026)
<i>L. canqueli</i>	2	0.0131 (\pm 0.030)	0.0021 (\pm 0.062)	0.0024 (\pm 0.000)	0.0115 (\pm 0.035)	0.029 (\pm 0.023)
<i>L. ceii</i>	2	0.0172 (\pm 0.057)	0.0039 (\pm 0.012)	0.0039 (\pm 0.049)	0.0175 (\pm 0.048)	0.0425 (\pm 0.049)
<i>L. ceii</i>	2	0.0138 (\pm 0.021)	0.0027 (\pm 0.051)	0.0034 (\pm 0.032)	0.0101 (\pm 0.025)	0.03 (\pm 0.019)
<i>L. coeruleus</i>	2	0.0067 (\pm 0.013)	0.0008 (\pm 0.053)	0.0020 (\pm 0.034)	0.0065 (\pm 0.030)	0.0160 (\pm 0.019)
<i>L. coeruleus</i>	2	0.0046 (\pm 0.007)	0.0008 (\pm 0.046)	0.001 (\pm 0.068)	0.0041 (\pm 0.031)	0.0105 (\pm 0.018)
<i>L. cuyanus</i>	2	0.0511 (\pm 0.008)	0.0079 (\pm 0.019)	0.0209 (\pm 0.073)	0.0420 (\pm 0.026)	0.1219 (\pm 0.021)
<i>L. cuyanus</i>	1	0.0325 (NA)	0.0035 (NA)	0.013 (NA)	0.0207 (NA)	0.0697 (NA)
<i>L. elongatus</i>	2	0.0138 (\pm 0.055)	0.0028 (\pm 0.114)	0.0031 (\pm 0.018)	0.0162 (\pm 0.041)	0.0359 (\pm 0.050)
<i>L. elongatus</i>	2	0.0101 (\pm 0.029)	0.002 (\pm 0.100)	0.0022 (\pm 0.026)	0.0097 (\pm 0.027)	0.0241 (\pm 0.034)
<i>L. escachardosi</i>	2	0.0118 (\pm 0.002)	0.0031 (\pm 0.029)	0.0030 (\pm 0.037)	0.0194 (\pm 0.009)	0.0373 (\pm 0.006)
<i>L. escachardosi</i>	1	0.0064 (NA)	0.0011 (NA)	0.0014 (NA)	0.0048 (NA)	0.0137 (NA)
<i>L. fitzingerii</i>	2	0.0156 (\pm 0.018)	0.0032 (\pm 0.011)	0.0022 (\pm 0.069)	0.0176 (\pm 0.036)	0.0387 (\pm 0.006)
<i>L. fitzingerii</i>	1	0.013 (NA)	0.0021 (NA)	0.0029 (NA)	0.0106 (NA)	0.0285 (NA)
<i>L. hatcheri</i>	2	0.0068 (\pm 0.071)	0.0007 (\pm 0.000)	0.0012 (\pm 0.039)	0.0063 (\pm 0.021)	0.0148 (\pm 0.030)
<i>L. hatcheri</i>	2	0.0096 (\pm 0.148)	0.0007 (\pm 0.055)	0.0009 (\pm 0.043)	0.0043 (\pm 0.035)	0.0155 (\pm 0.073)
<i>L. kingii</i>	2	0.0147 (\pm 0.000)	0.0043 (\pm 0.064)	0.0039 (\pm 0.048)	0.0251 (\pm 0.059)	0.0418 (\pm 0.000)
<i>L. kingii</i>	2	0.0115 (\pm 0.027)	0.0019 (\pm 0.056)	0.0024 (\pm 0.007)	0.0136 (\pm 0.007)	0.0294 (\pm 0.011)
<i>L. kolengh</i>	2	0.0059 (\pm 0.030)	0.0010 (\pm 0.075)	0.0010 (\pm 0.065)	0.0053 (\pm 0.066)	0.0132 (\pm 0.023)
<i>L. kolengh</i>	2	0.0047 (\pm 0.017)	0.0005 (\pm 0.069)	0.0005 (\pm 0.072)	0.004 (\pm 0.008)	0.0097 (\pm 0.011)
<i>L. lineomaculatus</i>	2	0.0066 (\pm 0.052)	0.0018 (\pm 0.034)	0.0018 (\pm 0.017)	0.0076 (\pm 0.011)	0.0177 (\pm 0.029)
<i>L. lineomaculatus</i>	2	0.005 (\pm 0.012)	0.001 (\pm 0.104)	0.0011 (\pm 0.008)	0.0043 (\pm 0.001)	0.0114 (\pm 0.013)
<i>L. magellanicus</i>	2	0.0053 (\pm 0.041)	0.0009 (\pm 0.053)	0.0008 (\pm 0.104)	0.0064 (\pm 0.051)	0.0134 (\pm 0.043)
<i>L. magellanicus</i>	2	0.0028 (\pm 0.102)	0.0003 (\pm 0.013)	0.0006 (\pm 0.055)	0.0032 (\pm 0.053)	0.0069 (\pm 0.069)
<i>L. mapuche</i>	2	0.0237 (\pm 0.085)	0.0042 (\pm 0.025)	0.0034 (\pm 0.084)	0.0198 (\pm 0.054)	0.0511 (\pm 0.014)
<i>L. mapuche</i>	2	0.0079 (\pm 0.032)	0.0016 (\pm 0.042)	0.0016 (\pm 0.055)	0.006 (\pm 0.041)	0.0171 (\pm 0.020)
<i>L. multimaculatus</i>	1	0.0064 (NA)	0.0013 (NA)	0.0023 (NA)	0.0053 (NA)	0.0152 (NA)
<i>L. multimaculatus</i>	2	0.0077 (\pm 0.006)	0.0011 (\pm 0.047)	0.0022 (\pm 0.055)	0.0071 (\pm 0.009)	0.0181 (\pm 0.004)
<i>L. petrophilus</i>	2	0.0146 (\pm 0.024)	0.0032 (\pm 0.005)	0.0039 (\pm 0.009)	0.0168 (\pm 0.038)	0.0386 (\pm 0.027)
<i>L. petrophilus</i>	2	0.0083 (\pm 0.000)	0.0024 (\pm 0.012)	0.0018 (\pm 0.000)	0.0104 (\pm 0.003)	0.023 (\pm 0.000)
<i>L. poecelochromus</i>	2	0.0091 (\pm 0.008)	0.0024 (\pm 0.056)	0.0026 (\pm 0.030)	0.0098 (\pm 0.010)	0.0239 (\pm 0.002)
<i>L. poecelichromus</i>	1	0.0062 (NA)	0.0009 (NA)	0.0006 (NA)	0.0062 (NA)	0.0139 (NA)
<i>L. pseudoanomalous</i>	2	0.0159 (\pm 0.056)	0.0026 (\pm 0.060)	0.0030 (\pm 0.051)	0.0158 (\pm 0.081)	0.0372 (\pm 0.066)
<i>L. pseudoanomalous</i>	1	0.0057 (NA)	0.0007 (NA)	0.0016 (NA)	0.0039 (NA)	0.012 (NA)
<i>L. rothi</i>	2	0.0152 (\pm 0.003)	0.0048 (\pm 0.005)	0.0039 (\pm 0.049)	0.0183 (\pm 0.020)	0.0422 (\pm 0.012)
<i>L. rothi</i>	2	0.01 (\pm 0.026)	0.0022 (\pm 0.000)	0.0019 (\pm 0.012)	0.0109 (\pm 0.005)	0.025 (\pm 0.007)
<i>L. scapularis</i>	1	0.0155 (NA)	0.0053 (NA)	0.0050 (NA)	0.0173 (NA)	0.0431 (NA)
<i>L. scapularis</i>	2	0.0061 (\pm 0.023)	0.0012 (\pm 0.062)	0.0016 (\pm 0.020)	0.0059 (\pm 0.057)	0.0148 (\pm 0.040)
<i>L. scrochii</i>	1	0.0187 (NA)	0.0003 (NA)	0.0036 (NA)	0.0252 (NA)	0.0477 (NA)
<i>L. scrochii</i>	2	0.0165 (\pm 0.005)	0.0025 (\pm 0.095)	0.0022 (\pm 0.026)	0.0124 (\pm 0.025)	0.0336 (\pm 0.017)
<i>L. tenuis</i>	2	0.0053 (\pm 0.005)	0.0015 (\pm 0.074)	0.0019 (\pm 0.003)	0.0059 (\pm 0.010)	0.0146 (\pm 0.002)
<i>L. tenuis</i>	2	0.0033 (\pm 0.027)	0.0008 (\pm 0.080)	0.0009 (\pm 0.002)	0.0032 (\pm 0.073)	0.0083 (\pm 0.047)
<i>L. xanthoviridis</i>	2	0.0170 (\pm 0.000)	0.0058 (\pm 0.034)	0.0036 (\pm 0.042)	0.0201 (\pm 0.010)	0.0438 (\pm 0.031)
<i>L. xanthoviridis</i>	3	0.0113 (\pm 0.071)	0.0019 (\pm 0.108)	0.0023 (\pm 0.067)	0.0107 (\pm 0.083)	0.0262 (\pm 0.078)
<i>L. zullyae</i>	2	0.0125 (\pm 0.082)	0.0029 (\pm 0.151)	0.0024 (\pm 0.022)	0.0132 (\pm 0.126)	0.0309 (\pm 0.099)
<i>L. zullyae</i>	2	0.0097 (\pm 0.046)	0.0016 (\pm 0.112)	0.002 (\pm 0.170)	0.0093 (\pm 0.184)	0.0226 (\pm 0.115)
<i>Phymaturus tenebrosus</i>	2	0.0117 (\pm 0.000)	0.0026 (\pm 0.011)	0.0033 (\pm 0.020)	0.0134 (\pm 0.007)	0.0311 (\pm 0.012)
<i>P. tenebrosus</i>	2	0.0101 (\pm 0.001)	0.0016 (\pm 0.059)	0.0029 (\pm 0.001)	0.0107 (\pm 0.005)	0.0253 (\pm 0.006)

