

Are there general laws for digit evolution in squamates? The loss and re-evolution of digits in a clade of fossorial lizards (*Brachymeles*, Scincinae)

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Abstract

Evolutionary simplification of autopodial structures is a major theme in studies of body-form evolution. Previous studies on amniotes have supported Morse's law, that is, that the first digit reduced is Digit I, followed by Digit V. Furthermore, the question of reversibility for evolutionary digit loss and its implications for "Dollo's law" remains controversial. Here, we provide an analysis of limb and digit evolution for the skink genus *Brachymeles*. Employing phylogenetic, morphological, osteological, and myological data, we (a) test the hypothesis that digits have re-evolved, (b) describe patterns of morphological evolution, and (c) investigate whether patterns of digit loss are generalizable across taxa. We found strong statistical support for digit, but not limb re-evolution. The feet of pentadactyl species of *Brachymeles* are very similar to those of outgroup species, while the hands of these lineages are modified (2-3-3-3-2) and have a reduced set of intrinsic hand muscles. Digit number variation suggests a more labile Digit V than Digit I, contrary to Morse's law. The observed pattern of digit variation is different from that of other scincid lizards (*Lerista*, *Hemiergis*, *Carlia*). Our results present the first evidence of clade-specific modes of digit reduction.

KEYWORDS

ancestral state reconstruction, digit evolution, Dollo's law, Morse's law, reversible evolution

1 | INTRODUCTION

The first comparative studies of animal and plant structure were motivated by a search for the "laws of nature" that would govern the origin and establishment of biological diversity. This was most obvious in the pre-Darwinian era of biology, where the hope was to find a system of regularities similar to, though more complex than, those established for crystals and chemical elements (Amundson, 2005; Nyhart, 1995; Rupke, 1994). After the establishment of evolutionary theory, the

search for patterns was re-oriented towards a search for regularities of evolutionary change (Gregory, 1933; Riedl, 1978; Sewerzoff, 1931), and a number of these regularities still influence evolutionary thinking today. Examples include Dollo's law concerning the irreversibility of evolutionary loss of complex characters (Collin & Miglietta, 2008; Gould, 1970), Morse's law of digit reduction (Morse, 1872; Shapiro, Shubin, & Downs, 2007; Shubin, Tabin, & Carroll, 1997), Haldane's rule of hybrid viability, Cope's law of body size increase, and Rensch's rule of sexual size dimorphism (Rensch, 1960). While the study of these

“laws” and rules is useful for systematizing knowledge about biological diversity, they need to be tested for each taxon, rather than assumed. The discovery of apparent or real violations of these rules can lead to deeper insights into the causal mechanisms that underlie them. In this study, we investigate limb evolution in the skink genus *Brachymeles* to test if two of these rules apply: Dollo's law of irreversibility and Morse's law of digit reduction.

Dollo's law is actually not an empirical generalization as explained lucidly by Stephen J. Gould (1970) in his review of the history of this concept. There are at least two ideas that Dollo himself used: (a) organisms never return to exactly the same state (phenotype) as represented by an ancestor, and (b) complex characters never return exactly to an ancestral state. Dollo never asserted, as is often ascribed to him, that complex characters, once lost, cannot re-evolve. In fact, many of the examples of apparent violations of Dollo's law are fully compatible with Dollo's writings, who acknowledged the reality of atavisms and the possibility of reactivating developmental vestiges to be expressed in the adult organism (Hall, 1984). There are now many well-documented cases of the re-evolution of complex characters, including teeth and nipples in mammals (Gilbert, 1986; Kurtén, 1963; Lihoreau et al., 2006; Sherman, Braude, & Jarvis, 1999), mandibular teeth in frogs (Wiens, 2011), wings in insects (Whitting, Bradler, & Maxwell, 2003), coiling in snails (Collin & Cipriani, 2003), sexuality in oribatid mites (Domes et al., 2007), muscles in primates (Diogo & Wood, 2012), oviparity in snakes (Lynch & Wagner, 2010), complex life cycles in marsupial frogs (Wiens et al., 2007), and phalanges and digits in squamate reptiles (Brandley et al., 2008; Kohlsdorf & Wagner, 2006; Siler & Brown, 2011). Nevertheless, the re-evolution of complex phenotypic traits remains controversial for a number of reasons, including technical issues and different opinions about mechanistic plausibility (Blackburn, 2015; Galis, Amtzen, & Lande, 2010; Goldberg & Igic, 2008; Griffith et al., 2015; Kohlsdorf, Lynch, Rodrigues, Brandley, & Wagner, 2010; Pyron & Burbrink, 2015; Skinner & Lee, 2010).

Dollo, however, did assert a subtler point that re-evolved structures do not recreate exactly the same ancestral character state. This has, indeed, sometimes been found to be the case. For instance, the re-evolution of oviparity in snakes is associated with the lack of egg teeth in species inferred to have re-evolved oviparity (Lynch & Wagner, 2010, but see Griffith et al., 2015). Also, cases of inferred digit re-evolution in *Bachia* and *Bipes* are associated with changes in the phalangeal formula and muscle anatomy (Abdala, Grizante, Diogo, Molnar, & Kohlsdorf, 2015; Brandley et al., 2008; Kohlsdorf & Wagner, 2006). In fact, the phenotypic differences between ancestral and re-evolved characters can be considered as ancillary evidence that re-evolution has occurred (Griffith et al., 2015), although there are also examples of reversion that are not associated with structural differences (Diogo & Wood, 2012). Here, we address both of these issues for scincid lizards of the genus *Brachymeles*: (a) we re-evaluate the strength of the statistical evidence that digit and limb re-evolution took place, and (b) we investigate whether the inferred re-evolution affected the morphology of the re-evolved digits.

The other evolutionary rule we address in this study is Morse's law, which describes the sequence of digit loss in amniotes and was first articulated in a study on the avian limb (Morse, 1872). Morse wrote

“[...] when the number of fingers or toes is reduced in Mammalia and Reptilia, they are always taken away from the sides of the member, the thumb first disappearing and then the little finger” (p. 153). There are several well-documented examples supporting this generalization (Shapiro, 2002; Shapiro et al., 2007). Against this background, the inference that the avian wing evolved by losing Digits V and IV has been received with skepticism, resulting in a continued, active area of research which is embedded in the controversy over the dinosaur origin of birds (for reviews see Feduccia, 2001; Prum, 2002; Wagner, 2005; Xu & Mackem, 2013; Young, Bever, Wang, & Wagner, 2011). Here, we test the generality of Morse's law by studying digit variation in *Brachymeles*.

There are several clades of lizards that comprise a broad diversity of limb morphologies that range from limbless, to limbed but non-pentadactyl, to pentadactyl. Among them, the genera *Brachymeles* and *Lerista* possess the greatest diversity of digit and limb states (and have 41 and 95 species recognized, respectively; Couper et al., 2016; Davis et al., 2016; Skinner, 2010; Skinner et al., 2008; Skinner & Lee, 2010; Siler et al., 2011, 2016). For example, among the 41 *Brachymeles* species so far described, the genus encompasses at least 18 limbed but non-pentadactyl species (representing a spectrum of different digit state combinations on the forelimb and hind limbs), and five externally limbless species (Couper et al., 2016; Davis et al., 2016; Siler et al., 2011, 2016). *Brachymeles* is found almost exclusively in the Philippines, with the exception of two limbless species known from Borneo and Thailand (Davis et al., 2016; Heyer, 1972; Hikida, 1982; Siler et al., 2016).

Although both clades, *Lerista* and *Brachymeles*, represent ideal systems for fine-scale studies of evolutionary patterns of limb and digit development (Greer, 1990, 1991; Skinner, 2010; Skinner et al., 2008; Skinner & Lee, 2009, 2010; Siler & Brown, 2011; Siler et al., 2016), *Lerista* appears to have evolved these phenotypes only through the loss of digits and limbs (Skinner, 2010; Skinner et al., 2008; Skinner & Lee, 2009, 2010), while phylogenetic evidence suggests that *Brachymeles* has lost and subsequently re-evolved digits and possibly limbs (Siler & Brown, 2011). Here, we re-assess the hypothesis that the pentadactyl limb in the genus *Brachymeles* results from digit re-evolution using an extended species phylogeny of *Brachymeles*. We test this using a robust and time-calibrated phylogenetic tree of 37 recognized species, statistical character model testing, and comparative evaluation of osteological and myological data. We use this system to test the generality of Dollo's and Morse's laws by describing the sequence of digit reduction and comparing the osteology of 10 species of *Brachymeles* and three pentadactyl outgroup species from the genera *Lygosoma* and *Eutropis*. Finally, we examine the myology of the large pentadactyl skink *Brachymeles kadwa*, to identify if there is evidence of a derived musculature consistent with a re-evolution of the pentadactyl limb.

