




SYMPOSIUM

Convergent Evolution of Elongate Forms in Craniates and of Locomotion in Elongate Squamate Reptiles

Philip J. Bergmann ,* Sara D. W. Mann,* Gen Morinaga,^{1,*†} Elyse S. Freitas[‡] and Cameron D. Siler[‡]

*Department of Biology, Clark University, Worcester, MA, USA; [†]Department of Integrative Biology, Oklahoma State University, Stillwater, OK, USA; [‡]Department of Biology and Sam Noble Oklahoma Museum of Natural History, University of Oklahoma, Norman, OK, USA

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¹E-mail: pbergmann@clarku.edu

Synopsis Elongate, snake- or eel-like, body forms have evolved convergently many times in most major lineages of vertebrates. Despite studies of various clades with elongate species, we still lack an understanding of their evolutionary dynamics and distribution on the vertebrate tree of life. We also do not know whether this convergence in body form coincides with convergence at other biological levels. Here, we present the first craniate-wide analysis of how many times elongate body forms have evolved, as well as rates of its evolution and reversion to a non-elongate form. We then focus on five convergently elongate squamate species and test if they converged in vertebral number and shape, as well as their locomotor performance and kinematics. We compared each elongate species to closely related quadrupedal species and determined whether the direction of vertebral or locomotor change matched in each case. The five lineages examined are obscure species from remote locations, providing a valuable glimpse into their biology. They are the skink lizards *Brachymeles lukbani*, *Lerista praepepedita*, and *Isopachys anguinoides*, the basal squamate *Dibamus novaeguineae*, and the basal snake *Malayotyphlops cf. ruficaudus*. Our results support convergence among these species in the number of trunk and caudal vertebrae, but not vertebral shape. We also find that the elongate species are relatively slower than their limbed counterparts and move with lower frequency and higher amplitude body undulations, with the exception of *Isopachys*. This is among the first evidence of locomotor convergence across distantly related, elongate species.

Introduction

Elongate forms have evolved many times in most major lineages of vertebrates (Lande 1978; Greer 1991; Caldwell 2003; Wiens et al. 2006; Brandley et al. 2008; Ward and Mehta 2014; Law et al. 2019). Therefore, their convergent evolution is a major theme in vertebrate evolution. However, despite much study of some focal clades of elongate species, their evolutionary distribution across vertebrates, and whether these independent instances are developmentally (Infante et al. 2018; Leal and Cohn 2018), functionally (Bergmann and Irschick 2010; Barros et al. 2011), and ecologically convergent (Kendrick 1991; Grizante et al. 2012) remains poorly understood.

Published work shows that elongate forms may have evolved differently across vertebrate clades. For example, in fishes, elongation can occur through elongation of the head, trunk, or tail, and by addition and/or lengthening of vertebrae (Ward and Brainerd 2007; Ward and Mehta 2014). Salamanders evolve body elongation through the lengthening of the trunk and tail and by either addition or elongation of the vertebrae (Parra-Olea and Wake 2001; Wake et al. 2011). In contrast, body elongation in squamates (lizards and snakes) occurs through the addition of vertebrae and the lengthening of the body or both the body and tail (Bergmann and Irschick 2012; Wiens et al. 2006; Brandley et al. 2008; Bergmann and Morinaga 2019). Finally, in

mustelids and whales, the regional lengthening of trunk vertebrae is responsible for elongation of the body rather than increasing the number of vertebrae (Buchholz 2001; Law et al. 2019).

Understanding the evolution of locomotion in elongate forms also remains poorly understood. The transition from quadrupedal to elongate and limb-reduced form involves a transition from limb-based to axial propulsion by body undulation (Gans 1986; Renous et al. 1998). This seems obvious, yet transitional forms use limb and axial propulsion simultaneously (Gans and Fusari 1994; Renous et al. 1999; Bergmann and Irschick 2010), and sprawling quadrupeds also undulate their bodies (Renous et al. 1995; Renous et al. 1998). Some evidence suggests that elongate forms with reduced or absent limbs undulate their bodies at lower frequencies and relatively larger amplitudes than quadrupedal forms (Gans and Fusari 1994; Renous et al. 1999; Morinaga and Bergmann 2019). However, it remains unknown whether convergent elongate limbless forms have also convergently evolved their locomotion relative to their limbed relatives.

The evolution of elongate body forms is also related to various ecological variables. For example, gymnophthalmid lizards that inhabit looser soil have longer, more tapering snouts and are more elongate with smaller limbs (Barros et al. 2011; Grizante et al. 2012). Australian *Lerista* species that are more snake-like tend to have smaller range sizes, possibly due to lower dispersal abilities (Lee et al. 2013). In lizards, elongate limbless forms that live in cluttered habitats tend to have long tails, while fossorial species tend to have shorter tails (Wiens et al. 2006). However, fossorial elongate lizards live in a wide diversity of habitats from arid deserts with loose sand substrates to wet tropical forests with packed, water-saturated soil (Kendrick 1991; Branch 1998; Siler et al. 2011; Grizante et al. 2012). These differences in habitat suggest that convergently elongate taxa may need to adopt divergent locomotor strategies to negotiate their respective habitats.

Here, we set the context for the study of elongate body forms by surveying their evolutionary distribution among vertebrates, and then focus on five convergently elongate squamate species to test for convergent evolution of their locomotion. First, we survey the distribution of elongate bodies in craniates (vertebrates and their sister group, the Agnatha). We provide a catalog of clades in which this body form has evolved, determine how many times it has evolved, and quantify its likelihood of being ancestral. Second, we test for convergence of surface

locomotor performance and kinematics in five focal examples of convergently elongate squamate species. Our five elongate species represent obscure and understudied taxa that live in relatively remote locations: *Lerista praepedita* from Australia, *Brachymeles lukbani*, *Dibamus anguinoides*, and *Malayotyphlops cf. ruficaudus* from the Philippines, and *Isopachys anguinoides* from Thailand. These five examples have evolved independently, sharing most recent common ancestor's 45–205 mya (Zheng and Wiens 2016).

Methods

Phylogeny and elongation data collection and analysis

We quantified the number of times elongate body forms evolved across the Craniata using evolutionary Markov models. We used a published family-level tree of all life (Scholl and Wiens 2016) and edited it in R v3.6.1 (R Core Team 2019) with the “geiger” (Pennell et al. 2014) and “phytools” (Revell 2012) packages. We pruned the phylogeny to include only vertebrates, manually added the Polypteridae as sister to the other ray-finned fishes (Rabosky et al. 2018) and combined our vertebrate phylogeny with that of the Agnatha (Kuraku and Kuratani 2006), resulting in a phylogeny of 783 families. Only nine nodes were unresolved, so we resolved them arbitrarily and set their branch lengths equal to 0.01 million years. We made small adjustments to branch lengths to ensure ultrametry by minimizing sum of square differences between non-ultrametric and ultrametric trees (Revell 2012).