N is the number of dissected specimens for muscle measurements for each species. Mame, MAMP, MPsT, MPt and closers are the mean cross-sectional areas (cm²) of the jaw muscles (Mame, m. adductor mandibulae externus; MAMP, m. adductor mandibulae posterior; MPsT, m. pseudotemporalis; MPt, m. pterygoideus, respectively). Data are presented for males (white rows) and females (grey rows).

Table 3. Phylogenetic generalized least squares (PGLS) models of different traits (phylogenetic residuals of muscle cross-sectional area and external head dimensions vs. bite force)

Model	r^2	Slope	P
All			
Maxbite ~ closers	0.352	0.959	0.001*
Maxbite ~ MPt	0.369	0.321	0.001*
<u>Maxbite ~ MPst</u>	<u>0.588</u>	0.578	0.001*
Maxbite ~ MAMP	0.246	0.528	0.009*
Maxbite ~ Mame	<u>0.383</u>	0.793	0.001*
Maxbite ~ Headl	0.031	0.202	0.565
Maxbite ~ Headw	0.065	-0.428	0.125
Maxbite ~ Headh	0.025	0.344	0.222
Maxbite ~ Close	0.028	-0.152	0.541
Males			
Maxbite ~ closers	<u>0.268</u>	0.653	0.006*
Maxbite ~ MPt	0.081	0.123	0.102
Maxbite ~ MPst	<u>0.227</u>	0.344	0.012*
Maxbite ~ MAMP	0.115	0.418	0.062
Maxbite ~ Mame	0.192	0.568	0.021*
Maxbite ~ Headl	0.013	0.191	0.411
Maxbite ~ Headw	0.019	0.117	0.457
Maxbite ~ Headh	0.001	0.211	0.328
Maxbite ~ Close	0.027	0.107	0.533
Females			
Maxbite ~ closers	0.088	0.503	0.091
<u>Maxbite ~ MPt</u>	<u>0.344</u>	0.692	0.001*
Maxbite ~ MPst	0.102	0.809	0.075
Maxbite ~ MAMP	<u>0.321</u>	0.465	0.002*
Maxbite ~ Mame	0.249	0.639	0.008*
Maxbite ~ Headl	0.172	0.545	0.027*
Maxbite ~ Headw	0.008	-0.391	0.288
Maxbite ~ Headh	0.216	0.631	0.014*
Maxbite ~ Close	0.009	0.230	0.385

We conducted these analyses for the species means based on all individuals, only males and only females. r^2 corresponds to the adjusted coefficient of determination. We also provide the slope of each variable in the models. Asterisks indicate significance. The two morphological traits with the highest slope (highest mechanical sensitivity) are indicated in bold. The two best predictors of bite force (highest r^2) are underlined. Mame, *m. adductor mandibulae externus*; MAMP, *m. adductor mandibulae posterior*; MPst, *m. pseudotemporalis*; MPt, *m. pterygoideus*; Headl, head length; Headw, head width; Headh, head height; Maxbite, maximum bite force; Close, in-lever for jaw closing.

analyses). To do so, we used the ratebytree module in 'phytools' v.6.99 (Revell, 2012). This procedure is based on contemporary phylogenetic studies in which the evolutionary rates between traits are compared using the rate parameter, σ^2 (O'Meara *et al.*, 2006; Revell, 2008; Revell *et al.*, 2008, 2018). This method also was used to compare the rate of evolution between sexes (Table 4). Here we compared the relationship between pairs of traits and their rate of change (σ^2). While doing so we consider error in the estimation of species means and error in the phylogeny following Revell

et al. (2018), and for this we used the standard errors (SE) shown in Tables 1 and 2. We used the function `nnls.tree` in 'phangorn' 4.0 (Schliep, 2011) to force the tree to be ultrametric.

Finally, we tested whether the rate of evolution and our proxies for mechanical sensitivity are associated with trait variance (quantified here as standard deviation; see Supporting Information, Tables S5 and S6).

DATA AVAILABILITY

Data of collection sites for the species studied here are given in Supporting Information Table S1. Mitochondrial DNA sequences representing the species reported here have been deposited in GenBank and the alignment used for phylogenetic tree reconstruction is available in TreeBASE (<https://www.treebase.org/treebase-web/search/study/summary.html?id=14692>). GenBank accession numbers for all species are available from Schulte (2013). Morphometric data are available upon reasonable request.

RESULTS

PHYLOGENETIC SIGNAL

Our analyses showed moderate phylogenetic signal in external head dimensions for both males and females but not in bite force nor the in-lever for jaw closing, a biomechanical trait calculated based on external head dimensions (Supporting Information, Table S2). Of the muscles only the cross-sectional area of the pterygoid muscle showed a moderate degree of phylogenetic signal in the overall analysis. Unexpectedly, there was no phylogenetic signal in the muscle and SVL data when analysing males only. In contrast, the signal was moderately high for most muscle traits except for the *m. adductor mandibulae posterior* in females. Phylogenetic signal in the external morphological traits was, on average, slightly higher for females than for males.

MECHANICAL SENSITIVITY

In the overall analyses, the summed cross-sectional area of all jaw closer muscle groups had the greatest mechanical sensitivity followed by the external adductors in terms of slope (see Table 3). However, the pseudotemporalis muscle showed the highest r^2 value and thus the tightest relationship with bite force. In females, the *m. pseudotemporalis* and the Mame showed the greatest mechanical sensitivity in terms of slope, with the *m. pterygoideus* showing the greatest r^2 . In males, the overall jaw closer

cross-sectional area showed the greatest mechanical sensitivity in terms of slope and r^2 , with the Mame also showing high sensitivity in terms of slope and the *m. pseudotemporalis* in terms of r^2 (Table 3). External head dimensions generally showed low sensitivity irrespective of whether using slope or r^2 as a metric of sensitivity. With the exception of the r^2 in males, none of our estimators of mechanical sensitivity was correlated with standard deviation (Supporting Information, Table S6).

DRIVERS OF BITE FORCE

Our model on the species means for females and males combined showed that the best model explaining variation in bite force retained the cross-sectional area of the *m. pseudotemporalis* only. For females, a model including the *m. adductor mandibulae posterior*, the *m. pterygoideus* and the summed cross-sectional area of all jaw closers was retained. For males, the best model retained both the external adductor and the *m. pseudotemporalis*. In contrast to the global analysis and the analysis for males, in females a significant model with external head dimensions was also retained and included head width and head height as best predictors of variation in bite force (Supporting Information, Table S3).

