



# Strange geckos in a strange land: did morphological evolution and climatic diversification lead to ecological radiation and speciation in Flap-footed Lizards (Pygopodidae) during the Miocene aridification of Australia?

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**Abstract.** Pygopodids are more than forty species of gekkonoid lizards with an elongated body, no forelimbs and rudimentary hindlimbs that are reduced to flap appendices, endemic to Australasia. I explored the process of morphological evolution and diversification of pygopodids from a biogeographic and ecological perspective, subjecting body size and shape to comparative methods along a phylogenetic tree of species. In particular, I analysed the tempo of morphological diversification and disparification of Flap-footed Lizards in relation to the aridification of Australia and the arrival of Asian reptile faunas to the continent, and whether it is related to its ecological radiation and speciation. The transition from an ancestor with a short body and four limbs to the final elongated and partially limbless body plan of Pygopodidae occurred before the Miocene aridification of Australia. However, the current morphological diversification of Flap-footed Lizards is concordant with the opening of new habitats and the spread of the new squamate fauna that arrived from Asia during the Miocene aridification of Australia. Pygopodid lizards diversified in body shape and size from an ancestral terrestrial form with tail that was longer than the body, leading to different morphologies that do not fully agree with previous categorisations of the species' ecologies. The evolution of body size in Flap-footed Lizards experienced an initial strong phenotypic rate that led to large disparity and was followed by a decline during the rest of its evolutionary history. Hence, the evolution of body size and the colonization of warm regions of Australia are positively associated with speciation whereas the diversity of body shapes could be linked with ecological divergences, thus suggesting that pygopodids deserve further investigation to test whether they must be considered an example of adaptive radiation.

Key words. Squamata, Gekkota, adaptive radiation, ecomorphology, speciation.

## Introduction

Australia harbours a singular fauna remarkably rich in large endemic radiations as a result of its particular paleogeologic history. Long-time isolation from the other Gondwana landmasses and its closeness to Asia caused by their northern drift were the major driving forces, explaining the origins of the Australian fauna (HEATWOLE 1987, BERRA 1998). Thus, Australia preserves ancient Gondwana faunas since their drift from Antarctica (55–34 mya, HUSTON et al. 2012), such as for instance, marsupial and monotreme mammals, myobatrachid frogs, ratite birds, and chelid turtles (VAN TUIEN et al. 1998, KEALY & BECK 2017). However, during the Miocene, the northward migration of Australia placed the continent progressively close to Asia, facilitating the arrival of new lineages by marine dispersal that enormously contributed to its squamate diversity (POWNEY et al. 2010). For instance, the colonization of

Australia by varanid, agamid and skincid lizards, pythons and elapid snakes resulted in a vast number of species, creating the richest arid reptile fauna on the planet (PIANKA 1972). The departure of Australia from Antarctica also produced a complete circumpolar current circa 25 mya, leading to the formation of shelf ice sheets and triggering dramatic climatic changes on this continent (BARKER & BURRELL 1977, MCGOWRAN et al. 2004). The drop of temperatures during this period led to an initial aridification of Australia and the regression of the original tropical forests before being followed by a partial recovery of a warmer climate from the early to mid Miocene (23–14 mya, MCGOWRAN et al. 2004, BRYNE et al. 2008). Subsequent episodes of Antarctic ice expansion resulted in increases of the degree of aridity on this continent that gave rise to the expansion of scrublands 14–5 mya and later to the formation of the central desert 5–2.5 mya (MCGOWRAN et al. 2004, FUJIOKA et al. 2009).

One of the most enigmatic and remarkable endemics of the Australopapuan region is the Flap-footed Lizards of the family Pygopodidae (KINGHORN, 1926), a sister group of the Diplodactylidae, both members of the Pygodomorpha, one of the major Australian Gondwana endemics. This group currently includes more than forty species arranged in seven genera (*Aprasia*, *Delma*, *Lialis*, *Ophidiocephalus*, *Paradelma*, *Pletholax* and *Pygopus*) in Papua New Guinea and all of Australia with the only exception of Tasmania. Despite the large number of species and worldwide distribution of geckos (UETZ et al 2020), Flap-footed Lizards are the only elongated and partially limbed ones. All the species included lack forelimbs although they have a vestigial pectoral girdle, while the pelvic girdle is more developed and supports hindlimbs that vary in their degree of development (STEPHENSON 1962), but always externally form flap-like structures. Some authors suggested that because of the snake-like appearance of the body, Flap-footed Lizards might be convergent with snakes, filling ecological niches that are occupied by this group on other continents (PACHELL & SHINE 1986) and even competing with them. It is possible that the morphological diversification of Flap-footed Lizards happened before diverse lineages of elongated squamates arrived in Australia from Asia, to occupy the vacant niches that the latter fill in other mainland habitats. Alternatively, if the morphological diversification of pygopodids occurred during the aridification of Australia, it might be a result of adaptation to the emergence of new ecological niches in combination with the avoidance of competition with these newly arrived immigrants. In this case, Flap-footed Lizards may have diversified in ecology and morphology to specialize and preclude competition against snakes and elongated skinks rather than converging with them.

Despite the low number of species, pygopodids exhibit a remarkable diversity in their morphology and ecology. For example, they exhibit a wide dietary diversification to adapt to different trophic regimes by means of changes in cranial shape and dentition (GURGIS et al. 2021). Fossorial *Aprasia* have small skulls with rounded and short snouts, and a reduced dentition adapted to foraging on ant larvae and pupae (WEBB & SHINE 1994), whereas *Delma* species predate upon different kinds of insects and spiders. However, some species of this genus, as well *Paradelma orientalis*, include plant material in their diets (TREMUL 2000). In contrast, the diets of *Pygopus lepidopodus* and *P. nigriceps* incorporate significant quantities of mygalomorph spiders and scorpions, respectively (PACHELL & SHINE 1986). Perhaps the most surprising trophic specialization is found in *Lialis* species, which predate upon lizards, in particular skinks, and have developed a kinetic skull and hinged teeth to subdue them (MURRAY et al. 1991, WALL et al. 2013). Pygopodids also dramatically diverge in habitat use. For example, *Delma* comprises species that dwell on the ground, in leaf litter, and in small bushes and are capable of moving quickly, leaping (BAUER 1986) and even climbing, the latter behaviour also observed in *Pletholax* (BAMFORD 1998). In contrast, *Ophidiocephalus* and *Aprasia* live in the upper strata of sand or soil, although their ability to

make excavations has so far remained unclear (EHMANN 1981, WONG et al. 2011). On the other hand, *Lialis*, *Pygopus* and *Paradelma* are mainly active on the ground and in leaf litter (PIANKA 2011), and while the members of the first genus act as sit-and-wait predators, the others actively search for prey (WALL & SHINE 2013).