We discretely categorized each family as either having evolved one or more elongate species or not by surveying the elongation literature (cited in “Introduction” section), and qualitatively surveying online resources. We did the latter by searching for each family using Google and observing a range of images for each. We vetted sources to ensure that observed images represented the identified clades. Then a visual determination of whether observed species were elongate was made. Any borderline phenotypes were considered not elongate. While this approach is not quantitative, most examples were obvious, and our intent was to provide a comprehensive starting point to spur research in previously overlooked clades. These data (Supplementary Table S1) and the phylogeny are available in Supplementary Material.

We modeled the evolution of elongation in craniates by fitting two discrete Markov models to the data with likelihood (Schluter et al. 1997) using the “ape” package (Paradis and Schliep 2019). The

equal rates model estimated a single rate of evolution from elongate to non-elongate and vice versa, while the unequal rates model estimated a different rate for each direction. We used AIC to select the best model (Burnham and Anderson 2002).

We reconstructed ancestral character states and the evolution of elongation using stochastic character mapping (Huelsenbeck et al. 2003; Bollback 2006) as implemented in phytools (Revell 2012). We ran 1000 stochastic character maps under the selected equal rates model while estimating the root state empirically. This analysis quantified uncertainty by providing a probability of each state at each node.

Morphological and locomotor convergence in five squamate taxa

During fieldwork in Western Australia, the Philippines, and Thailand between 2006 and 2017, we collected locomotion data for five squamate species representing independent origins of elongate limbless (or almost limbless) body forms (Zheng and Wiens 2016). These were the scincid lizards *B. lukbani*, *L. praepedita*, *I. anguinoides*, the basal squamate *D. novaeguineae*, and the basal snake *M. cf. ruficaudus*. These data allow us to test for the first time whether multiple independent origins of this body form are convergent in their locomotion.

We tested for convergence in vertebral and locomotor variables by using a sister group comparison approach, comparing each elongate species to a short-bodied quadrupedal species. The quadrupedal comparison species were *B. kadwa*, *L. elegans*, and *Parvosцинus steerei*, respectively, for the first three comparisons. *Dibamus* and *Malayotyphlops* have no close relatives that have limbs because *Dibamus* is sister to all other squamates and *Malayotyphlops* is a member of Serpentes (snakes), sister to Iguanian + Anguimorph lizards (Zheng and Wiens 2016). Hence, we included two lizard species with generalized pentadactyl morphology for comparison: the lacertid *Eremias arguta* and the phrynosomatine *Uta stansburiana*. We compared both *Uta* and *Eremias* to both *Dibamus* and *Malayotyphlops* to bracket possible comparisons. In all of our analyses, we tested for convergence by comparing the change in morphological and locomotor variables from quadrupedal to elongate species within each comparison and evaluate if the direction of change is the same in each instance.

Morphological data collection and analysis

To test whether the five limbless species evolved to be elongate in the same way, we collected data on

vertebral number and dimensions for all quadrupedal and elongate species. We used X-rays to count the number of trunk and caudal vertebrae, and measured length and width of a subset of vertebrae using ImageJ (Rasband 2019). We measured the two posterior cervical vertebrae (defined as those without ribs), the fourth and fifth ribbed vertebrae anterior to the sacral vertebrae, the two anterior caudal vertebrae, and the fifth and sixth caudal vertebrae without enlarged transverse processes. These sets of vertebrae are distinct and distributed along the length of the vertebral column (Bergmann and Morinaga 2019).

Obtaining these data was challenging because they require dorsal view X-rays of sufficient quality to measure vertebrae that are frequently less than a millimeter in length. Hence, sample sizes were one to two individuals per species. We also had to substitute several species that we had locomotor data for species that we had vertebral data for: we substituted *P. steerei* with *Pinoyscincus jagori*, *Malayotyphlops* with *Rhamphotyphlops ericinus*, and *E. arguta* with *E. strauchi*. The substituted species are closely related to those they replaced relative to the distant relationship of our five examples (Zheng and Wiens 2016), so these substitutions are unlikely to bias results. The morphological data are available in Supplementary Table S2.

We calculated the aspect ratio of each vertebra as length/width and the length ratio as vertebral/head length (HL). We used HL as a proxy for animal size, given that it is independent of vertebral length, unlike snout–vent length (SVL), and because it is used in past studies of elongation (Stokely 1947; Wiens and Slingluff 2001; Siler and Brown 2011). We compared vertebral numbers, aspect ratios, and length ratios for all quadrupedal to all elongate species using Mann–Whitney U tests because the data had small sample sizes and were not normally distributed.

Locomotor trials

The procedures that we used for capturing animals, running locomotor trials, and releasing or preserving animals are published elsewhere (Bergmann and Irschick 2010; Bergmann et al. 2017). Appropriate government agencies and IACUC at our respective universities approved all procedures with live animals. Prior to trials, we painted dots on each animal dorsally at the level of the occiput, pectoral girdle, midback, and pelvic. Limbless lizards had subtle external depressions at the location of the pectoral girdle. *Malayotyphlops* lacked these, so we placed the

pectoral dot at 15% of SVL posterior to the occiput, and the pelvic dot just anterior to the cloaca. We conducted locomotor trials on a field-portable race-track with a sieved substrate of 0.25–0.50 mm diameter particles to a depth of 5 mm. For *Lerista*, *Uta*, and *Eremias*, the substrate consisted of sand, whereas for the other species, it consisted of sieved soil from the local environment. These substrates have different properties but represent natural substrates that each species moves on. We recorded trials from dorsal views at 240–250 Hz (Supplementary Table S3). Trials were conducted during the afternoon, with ambient temperatures $>26^{\circ}\text{C}$. We recorded two to five trials per individual, retaining one trial per individual with the highest maximal velocity, in which it moved with the fewest pauses and without touching the sides of the racetrack.

Quantification of locomotion from videos

We digitized painted dots using DLTdv5 (Hedrick 2008) in MATLAB v2017a (MathWorks, Natick, MA, USA) to obtain xy coordinates for each dot. We calculated the cumulative distance moved by the occiput during and fit a sixth order smoothing spline to the cumulative distance and time data in MATLAB, using the first derivative as an estimate of frame-by-frame velocity (Bergmann et al. 2017). From this, we calculated average velocity while the animal was moving and maximum velocity as measures of locomotor performance.

We also used digitized dot data to obtain kinematic variables. To do this, we used R code we developed previously (Morinaga and Bergmann 2019) to quantify the amplitude, spatial wavelength, and frequency of undulations at the occiput, pectoral, midback, and pelvic dots. We divided velocities, amplitudes, and wavelengths by HL, our proxy for size, to make these values relative and comparable among species. Locomotor data are available in Supplementary Table S4.