COMPARISON OF RATES OF EVOLUTION

Overall, the rates of evolution of the cross-sectional areas of muscles and body mass were higher than those for external measurements of the head and SVL (Supporting Information, Table S4; Fig. 3). Bite

force showed a similar (albeit slightly higher) rate of evolution compared to the muscular cross-sectional areas and body mass, yet showed a significantly higher rate than the one observed for head dimensions and SVL (see Table S4, Fig. 3). We did not observe differences between males and females in the rate of evolution of muscle cross-sectional area, head dimensions or bite force, except for the rate of evolution of the *m. pseudotemporalis* which was higher in females than in males (Table 4). Rates of evolution were significantly associated with trait variance when considering both the overall sample and data sets separated by sex (Table S6). This suggests that trait variance impacts our measures of the rate of evolution and that these should be interpreted with caution.

DISCUSSION

The phylogenetic signal in the muscular cross-sectional area was generally low. This is common for traits that are thought to be adaptive and evolve convergently (Mahler *et al.*, 2013). Alternatively, the lack of phylogenetic signal in some traits may suggest that Brownian motion is not a good model for representing the evolution of these traits, but this remains to be tested.

Our results showed that mechanical sensitivity was greatest for either the total summed cross-sectional area of the jaw adductors (overall analysis and males) or the cross-sectional area of the *m. pseudotemporalis* in the case of females. This is not unexpected as the total summed cross-sectional area should be driving variation in bite force and small changes in this trait should have a large impact on bite force. For females,

Table 4. Rate comparison between males (δ) and females (φ) in the Liolaemidae species studied

Trait	$\sigma^2\delta$	$\sigma^2\varphi$	$\sigma^2\delta/\sigma^2\varphi$	r likelihood	$P(\chi^2)$
Mame	0.298	0.322	0.925	0.023	0.878
MAMP	0.713	0.29	2.459	2.861	0.091
MPsT	0.128	0.568	0.225	6.436	0.011*
MPt	0.323	0.255	1.267	0.201	0.653
Closers	0.276	0.28	0.986	0.007	0.933
SVL	0.032	0.023	1.391	0.331	0.565
Body m	0.318	0.242	1.314	0.265	0.606
HeadL	0.022	0.014	1.571	0.563	0.452
HeadW	0.029	0.021	1.381	0.25	0.616
HeadH	0.042	0.035	1.200	0.184	0.667
Close	0.063	0.039	1.615	0.192	0.661
Maxbite	0.325	0.152	2.072	1.355	0.244

Rate of change = σ^2 , the relationship between rates in males and females, the relative likelihood (r likelihood) and P -values corresponding to χ^2 . Asterisks indicate significant differences between males and females. Mame, *m. adductor mandibulae externus*; MAMP, *m. adductor mandibulae posterior*; MPsT, *m. pseudotemporalis*; MPt, *m. pterygoideus*; SVL, snout–vent length; Body m, body mass; HeadL, head length; HeadW, head width; HeadH, head height; Maxbite, maximum bite force; Close, in-lever for jaw closing.

as predicted, the muscle with the greatest moment arm, the *m. pseudotemporalis* (Fig. 1), appeared to have the greatest mechanical sensitivity. This muscle was the only muscle showing differences in the rate of evolution between sexes, evolving faster in females than in males (Table 4). This observation may be explained by the fact that males tend to invest more in muscles positioned lateral to the head such as the external adductor or the *m. pterygoideus* (Herrel *et al.*, 1996, 1999). In addition to allowing for the presence of larger muscles as they are not constrained by the space available inside the adductor chamber, these muscles also make the head look wider. This has been shown to be an important visual cue in many lizards, often accentuated with bright colours (Carothers, 1984; Cooper & Vitt, 1988; Molina-Borja *et al.*, 1998; Huyghe *et al.*, 2005). The difference between males and females may thus reflect the role of sexual selection in driving the jaw system in males.

Differences between the sexes were also evident in our model selection analysis (Table 2). Whereas in the overall analysis and the analysis for males only muscles were the sole significant predictors of bite force, in females, external head dimensions were also good predictors of bite force (Table 3) and explained even more of the variation in residual bite force than muscles. Specifically, females with tall, narrow heads showed greater bite force (Table 3). Interestingly, the muscles that best predicted bite force also were different between the sexes. Whereas the external adductor and to some degree also the *m. pseudotemporalis* were the best predictors of bite force in males, in females the *m. adductor posterior*, the *pterygoideus* and the summed cross-sectional area of the adductors were the best predictors. Male–male interactions in *Liolaemus* lizards are mainly visual (Martins *et al.*, 2004) and there are many species that are dichromatic; however, male combats can be observed in both *Liolaemus* and *Phymaturus* species (F. B. Cruz *et al.*, unpubl. data). Additionally, males show larger and broader heads in different groups of *Liolaemus* and *Phymaturus* lizards (for details, see Pincheira-Donoso & Tregenza, 2011; Cabrera *et al.*, 2013; Valdecantos *et al.*, 2019) and sexual head size dimorphism is mostly male-biased. In our sample, males were slightly larger than females in all cases.