While the phylogenetic relationships among pygopodids have received much attention (KLUGE 1976, JENNINGS et al. 2003, LEE et al. 2009, OLIVER & SANDERS 2009, GARCIA-PORTA & ORD 2013, BRENNAN & OLIVER 2017, JENNINGS 2021), the process of morphological evolution and diversification of these geckos has remained barely explored.

The main goal of this study is to analyse the morphological diversification of Flap-footed Lizards from evolutionary, ecological and climatic perspectives by addressing several issues: i) whether morphological diversification in Flap-footed Lizards is related to the aridification of Australia and the arrival of new reptile lineages from Asia during the Miocene; ii) how morphological and ecological diversification happened through the history of Flap-footed Lizards and whether they co-evolved; and iii) which roles played morphological and climatic diversifications in the speciation of pygopodid lizards

## Material and methods

I reconstructed the evolutionary history of pygopodid geckos by building a phylogenetic tree using the Bayesian analysis incorporated in BEAST v2.6.4 (BOUCKAERT et al. 2014) with a dataset of sequences of 3741 nuc in maximum length (Supplementary document I). The dataset comprised forty-two species of Flap-footed Lizards plus eighteen Gekkota outgroups, assembling mitochondrial ND2 and nuclear CMOS, RAG1, DYNLL and PDC genes. After using jModelTest v2.1.10 (DARRIBA et al. 2021) to evaluate the fit of several nucleotidic models for each gene, I partitioned the dataset by assigning the GTR model to ND2, DYNLL and PDC, and the JC69 to CMOS and RAG1 genes. A log-normal molecular clock was applied to the Bayesian analysis, using five points of calibration based on the fossil record of Gekkota (Table 1).

The separation between *Euleptes* and *Teratoscincus* dating to the early Miocene (based on the fossil record, MÜLLER 2001, MÜLLER & MÖDDEN 2001) was used to model an exponential distribution of mean 25.0 and offset 22.5. The finds of fossils from the Miocene in New Zealand (LEE et al. 2009) allowed to date the divergence between those (*Naultinus*) and Australian (*Oedura*) geckos and was used to set an exponential distribution of mean 17.0 and offset 16.0. I modelled the divergence between the Caribbean geckos *Sphaerodactylus dommeli* and *S. ciguapa* by means of a log-normal distribution (mean = 15.0 and standard deviation = 34.0) according to Miocene fossils preserved in amber (DAZA & BAUER 2012, DAZA et al. 2013). A fossil record of pygopodid geckos assigned to the clade *Pygopus* plus *Paradelma* (20 mya, HUTCHINSON 1998) was used

Table 1. Points of calibration based on the fossil record of Gekkota used for estimation of divergences between Flap-footed Lizards under relaxed log normal molecular clock.

Divergence	Mean (offset)	References
<i>Euleptes / Teratoscincus</i>	Exponential mean 25.0 offset 22.5	MÜLLER (2001) MÜLLER & MÖDDEN (2001)
<i>Naultinus / Oedura</i>	Exponential mean 17.0 offset 16.0	LEE et al. (2009)
<i>Sphaerodactylus dommeli / S. ciguapa</i>	LogNormal mean 15.0 sd 34.0	DAZA & BAUER (2012, 2013)
<i>Pygopus / Paradelma</i>	Exponential mean 20.0 offset 0	HUTCHINSON (1998)
Gekkota / Unidentata	Uniform 145.5 – 251.0	DRUMMOND et al. (2006), VIDAL & HEDGES (2009)

to set an exponential distribution (mean 20.0, offset 0) for modelling the divergence between these two genera. Finally, I set the divergence between Gekkota and Unidentata to a uniform distribution of 145–251 based on previous results (DRUMMOND et al. 2006, VIDAL & HEDGES 2009).

I ran three chains of  $10^8$  iterations, sampling the posterior distribution each  $10^6$  times and assessing them for convergence and minimum sample sizes  $< 200$  with the aid of Tracer v1.7.1. (RAMBAUT et al. 2018). After discarding 99.9% of the posterior samples, I used the resulting 1000 trees to compute a maximum-credibility tree. In order to increase taxon sampling, I included *Pletholax edelensis* in the tree by creating a bifurcation between this species and the congeneric *P. gracilis* at 11.5 mya, using the percentage of ND4 divergence from KEALLEY et al. (2020) and a mean estimated rate of 1.3% per million years as computed using the four Gekkota points of calibration. The resulting tree with its complete taxonomic sampling was used in the analysis, and pruned to delete species that were not sampled in the morphological analysis. In order to study the ecological diversification of pygopodid lizards, I compiled information from bibliographic sources and following the schemes of GURGIS et al. (2021), I assigned the forty-eight species of Flap-footed Lizards to three main categories of habitat use: generalised fossorial, ground-dwelling, and grass-dwelling (Supplementary document II). The process of ecological diversification of pygopodid geckos was reconstructed by means of stochastic mapping, using a continuous time-reversible Markov chain on discrete traits by means of the simmap function in R Phytools v0.7 (REVELL, 2012). Prior to the analysis, I computed the fit of equal-rate, asymmetric-rate, and different-rate models to choose the best for stochastic mapping of the species' ecologies, using R Ape v5.5 (PARADIS & SCHLIEP 2019). In this and all the selected procedures of modelling, I compared models using the Akaike weights corrected by small sample sizes given the low number of pygopodid species.

Morphological data on Flap-footed Lizards from twenty-eight species representative of all the ecologies and genera was gathered from KLUGE (1974, see Supplementary document III for details). I computed the central value of nine linear measurements: SVL (snout–vent length), snout, postorbital and tail lengths, eye, head and rostral widths, head depth, and the number of hindlimb scales, with the latter being used as a surrogate of hindlimb length. In order to analyse separately the evolution of body size and

shape, I obtained a measure of size-free variation on the variables by performing an ordinary least-squared (OLS) regression of the other seven log-transformed variables, and trunk length, the latter was calculated as the difference between SVL and head length on the log-transformed head length using PAST v3.24. (HAMMER et al. 2001, Supplementary document III). Patterns of covariation on size-free morphologic variables were examined by means of a principal component analysis (PCA) using PAST v3.24 in order to characterize the morphological diversity of Pygopodidae. To reconstruct morphological evolution, I constructed a phylogenetic morphospace generated by body size (SVL) and shape (first size-corrected PC) in R Phytools v0.7. Prior to the analysis, I checked whether the morphological variables exhibited phylogenetic signals by computing lambda and K tests with R Phytools v0.7. To analyse how morphology evolved through the history of Flap-footed Lizards, I created several models of evolution of body size and shape (Brownian motion, Ornstein-Uhlenbeck, early-burst, lambda, delta, kappa, mean and rate trend, and white noise) with the aid of R Geiger v2.0.7. I performed a maximum likelihood reconstruction of the ancestral state under the best model by using the estimates of the parameters obtained in the previous procedure of modelling. Thus, for body size I used the early-burst model reconstruction in R Phytools v0.7 and for body shape, I rescaled the tree based on the Delta model with R Geiger v2.0.7 and reconstructed the ancestral values from the residual log-likelihood estimation function in R Ape v5.5.