2 | MATERIALS AND METHODS

2.1 | Phylogenetic relationships and timing of diversification

We sampled 39 individuals, representing all but two of the 41 species of *Brachymeles* currently recognized for our ingroup (Figure 1;

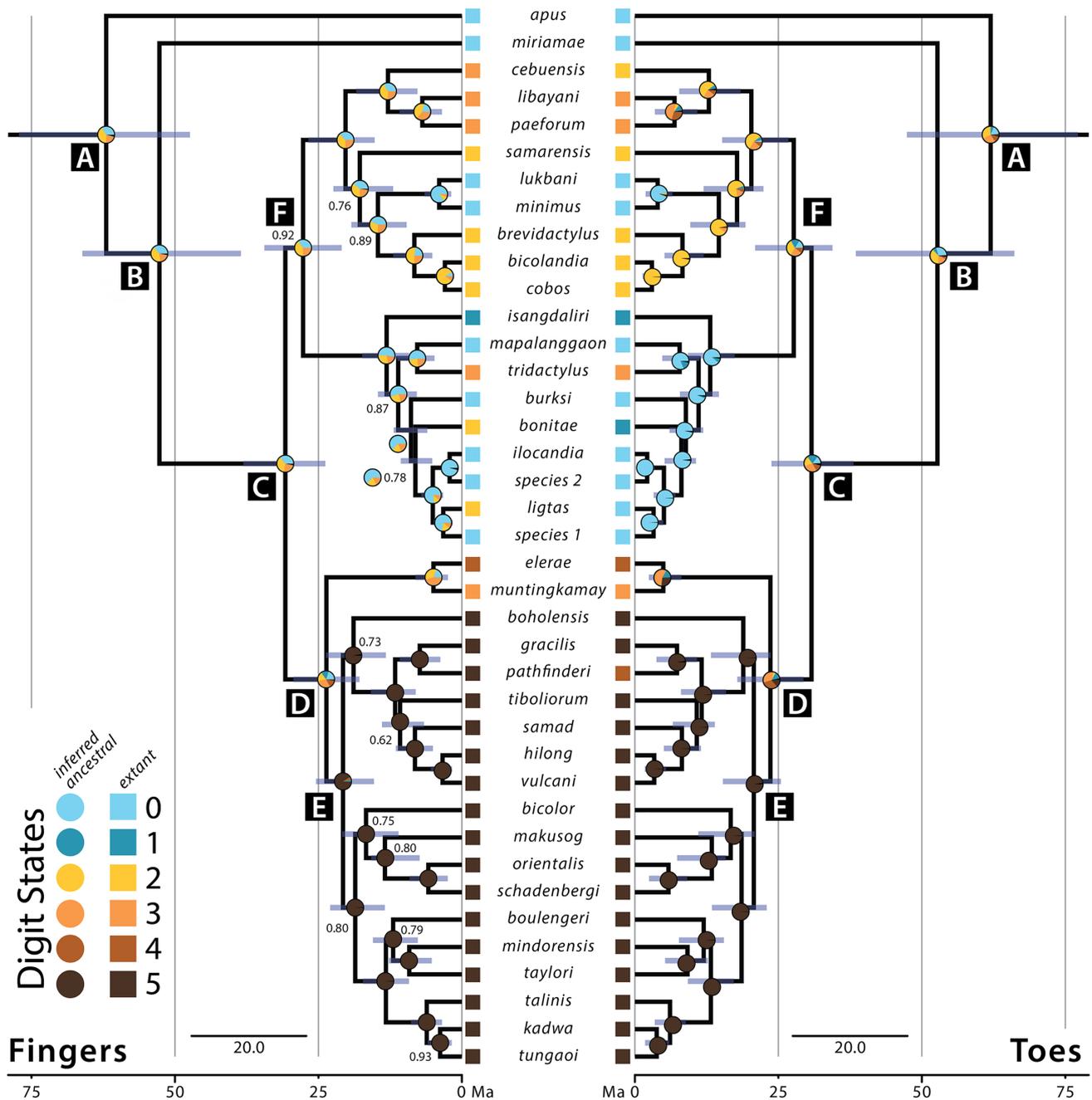


FIGURE 1 Timed phylogeny of the genus *Brachymeles* showing ancestral state reconstructions for fore limb (left) and hind limb (right) digit evolution. Pie charts indicate the probability distribution of ancestral states based on stochastic character mapping. The major clades are indicated with capital letters: A is the crown group *Brachymeles*; B is the Philippine *Brachymeles* with *B. miriamae*; C is all Philippine *Brachymeles* species; D represents the pentadactyl clade with its sister group of partially digit reduced *B. elerae* and *B. muntingkamay*; E is the pentadactyl *Brachymeles* clade; F is the digit-reduced clade of *Brachymeles*. Note that nodes A, B, C, D, and F all have digit-reduced states, which implies that the five-digitated clade E has re-evolved digits

supporting information Table S1; Davis et al., 2016; Siler et al., 2016). Our outgroup sampling included 30 individuals, representing 30 outgroup species sampled across squamata, which include 22 species in the family Scincidae. Following the methods of Brandley et al. (2011), we conducted divergence dating analyses by employing three fossil calibrations based on phylogenetic inferences of the placement of three non-scincid fossil taxa in the squamate tree of life (Conrad, 2008).

Multilocus phylogenetic datasets were available for most species from previously published work for the coding region of the mitochondrial gene NADH dehydrogenase subunit 1 (*ND1*), and three nuclear loci: brain-derived neurotrophic factor (*BDNF*), RNA fingerprint protein 35 (*R35*), and prostaglandin E receptor 4 (*PTGER4*) (Davis et al., 2014; Siler et al., 2011a, 2011b, 2012). We collected near complete sequence datasets for all four loci for all remaining ingroup taxa (supporting

information Table S1). We carried out polymerase chain reaction (PCR) and sequencing protocols following Siler et al. (2011) and Siler and Brown (2011), deposited all novel sequences in GenBank (supporting information Table S1), made initial alignments using MUSCLE 3.8.31 (Edgar, 2004) with minimal manual adjustments, and deposited those in Dryad.

To estimate an ultrametric, time-calibrated phylogeny, we used a Bayesian relaxed molecular clock model implemented in BEAST 2.4.2 (Bouckaert et al., 2014). The absence of any fossil record for *Brachymeles* and a poor scincid fossil record, in general (Brandley et al., 2011; Evans, 2003), prevents the use of internal, fossil-based node-age calibrations within the ingroup. Instead, we used three higher level squamate fossil calibrations that have previously been used to estimate timing of divergences in the genus *Plestiodon* (Brandley et al., 2011), a clade that is closely related to *Brachymeles* (Brandley et al., 2008; Siler & Brown, 2011; Siler et al., 2011). These fossil calibrations were based on the inferred age of (1) the crown Episquamata, (2) the divergence between Amphisbaenia and Teiidae, and (3) Scinciformata (Conrad, 2008). Our fossil calibration parameters follow Brandley et al. (2011).

We allowed branch-specific rates of substitution to vary across the tree according to uncorrelated log-normal distributions (UCLDs) (Drummond, Ho, Phillips, & Rambaut, 2006). Data was partitioned by codon position for ND1, and codon positions were pooled across the nuclear genes, for a total of six partitions, each with separate UCLDs applied. We employed the Akaike Information Criterion (AIC), as implemented in jModelTest v2.1.10 (Darriba et al., 2012), to determine the best-fit models of nucleotide substitutions for each partition (see supporting information Table S2). For all UCLD relaxed-clocks, we used an exponential prior distribution ($SE = .01$). We used a Yule process tree prior and left all remaining priors at default values. We ran two independent analyses of 50 million generations, sampling the parameter values every 20,000 generations. We assessed stationarity of the chains by plotting parameter values and likelihood scores of all four chains over generations and confirmed congruence among consensus trees across the four chains. We conservatively discarded the first 20% of samples as burn-in and combined the remaining 2,000 samples across all four independent MCMC chains.

2.2 | Morphological data

We recorded the presence or absence of limbs and counted the number of digits on the *manus* and *pes* for 39 species of *Brachymeles* and 30 outgroups taxa and collected phalangeal formulae and measured the bones of the *manus* and *pes* for 11 species of *Brachymeles* and three outgroup taxa (see Table 3). We observed and described the osteology of the two tetradactyl species, *Brachymeles pathfinderi* (5/4) and *Brachymeles elerae* (4/4), to test Morse's law by determining whether Digit I or V was lost first in *Brachymeles*. Finally, we dissected the forelimb and hind limbs of the pentadactyl *B. kadwa* to describe their muscle anatomy. To study osteology, we cleared and stained or microCT scanned forelimb and hind limbs. For clearing and staining, we removed the limbs from the body and skinned them. Limbs were then (a) stained in Alcian Blue for 12–24 hr, (b) macerated in trypsin, (c)

stained in Alizarin Red S for 24 hr, and (d) stepped into 100% glycerol and preserved with phenol (Hancken & Wassersug, 1981). Finally, we photographed cleared and stained limbs. For microCT scanning, we embedded whole limbs in agarose and scanned them with an XRA-002 microCT (X-Tek; Tring, United Kingdom) at the Center for Nanoscale Systems at Harvard University. We produced 3D reconstructions using VGStudio Max 2.0 (Volume Graphics).