Our comparison between traits for the overall data set (rate by tree analyses) demonstrates that both bite force and muscular cross-sectional area show a rate that was 2.5–10 times higher than the rate of external head dimensions and SVL (see Supporting Information, Table S4, Fig. 3). However, as rates are strongly correlated with trait variance this may underlie this observation (Table S6). Interestingly, no major differences in the rate of change between males and females were observed, except for the

pseudotemporalis muscle as mentioned above. Overall, these data suggest that bite force and muscular cross-sectional area had the higher rates of evolution, above those of external measurements (Table S4). These results make intuitive sense as external head dimensions and SVL are constrained by features other than those purely related to bite force. Muscle cross-sectional areas, on the other hand, are direct drivers of variation in bite force and can be expected to evolve faster than external head dimensions. As bite force is the combination of variation in muscle cross-sectional area, the space available for jaw muscles and the lever arms of the jaw system, we expected that rates would have been higher for bite force. Yet, this was not the case and rates of evolution for bite force and muscle cross-sectional areas are of similar magnitude. Muscle cross-sectional areas showed the highest mechanical sensitivity and were good predictors of bite force, in accordance with our predictions which may explain the similarity in rate. This suggests that variation in lever arms or external head dimensions are less important as drivers of variation in bite force. Our a priori predictions suggesting that in systems where variation in multiple traits impact a single performance trait, and where rates of change are lower for morphology than for performance are thus only partly confirmed.

Yet, our results are coherent from a biomechanical perspective. Muscles such as the *m. pseudotemporalis* or the external adductors that are well positioned to generate bite force showed generally high sensitivity and were good predictors of bite force. This is in accordance with Muñoz *et al.* (2018), which suggests (based on linkage systems) that traits with greater sensitivity evolve faster. Mechanical sensitivity is greater for muscle cross-sectional areas. Indeed, whereas force increases with the square of linear dimensions such as external head dimensions, it is directly proportional to muscle cross-sectional area. As suggested previously (Muñoz *et al.*, 2017), we found an association between mechanical sensitivity and rate of evolution, with rates being higher for those traits that have the greatest impact on the mechanical output of the system (i.e. muscle cross-sectional areas). Our results further showed rather high evolutionary rates, at least for some traits. Biting is known to relax trait integration in some taxa and may increase the evolvability of the trait (Collar *et al.*, 2014). A more modular structure also increases the potential for many-to-one mapping and may augment rates of evolution in cranial shape. However, in some cases greater modularity may reduce the rate of morphological evolution (Claverie & Patek, 2013) at an interspecific level as it relaxes constraints on individual structures.