I examined how fast morphology changed along the evolutionary timeline of Flap-footed Lizards by reconstructing ancestral phenotypic rates by means of a Bayesian Analysis of Macroevolutionary Mixtures (BAMM, RABOSKY 2014) using four chains of  $2 \times 10^9$  iterations and sampling them each  $10^5$  times. Before running BAMM, priors were calculated with R BAMMtools (RABOSKY et al. 2014) that were used to analyse the results. Morphological disparities of body size and shape were calculated as Euclidean distances within clades and between clades as computed in the nodes of the phylogenetic tree with R Geiger v2.0.7 (PENNELL et al. 2014). I performed 1000 simulations under Brownian motion to build a null model of morphological disparity and computed the mean expected values of random disparity.

I analysed the climatic diversity of Flap-footed Lizards based upon data of the most representative bioclimatic

variables, i.e., mean annual temperature (bio1), and annual precipitation (bio12), gathered from the World Climate database (FLICK & HIJMANS 2017). Thus, I compiled 14,863 distributional records of forty-three species from several databases (VertNet, Atlas of Living Australia and GBIF) and publications (e.g., KEALLEY et al. 2020) to extract the two selected variables and compute their means. I tested for phylogenetic signals in the two bioclimatic variables and evaluated which model of evolution fitted each of them best. For the annual mean temperature, the only bioclimatic variable showing a phylogenetic signal (see Results), I used the Kappa transformation of the branch lengths of the complete taxonomically sampled phylogeny, because it is the best model to reconstruct its evolution, by performing the same procedure as used for the morphological variables.

In order to evaluate the roles of morphology and climate in promoting speciation in Flap-footed Lizards, I performed quantitative-state speciation and extinction analyses (QuaSEE, FITZJOHN 2010) on body size and shape, and mean annual temperature by means of R package DiversiTree v0.9 (FITZJOHN 2012). Analysis was conducted using a sampling fraction of 0.6 and the variance of body size (SVL), first size-free PC and mean annual temperature of the range computed across species and evaluating constant, linear, sigmoid and hump-shaped distributions with and without drift.

## Results

Bayesian phylogenetic analysis placed the rise of Flap-footed Lizards from the other diplodactyloid geckos at after the drift of Australia from Antarctica at circa 47 mya (95% confidence interval, 56–39 mya, Fig. 1). Despite this ancient origin, the emergence of the current lineages of the family was more recent, at 22 mya (26–18 mya), and roughly overlapped with the early phase of Australia aridification. The maximum-credibility tree showed a well-supported basal split between *Lialis* and the other pygopodids and the colonization of Papua New Guinea by this genus at an estimated 11 mya (14–6 mya). The timespan of the emergence of *Delma* species, which diverged from their sister lineage formed by the remaining genera *Ophidiocephalus*, *Pygopus*, *Pletholax* and *Aprasia* 24–16 mya, encompassed 17–11 mya. Phylogenetic relationships between genera were found to be well supported by high posterior probabilities with the exception of the placement of *Ophidiocephalus* and the speciose genus *Aprasia*, with this latter sister group of the clade being formed by *Pygopus* plus *Paradelma*, which started its diversification 15–9 mya.

Stochastic character mapping of ecological diversification was performed using the preferred model (equal rates, AICc weight 0.738), and despite that the weight differences between this and the second best model were not large, ancestral reconstructions did not differ substantially. The ancestor of the recent Flap-footed Lizards was reconstructed as a ground-dweller (Fig. 2) with high confidence and

Table 2. Structure of the first two factors of the size-corrected PCA using variables obtained as residuals from OLS regression of each one on the head length. Total explained variability by the two first factors: 81.3%.

Variable	1 <sup>st</sup> PC	2 <sup>nd</sup> PC
Snout length	-0.027	0.014
Eye width	0.053	0.273
Postorbital length	0.054	-0.049
Head width	0.126	0.367
Head depth	0.107	0.312
Rostral width	0.333	0.360
Trunk length	0.470	-0.323
Tail length	-0.347	0.635
Number of hindlimb scales	0.715	0.225
Eigenvalues	0.073	0.016
% Variance accounted	66.8	14.5

evolved twice into fossorial lineages (*Ophidiocephalus* and *Aprasia*) and four times as grass-dwellers in *Pletholax* and some *Delma*.

All the morphologic variables and the two size-free PCs produced significant phylogenetic signals in both K and Lambda test. A PCA on the size-corrected morphologic variables revealed reasonably well patterns of covariation in morphologic variables, accounting for 81.3% of the total variation in only the first two factors (Table 2). Morphologic diversification in Flap-footed Lizards was characterized by opposite roles of the trunk elongation, limb scalation, and rostral width, and on the other hand of tail length, as is indicated by the first size-free PC. In the second, trunk length had a large negative coefficient in contrast to the positive coefficients of tail length and head and rostral widths, but their contribution to the variation was decidedly minor. Therefore, the first PC of size-free morphologic variables likely represents the evolution of diversity of body shapes seen in the recent pygopodid lizards.

Results of modelling show that body size (SVL) evolved according to an early-burst model (corrected Akaike weight 0.742), indicating a fast morphological diversification during the first stages of pygopodid evolutionary history. The evolution of body shape was better represented by the Delta model (corrected Akaike weight 0.806) with an estimated value of 0.126, suggesting that changes in shape became sharply decelerated in the latest stages of the evolutionary history (see Supplementary document V for details). Maximum-likelihood reconstruction of ancestral traits revealed that the common ancestor of the recent pygopodids was a large-sized gecko (SVL 132 mm, 95% confidence interval 76–189 mm, Fig. 3). From this ancestor, body size became larger in *Lialis*, but experienced a trend towards decrease in all other lineages, especially marked so in *Delma*, with the only exception of a secondary increase in the lineage of *Pygopus* plus *Paradelma*. The evolution of body shape in Flap-footed Lizards progressed from the ancestral bauplan characterized by an estimated

tail length that was to reach twice the SVL. *Aprasia* experienced a reduction in trunk length and more dramatically in tail length, whereas in *Ophidiocephalus* and especially in *Delma* and *Pletholax*, tail length increased. In *Lialis*, *Pygopus* and *Paradelma* body proportions changed by the elongation of trunk length in *Lialis*, although the tail remained longer than the trunk length.