Limb photographs and 3D models were used to measure the lengths and widths of each phalanx, except the terminal, claw-bearing one, and the lengths and widths of each metapodial (metacarpal in the *manus* and metatarsal in the *pes*) using ImageJ (Rasband, 2016). Lengths were straight distances from proximal to distal end of each bone. The widths were measured as straight distances at the midpoint of the shaft, which corresponded closely with the narrowest point of the shaft. We then calculated the aspect ratio of each of these long bones as the length divided by the width. From these, we calculated the average aspect ratios of all long bones, all phalanges (except the terminal ones), and all metapodials for forelimb and hind limbs. We then analyzed these average aspect ratios. We used aspect ratios in our analysis because they were normally distributed (Shapiro–Wilk test $p > .062$), represent intuitive descriptions of the shape of the bones and are comparable across studies (Smith, 1999; Sokal & Rohlf, 1995).

To study limb myology, we made gross anatomical dissections of the forelimb and hind limb musculature of five specimens of *B. kadwa*. We wrote detailed descriptions and took photographs using a SPOT Insight digital camera mounted on a Wild M3Z stereo microscope to document limb anatomy. We cleaned each muscle layer of all fascia, before removing individual muscles, allowing us to observe deeper layers. We used the nomenclature of Abdala and Diogo (2010) for the hand, which takes into account the evolution and homologies of the forelimb and hand muscles of all the major tetrapod groups. We used the nomenclature of Snyder (1954) and Russell and Bauer (2008) for the foot muscles.

2.3 | Statistical analyses

To quantitatively study limb evolution in *Brachymeles*, we applied four complementary statistical analyses: (a) we tested alternate evolutionary hypotheses based on models of digit number evolution; (b) we reconstructed ancestral states on the phylogeny; (c) we tested for associations between bone aspect ratios and digit number; and (d) we tested for differences in pentadactyl morphology between burrowing and non-burrowing species. All statistical analyses were performed in R 3.2.2 (R Core Team 2016).

We modeled presence/absence of limbs and number of digits (0–5) on the *manus* and *pes* as discrete traits and reconstructed ancestral character states based on likelihood (Schluter, Price, Mooers, & Ludwig, 1997) using the package “ape” (Paradis, Claude, & Strimmer, 2004). We started by fitting a series of discrete Markov evolutionary models to each trait. We used AIC to select the most parsimonious model in each case, and calculated evidence ratios $[ER = \exp(-\Delta_i/2)/\exp(-\Delta_j/2)]$ where, i is the better model and j is the worse model] to quantify how much better one model fit data than another (Anderson & Burnham,

TABLE 1 Model comparison for limb presence/absence evolution. The number of parameters (K), log likelihood, AIC, Δ values, and evidence ratios relative to the best model are shown for each model. The best model is in bold font

Data	Model	K	LogL	AIC	Δ	ER
Limbs	Equal rates	2	-13.0	28.1	0.9	1.54
Limbs	All rates different	3	-11.6	27.2	0.0	1.00
Limbs	Dollo	2	-13.3	28.5	1.3	1.92

2002). For limb presence/absence, we fit Dollo, equal, and unequal rates models. The Dollo model allowed digit loss but not gain, the equal rates model estimated a single rate for limb gain and loss, and the unequal rates model allowed for different rates of gain and loss. For the digit number variables, we fitted an equal rates model; a model that applied one rate to all digit gains and another for all digit losses (gain/loss); a model that estimated a different rate for every possible character state transition but applied the same rate to the gain and loss of digits (symmetrical model); a model that was asymmetrical, in that a different rate was estimated for every possible transition (all rates different); and an ordered model that allowed digits to be gained or lost only one at a time. We also fitted two versions of a Dollo's law model: the strict Dollo model allowed digit losses but not gains, and a relaxed Dollo model that allowed digit losses and gains, but if all digits were lost, they could not be regained. We compared these models using AIC to test hypotheses about limb and digit evolution.

In the second approach, we reconstructed ancestral states at each node on the phylogeny using the "all rates different" model because it returned the greatest likelihood, even if not the lowest AIC. We used this model because it fit the data the best and allowed us to use the

same model for forelimb and hind limbs, making the analyses comparable. We reconstructed ancestral character states using stochastic character mapping (Bollback, 2006; Huelsenbeck et al., 2003) as implemented in the R package "phytools" (Revell, 2011). We based reconstructions on 1000 stochastic character maps with estimated character states at the root node of the phylogeny.

In order to test whether the long bones of more limb-reduced species were more robust (lower aspect ratio), we used phylogenetic generalized least squares regression (Pagel, 1999), as implemented in the package "caper" (Orme, 2013), while taking the phylogenetic signal in the residuals into account (Revell, 2010). We tested for a relationship between the number of long bones in the autopodium of each limb and the aspect ratio of all the long bones, just the metapodials, or just the phalanges. We used raw average aspect ratios for these analyses, and both the aspect ratios and the model residuals were normally distributed.

Finally, to test whether the limbs of more fossorial species had autopodial long bones that were more robust than those of surface-dwelling species, we used phylogenetic ANOVA (Garland et al., 1993) implemented in "phytools" (Revell, 2011). We ran one ANOVA comparing average aspect ratios between *Brachymeles* and the three outgroup taxa. However, since two of the outgroup taxa (*Lygosoma bowringii* and *Lygosoma quadrupes*) are also semi-fossorial, we also ran the ANOVAs comparing all + *Lygosoma* to the single *Eutropis* species, essentially reducing the test to a phylogenetic one-sample t test. For these analyses, we only included pentadactyl species, which allowed us to control for digit number, manipulating only the species classification as semi-fossorial or epigeal. For each phylogenetic ANOVA, we used 10,000 simulated null datasets that followed Brownian motion evolution to calculate p values.

TABLE 2 Model comparison for evolution of number of fingers and toes. The number of parameters (K), log likelihood, AIC, Δ values, and evidence ratios relative to the best model are shown for each model

Data	Model	K	LogL	AIC	Δ	ER
Fingers	Equal rates	2	-66.4	134.8	27.2	8.18E + 05
Fingers	Symmetric	16	-43.8	117.6	10.1	1.57E + 02
Fingers	All rates different	31	-44.5	148.9	41.4	9.70E + 08
Fingers	Ordered	2	-80.0	162.0	54.5	6.92E + 11
Fingers	Gain/Loss	3	-51.8	107.5	0.0	1.00
Fingers	Relaxed Dollo	3	-67.8	139.5	32.0	8.97E + 06
Fingers	Strict Dollo	2	-79.2	160.3	52.8	2.94E + 11
Toes	Equal rates	2	-66.7	135.5	19.1	1.42E + 04
Toes	Symmetric	16	-48.5	127.0	10.7	2.09E + 02
Toes	All rates different	31	-45.1	150.2	33.8	2.19E + 07
Toes	Ordered	2	-74.3	150.6	34.2	2.72E + 07
Toes	Gain/Loss	3	-56.2	116.4	0.0	1.00
Toes	Relaxed Dollo	3	-66.0	135.9	19.6	1.79E + 04
Toes	Strict Dollo	2	-81.3	164.5	48.1	2.86E + 10

Note. The best model is in bold

3 | RESULTS

3.1 | Phylogenetics and divergence dating

At least 37 lineages of *Brachymeles* occur in the Philippines, with two basally branching lineages of limbless species known from Sabah, Malaysia (*B. apus*) and Thailand (*B. miriamae*), respectively (Figure 1). The most recent common ancestor of *Brachymeles* crown taxon is estimated to have lived about 60 Mya (47.4–77.2 Ma, 95% highest posterior density). The Philippine *Brachymeles* are divided into two major, well-supported clades that diverged about 32 Mya (23.8–38.1 Ma; Figure 1, Clades D and F). The first consists only of digit or limb reduced species (Figure 1, Clade F), with two well-supported subclades, each comprising nine species. The second major clade consists of all pentadactyl species, in addition to *B. elerae* (4/4), *B. pathfinderi* (5/4) and *Brachymeles muntingkamay* (3/3) (Figure 1, Clade D; Siler et al., 2011). Interestingly, the subclade of *B. elerae* + *B. muntingkamay* is sister to the clade of *B. pathfinderi* + all pentadactyl species (Figure 1, Clade E). This contrasts with previous studies based on fewer taxa, which suggested these non-pentadactyl species were nested within the pentadactyl clade (Siler & Brown, 2011; Siler et al., 2011). Among the pentadactyl species, the results provide moderate to high support for three subclades.

3.2 | Limb and digit evolution models and ancestral reconstructions

The model with different rates of limb gain and loss was marginally more parsimonious than the equal rates model and the Dollo model of irreversible limb loss (Table 1). However, the low Δ_{AIC} and evidence ratio (ER = 1.92, which is the ratio of the probabilities of the data given the two models for the Dollo model compared to the reversible model indicated no evidence against unidirectional limb loss. The ancestral state reconstructions of limb presence/absence showed that the presence of limbs was more likely for most ancestors within *Brachymeles*, except for the ancestor of *B. lukbani* and *B. minimus* (see Figure 1), which are both limbless. Overall this evidence does not support limb re-evolution.

Regarding digit number evolution, in both the *manus* and the *pes*, the unordered gain/loss model with different rates for gains and losses (GL) had unequivocally the strongest support (Table 2, minimum $\Delta_{AIC} = 10.1$, ER = 157). Estimated gain and loss rates for fingers and toes were similar: on average, loss of digits was estimated to be about 22.5 times more likely than digit re-evolution by this model. High Δ_{AIC} values for both Dollo models relative to the gain/loss model strongly rejects irreversible digit loss (Δ_{AIC} : 19.6–52.8, ER = 1.8×10^4 – 2.9×10^{11}), so digit re-evolution is much more likely than unidirectional digit loss in the evolution of *Brachymeles*.