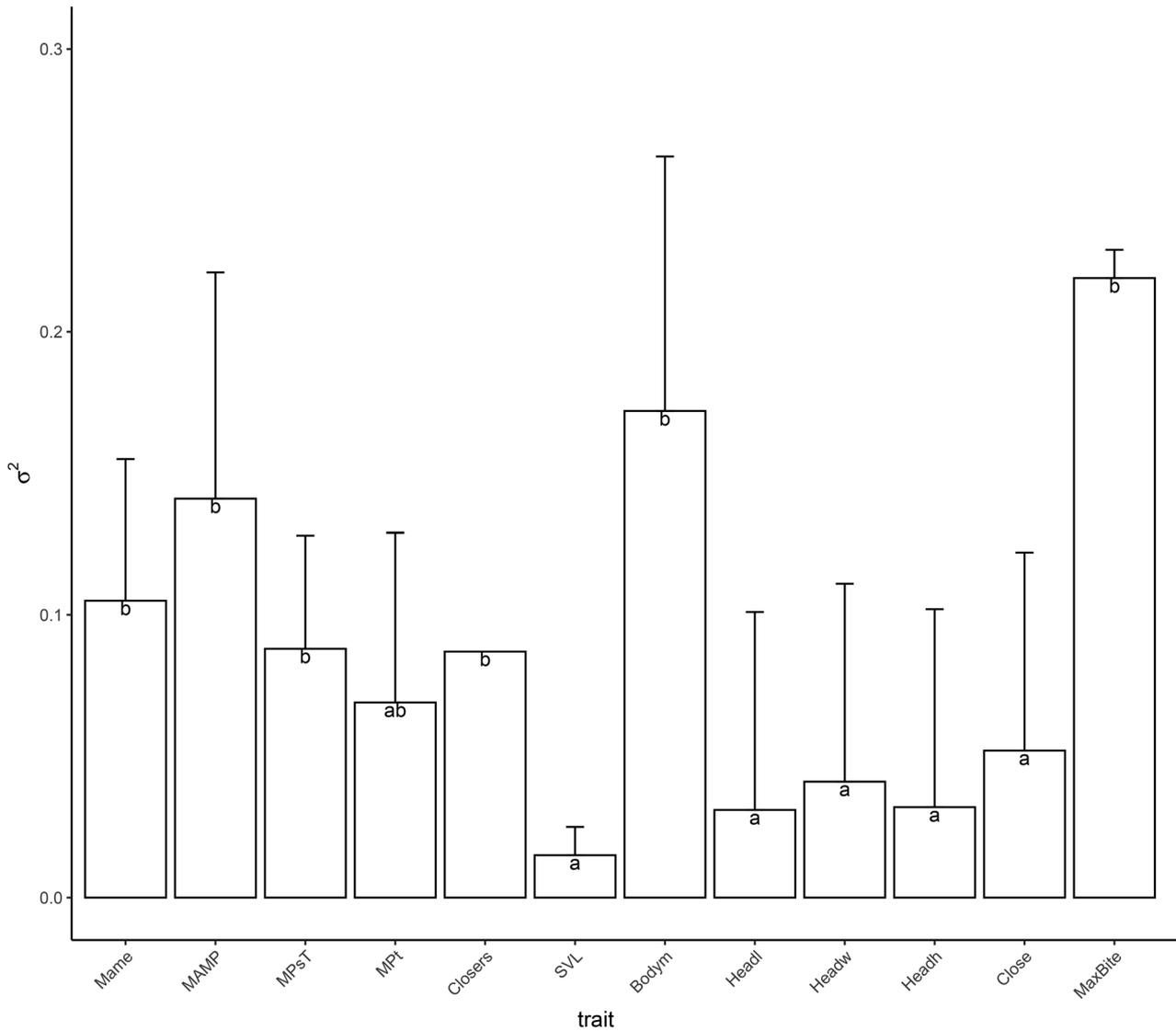


Figure 3. Histogram showing the magnitude of rate of evolution (bars, σ^2) and standard error of each trait studied here (abbreviations as in Table 1). Letters denote homogeneous groups based on ratebytree analyses ('phytools', Revell, 2012) comparisons based on χ^2 sensu Revell et al. (2018). Mame, *m. adductor mandibulae externus*; MAMP, *m. adductor mandibulae posterior*; MPST, *m. pseudotemporalis*; MPt, *m. pterygoideus*; Closers, summed muscle cross-sectional area for all closer muscles; SVL, snout-vent length; Bodym, body mass; Headl, head length; Headw, head width; Headh, head height; Close, length of the jaw closing in-lever; Maxbite, maximum bite force.

In conclusion, our results show high rates of evolution for performance and some morphological traits in a system where many-to-one mapping is common. Furthermore, traits showing greater mechanical sensitivity, such as muscle cross-sectional areas, generally showed higher rates than those involved in other functions (external head dimensions). Finally, few differences between males and females are observed in how fast the cranial system evolves, suggesting strong selection for bite force in both sexes, probably related to diet. Yet, differences in which muscles showed greater

mechanical sensitivity and the predictors of variation in bite force between sexes suggest an additional role of sexual selection in driving variation in the jaw system in males.

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REFERENCES

- Adams DC. 2012.** Comparing evolutionary rates for different phenotypic traits on a phylogeny using likelihood. *Systematic Biology* **62**: 181–192.
- Aguirre LF, Herrel A, Van Damme R, Mathysen E. 2003.** The implications of food hardness for diet in bats. *Functional Ecology* **17**: 201–212.
- Alfaro ME, Bolnick DI, Wainwright PC. 2004.** Evolutionary dynamics of complex biomechanical systems: an example using the four-bar. *Evolution* **58**: 495–503.
- Alfaro ME, Bolnick, DI, Wainwright, PC. 2005.** Evolutionary consequences of many-to-one mapping of jaw morphology to mechanics in labrid fishes. *The American Naturalist* **165**: 140–154.
- Anderson PSL, Patek S. 2015.** Mechanical sensitivity reveals evolutionary dynamics of mechanical systems. *Proceedings of the Royal Society of London B* **282**: 20143088.
- Anderson R, McBrayer LD, Herrel A. 2008.** Bite force in vertebrates: opportunities and caveats for use of a nonpareil whole-animal performance measure. *Biological Journal of the Linnean Society* **93**: 709–720.
- Arnold SJ. 1983.** Morphology, performance and fitness. *American Zoologist* **23**: 347–361.
- Arnold SJ. 1993.** Constraints on phenotypic evolution. *The American Naturalist* **140**: S85–107.
- Blomberg SP, Garland Jr T, Ives AR. 2003.** Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**: 717–745.
- Burnham KP, Anderson DR. 2004.** Multimodel inference: understanding AIC and BIC in model selection. *Sociological Methods and Research* **33**: 261–304.
- Cabrera MP, Scrocchi G, Cruz FB. 2013.** Sexual size dimorphism and allometry in *Liolaemus* of the *L. laurenti* group (Sauria: Liolaemidae): morphologic lability in a clade of lizards with different reproductive modes. *Zoologischer Anzeiger* **252**: 299–306.