These processes of size and shape evolution led to the rise of five distinctive morphologies in Flap-footed Lizards, as the phylomorphospace revealed (Fig. 4): *Lialis* had the largest sizes, larger trunk, and small hindlimbs and eyes; *Delma* and *Pletholax* had very small sizes with longer tails and hindlimbs, and heads with relative large eyes only in

the first genus; *Ophidiocephalus* was similar to this last group although slightly larger in size and its tail was comparatively shorter; *Pygopus* and *Paradelma* were characterized by their larger sizes, more developed hindlimbs, larger eyes and shorter tails compared with the other pygopodids with the exception of *Aprasia* and *Lialis*; and *Aprasia* were of small size, had very short tails relative to the SVL, little developed hindlimbs, and small eyes. These groupings did not match completely the main ecological diversification of Pygopodidae. Thus, ground-dwelling forms (*Lialis*, *Paradelma*, *Pygopus* and *Delma*) are not clustered, occupying different areas of the morphospace, as is the case in the generalised burrowers *Aprasia* and *Ophidiocephalus*. How-

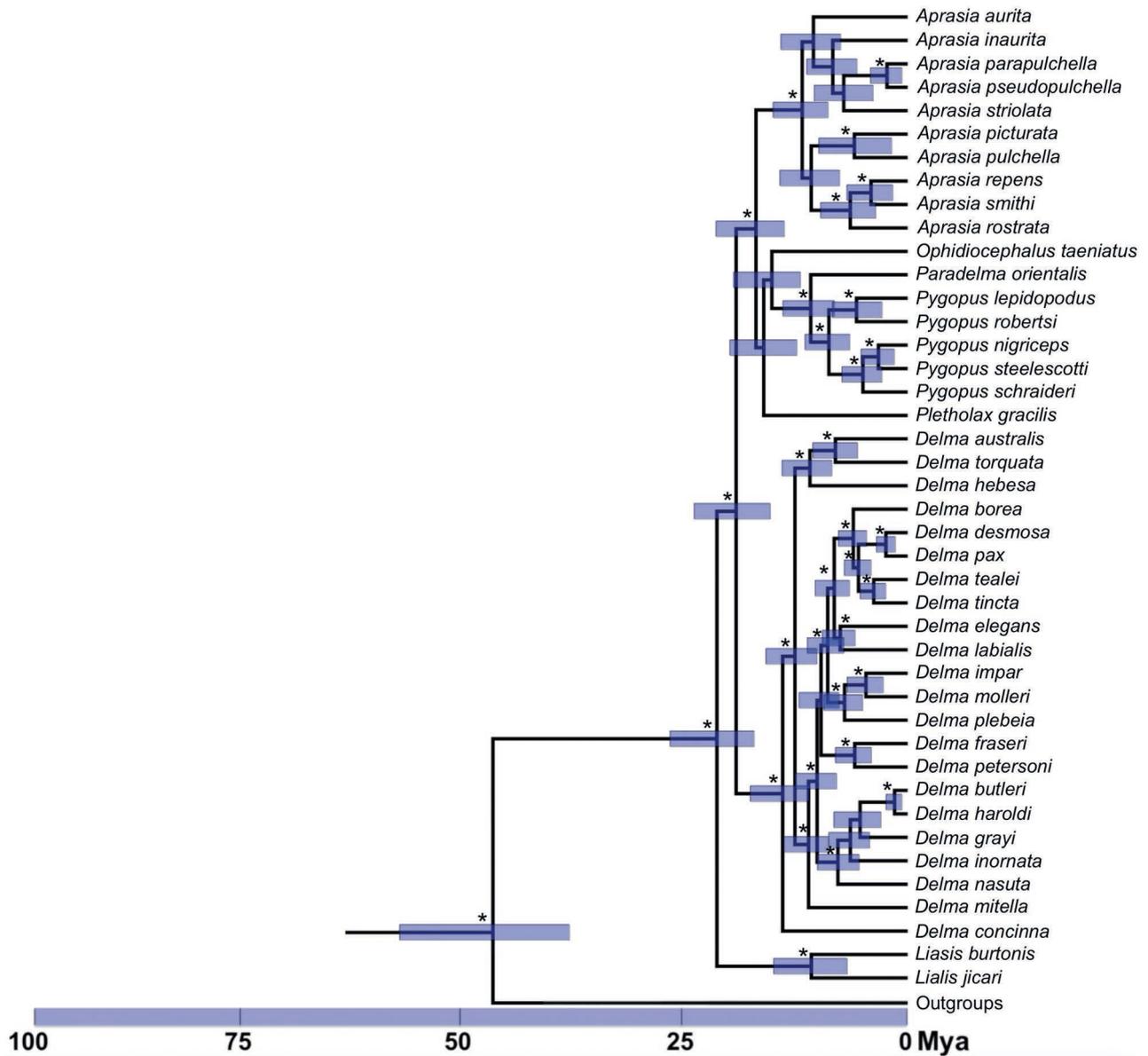


Figure 1. Maximum-credibility tree depicting the phylogenetic relationships between pygopodid geckos as estimated by means of a Bayesian analysis. Bars on the nodes represent the 95% confidence intervals of the divergences between lineages in million years. Nodes marked with asterisks are supported by posterior probabilities equal to or larger than 0.9.

ever, the grass-dwellers *Delma* and *Pletholax* were mixed with ground-dweller *Delma* species, indicating the lack of apparent morphological differences on habitat use in this genus.

BAMM analysis revealed a similar pattern of changes in phenotypic rates of body size and shape through time (Fig. 5). Rates in both traits were higher at the beginning of pygopodid diversification, approximately during a framespan of 22–15 mya, and suddenly declined thereafter. All pygopodid lineages followed this pattern with the only exception being *Lialis*, which phenotypically developed at moderate rates during most of their evolutionary history. In accordance with that, morphological disparity through time both in body size and shape produced a parallel pattern of sudden decreases after maximum initial values departing from the expectations derived from the null model of a random pattern (Fig. 6). The only difference between the two morphologic variables was a small increase of disparity in body shape that occurred circa 4 mya.