Analysis of locomotor data

All analyses were done in R v3.6.1. We analyzed each locomotor variable separately and ran a principal component analysis (PCA) on a correlation matrix that included all 14 locomotor variables (Supplementary Table S5) to understand how locomotion compared among species. Values for the occiput point were missing for two trials, so we imputed them with a PCA model using the “missMDA” package (Josse and Husson 2016), allowing us to make best use of data from rare specimens.

We analyzed locomotor variables using mixed effects modeling (Bolker et al. 2009; Crawley 2012) followed by contrasts for specific comparisons between quadrupedal and elongate species (Bretz et al. 2010). We fitted a mixed effects model for each locomotor variable and Principal Component (PC) factor scores for the first two PCs using the “lme4” package (Bates et al. 2015). We defined a variable called “comparison” that represented the phylogenetic comparisons: *Brachymeles*, *Lerista*, *Isopachys/Parvosцинus*, and the remaining species. For each model, the locomotor or PC variable was the response, comparison was a random effect, and species nested in comparison was a fixed effect. Body temperature was included as a random factor but was eliminated from all models because of its negligible effect. To evaluate model fit, we calculated conditional and marginal R^2 using the “MuMIn” package (Barton 2009). Conditional R^2 (R_c^2) is the proportion of response variance explained by fixed and random effects, while marginal R^2 (R_m^2) is the proportion explained by fixed effects only (Nakagawa and Schielzeth 2013).

For the a priori defined contrasts, we calculated estimated marginal means and used t -tests, as implemented in the “emmeans” package (Searle et al. 1980). We corrected for multiple comparisons using the false discovery rate (Williams et al. 1999).

Results

Evolution of body elongation in the Craniata

We found that body elongation evolved and reverted to non-elongate forms with approximately equal rates in the 783 family-level clades of craniates surveyed ($\Delta_{\text{AIC}}=1.2$). Stochastic character mapping suggested that body elongation evolved an average of 68 times and reverted back to a non-elongate form 14 times on the phylogeny (Fig. 1).

All Agnatha are elongate, and this resulted in a $P=0.21$ that the ancestor of craniates was elongate. The ancestor of vertebrates had no probability of being elongate. We classified 130 of 783 clades of craniates as containing elongate species, whether due to elongation of the body, head, and/or tail (all identified in Supplementary Table S1). Many of these taxa were members of larger, elongate clades, resulting in fewer independent origins. We identified 7 independent origins of elongation in the Chondrichthyes and 32 origins in the Actinopterygii. We note no origins in Aves and only two origins in the Mammalia: the Mustelidae and Cetacea. The three origins of elongation in the Amphibia and few origins in the Squamata are underestimates because elongation has evolved

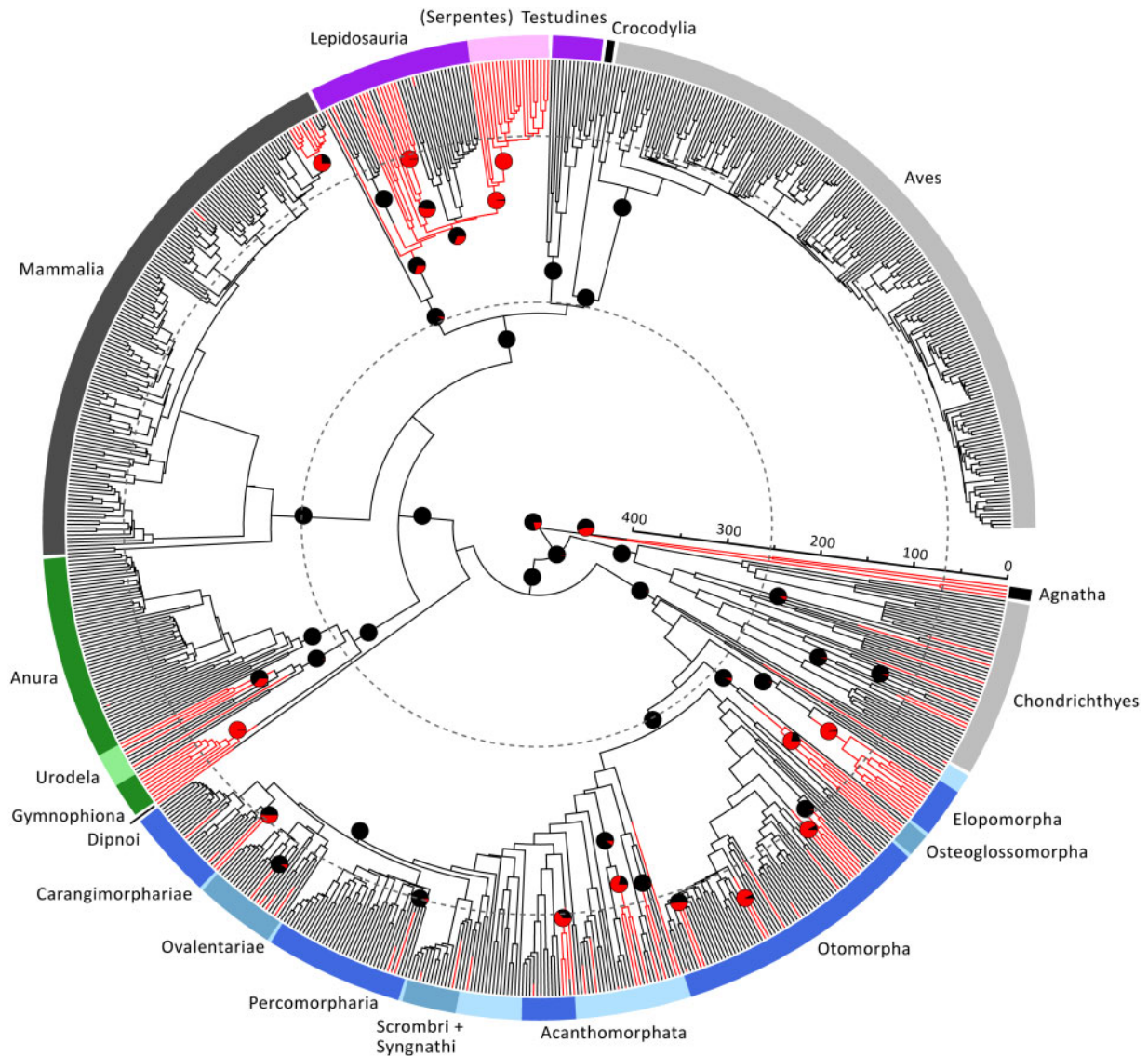


Fig. 1 Family-level phylogeny of the Craniata showing the distribution and evolution of elongate body forms. Phylogenetic branches show one stochastic character map with red branches indicating elongate body forms and black representing non-elongate forms. Pie charts represent the probability of each body form as ancestral at select nodes based on 1000 stochastic character maps. Some pies have been moved slightly for clarity. Colored ring around the phylogeny and labels identify higher level taxa. Note that blues identify the Actinopterygii and that Serpentes is nested within the Lepidosauria.

multiple times within numerous families, particularly Plethodontidae, Anguidae, Scincidae, and Gymnophthalmidae (Greer 1991; Parra-Olea and Wake 2001; Wiens and Slingluff 2001; Wiens et al. 2006). Other sources have suggested at least 6 origins in the Amphibia, and 25 in the Squamata (Parra-Olea and Wake 2001; Wiens et al. 2006; Pough et al. 2016).