Ancestral state reconstructions for the number of digits in the forelimb and hind limbs were similar (Figure 1) but showed some important differences in detail. A digit reduced state was the most likely phenotype of the common ancestor of crown group *Brachymeles*, and a pentadactyl ancestor was the most likely state for the pentadactyl clade of *Brachymeles* (Clade E), but not for intervening nodes, suggesting

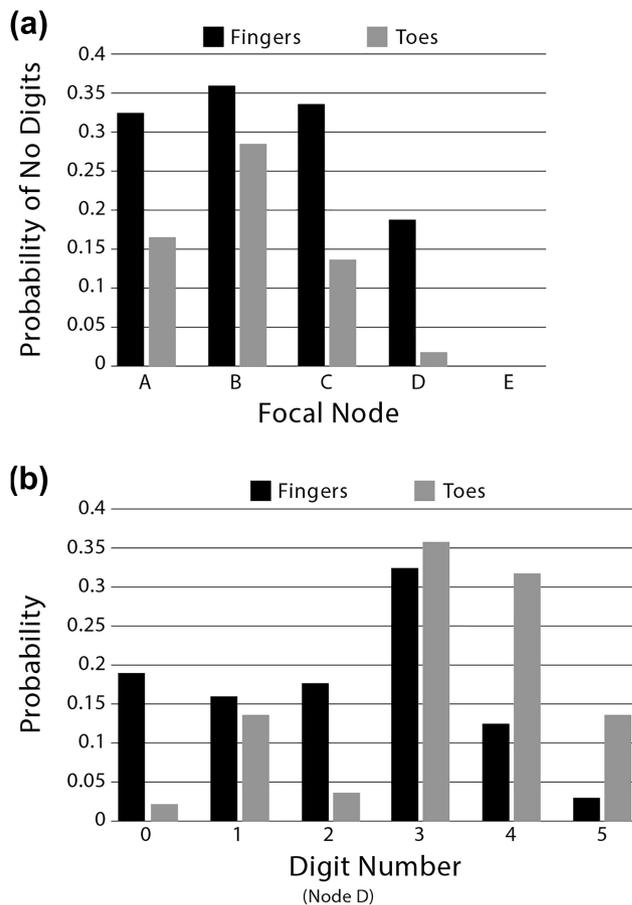


FIGURE 2 Estimated digit number phenotype probabilities of the fore and hind limb basal to the pentadactyl clade E. (a) Probabilities of zero digits inferred for nodes A–E, from the most recent common ancestor of *Brachymeles* (node A) to the base of the pentadactyl clade (node E). For nodes A–D, the likelihood of the hand to have no digits is higher than in the foot, with the greatest discrepancy in node D (i.e., just before the re-evolution of the pentadactyl state). (b) Probability distribution at node D for different digit number configurations in the forelimbs and the hind limbs. Note that for the forelimb the most likely character states are 3, 0, and 2, while those for the hind limb are 3, 4, and 5. These reconstructions thus suggest a more digit-reduced state in the forelimb than in the hind limb

pentadactyly as a derived state in this clade. Ancestral states for the intervening nodes were reconstructed with much greater probabilities of being digit-reduced, including the most recent common ancestor of members of Clade D (Front: $P_5 = .029$, Hind: $P_5 = .135$), the limb-reduced Clade F (Front: $P_5 < .001$, Hind: $P_5 < .001$), the Philippine radiation of *Brachymeles* (Clade C; Front: $P_5 < .001$, Hind: $P_5 = .007$), and all *Brachymeles* (Clade A; Front: $P_5 = .024$, Hind: $P_5 = .040$; Figure 1). Hence, four consecutive nodes were reconstructed with digit-reduced states that separated an ancestral and a derived pentadactyl condition (Figure 1, Clades A–D). Hence, ancestral state reconstruction and model testing strongly support digit re-evolution.

Digit number ancestral state reconstructions were largely consistent between forelimb and hind limbs, except for the most recent common ancestor of Clade D. This node, uniting *B. elerae* + *B.*

TABLE 3 List of species, specimen, and phalangeal formulae included in the morphological comparison for this study. *Eutropis* and *Lygosoma* were included as outgroup species. F-AR and H-AR are the average aspect ratios for the fore- and hind limb, respectively. 0 indicates that the digit lack phalanges but still has its metapodial element; X means that the phalanges and the metapodial is missing

Species	Fore limb	Hind limb	F-AR	H-AR	Specimens
<i>Eutropis longicaudata</i>	2-3-4-5-3	2-3-4-5-4	7.28	7.98	YPM4757, YPM7620
<i>Lygosoma bowringii</i>	2-3-4-5-3	2-3-4-5-4	4.91	5.08	CAS157414
<i>Lygosoma quadrupes</i>	2-3-3-3-3	2-3-3-3-3	3.58	4.35	KU157382, CAS157383
<i>Brachymeles talinis</i>	2-3-3-3-2	2-3-4-4-2	2.21	2.91	KU306762
<i>Brachymeles boulengeri</i>	2-3-3-3-2	2-3-4-4-3	2.33	3.05	KU330820
<i>Brachymeles boholensis</i>	2-3-3-3-2	2-3-4-4-2	2.38	2.85	KU323990, KU323981
<i>Brachymeles gracilis</i>	2-3-3-3-2	2-3-4-4-2	2.31	2.95	KU326107
<i>Brachymeles pathfinderi</i>	2-3-3-3-2	2-2-3-4-0	2.32	2.85	KU324061, KU324094
<i>Brachymeles elerae</i>	2-2-2-2-0	1-2-3-3-0	2.59	2.95	KU326562, KU326567
<i>Brachymeles mutingkamay</i>	X-2-2-2-0	X-2-3-3-0	2.59	2.41	KU32579,
<i>Brachymeles tridactylus</i>	X-2-2-2-0	X-2-2-2-0	1.42	1.51	KU327348, KU307727
<i>Brachymeles cebuensis</i>	X-2-2-2-0	X-2-2-0-0	0.94	0.95	KU320420, KU32042
<i>Brachymeles libayani</i>	X-2-2-2-0	X-2-2-2-0	1.26	1.67	KU320442, KU320462
<i>Brachymeles paeorum</i>	X-2-2-2-0	X-2-2-2-X	0.95	1.34	CAS26122, CAS26120

mutingkamay with the pentadactyl species, showed a nearly 10-fold discrepancy between the forelimb ($P_0 = .189$) and hind ($P_0 = .020$) limb in the probability of having zero digits (Figure 2a). Inspection of probabilities of all digit numbers from both limbs at this node showed 0-3 digits being most probable for the forelimb limb, and 3-5 digits being most probable for the hind limb (Figure 2b). It is thus likely that the hand was reduced more severely than the foot immediately before the re-evolution of pentadactyly. This observation will be important when we consider the FL-HL asymmetry in morphology.

3.3 | Patterns of digit evolution in *Brachymeles*

In our sample of species for which we obtained phalangeal formulae, *Eutropis longicaudata* is not fossorial, while both species of *Lygosoma* and all species of *Brachymeles* are semi-fossorial. The species *E. longicaudata* and *L. bowringii* had the ancestral phalangeal formulae in both forelimb and hind limbs (Table 3; Figure 3). In contrast, *L. quadrupes* had a reduced phalangeal formula of 2-3-3-3-3 in both sets of limbs (Table 3; Figure 3), and so this is likely an autapomorphic, that is, a unique derived, condition. The pentadactyl *Brachymeles* species examined here all had a reduced forelimb phalangeal formula (2-3-3-3-2), but a hind limb formula (2-3-4-4-3) close to the ancestral condition (Figure 4 with *B. boholensis* as an example), although *Brachymeles gracilis* lost an additional phalanx on Digit V (Table 3).

Species exhibiting more reduced digit numbers on both forelimb and hind limbs had reduced numbers of phalanges on those digits that were present on both sets of limbs (Table 3). The mesopodial elements (wrist and ankle) of pentadactyl outgroup and ingroup species were overall conserved. In *Brachymeles* species lacking a Digit I, metapodial I was also missing (Table 3), but those that lacked a Digit V retained

metapodial 5 (also see below), with the exception of the hind limb of *B. paeorum* (Table 4).