Climatic variables greatly differed in the strength with which the phylogenetic relationships between species constrained the evolution of these traits. Thus, mean annual temperatures produced a strong phylogenetic signal in the two tests, whereas in the case of annual accumulated precipitation, only the K test detected a significant phyloge-

netic effect (Supplementary document IV). Congruently, modelling of the two bioclimatic variables led to different results. The evolution of the mean annual temperatures in pygopodid species ranges was best represented by the Kappa model followed by the Delta one (Akaiké weights 0.508 and 0.140, respectively). The estimated value of Kappa (0.138) supported the concentration of changes in this trait in speciation events during the evolution of Flap-footed Lizards. The second best model, which had a high estimated value of the Delta parameter (2.999), indicated that changes in mean annual temperatures in the species' ranges were concentrated in the tips of the phylogeny, suggesting a late diversification of this trait in the evolutionary history of pygopodid geckos. Based on the best model, the ancestor of recent Flap-footed Lizards was reconstructed as having been a mesophilous species that experienced several independent transitions towards warm climates in some *Aprasia* and *Delma* species and temperate climates in other species of these genera, as well in the ancestral *Pygopus* species (Fig. 7). In contrast, white noise followed by the OU were the best-fitted model to the evolution of annual accumulated precipitation (Akaiké weights 0.404 and 0.183), indicating that this trait changes without constraints from the phylogenetic relationships between spe-

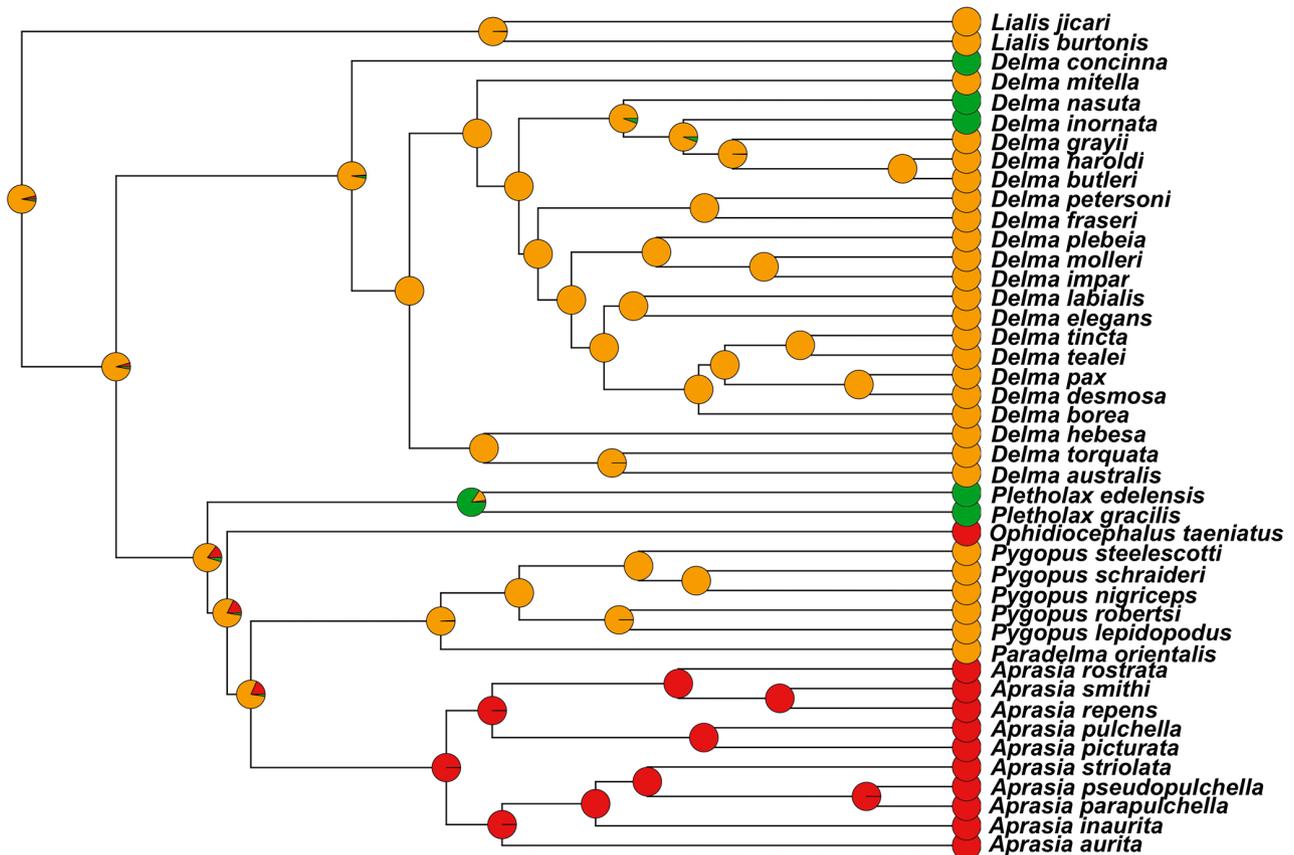


Figure 2. Stochastic-mapping reconstruction of the ecological diversification of Flap-footed Lizards: orange, ground-dweller; red – generalised fossorial; green – grass-dweller. Based on the equal-rates model, transition rate is  $0.0118 \pm 0.0037$ .