Convergence of vertebral morphology in five focal squamate taxa

In focusing on the five examples of elongate squamates, we found evidence of convergence in vertebral

numbers but not dimensions. All five elongate species had more presacral (diff = 27.0, $U = 25$, $P = 0.012$) and fewer caudal (diff = 10, $U = 6$, $P = 0.207$, not significant) vertebrae than their quadrupedal relatives (Fig. 2A). There were no significant differences in vertebral aspect ratio between quadrupedal and elongate species (Fig. 2B). This may be due to low sample size because our graphs suggest that cervical vertebrae are relatively more elongate in snake-like taxa and caudal vertebrae are relatively less elongate (Fig. 2B). No significant differences or patterns were observed among vertebral length ratios (Supplementary Fig. S1).

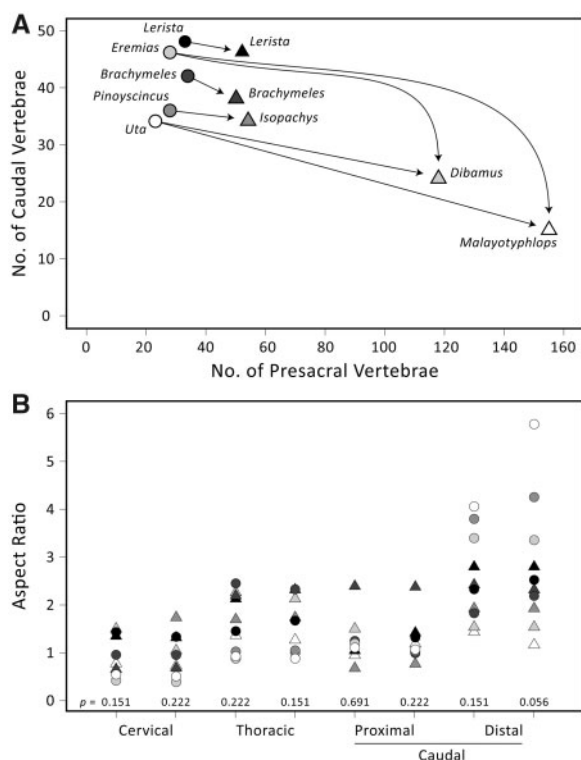


Fig. 2 Vertebral numbers (A) and aspect ratios (length/width) (B) in five snake-like species (triangles) and their lizard-like relatives (circles). In (A), species comparisons are connected with arrows and labeled. Note that both *Eremias* and *Uta* are compared to both *Dibamus* and *Rhamphotyphlops* (see text). In (B), species are organized from darkest to lightest gray: *Lerista*, *Brachymeles*, *Pinosincus*/*Isopachys*, *Eremias*/*Dibamus*, and *Uta*/*Rhamphotyphlops*. P-values are for Mann–Whitney U tests comparing lizard-like and snake-like species.

Convergence of locomotion in five focal squamate taxa

We also found evidence of convergence in locomotion of the five focal elongate species. The first three PCs of our PCA explained 76% of the variance (Supplementary Table S5). PC-1 represented a positive relationship between velocity and frequency, trading off with amplitude and wavelength; high values of PC-1 corresponded with high velocities and frequencies of undulation but low amplitudes and wavelengths (Supplementary Table S5). PC-2 characterized a trade-off between amplitude and wavelength, with high values indicating higher amplitudes and lower wavelengths (Supplementary Table S5). PC-3 was difficult to interpret and is not considered further.

Factor scores for the first two PCs showed clear differences in locomotion between quadrupedal and elongate species, with the two groups occupying non-overlapping areas of locomotion space (Fig. 3).

Mixed effects models of PC factor scores provided more detail because they considered the paired nature of the species. These models explained 68% and 75% of variance in PC-1 and PC-2, respectively, with the fixed effect of species nested in comparison explaining most of this (Supplementary Table S6). In all comparisons except that of *Isopachys* to *Parvosincus*, we found that elongate species had significantly lower PC-1 factor scores than their quadrupedal relatives (Fig. 4A). Elongate species had significantly higher PC-2 values than quadrupedal species, except for *Brachymeles* (Fig. 4B).

To test these patterns more directly, we made the same comparisons for each locomotor variable. These mixed effects models explained 36–71% of variance, the majority of which was due to fixed effects (Supplementary Table S6). Elongate species tended to have convergently lower relative average velocities than quadrupedal species in most comparisons (Fig. 5A), but no differences in relative maximum velocity (Supplementary Fig. S2). We found similar patterns for occipital, pectoral, midbody, and pelvic points, so we present data for the occiput in Fig. 5 and the other points in Supplementary Figs S3–S5. Elongate species, with the exception of *Isopachys*, convergently adopted lower undulatory frequencies than quadrupedal species, although some of these comparisons were not significant (Fig. 5B). Additionally, all elongate species had significantly higher relative amplitudes than quadrupedal species (Fig. 5C). They also tended to have lower relative wavelengths than the quadrupeds (but none of these comparisons were significant; Fig. 5D).

Discussion

Evolutionary distribution of elongate forms

Here we presented the first craniate-wide survey of elongate body forms. We found that although elongation occurred in at least 130 family-level clades (Fig. 1), it evolved about 75 times in craniates, and may have reverted back to non-elongate form up to 14 times. This difference in numbers is due to some families forming larger clades that are elongate (e.g., Serpentes, Gymnophiona, Anguilliformes, and Agnatha) and others in which this phenotype has evolved multiple times independently (e.g., Squamata and Amphibia). Many of these taxa should be further studied with respect to elongation, including Amphibia, Chondrichthyes, and Actinopterygii (Fig. 1). Elongate body forms have evolved only twice in mammals and never in birds, a pattern that is strikingly apparent in our analysis (Fig. 1). Equally interesting is the reversion of body form