We tested whether species with fewer autopodial long bones had more robust long bones. More robust bones would be indicative of an adaptation for burrowing, as seen in some burrowers in a variety of taxa (Kley & Kearney, 2007), and phalangeal loss could also be adaptive for burrowing (Gans, 1986). In particular, we were interested in whether the fore-hind limb asymmetry in digit morphology could be explained by a more important role of the forelimb in burrowing. Direct observations of limb use of animals under the substrate are impossible under field conditions, although *Brachymeles* do seem to use both sets of limbs while on the surface and initiating burrowing (CDS and PJB, personal observation), and so we approached this question indirectly by testing whether forelimb bones were more robust than those of the hind limb in the semi-fossorial species. We found a strong positive relationship between long-bone aspect ratio and number of long bones for both the forelimb and hind limbs (Table 4), indicating that the fewer the number of long bones, the more robust they were. This was the case when all long bones or only the metapodials were used to calculate aspect ratio. For phalanges, the relationship was strong for hind limbs and approached significance for forelimb (Table 4). We also compared long bone robustness between pentadactyl *Brachymeles* and the three outgroups, and between all pentadactyl semi-fossorial species and the surface-dwelling *E. longicaudata*. There were no differences in aspect ratio for any long bones when comparing pentadactyl *Brachymeles* to all the outgroups (Table 5). However, when comparing semi-fossorial species and the surface-dwelling *E. longicaudata*, the hind limb metapodials, phalanges, and all bones combined were more robust in the semi-fossorial species (Table 5). For the forelimbs, only phalanges were more robust in the semi-fossorial species (Table 5). Overall this

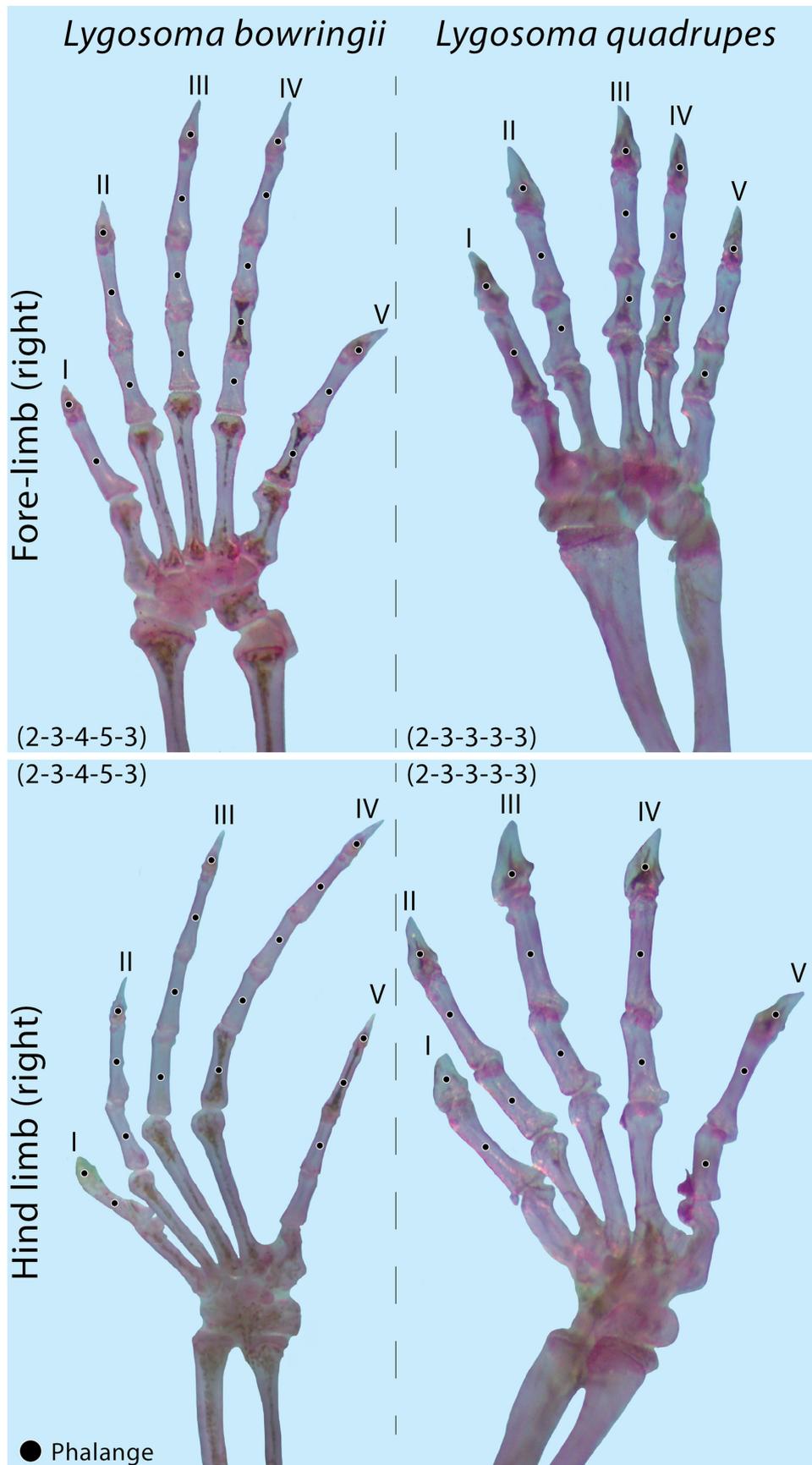


FIGURE 3 Manus and pes of two outgroup species, *L. bowringii* (CAS157414) and *Lygosoma quadrupes* (CAS157383). Left panels: *L. bowringii*, the phalangeal formulas are very close to the ancestral condition for squamates, with the exception of only three phalanges in Digit 5. Right panels: *L. quadrupes*, this species is strongly phalanx reduced in both fore and hind limb with a phalangeal formula of 2-3-3-3-3

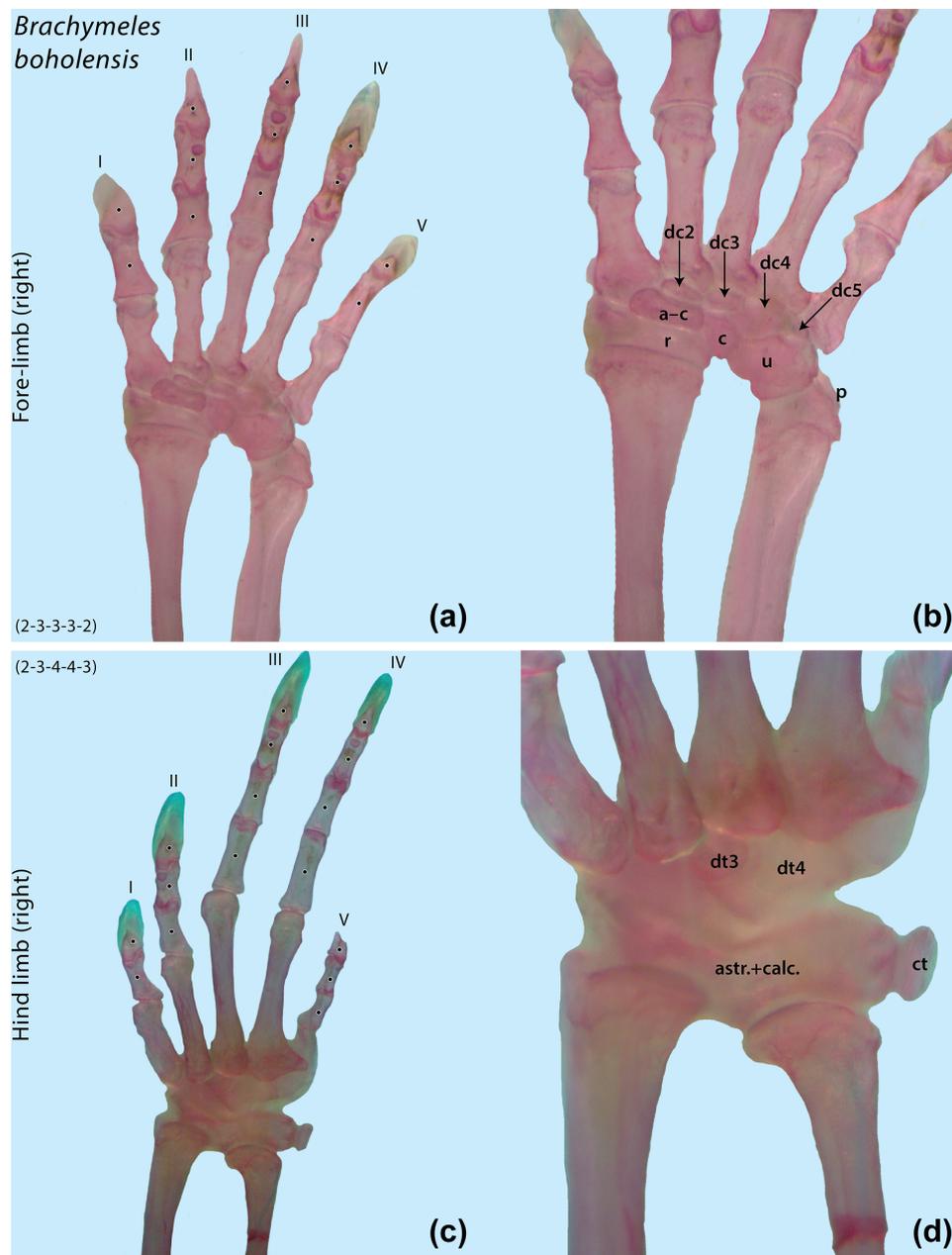


FIGURE 4 *Manus* and *pes* of a pentadactyl *Brachymeles* species, *B. bohollensis* (KU323990). (a) Overview of right manus; the phalangeal formula is 2-3-3-3-2. (b) Carpus of right FL; seven separate elements are visible with a centrale (c), a radiale (r), an ulnare (u), and distal carpals 2-5. The element that is subtending the dc-2 and also contacts the first metacarpal, here identified as an anterior centrale (a-c), rather than a first distal carpal because in squamates the dc-1 is usually found to fuse with mc1 and a dc does not come to lie proximal of other distal carpals. As usual, dc-4 is the largest. (c) Overview of the right *pes*; the phalangeal formula is 2-3-4-4-3. (d) Tarsus of right HL. Only three elements can be seen, a distal tarsal (dt) 3 and a large dt-4. The proximal tarsus is occupied by a large combined astragalus and calcaneum. Posterior there is a likely calcaneal tuberosity (ct)

supports an association between bone robustness and fossoriality but does not provide a functional explanation for greater digit reduction in the hand compared to the foot.