- Carothers JH. 1984.** Sexual selection and sexual dimorphism in some herbivorous lizards. *The American Naturalist* **124**: 244–254.
- Claverie T, Patek SN. 2013.** Modularity and rates of evolutionary change in a power-amplified prey capture system. *Evolution* **67**: 3191–3207.
- Collar DC, Wainwright, PC. 2006.** Discordance between morphological and mechanical diversity in the feeding mechanism of centrarchid fishes. *Evolution* **60**: 2575–2584.
- Collar DC, Wainwright PC, Alfaro ME, Revell LJ, Mehta RS. 2014.** Biting disrupts integration to spur skull evolution in eels. *Nature Communications* **5**: 5505.
- Cooper WE Jr, Vitt LJ. 1988.** Orange head coloration of the male broad-headed skink *Eumeces laticeps*, a sexually selected social cue. *Copeia* **1988**: 1–6.
- Donihue CM, Brock KM, Foufopoulos J, Herrel A. 2016.** Feed or fight: testing the impact of food availability and intraspecific aggression on the functional ecology of an island lizard. *Functional Ecology* **30**: 566–575.
- Dufour CMS, Losos JB, Herrel A. 2018.** Do differences in bite force and head morphology between a native and an introduced species of anole influence the outcome of species interactions? *Biological Journal of the Linnean Society* **125**: 576–585.
- Felsenstein J. 1985.** Phylogenies and the comparative method. *The American Naturalist* **125**: 1–15.
- Haas G. 1973.** Muscles of the jaws and associated structures in the Rhynchocephalia and Squamata. In: Gans C, Parsons T, eds. *Biology of the Reptilia*, vol. 4. London: Academic Press, 285–490.
- Herrel A, Andrade DV, de Carvalho JE, Brito A, Abe A, Navas C. 2009.** Aggressive behavior and performance in the tegu lizard *Tupinambis merianae*. *Physiological and Biochemical Zoology* **82**: 680–685.
- Herrel A, Cleuren J, De Vree F. 1996.** Kinematics of feeding in the lizard *Agama stellio*. *Journal of Experimental Biology* **199**: 1727–1742.
- Herrel A, De Grauw E, Lemos-Espinal JA. 2001.** Head shape and bite performance in xenosaurid lizards. *Journal of Experimental Zoology* **290**: 101–107.
- Herrel A, De Smet A, Aguirre LF, Aerts P. 2008b.** Morphological and mechanical determinants of bite force in bats: do muscles matter? *Journal of Experimental Biology* **211**: 86–91.
- Herrel A, Holanova V. 2008.** Cranial morphology and bite force in *Chamaeleolis* lizards, adaptations to molluscivory? *Zoology* **111**: 467–475.
- Herrel A, Huyghe K, Vanhooydonck B, Backeljau T, Breugelmans K, Grba, I, Van Damme R, Irschick DJ. 2008a.** Rapid large-scale evolutionary divergence in morphology and performance associated with exploitation of a different dietary resource. *Proceedings of the National Academy of Sciences USA* **105**: 4792–4795.
- Herrel A, James RS, Van Damme R. 2007.** Fight versus flight: physiological basis for temperature dependent behavioral shifts in lizards. *Journal of Experimental Biology* **210**: 1762–1767.
- Herrel A, Spithoven L, Van Damme R, De Vree F. 1999.** Sexual dimorphism of head size in *Gallotia galloti*; testing the niche divergence hypothesis by functional analyses. *Functional Ecology* **13**: 289–297.
- Herrel A, Van Damme R, De Vree F. 1996.** Sexual dimorphism of head size in *Podarcis hispanica atrata*: testing the dietary divergence hypothesis by bite force analysis. *Netherlands Journal of Zoology* **46**: 253–262.