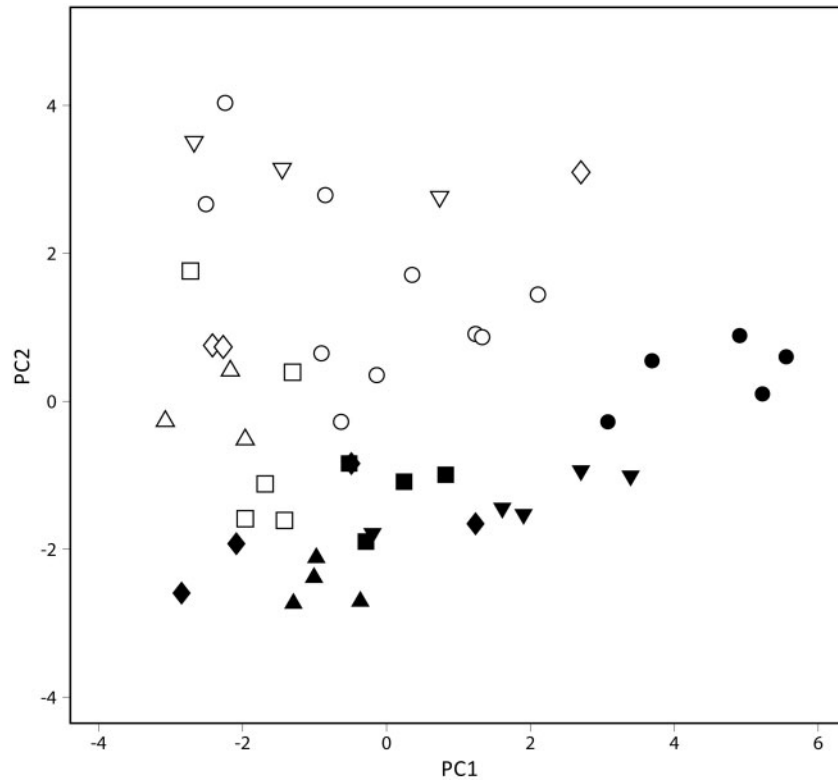


Fig. 3 Principal component factor scores for PC-1 and PC-2 based on all locomotor variables (relative velocities, amplitudes, and wavelengths, and absolute frequencies). Data are presented for lizard-like (closed symbols) and snake-like (open symbols) species. Squares: *B. kadwa* and *B. lukbani*; circles: *L. elegans* and *L. praepedita*; diamonds: *P. steerei* and *I. anguinoidea*; upward triangles: *E. arguta* and *D. novaeguineae*; downward triangles: *U. stansburiana* and *Malayotyphlops* sp.

from elongate to not, and this has been documented in *Brachymeles* skink lizards (Siler and Brown 2011). Other identified reversions have focused primarily on the number of digits, which correlate with body elongation, such as in gymnophthalmid lizards and the amphisbaenian, *Bipes* (Brandley et al. 2008). The mechanisms of such evolutionary reversals are an area of needed future study.

Our analysis firmly rejected an elongate vertebrate ancestor and assigned a probability of only 0.21 for elongation in the ancestral craniate (Fig. 1). However, there are a number of challenges in determining this. The Agnatha diverged from vertebrates ~500 mya, making ancestral reconstruction challenging, and all extant agnathans are specialized ectoparasites (Gill et al. 2003), a lifestyle that may be associated with an elongate body form. Many fossil agnathans are less elongate than extant species (Janvier 1981; Miyashita et al. 2019), lending support to a low probability of ancestral elongation. Successive outgroups are difficult to compare to craniates due to fundamentally different body plans. For example, the craniate sister group, the Urochordata, has a larval stage that is not very elongate and a sessile and bulbous adults (Satoh et al. 2014). Further successive

sister groups are the Cephalochordata, which are elongate, and the Ambulocaria (Echinoderms + Hemichordates), which are pentamerously symmetrical. More broadly speaking, elongation also appears frequently outside the craniates in the animal tree of life (Brusca et al. 2016).

Convergent evolution of morphology and locomotion in five squamate taxa

We showed that the five focal elongate squamate species were convergent in aspects of their vertebral phenotype and locomotion. Morphologically, all five species convergently increased the number of presacral vertebrae and decreased the number of caudal vertebrae compared to quadrupedal species (Fig. 2A). However, we found no evidence of convergent changes in vertebral dimensions. Published work on six other squamate clades with elongate and extant transitional species, including *Brachymeles* and *Lerista*, also found strong evidence that body elongation evolved through the addition but not lengthening of vertebrae (Bergmann and Morinaga 2019). Our three additional examples suggest a common mechanism across fossorial

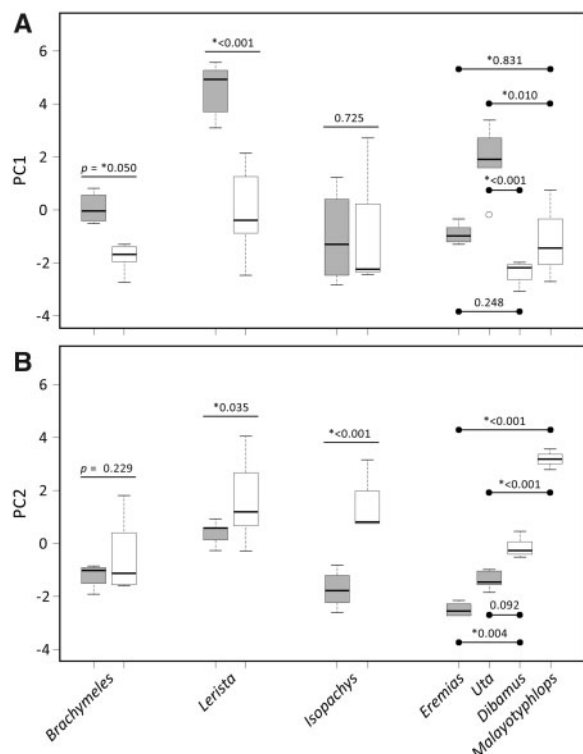


Fig. 4 Principal component factor scores for PC-1 (A) and PC-2 (B) for lizard-like (gray) and snake-like (white) species.

Comparisons are *B. kadwa* to *B. lukbani*; *L. elegans* to *L. praepedita*; *P. steerei* to *I. anguinoides*; and *E. arguta* and *U. stansburiana* to *D. novaeguineae* and *Malayotyphlops* sp. *P*-values from post hoc contrasts of mixed effects models are presented, with asterisks indicating significant differences and lines connecting species being compared.

squamates. All five of our examples are at least semi-fossorial, and even the three skink species with intermediate tail lengths have lost caudal vertebrae. This is predicted by the hypothesis of snake-like forms evolving to be either short-tailed burrowers or long-tailed surface dwellers (Wiens et al. 2006).

This study is the first to show convergence in locomotion among elongate forms. The snake-like species that we studied convergently moved slower, with lower frequency and higher amplitude undulations than their lizard-like relatives, with *I. anguinoides* being the exception (Fig. 5). Our findings may also apply to long-tailed surface dwellers because the European glass lizard, *Ophisaurus apodus*, uses undulations that are lower frequency, higher amplitude, and lower wavelength than the limbed skink, *Mochlus fernandi* (Morinaga and Bergmann 2019). However, in three species of intermediate/reduced-limbed *Lerista*, one of the more elongate species adopted higher amplitude undulations, but the other did not (Gans and Fusari 1994). However, that study only considered three intermediate species.