Evolution and speciation in Pygopodidae

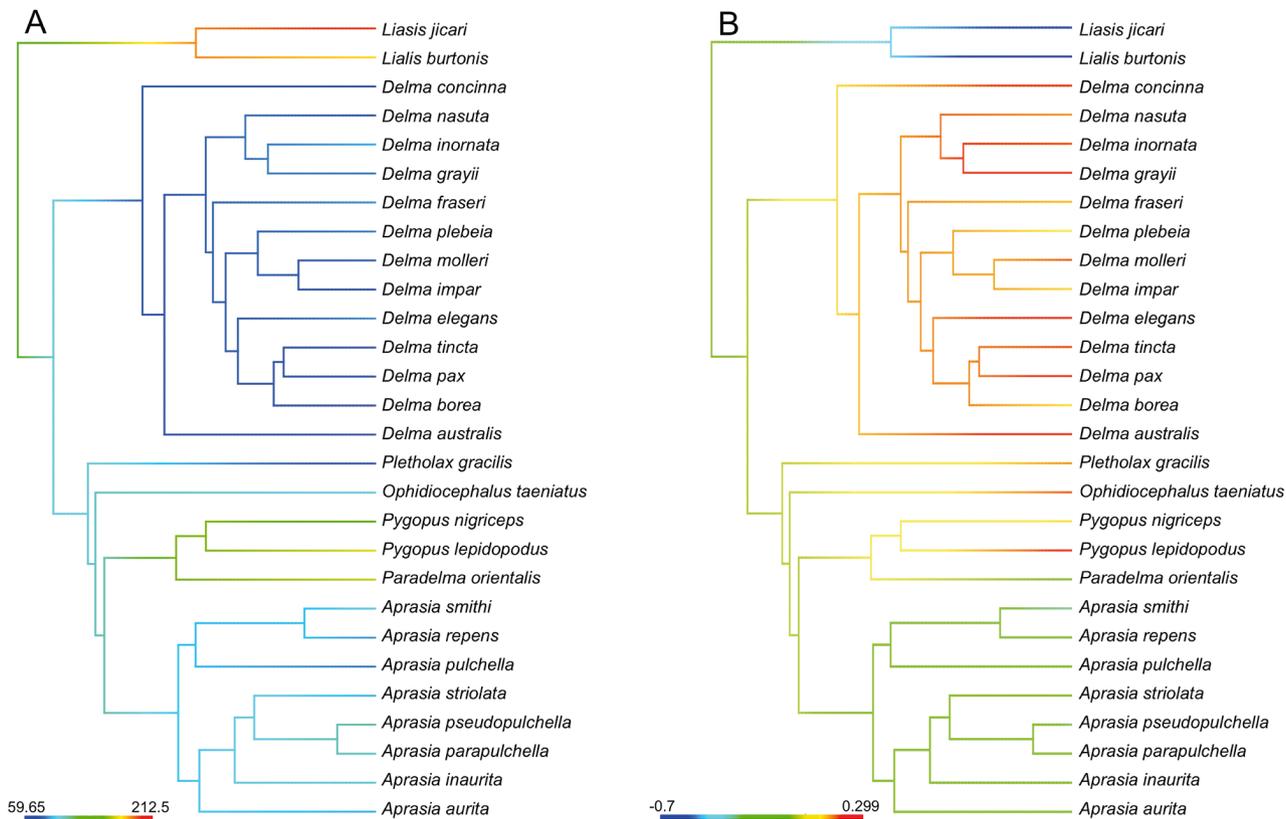


Figure 3. Maximum-likelihood reconstruction of size (a – SVL) and body shape (b – first size-corrected PC) evolution of twenty-eight species of Flap-footed Lizards.

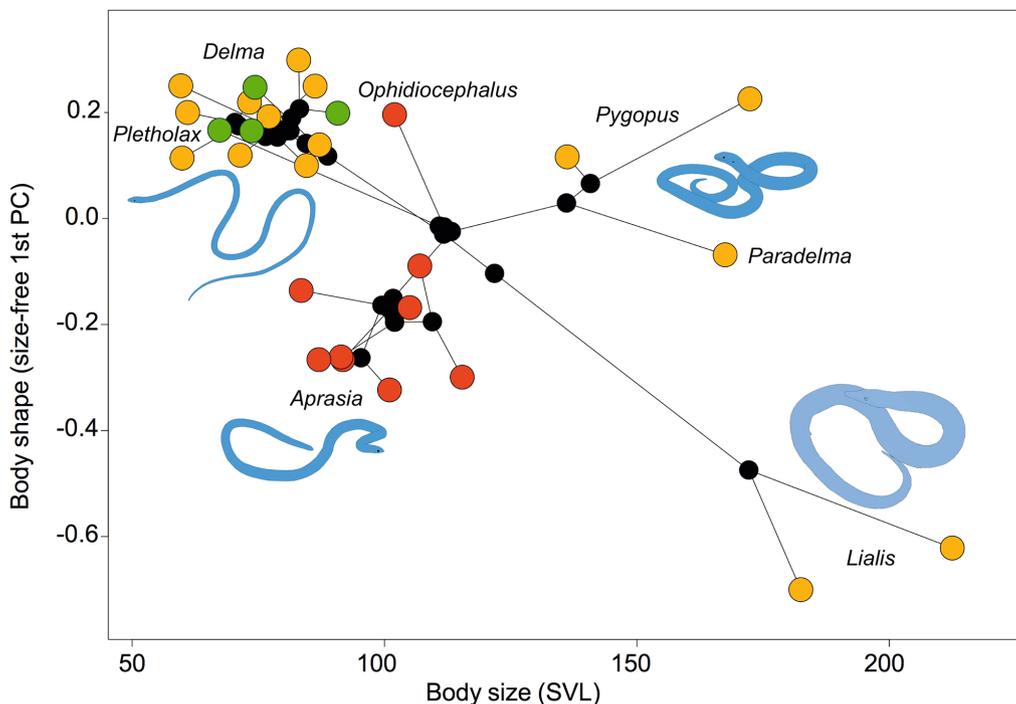


Figure 4. Phylomorphospace generated by size and body shape encompassing the morphological diversity of pygopodid lizards and the main ecologies in this family: orange – ground-dweller; red – generalised fossorial; green – grass-dweller; black – ancestral nodes.

QuaSSE analysis revealed a clear linear effect of body size on speciation (AICc weight = 0.974, Supplementary document VI) characterized by an increased speciation rate associated with the decreases in body size (coefficient = -0.001). Although I found evidence that body shape could have promoted speciation, the weight of the preferred model was not very high (sigmoid distribution, AICc weight = 0.566, Supplementary document VI) with respect to the second best and model that in this case was the null model of constant effect. My results also indicate that the occupancy of the warm regions of Australia promoted speciation in Flap-footed Lizards. The best model examined was the linear one with a drift that fitted considerably better than all others (AICc weight = 0.999, Supplementary document VI) and had a positive coefficient (0.02), thus evidencing an increased speciation in arid and warm Australia.

**Discussion**

This study is the first attempt to elucidate the tempo and mode of the morphological evolution and diversification of pygopodid lizards. Concordantly with previous studies (LEE et al. 2009, OLIVER & SANDERS 2009, BRENNAN & OLIVER 2017), I found that Flap-footed Lizards have a long independent evolutionary history since they diverged from diplodactyloid geckos. Their evolution towards body elongation and limblessness falls within the period between the separation of Australia from Antarctica and the beginning of the aridification of the continent in Miocene times. During the long period of isolation of Australia from other land masses, the fauna of elongated squamates on the continent was likely made up only by pygopodids and the now-extinct, large madtsoiid snakes (SCANLON 2005). Molecular clock analysis indicates that the arrival of the Aus-