3.4 | Osteology of tetradactyl *brachymeles*

B. pathfinderi has five digits on the forelimb and four on the hind limb. In the hind limb, the most posterior digit variably has three to four phalanges and a massive metatarsal articulating with a large distal tarsal

element, a feature that is typical for distal tarsal 4 (Figure 5a). Posterior to this digit is a likely vestige of metatarsal 5 because its distal end extends beyond the proximal articulation of the other metatarsals, and proximally broadens and assumes a hook shape (Figure 5b). This is the characteristic shape of metatarsal 5 of many squamates (Romer, 1956) including pentadactyl *Brachymeles* species, as exemplified by *B. bohollensis* in Figure 5d. The anterior digit has two phalanges and no associated distal tarsal, consistent with Digit I identity in squamates (Romer, 1956).

TABLE 4 Results of phylogenetic least squares regression relating the y -variable to the number of autopodial long bones. λ is the measure of phylogenetic signal in the residuals

Limb	y -variable	λ	R^2	Slope \pm SE	t	p
Front	All	.94	.526	.246 \pm .067	3.65	.003
Front	Metacarpals	.00	.829	.503 \pm .066	7.64	.000
Front	Phalanges	1.00	.231	.122 \pm .064	1.90	.082
Hind	All	1.00	.476	.166 \pm .050	3.30	.006
Hind	Metatarsals	.67	.798	.402 \pm .059	6.87	.000
Hind	Phalanges	1.00	.438	.145 \pm .047	3.06	.010

This suggests that Digit V is missing with a vestigial metatarsal 5 retained.

The tetradactyl *B. elerae* has four digits both in forelimb and hind limbs, with a vestigial metacarpal and metatarsal 5, based on the same morphological criteria as discussed above (Figure 6). In the hand, the anterior-most digit has two phalanges and no distal carpal, consistent with it being a Digit I. The remaining posterior-most digit is associated with the largest distal carpal, characteristic of distal carpal 4. Together, these observations suggest that the missing digit in *B. elerae* is Digit V. The sister species of *B. elerae*—*B. muntingkamay*—has three digits and also exhibits distinct metacarpal and metatarsal 5. The most posterior remaining digit in both the forelimb and hind limb articulates with a large distal carpal, and thus most likely is Digit IV. These data show that, in *Brachymeles*, the tetradactyl configuration arises through a missing Digit V rather than Digit I.

3.5 | Myology of the pentadactyl *B. kadwa*

The phylogenetic position of *B. kadwa* suggests that this species represents the re-evolved pentadactyl morphology of *Brachymeles*; therefore, its muscle anatomy can be compared with the plesiomorphic condition for Squamata (Abdala & Diogo, 2010). The overall myology of the hind limb of *B. kadwa* was typical of other pentadactyl lizards (Snyder, 1954) and was consistent with the plesiomorphic condition. However, the forelimb lacked or had highly reduced versions of six muscles normally associated with Digit V (Figure 7). This is a derived condition, suggesting more severe limb reduction of the forelimbs than the hind limbs. On the ventral/palmar surface, the *m. flexor digitorum longus* originated from the medial epicondyle of the humerus and the proximal end of the ulna and sent tendons to the distal phalanges of all five digits. The *mm. flexores breves superficiales* originated from the sesamoid bones of the flexor plate of the *m. flexor digitorum longus* and inserted via long tendons onto the distal phalanges of Digits II–IV, but the tendon for Digit V was absent. The *mm. lumbricales* were associated with the tendons of the *m. flexor digitorum longus*, and also inserted on Digits II–IV, with that for Digit V missing. Deep to the *m. contrahentes* of Digits I and V, the *mm. flexores breves profundi* originated from the carpal bones of each digit and inserted on the proximal phalanges of Digits I–IV. Again, the insertion on Digit V was missing in this species. On the dorsal side of the manus, the *m. extensor digitorum* originated from the lateral epicondyle of the humerus and inserted superficially on the origin of the *mm.*

extensores breves at the base of metacarpals II–IV. The *mm. extensores breves* originated from the most ulnar carpal bones and inserted via long tendons on the distal phalanges of Digits I–IV, with that going to Digit V being absent. Deeper, the *mm. dorsometacarpales* originated from the base of its respective metacarpal and inserted on the distal phalanges of Digits I–IV, again with that for Digit V being absent. A very thin *m. abductor digiti minimi* originated from the pisiform and inserted on the ulnar side of the proximal phalanx of Digit V. The more reduced configuration of hand muscles in *B. kadwa* parallels the more reduced osteology of the hand compared to that of the foot.

4 | DISCUSSION

The results of this study reveal that although there is little evidence of limb re-evolution in *Brachymeles*, there is strong statistical support for the re-evolution of a pentadactyl limb from a digit-reduced form. This phylogenetic evidence is corroborated with osteological and myological data, which show that the pentadactyl forelimb in *Brachymeles* exists in a structurally less complex form with fewer phalanges and fewer muscles, at least in *B. kadwa*, than what is typical of other pentadactyl skinks. Our data are consistent with Dollo's law, as Dollo himself meant it, and we show that although complex structures once lost (i.e., digits), can re-evolve, they do not re-evolve in the same form as the ancestral state (but see Diogo & Wood, 2012). As a test of Morse's law, we investigated the order of digit reduction established during the processes of digit loss in *Brachymeles*. Counter to Morse's assertion, Digit V appears to be the most readily absent digit in this clade of scincid lizards, which seems to be a peculiar pattern of this lineage among Squamata (Shapiro et al., 2007). These results suggest differences in the organization of digit loss between *Brachymeles* and other digit-reduced lizards, including *Lerista* and *Hemiergis* (Greer, 1991; Shapiro, 2002; Skinner, 2010).

4.1 | *Brachymeles* and Dollo's law

This study provides strong evidence for digit loss and re-evolution in *Brachymeles* lizards, but lack of support for limb loss and re-evolution. The digit-reduced phase in the lineage leading to the pentadactyl clade lasted about 38 Ma (Figure 1, nodes A–D). During that time, the most likely character state was two to three digits, while complete absence

TABLE 5 Results of phylogenetic ANOVA with 1,000 null simulations comparing aspect ratio of bones specified in the y -variable between the specified taxa

Limb	y -variable	<i>Brachymeles</i> vs. outgroups		Fossorial vs. not fossorial	
		F	p	F	p
Front	All	8.95	.345	13.47	.060
Front	Metacarpals	2.88	.570	3.69	.249
Front	Phalanges	13.87	.255	16.39	.046
Hind	All	9.10	.334	28.42	.017
Hind	Metatarsals	4.15	.501	21.18	.029
Hind	Phalanges	12.62	.276	22.20	.027