As we show on our phylogeny, there are many non-squamate examples of elongate body forms, but convergence in locomotion has not been tested in these clades. Nevertheless, the locomotion of some clades has been studied, allowing for comparison. Congruent with our findings, elongate fishes exhibit slower speeds than non-elongate fishes during both swimming and terrestrial excursions (Pace and Gibb 2009; Ward et al. 2015). Elongate fishes also tend to use lower frequency, higher amplitude undulations than non-elongate species (Gillis 1998; Horner and Jayne 2008; Herrel et al. 2011; Pace and Gibb 2011). These findings are limited to anguilliform swimmers such as eels and ropefish, and other elongate taxa that use different modes of swimming also exist (Sfakiotakis et al. 1999; Blake 2004). Locomotion has also been studied in ferrets (*Mustela putorius furo*) traveling in unconstrained racetracks and simulated burrows, showing that they moved in similar ways and at comparable speeds in both situations, suggesting adaptation of body form to fossorial locomotion (Horner and Biknevicius 2010).

Our work shows convergence at a number of biological levels of organization in the evolution of elongate squamates. Past work has shown that elongate forms have evolved convergently (Lande 1978; Greer 1991; Wiens et al. 2006), sometimes through convergent changes in body parts (Brandley et al. 2008; Morinaga and Bergmann 2017; Bergmann and Morinaga 2019), and sometimes through divergent changes (Ward and Brainerd 2007; Wake et al. 2011). We showed that the convergent elongate forms arose through convergent changes in vertebral characteristics and locomotor kinematics, leading to slower locomotor performance. This matches predictions that convergence at a particular level of biological organization will result in convergence at higher levels (Losos 2011). However, our findings for *Isopachys* go against these predictions because although it is convergent in body form and vertebral morphology with the other elongate species, its locomotion is not. This may be because of a lack of closely related quadrupedal species for comparison, or because of actual non-convergence in locomotion.

Unanswered questions in the evolution of elongate forms

Our work provides the first craniate-wide analysis of evolution of elongate forms and the first test of the hypothesis that convergent elongate forms move convergently. Nevertheless, our work reveals numerous gaps in the current understanding of the evolution of this phenotype. Although we document at

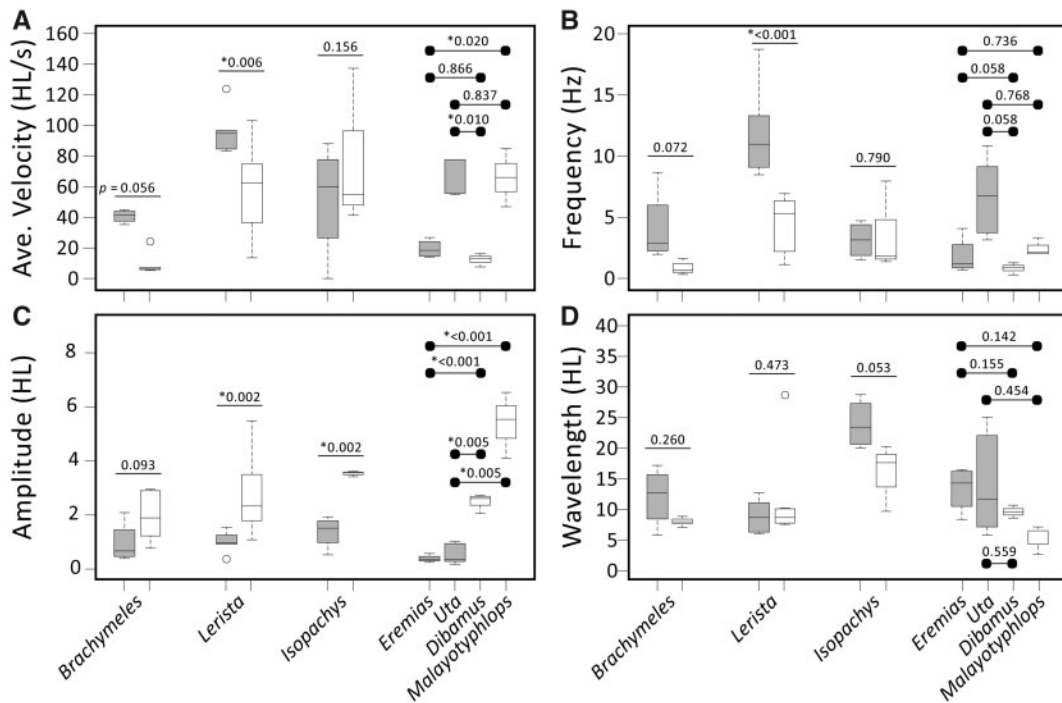


Fig. 5 Locomotor performance and kinematics for limbed (gray) and snake-like (white) species. Data are presented for (A) average relative velocity and (B) frequency, (C) Amplitude and (D) wavelength of undulations of the occipital point. Comparisons are *B. kadwa* to *B. lukbani*; *L. elegans* to *L. praepedita*; *P. steerei* to *I. anguinoides*; and *E. arguta* and *U. stansburiana* to *D. novaeguineae* and *Malayotyphlops* sp. *P*-values from post hoc contrasts of mixed effects models are presented, with asterisks indicating significant differences and lines connecting species being compared.

least 75 instances of elongate forms evolving among craniates, most remain poorly studied. There is a need for tests of how elongate forms have evolved in each lineage and whether those forms coincide with fin/limb reduction. Even large and recognizable clades like salamanders (Urodela) remain poorly characterized in terms of body elongation. Comparing how body form, body parts, and function have evolved in the range of elongate taxa from short-tailed to long-tailed, a pattern also seen in fishes (Ward and Brainerd 2007), would provide a more nuanced understanding of how this form evolves divergently. More species-level, phylogenetically informed analyses of morphological evolution will open research opportunities across biology, including developmental mechanism (Bejder and Hall 2002; Shapiro et al. 2003; Young et al. 2009; Leal and Cohn 2018), locomotion (Gans and Fusari 1994; Renous et al. 1998; Bergmann and Irschick 2010; Ward et al. 2015), and ecology (Kendrick 1991; Pough et al. 1997; Barros et al. 2011).

The non-convergence in locomotion of *Isopachys* with our other focal species may lie in the niches used by each, illustrating another understudied area. Although many elongate taxa are convergently fossorial, closer examination shows large differences

in their respective habitats. In our five focal species, the substrate ranges from loose, dry sand (*Lerista*) to wet rainforest soil (*Brachymeles*). These substrates differ in the materials that they are made of, particle size, amount of plant debris mixed into them, degree of packing, and moisture content (Branch 1998; Wilson and Swan 2005; Siler et al. 2011). We collected *Isopachys* in dry beach sand at the base of coniferous trees, a distinct habitat from the other examples, suggesting a lack of convergence in habitat use despite all lineages being semi-fossorial. Ultimately, the framework for studies of elongate taxa is convergent evolution and understanding how predictable or historically contingent evolution is (Gould 2002; Losos 2011; Muschick et al. 2012).