- Huey RB, Stevenson D. 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *American Zoology* **19**: 357–366.
- Husak JF, Lappin AK, Fox SF, Lemos-Espinal JA. 2006. Bite-force performance predicts dominance in male venerable collared lizards (*Crotaphytus antiquus*). *Copeia* **2006**: 301–306.
- Huyghe K, Vanhooydonck B, Scheers H, Molina-Borja M, Van Damme R. 2005. Morphology, performance and fighting capacity in male lizards, *Gallotia galloti*. *Functional Ecology* **19**: 800–807.
- Lappin AK, Husak JF. 2005. Weapon performance, not size, determines mating success and potential reproductive output in the collared lizard *Crotaphytus collaris*. *The American Naturalist* **166**: 426–436.
- López-Darias M, Vanhooydonck B, Cornette R, Herrel A. 2015. Sex-specific differences in ecomorphological relationships in lizards of the genus *Gallotia*. *Functional Ecology* **29**: 506–514.
- Mahler DL, Ingram T, Revell LJ, Losos JB. 2013. Exceptional convergence on the macroevolutionary landscape in island lizard radiations. *Science* **341**: 292–295.
- Martins EP, Labra A, Halloy M, Thompson JT. 2004. Large-scale patterns of signal evolution: an interspecific study of *Liolaemus* lizard headbob displays. *Animal Behaviour* **68**: 453–463.
- McBrayer LD, White TD. 2002. Bite force, behavior, and electromyography in the teiid lizard, *Tupinambis teguixin*. *Copeia* **2002**: 111–119.
- McHenry MJ. 2012. When skeletons are geared for speed: the morphology, biomechanics, and energetics of rapid animal motion. *Integrative and Comparative Biology* **52**: 588–596.
- Medina M, Ibargüengoytia NR. 2010. How do viviparous and oviparous lizards reproduce in Patagonia? A comparative study of three species of *Liolaemus*. *Journal of Arid Environments* **74**: 1024–1032.
- Meyers JJ, Nishikawa KC, Herrel A. 2018. The evolution of bite force in horned lizards: the influence of dietary specialization. *Journal of Anatomy* **232**: 214–226.
- Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop GCE*. New Orleans, LA: IEEE, 1–8.
- Molina-Borja M, Padrón-Fumero M, Alfonso-Martín MT. 1998. Morphological and behavioral traits affecting the intensity and outcome of male contests in *Gallotia galloti* family Lacertidae. *Ethology* **104**: 314–322.
- Muñoz MM. 2019. The evolutionary dynamics of mechanically complex systems. *Integrative and Comparative Biology* **59**: 705–715.
- Muñoz MM, Anderson PSL, Patek SN. 2017. Mechanical sensitivity and the dynamics of evolutionary rate shifts in biomechanical systems. *Proceedings of the Royal Society of London B* **284**: 20162325.
- Muñoz MM, Hu Y, Anderson PSL, Patek SN. 2018. Strong biomechanical relationships bias the tempo and mode of morphological evolution. *E-life* **7**: e37621.
- O'Meara BC, Ane C, Sanderson MJ, Wainwright PC. 2006. Testing for different rates of continuous trait evolution using likelihood. *Evolution* **60**: 922–933.
- Orme CDL, Freckleton RP, Thomas GH, Petzold, T, Fritz SA, Isaac NJB. 2012. CAPER: comparative analyses of phylogenetics and evolution in R. *Methods in Ecology and Evolution* **3**: 145–151.
- Paradis E, Claude J, Strimmer K. 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**: 289–290.
- Pincheira-Donoso D, Tregenza T. 2011. Fecundity selection and the evolution of reproductive output and sex-specific body size in the *Liolaemus* lizard adaptive radiation. *Evolutionary Biology* **38**: 197–207.
- Pitchers W, Wolf JB, Tregenza T, Hunt J, Dworkin I. 2014. Evolutionary rates for multivariate traits: the role of selection and genetic variation. *Philosophical Transactions of the Royal Society of London B Biology Sciences* **369**: 20130252.
- R Core Development Team. 2014. *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.
- Revell LJ. 2008. On the analysis of evolutionary change along single branches in a phylogeny. *The American Naturalist* **172**: 140–147.
- Revell LJ. 2010. Phylogenetic signal and linear regression on species data. *Methods in Ecology and Evolution* **1**: 319–329.
- Revell LJ. 2012. Phytools: an R package for phylogenetic comparative biology and other things. *Methods in Ecology and Evolution* **3**: 217–223.
- Revell LJ, González-Valenzuela LE, Alfonso A, Castellanos-García LA, Guarnizo CE, Crawford AJ. 2018. Comparing evolutionary rates between trees, clades, and traits. *Methods in Ecology and Evolution* **9**: 994–1005.
- Revell LJ, Harmon LJ, Collar DC. 2008. Phylogenetic signal, evolutionary process, and rate. *Systematic Biology* **57**: 591–601.
- Sagonas K, Pafilis P, Lymberakis P, Donihue CM, Herrel A, Valakos ED. 2014. Insularity affects head morphology, bite force and diet in a Mediterranean lizard. *Biological Journal of the Linnean Society* **112**: 469–484.
- Sanderson MJ. 2003. r8s: inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. *Bioinformatics* **19**: 301–302.
- Schliep MK. 2011. Phangorn: phylogenetic analysis in R. *Bioinformatics* **27**: 592–593.
- Schulte JA II. 2013. Undersampling taxa will underestimate molecular divergence dates: an example from the South American lizard clade *Liolaemini*. *International Journal of Evolutionary Biology* **2013**: 1–12.
- Schulte JA II, de Queiroz K. 2008. Phylogenetic relationships and heterogeneous evolutionary processes among phrynosomatine sand lizards (Squamata, Iguanidae) revisited. *Molecular Phylogenetics and Evolution* **47**: 700–716.
- Schulte JA II, Macey JR, Espinoza RE, Larson A. 2000. Phylogenetic relationships in the iguanid lizard genus