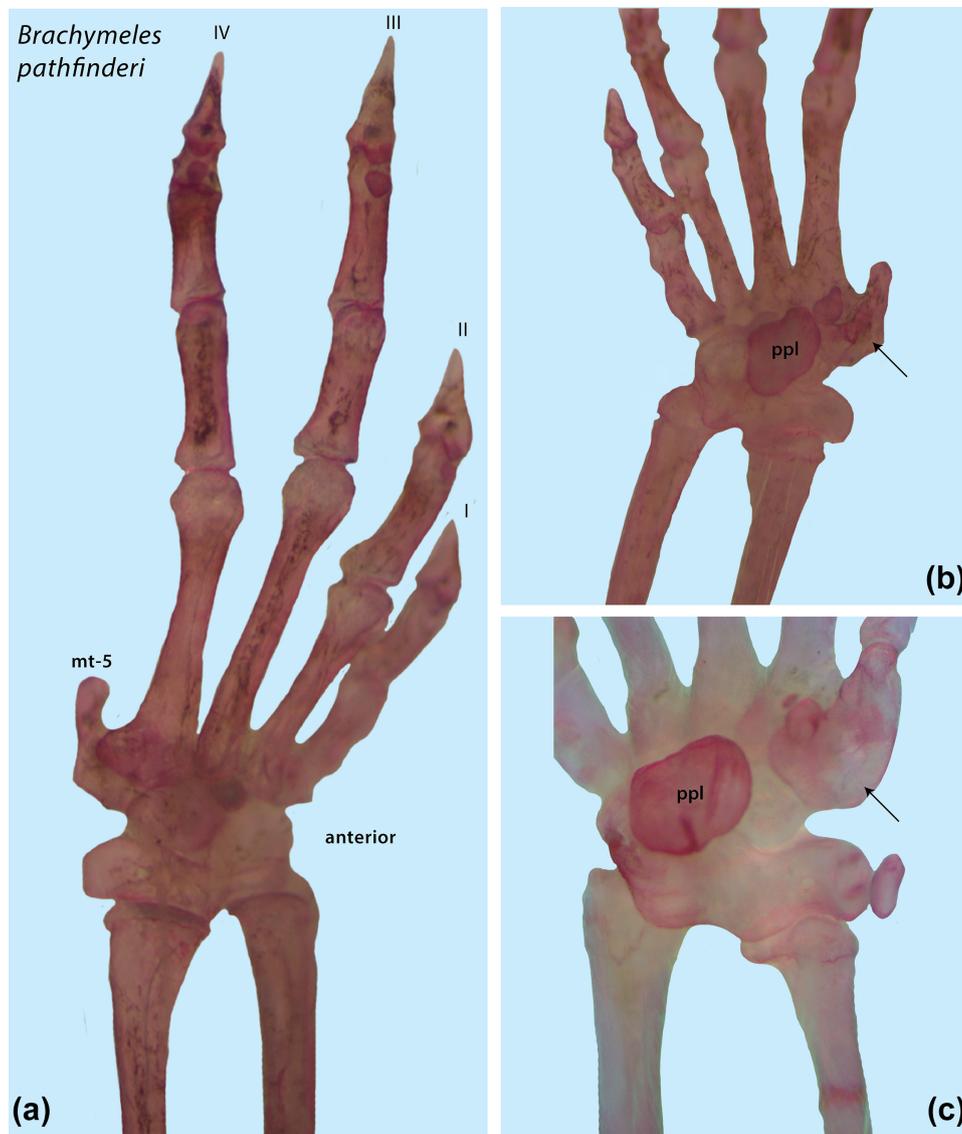


FIGURE 5 Evidence that the missing digit in the tetradactyl HL of *B. pathfinder* is D-5. (a) Dorsal aspect of the left foot of *B. pathfinder* (KU 324094). The foot has four digits with 2, 2, 3, and 4 phalanges and at the posterior margin a rudimentary elongated bone that we identify as metatarsal 5. (b) Ventral aspect of the tarsal region of the left foot of *B. pathfinder*. Note the putative posterior vestigial mt-5 with the typical hooked proximal shape. (c) Ventral aspect of the tarsal region of left hind limb of *B. bohollensis* (KU 323990), a pentadactyl species. Note that the shape of the proximal end of mt-5 is extended anteriorly similar to the putative vestigial mt-5 in panel B. In panels b and c, the dark stained plate is the plantar plate (ppl)

of digits also had a nonnegligible probability (Figure 2). Digits are serially homologous characters, and thus even after the loss of several digits the developmental program for forming a digit may be maintained (for discussion, see Kohlsdorf & Wagner, 2006). Therefore, our evidence for digit re-evolution is consistent with the idea that serially repeated elements can increase in number to reverse evolutionary loss.

Although ancestral state reconstructions are model-dependent inferences and prone to error, we conclude that the evidence for digit re-evolution in the case of *Brachymeles* is strong. Model testing showed that models assuming irreversible digit loss (Dollo's law) had 4–11 orders of magnitude less support than models allowing gain and loss of digits (Table 2). Thus, our conclusion is based on the statistical rejection of models of irreversible digit loss rather than the ancestral state reconstructions alone, an approach applied in other lizard taxa to test for

reversibility of digit loss (Kohlsdorf & Wagner, 2006; Kohlsdorf et al., 2010; Skinner, 2010). In contrast, model testing for limb evolution could not reject the Dollo model, and thus our data are equivocal in terms of whether limb loss was reversed or not.

We inferred digit re-evolution for both the forelimbs and hind limbs but with slightly different morphological outcomes. Ancestral character state reconstruction suggests a more severe digit reduction in the hand than the foot immediately before the re-establishment of the pentadactyl configuration. The more reduced ancestral states in the hand are associated with the more derived osteology and myology of the hand compared to the foot, similar to the derived osteology and myology of re-evolved digits in *Bachia* (Abdala et al., 2015; Kohlsdorf & Wagner, 2006) and *Bipes* (Brandley et al., 2008). Our results suggest that re-evolved digits assume a derived morphology only if the degree of reduction went

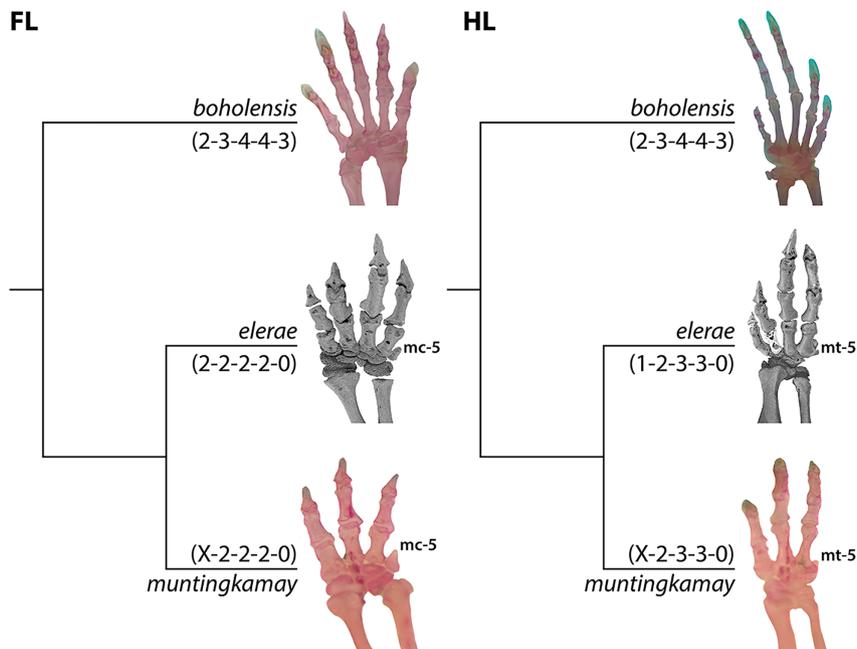


FIGURE 6 Digit number variation in the *B. elerae* + *B. muntingkamay* clade, here presented with the pentadactyl *B. boholensis* as an outgroup. Note the vestigial mc/mt-5 in *B. elerae* and *B. muntingkamay*. Both limbs of *B. elerae* have four digits, and a putative metapodial 5 indicating that Digit V is missing. *B. muntingkamay* has three digits with a vestigial metapodial 5 and thus potentially missing Digit I. The remaining most anterior digit has the morphology of a Digit I, with two phalanges and a missing dc-1

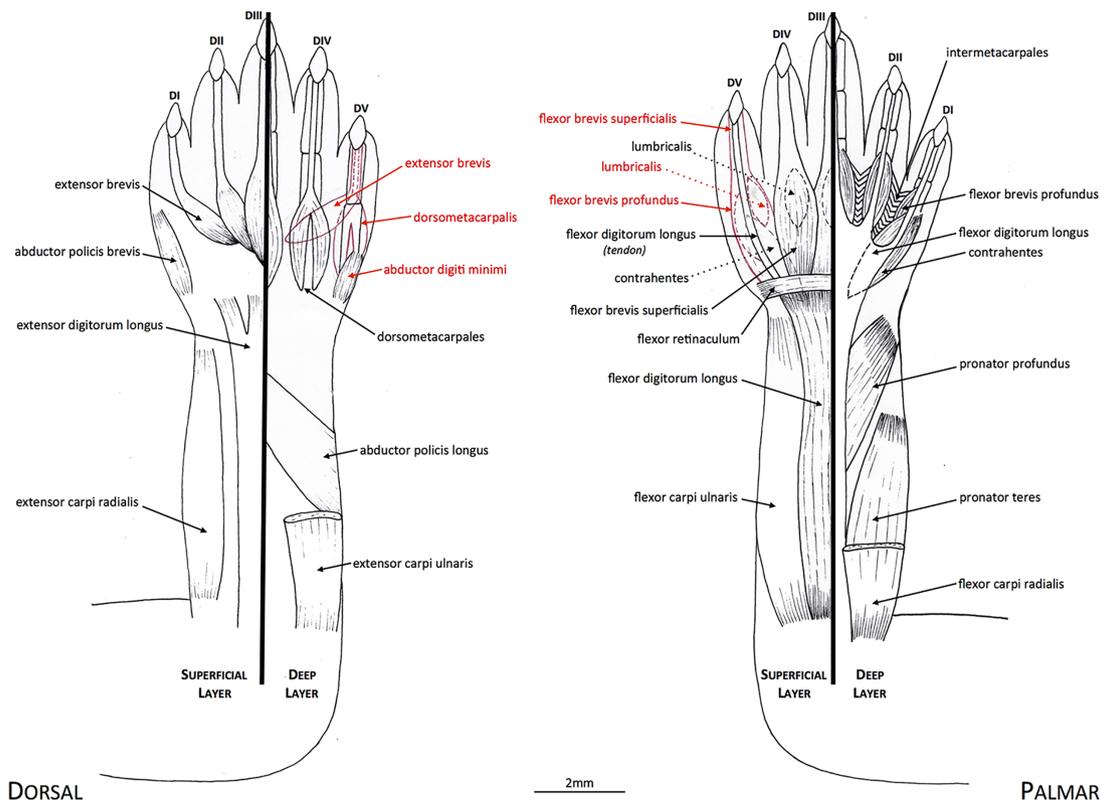


FIGURE 7 Anatomical drawings illustrating the musculature of the dorsal (left) and the palmar (right) aspects of the right forearm of *B. kadwa*. Six muscles behave differently to the typical pentadactyl plesiomorphic pattern; in red, we indicate muscles from the plesiomorphic pattern that are absent in *B. kadwa*. On its palmar side, Digit V is missing the *m. flexor brevis superficialis*, the *m. lumbricalis*, and the *m. flexor brevis profundus*, normally present on its plesiomorphic condition. On the dorsal aspect, the *m. extensor brevis* and the *dorsometacarpal* of Digit V are missing as well, representing a derived condition. (drawing by Bianca Bonini Campos)