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Supplementary data

Supplementary data available at *ICB* online.

References

- Barros FC, Herrel A, Kohlsdorf T. 2011. Head shape evolution in gymnophthalmidae: does habitat use constrain the evolution of cranial design in fossorial lizards? *J Evol Biol* 24:2423–33.
- Barton K. 2009. Mu-min: multi-model inference (<http://R-ForgeR-project.org/projects/mumin/>).
- Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. *J Stat Softw* 67:48.
- Bejder L, Hall BK. 2002. Limbs in whales and limblessness in other vertebrates: mechanisms of evolutionary and developmental transformation and loss. *Evol Dev* 4:445–58.
- Bergmann PJ, Irschick DJ. 2010. Alternate pathways of body shape evolution translate into common patterns of locomotor evolution in two clades of lizards. *Evolution* 64:1569–82.
- Bergmann PJ, Irschick DJ. 2012. Vertebral evolution and the diversification of squamate reptiles. *Evolution* 66:1044–58.
- Bergmann PJ, Morinaga G. 2019. The convergent evolution of snake-like forms by divergent evolutionary pathways in squamate reptiles. *Evolution* 73:481–96.
- Bergmann PJ, Pettinelli KJ, Crockett ME, Schaper EG. 2017. It's just sand between the toes: how particle size and shape variation affect running performance and kinematics in a generalist lizard. *J Exp Biol* 220:3706–16.
- Blake RW. 2004. Fish functional design and swimming performance. *J Fish Biol* 65:1193–222.
- Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White J-S. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol Evol* 24:127–35.
- Bollback JP. 2006. Simmap: stochastic character mapping of discrete traits on phylogenies. *BMC Bioinformatics* 7:88.
- Branch W. 1998. Field guide to the snakes and other reptiles of Southern Africa. Sanibel Island (FL): Ralph Curtis Publishing.
- Brandley MC, Huelsenbeck JP, Wiens JJ. 2008. Rates and patterns in the evolution of snake-like body form in squamate reptiles: evidence for repeated re-evolution of lost digits and long-term persistence of intermediate body forms. *Evolution* 62:2042–64.
- Bretz F, Hothorn T, Westfall P. 2010. Multiple comparisons using R. Boca Raton (FL): CRC Press.
- Brusca RC, Moore W, Shuster SM. 2016. Invertebrates. 3rd ed. Sunderland (MA): Sinauer Associates.
- Buchholtz EA. 2001. Vertebral osteology and swimming style in living and fossil whales (Order: Cetacea). *J Zool* 253:175–90.
- Burnham KP, Anderson DR. 2002. Model selection and multimodel inference: a practical information-theoretic approach. New York: Springer.
- Caldwell MW. 2003. “Without a leg to stand on”: on the evolution and development of axial elongation and limblessness in tetrapods. *Can J Earth Sci* 40:573–88.
- Crawley MJ. 2012. The R book. 2nd ed. West Sussex: John Wiley & Sons.
- Gans C. 1986. Locomotion of limbless vertebrates: pattern and evolution. *Herpetologica* 42:33–46.
- Gans C, Fusari M. 1994. Locomotor analysis of surface propulsion by three species of reduced-limbed fossorial lizards (*Lerista*: Scincidae) from Western Australia. *J Morphol* 222:309–26.
- Gill HS, Renaud CB, Chapleau F, Mayden RL, Potter IC. 2003. Phylogeny of living parasitic lampreys (Petromyzontiformes) based on morphological data. *Copeia* 2003:687–703.
- Gillis GB. 1998. Environmental effects on undulatory locomotion in the American eel *Anguilla rostrata*: kinematics in water and on land. *J Exp Biol* 201:949–61.
- Gould SJ. 2002. The structure of evolutionary theory. Cambridge (MA): Belknap Press.
- Greer AE. 1991. Limb reduction in squamates: identification of the lineages and discussion of the trends. *J Herpetol* 25:166–73.
- Grizante MB, Brandt R, Kohlsdorf T. 2012. Evolution of body elongation in gymnophthalmid lizards: relationships with climate. *PLoS One* 7:e49772.
- Hedrick TL. 2008. Software techniques for two- and three-dimensional kinematic measurements of biological and biomimetic systems. *Bioinspir Biomim* 3:034001.
- Herrel A, Choi H-F, De Schepper N, Aerts P, Adriaens D. 2011. Kinematics of swimming in two burrowing anguilliform fishes. *Zoology* 114:78–84.
- Horner AM, Biknevicius AR. 2010. A comparison of epigeal and subterranean locomotion in the domestic ferret (*Mustela putorius furo*: Mustelidae: Carnivora). *Zoology* 113:189–97.
- Horner AM, Jayne BC. 2008. The effects of viscosity on the axial motor pattern and kinematics of the African lungfish (*Protopterus annectens*) during lateral undulatory swimming. *J Exp Biol* 211:1612–22.
- Huelsenbeck JP, Nielsen R, Bollback JP. 2003. Stochastic mapping of morphological characters. *Syst Biol* 52:131–58.
- Infante CR, Rasys AM, Menke DB. 2018. Appendages and gene regulatory networks: lessons from the limbless. *Genesis* 56:e23078.
- Janvier P. 1981. The phylogeny of the craniata, with particular reference to the significance of fossil “agnathans”. *J Vertebr Paleontol* 1:121–59.
- Josse J, Husson F. 2016. Missmda: a package for handling missing values in multivariate data analysis. *J Stat Softw* 70:1–31.
- Kendrick PG. 1991. The phylogenetics and comparative ecology of *Lerista* Bell, 1983: patterns of evolution in a genus of sand-swimming skinks [doctoral]. Perth, Australia: University of Western Australia.
- Kuraku S, Kuratani S. 2006. Time scale for cyclostome evolution inferred with a phylogenetic diagnosis of hagfish and lamprey cDNA sequences. *Zool Sci* 23:1053–64.