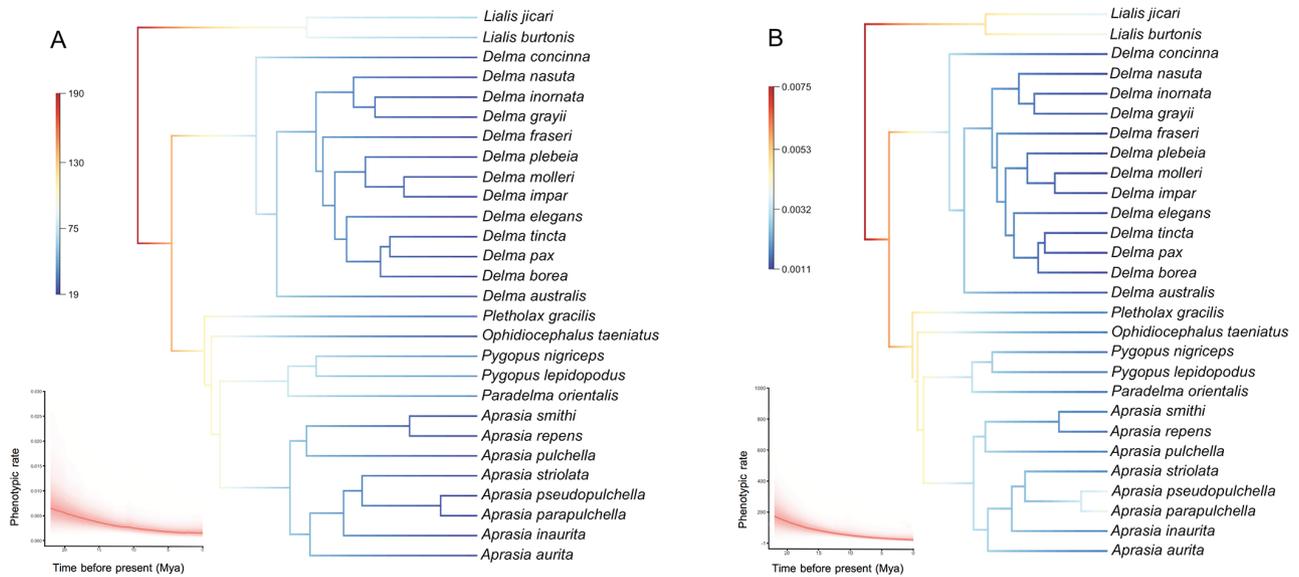


Figure 5. BAMM reconstruction of the phenotypic rates of evolution of size (a – SVL) and body shape (b – 1<sup>st</sup> size-corrected PC) and variation of phenotypic rates through time for each trait.

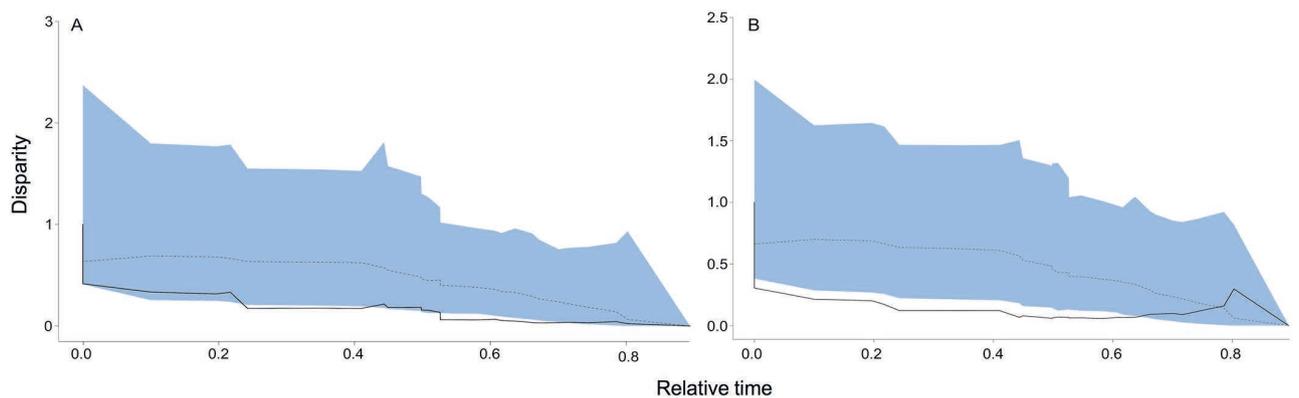


Figure 6. Morphologic disparities through time of size (a) and body shape (b) depicted by the continuous black line. For comparison, 1,000 disparities simulated under a Brownian motion null model were marked in blue and the mean disparity of the simulations in a discontinuous thin black line.

tralian snake fauna of elapid, python and blind snakes and sphenomorphine skinks from Asia may be timed at during the period between the Oligocene and the middle Miocene (RABOSKY et al. 2007, RAWLINGS et al. 2008, SANDERS & LEE 2008, SKINNER et al. 2008, VIDAL et al. 2010, RABOSKY et al. 2014). Thus, prior to the Asian squamate invasion, Australia had a multitude of unoccupied ecological niches, given their depauperate fauna of squamates, such as those requiring body elongation (WIENS et al. 2006). Thus, I hypothesize that the isolation of Australia and this faunistic anomaly could have generated ecological opportunity (STROUD & LOSOS 2016), one of the signatures of insularity, promoting the evolution of body elongation and limblessness in the ancestral pygopodid.

Accordingly to the results of molecular dating, the origin of the recent Flap-footed Lizards as well as the onset of their ecological diversification (26–18 mya) roughly matches the early phase of the process leading to the Aus-

tralian aridity and the invasion of the continent by lineages of squamates originating in Asia. I tentatively suggest that Flap-footed Lizards might have diversified to fill the new ecological niches provided by the opening and aridification of Australia during the Miocene in a quest to avoid competition with other elongated squamates. In addition, molecular dating is congruent with some adaptive inter-relationships between pygopodids and other Australian squamates. For example, the origin of Flap-footed Lizards exhibiting morphological (*Delma*, HALL 1905) and behavioural (*Paradelma* and *Pygopus*, GREER 1989) mimicry overlaps through time with the emergence of its model, the venomous elapid snakes of the genus *Pseudonaja* (SANDERS & LEE 2008).

The analysis of the body plan and morphology in Flap-footed Lizards indicates that previously defined ecomorphs (GURGIS et al. 2021) were an oversimplification in burden categories based on scarce ecological observations, lack-