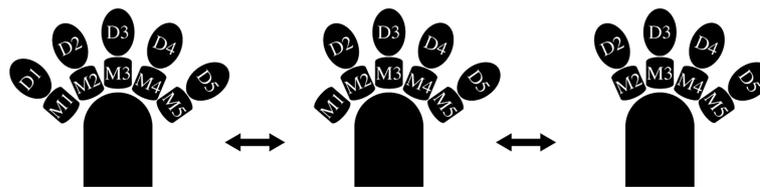
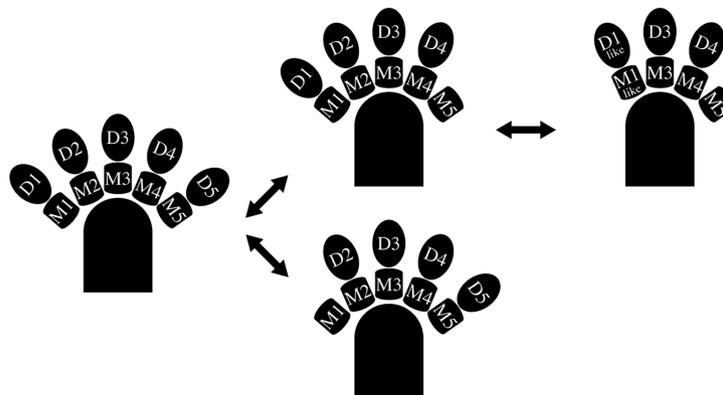
(a) Lygosominae mode of digit variation:**(b) Scincinae mode of digit variation:**

FIGURE 8 Schematic representation of the hypothesized Lygosominae and Scincinae modes of digit variation. (a) The Lygosominae mode of digit variation has been described in *Lerista*, *Hemiergus*, and *Carlia*. In these taxa, the first digit to become reduced is Digit I (D1). Only a few cases have been reported where metapodial 1 (M1) has been maintained in the absence of D1. This likely indicates that the loss of metapodial 1 soon follows the loss of Digit I phalanges. (b) The Scincinae mode of digit variation is found in *Brachymeles* and *Chalcides* and includes both examples of loss of Digit V or loss of Digit I phalanges. Absence of Digit V with the retention of Digit I has been documented in three species, *B. elerae*, *B. pathfinderi* hind limb and *Chalcides mionecton* as a polymorphism. An isolated loss of Digit I phalanges has been found in *C. sepsoides* as a bilateral polymorphism of the forelimb (YPM1865, Wagner, personal observation)

beyond a certain threshold. It is possible that reduced states may be associated with a more severe degeneration of the developmental program or larger changes in selective pressures, both of which could result in re-evolved digits that do not correspond exactly to the ancestral phenotype.

4.2 | *Brachymeles* and Morse's law

Morse's law, based on observations in mammals and reptiles, stipulates that the anterior Digit I is more likely to be lost than the posterior Digit V (Morse, 1872). In *Brachymeles*, a tetradactyl condition evolved twice independently, in *B. pathfinderi* (5/4) and *B. elerae* (4/4) (Figure 1). In both cases, a morphological analysis shows clearly that Digit V is missing, although both species retain a vestigial metapodial 5, which contradicts Morse's law.

These findings raise the question of how the *Brachymeles* configurations compare to the well-documented patterns of digit variation in *Lerista* and *Hemiergus* (Greer, 1991; Shapiro, 2002; Shapiro et al., 2007). In *Lerista*, there is a stereotypical sequence of digit loss that invariably started with Digit I, then Digit V, and finally Digits II and III, although the order of digit loss was postulated in the absence of a phylogeny (Greer, 1987, 1990). More recent phylogenetic data support this sequence of loss in *Lerista* (Skinner, 2010; Skinner et al., 2008; Skinner & Lee, 2009, 2010). The mode of digit reduction is also exemplified in the tetradactyl morphotype of *Hemiergus peronii*, where the anterior digit has the morphology of Digit II, and Digit V is clearly retained (Shapiro, 2002). The tridactyl *H. peronii* morphotype lacks Digit V but retains a vestigial

metapodial 5. A vestigial metacarpal 1, supporting the inference that Digit I was lost, has been described in *Carlia schmeltzii* based on the morphology of the remaining anterior most digit that resembles Digit II (Greer, 1991). In contrast, *Chalcides* skinks from North Africa and the Middle East are mostly pentadactyl or tridactyl (Caputo, Lanza, & Palmieri, 1995; Caranza et al., 2008). *Chalcides mionecton* has pentadactyl and tetradactyl morphs, with the latter missing Digit V (Caputo et al., 1995). Interestingly, we found a polymorphism in *Chalcides sepsoides* where the phalanges of the first digit were lost in the forelimb on both sides but metacarpal 1 was retained (0–2–3–4–2, YPM18065, Wagner, personal observation). Hence, while *Lerista* and *Hemiergus* do follow the predictions of Morse's law, *Brachymeles* and *Chalcides* seem to violate it. These differences may have a phylogenetic component because *Brachymeles* and *Chalcides* belong to the Scincinae and so are more closely related to one another than they are to *Lerista*, *Hemiergus*, and *Carlia*, which belong to the Lygosominae (Pyron & Burbrink, 2014). These comparisons suggest that the patterns of digit variation in skinks may differ among clades (Figure 8). A more extensive study is necessary to rigorously test the validity of this pattern.

4.3 | Digit reduction and the enigmatic metapodial 5

Shapiro et al. (2007) showed that in lepidosaurs, when Digit I is lost, its metapodial is also lost, but when Digit V is lost, its metapodial tends to be retained. Our findings from *Brachymeles* support this conclusion. One possible explanation is biomechanical, and postulates that metapodial 5

and metapodial 1 play different roles during lizard locomotion (Brinkman, 1980; Miles, 1994; Robinson, 1975; Russell, Bauer, & Laroia, 1997), leading to the retention of metapodial 5 despite loss of the phalanges. Lizards rotate their feet during horizontal locomotion using the metatarsal 5 as a lever (Robinson, 1975). Metapodial 5 of both the hand and foot is the insertion point for muscles moving the autopodium. In the *manus*, the *m. extensor carpi ulnaris* inserts on the pisiform and the base of metacarpal 5 (Abdala & Moro, 2006; Gaelle Bello-Hellegouarch, personal observation; Haines, 1950; Herrel, Vandooydonck, Prock, & Irschick, 2008; Russell & Bauer, 2008). In the *pes*, metatarsal 5 is the bone of insertion for the *mm. peroneus brevis*, *peroneus longus*, and the deep part of the *m. gastrocnemius* (Russell & Bauer, 2008). None of the other metapodials serve a similar function of moving the entire autopodium.

Given the role of metapodial 5 in autopodial movement, it seems likely that such function is retained in moderately digit-reduced limbs (i.e., tetra- and tridactyl limbs). Only if the limb is extremely reduced is metapodial 5 lost as well, consistent with Shapiro et al. (2007). To our knowledge, however, the muscle insertions on vestigial metapodials V have not been documented in skinks, and muscle descriptions in digit reduced species are rare for Squamata in general, but see Abdala et al. (2015) for some examples. A detailed analysis of the myology of moderately digit-reduced forms would further test this hypothesis.

5 | CONCLUSIONS

Our data support several conclusions. First, they support the hypothesis that digits have re-evolved in *Brachymeles*, and particularly in the pentadactyl clade. Second, they indicate that the hand of the pentadactyl species of *Brachymeles* is simplified, both in terms of its phalangeal formula (2-3-3-3-2), and its myology, with the loss or extreme reduction of six muscles that commonly insert to Digit 5 in *B. kadwa*. The most likely explanation for this is that the forelimb underwent a more severe digit reduction than the hind limb in the stem lineage of the pentadactyl *Brachymeles* clade, consistent with the results of ancestral character state reconstructions. Third, Digit I is more likely present than Digit V, contrary to Morse's law and contrary to the patterns found in *Lerista* and *Hemiergis* (Greer, 1987, 1990; Shapiro, 2002; Shapiro et al., 2007). Both, anterior-first and posterior-first modes of digit reduction are possible in lizards and may be clade-specific. Detailed phylogenetically informed studies of morphological disparity are essential for evaluating the generality of proposed patterns and laws of evolutionary change.

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DATA ARCHIVING

Sequence data accession numbers are listed in supporting information Table S1.

CONFLICT OF INTERESTS

The authors have no conflict of interests to declare.

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SUPPORTING INFORMATION

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