- Lande R. 1978. Evolutionary mechanisms of limb loss in tetrapods. *Evolution* 32:73–92.
- Law CJ, Slater GJ, Mehta RS. 2019. Shared extremes by ectotherms and endotherms: body elongation in musteloids is associated with small size and reduced limbs. *Evolution* 73:735–49.
- Leal F, Cohn MJ. 2018. Developmental, genetic, and genomic insights into evolutionary loss of limbs in snakes. *Genesis* 56:e23077.
- Lee MSY, Skinner A, Camacho A. 2013. The relationship between limb reduction, body elongation and geographical range in lizards (*Lerista*, Scincidae). *J Biogeogr* 40:1290–7.
- Losos JB. 2011. Convergence, adaptation, and constraint. *Evolution* 65:1827–40.
- Miyashita T, Coates MI, Farrar R, Larson P, Manning PL, Wogelius RA, Edwards NP, Anné J, Bergmann U, Palmer AR, et al. 2019. Hagfish from the cretaceous tethys sea and a reconciliation of the morphological–molecular conflict in early vertebrate phylogeny. *Proc Natl Acad Sci U S A* 116:2146–51.
- Morinaga G, Bergmann PJ. 2017. Convergent body shapes have evolved via deterministic and historically contingent pathways in *Lerista* lizards. *Biol J Linn Soc Lond* 121:858–75.
- Morinaga G, Bergmann PJ. 2019. Angles and waves: intervertebral joint angles and axial kinematics of limbed lizards, limbless lizards, and snakes. *Zoology* 134:16–26.
- Muschick M, Indermaur A, Salzburger W. 2012. Convergent evolution within an adaptive radiation of cichlid fishes. *Curr Biol* 22:2362–8.
- Nakagawa S, Schielzeth H. 2013. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods Ecol Evol* 4:133–42.
- Pace CM, Gibb AC. 2009. Mudskipper pectoral fin kinematics in aquatic and terrestrial environments. *J Exp Biol* 212:2279–86.
- Pace CM, Gibb AC. 2011. Locomotor behavior across an environmental transition in the ropefish, *Erpetoichthys calabaricus*. *J Exp Biol* 214:530–7.
- Parra-Olea G, Wake DB. 2001. Extreme morphological and ecological homoplasy in tropical salamanders. *Proc Natl Acad Sci U S A* 98:7888–91.
- Paradis E, Schliep K. 2019. Ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35:526–8.
- Pennell MW, Eastman JM, Slater GJ, Brown JW, Uyeda JC, FitzJohn RG, Alfaro ME, Harmon LJ. 2014. Geiger v2.0: an expanded suite of methods for fitting macroevolutionary models to phylogenetic trees. *Bioinformatics* 30:2216–8.
- Pough FH, Andrews RM, Crump ML, Savitzky AH, Wells KD, Brandley MC. 2016. *Herpetology*. 4th ed. Sunderland (MA): Sinauer Associates.
- Pough FH, Preest MR, Fusari M. 1997. Prey-handling and the evolutionary ecology of sand-swimming lizards (*Lerista*: Scincidae). *Oecologia* 112:351–61.
- Rabosky DL, Chang J, Title PO, Cowman PF, Sallan L, Friedman M, Kaschner K, Garilao C, Near TJ, Coll M, et al. 2018. An inverse latitudinal gradient in speciation rate for marine fishes. *Nature* 559:392–5.
- Rasband WS. 2019. Image J. Bethesda (MD): National Institutes of Health. (<http://imagej.nih.gov/ij/>).
- Renous S, Hofling E, Gasc J-P. 1995. Analysis of the locomotion pattern of two microteiid lizards with reduced limbs, *Calyptommatus leiolepis* and *Nothobachia ablephara*. *Zoology* 99:21–38.
- Renous S, Hofling E, Gasc JP. 1998. Respective role of the axial and appendicular systems in relation to the transition to limblessness. *Acta Biotheor* 46:141–56.
- Renous S, Hofling E, Gasc JP. 1999. On the rhythmical coupling of the axial and appendicular systems in small terrestrial lizards (Sauria: Gymnophthalmidae). *Zoology* 102:31–49.
- Revell LJ. 2012. Phytools: an r package for phylogenetic comparative biology (and other things). *Methods Ecol Evol* 3:217–23.
- Satoh N, Rokhsar D, Nishikawa T. 2014. Chordate evolution and the three-phylum system. *Proc Biol Sci* 281:20141729.
- Schluter D, Price T, Mooers AO, Ludwig D. 1997. Likelihood of ancestor states in adaptive radiation. *Evolution* 51:1699–711.
- Scholl JP, Wiens JJ. 2016. Diversification rates and species richness across the tree of life. *Proc Biol Sci* 283:20161334.
- Searle SR, Speed FM, Milliken GA. 1980. Population marginal means in the linear model: an alternative to least squares means. *Am Stat* 34:216–21.
- Sfakiotakis M, Lane DM, Davies J. 1999. Review of fish swimming modes for aquatic locomotion. *IEEE J Ocean Eng* 24:237–52.
- Shapiro MD, Hanken J, Rosenthal N. 2003. Developmental basis of evolutionary digit loss in the Australian lizard *Hemiergis*. *J Exp Zool* 297B: 48–56.
- Siler CD, Brown RM. 2011. Evidence for repeated acquisition and loss of complex body-form characters in an insular clade of southeast Asian semi-fossorial skinks. *Evolution* 65:2641–63.
- Siler CD, Diesmos AC, Alcalá AC, Brown RM. 2011. Phylogeny of Philippine slender skinks (Scincidae: *Brachymeles*) reveals underestimated species diversity, complex biogeographical relationships, and cryptic patterns of lineage diversification. *Mol Phylogenet Evol* 59:53–65.
- Stokely PS. 1947. Limblessness and correlated changes in the girdles of a comparative morphological series of lizards. *Am Midl Nat* 38:725–54.
- R Core Team. 2019. R: a language and environment for statistical computing. Vienna.
- Wake DB, Wake MH, Specht CD. 2011. Homoplasy: from detecting pattern to determining process and mechanism of evolution. *Science* 331:1032–5.
- Ward AB, Brainerd EL. 2007. Evolution of axial patterning in elongate fishes. *Biol J Linn Soc Lond* 90:97–116.
- Ward AB, Costa A, Monroe SL, Aluck RJ, Mehta RS. 2015. Locomotion in elongate fishes: a contact sport. *Zoology* 118:312–9.
- Ward AB, Mehta RS. 2014. Differential occupation of axial morphospace. *Zoology* 117:70–6.
- Wiens JJ, Brandley MC, Reeder TW. 2006. Why does a trait evolve multiple times within a clade? Repeated evolution of snakelike body form in squamate reptiles. *Evolution* 60:123–41.
- Wiens JJ, Slingluff JL. 2001. How lizards turn into snakes: a phylogenetic analysis of body form evolution in anguillid lizards. *Evolution* 55:2303–18.

- Williams VSL, Jones LV, Tukey JW. 1999. Controlling error in multiple comparisons, with examples from state-to-state differences in educational achievement. *J Educ Behav Stat* 24:42–69.
- Wilson S, Swan G. 2005. *A complete guide to the reptiles of Australia*. Sydney: Reed New Holland Publishers Ltd.
- Young RL, Caputo V, Giovannotti M, Kohlsdorf T, Vargas AO, May GE, Wagner GP. 2009. Evolution of digit identity in the three-toed italian skink *Chalcides chalcides*: a new case of digit identity frame shift. *Evol Dev* 11:647–58.
- Zheng Y, Wiens JJ. 2016. Combining phylogenomic and supermatrix approaches, and a time-calibrated phylogeny for squamate reptiles (lizards and snakes) based on 52 genes and 4162 species. *Mol Phylogenet Evol* 94:537–47.