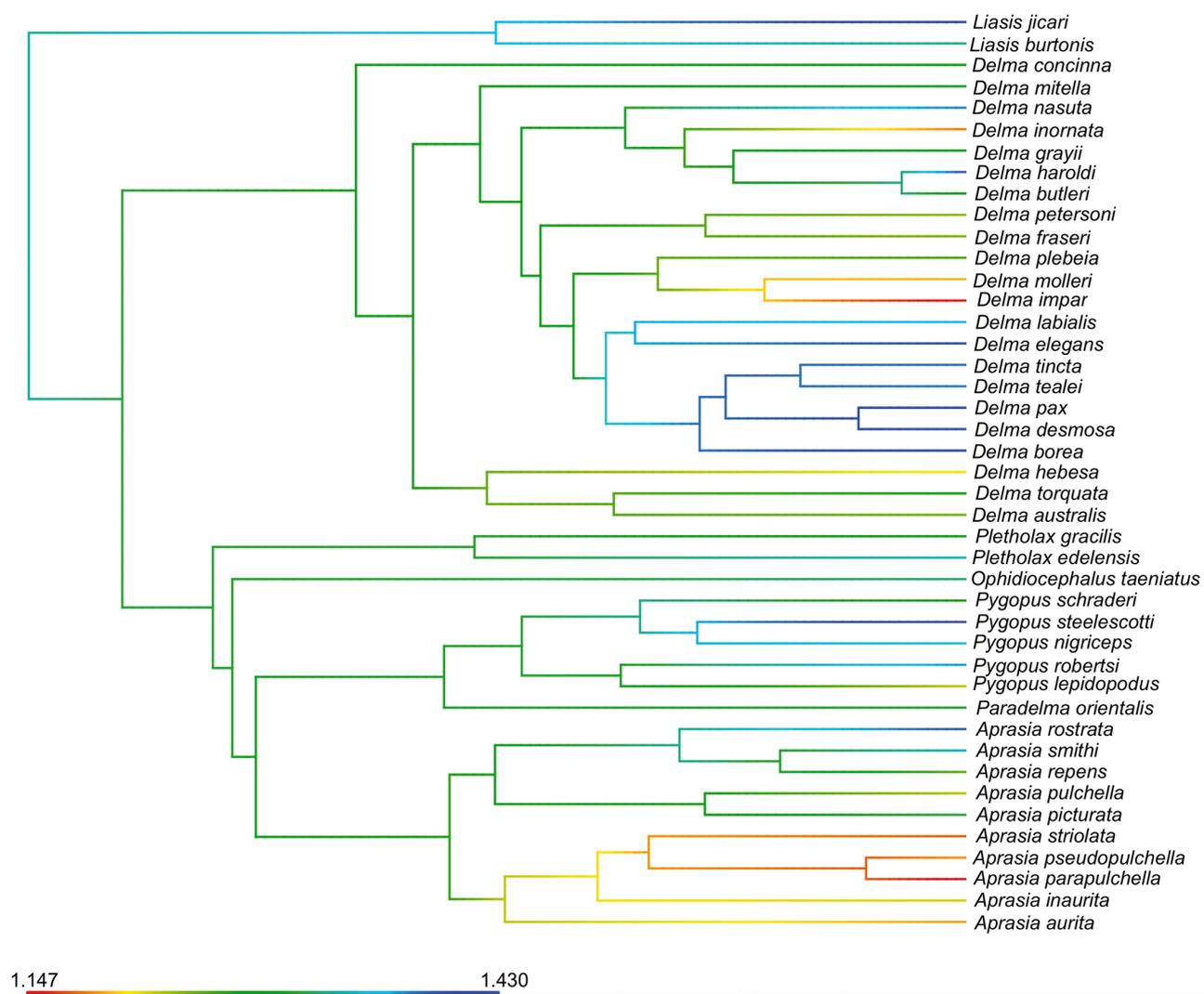


Figure 7. Maximum-likelihood reconstruction of log-transformed mean annual temperatures of the species ranges in Flap-footed Lizards.

ing accurate information about the interplay between the behaviour and habitat use of species. For instance, body proportions dramatically differ between the two burrowing lineages, *Aprasia* and *Ophidiocephalus*, suggesting that they move about in different manners, despite both using sandy substrates. *Aprasia* corresponds to the burrowing ecomorph (WIENS et al. 2006), but in contrast to other burrowing squamates (scolecophidians, dibamids or amphisbaenians), their eyes have a proportional size similar to other Flap-footed Lizards. *Ophidiocephalus*, which was not examined by WIENS et al. (2006), does not fit this classification due to its long tail. Aside from their general body proportions, *Ophidiocephalus* also depart from *Aprasia* by their proportionally small eyes and more developed hindlimbs, suggesting differences in ecology between them. Although they may be considered surface dwellers (WIENS et al. 2006), *Pletholax*, *Delma*, *Paradelma*, *Pygopus* and *Lialis* diverge in some morphological traits that can offer clues as to their habits. *Lialis* have a combination of traits, i.e., proportionally small eyes and longer heads, that might be related to their ambush predatory behaviour and the capture of elongated prey such as skinks by means of snout flexion (GREER 1989). In addition, *Lialis* have a less long tail than other Flap-footed Lizards, with the sole exception of *Aprasia*, likely to facilitate writhing its tail to attract prey (MURRAY et al. 1991). By comparison, *Delma* species are able to move fast across dense vegetation, search actively for prey (BAUER 1986), and their comparatively large eyes and very long tails might be an adaptation to this behaviour. Although *Pletholax* is very similar to most *Delma*, this genus and *D. concinna* have proportionally small eyes, suggesting that they might have a different foraging strategy. Species of these genera use their long muscular tails to leap (BAUER 1986) and the longest-tailed species, *D. concinna*, is able to scale tall shrubs (BAMFORD 1998). Remarkably, *Paradelma* and *Pygopus* roughly resemble *Delma*, but they possess proportionally longer hindlimbs, even if the functional significance of this trait is still unclear.

Some features of the evolutionary history of Flap-footed Lizards matches the expectations derived from a generous definition of adaptive radiation (SIMPSON 1953, WELLBORN & LANGERHANS 2015). Hence, despite the relatively low number of species, Flap-footed Lizards have undergone a fast diversification in bursts that led to divergent morphologies that could facilitate the exploitation of different habitats mediated by morphological and behavioural adaptations. Thus, extensive study of the species' functional and behavioural ecologies is crucial to test whether the distinct areas of the species' morphospaces can be confidently identified as ecomorphs (SCHLUTER 2001). Fast body size disparification seems to have played a fundamental role during the initial phases of the evolution of pygopodids and their speciation. Despite the little evidence found in this study, the importance of body shape cannot be ruled out, because its contribution to the morphological disparification of the family occurred shortly before the last phase of the formation of the central Australian desert. Speciation of pygopodid geckos exhibits an initial high rate followed

by posterior attenuation (JENNINGS et al. 2003, BRANDLEY et al. 2008) in agreement with the expected pattern in adaptive radiation (SIMPSON 1953, SCHLUTER 2000). However, physiological adaptation to tight thermal ranges could have favoured speciation in Flap-footed Lizards during the aridification of Australia, although this hypothesis is difficult to test due to the scarcity of data on this aspect of species ecology (CLUSELLA-TRULLAS & CHOWN 2014). In summary, Flap-footed Lizards could be a good example of condensed adaptive radiation, a fast early speciation of an old Gondwana lineage in a low number of, yet highly divergent, species. It could have been driven by the co-evolution of ecology and morphology to elude competition with novel immigrant squamate lineages from Asia and adapt to emergent niches produced by the aridification of Australia.

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### Supplementary data

The following data are available online:

Supplementary document I. Codes of gene bank DNA sequences used in this study.

Supplementary document II. Ecological characterization of pygopodid species.

Supplementary document III. Morphometric data used in morphologic analysis.

Supplementary document IV. Results of the test for phylogenetic signal on morphological variables.

Supplementary document V. Corrected Akaike values of the examined models of morphological evolution for body size and shape, and climate.

Supplementary document VI. Models examined in QuaSSE analysis of effects of SVL and body shape.