- Liolaemus*: multiple origins of viviparous reproduction and evidence for recurring Andean vicariance and dispersal. *Biological Journal of the Linnean Society* **69**: 75–102.
- Stamatakis A, Hoover P, Rougemont J. 2008.** A rapid bootstrap algorithm for the RAxML web-servers. *Systematic Biology* **57**: 758–771.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011.** MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28**: 2731–2739.
- Tavaré S. 1986.** Some probabilistic and statistical problems in the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences* **17**: 57–86.
- Valdecantos S, Lobo F, Perotti MG, Moreno Azócar DL, Cruz FB. 2019.** Sexual size dimorphism, allometry and fecundity in a lineage of South American viviparous lizards (*Liolaemidae*: *Phymaturus*). *Zoologischer Anzeiger* **279**: 152–163.
- Vanhooydonck B, Cruz FB, Abdala CS, Moreno Azócar DL, Bonino MF, Herrel A. 2010.** Sex-specific evolution of bite performance in *Liolaemus* lizards (Iguania: Iguanidae): the battle of the sexes. *Biological Journal of the Linnean Society* **101**: 461–475.
- Vervust B, Van Dongen S, Van Damme R. 2009.** The effect of preservation on lizard morphometrics – an experimental study. *Amphibia-Reptilia* **30**: 321–329.
- Wainwright PC. 2007.** Functional versus morphological diversity in macroevolution. *Annual Review in Ecology Evolution and Systematics* **38**: 381–401.
- Wainwright PC, Alfaro ME, Bolnick DI, Hulseley CD. 2005.** Many-to-one mapping of form to function: a general principle in organismal design? *Integrative and Comparative Biology* **45**: 256–262.
- Wittorski A, Losos JB, Herrel A. 2016.** Proximate determinants of bite force in *Anolis* lizards. *Journal of Anatomy* **228**: 85–95.
- Zablocki-Thomas PB, Karanewsky CJ, Pendleton JL, Aujard F, Pouydebat E, Herrel A. 2018.** Drivers of in vivo bite performance in wild brown mouse lemurs and a comparison with the grey mouse lemur. *Journal of Zoology* **305**: 180–187.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Liolaemid lizard species studied here.

Table S2. Summary of phylogenetic signal for the muscle cross-section area, head dimensions, body size, bite force, and proportion of plants and hard parts in the diet.

Table S3. Phylogenetic generalized least squares models (PGLS) evaluating the phylogenetically based residuals of morphological traits (cross-section area of muscles and head dimensions) on phylogenetically based residuals of bite force in the Liolaemidae species studied here (snout–vent length was the independent variable).

Table S4. Comparison between morphological variables (cross-sectional area of muscle, snout–vent length, body mass and head dimensions) of the rate of change σ^2 , the relationship between them (folding times), the relative likelihood (r likelihood) and P -values corresponding to χ^2 comparison.

Table S5. Interspecific standard deviation (SD) of traits from the complete sample (all) males (δ) and females (δ) in the Liolaemidae species studied.

Table S6. Generalized least squares models (GLS) of rates across traits (Rate) and the corresponding standard deviations (SD) and from mechanical sensitivity (MS, the relationship between morphological traits and bite force) indicators (r^2 and slope).