

A farewell to arms and legs: a review of limb reduction in squamates

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ABSTRACT

Elongated snake-like bodies associated with limb reduction have evolved multiple times throughout vertebrate history. Limb-reduced squamates (lizards and snakes) account for the vast majority of these morphological transformations, and thus have great potential for revealing macroevolutionary transitions and modes of body-shape transformation. Here we present a comprehensive review on limb reduction, in which we examine and discuss research on these dramatic morphological transitions. Historically, there have been several approaches to the study of squamate limb reduction: (i) definitions of general anatomical principles of snake-like body shapes, expressed as varying relationships between body parts and morphometric measurements; (ii) framing of limb reduction from an evolutionary perspective using morphological comparisons; (iii) defining developmental mechanisms involved in the ontogeny of limb-reduced forms, and their genetic basis; (iv) reconstructions of the evolutionary history of limb-reduced lineages using phylogenetic comparative methods; (v) studies of functional and biomechanical aspects of limb-reduced body shapes; and (vi) studies of ecological and biogeographical correlates of limb reduction. For each of these approaches, we highlight their importance in advancing our understanding, as well as their weaknesses and limitations. Lastly, we provide suggestions to stimulate further studies, in which we underscore the necessity of widening the scope of analyses, and of bringing together different perspectives in order to understand better these morphological transitions and their evolution. In particular, we emphasise the importance of investigating and comparing the internal morphology of limb-reduced lizards in contrast to external morphology, which will be the first step in gaining a deeper insight into body-shape variation.

Key words: body elongation, internal morphology, limb-reduced squamates, limb reduction, macroevolution, morphological evolution, snake-like lizards

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I. INTRODUCTION

Reconstructing the history of life, and consequently understanding how phenotypes evolve in response to varying environmental stimuli, are topics of primary interest in evolutionary biology. Dramatic transitions in body shapes and structures have historically represented a window into the interconnected factors that define evolutionary processes (Gould, 1990). Evolutionary theory has often benefitted from the study of morphological change: modifying biological structures involves changes at multiple organisational levels, from the genes involved in the coding of such structures, to the developmental mechanisms that generate them (Wiens & Slingsluff, 2001). In turn, the study of these structures, and their morphological patterns across taxa, can inform us of the selective environments that prompted their evolution and the modes of macroevolutionary transitions (Bergmann & Morinaga, 2019).

One of the most dramatic examples of morphological change in tetrapods is limb reduction, which has evolved independently several times in various lineages including mammals (cetaceans), birds (ratites), amphibians (caecilians and urodeles) and non-avian reptiles (squamates) (Gans, 1975; Lande, 1978; Greer, 1991; Bejder & Hall, 2002). Among tetrapods, no group surpasses squamate reptiles in the number and variability of limb-reduced forms (Gans, 1975; Lande, 1978; Brandley, Huelsenbeck & Wiens, 2008). In squamates, limb reduction is generally associated with the acquisition of a snake-like body shape (Lande, 1978; Greer, 1991). The transition to this body plan has occurred at least 26 times in squamates (Wiens, Brandley & Reeder, 2006) and is thought to involve progressive evolutionary transformations marked by the loss of digits, reduction in elements of the limbs, and elongation of the body. Its repeated occurrence across different groups, as well as the striking variability of limb-reduced forms (Fig. 1), makes it an invaluable tool to investigate the nature and mode of macroevolutionary transitions and how they relate to the patterns observed today. For this reason, limb-reduced squamates represent an ideal system to study the evolution of dramatic morphological modifications in the body plans of tetrapods (Wiens, 2009).

Here, we present a review of squamate limb reduction, providing a comprehensive overview that addresses multiple lines of research and their progress through time. We discuss the influence of the many scientific approaches that consider the evolution of limb reduction on our present understanding of this phenomenon. Finally, we outline recommendations for future work and highlight the key questions that still require answers in the context of squamate limb reduction.

II. HISTORICAL OVERVIEW

The study of squamate limb reduction has a rich history dating back more than 150 years, during which several different perspectives gained prominence with the development of

new analytical techniques and technologies (Table 1). Since an integration with palaeontological data has been hindered by the general lack of fossils for these lineages, the field relied upon examination of the patterns within extant organisms to infer information about their evolution (Brandley *et al.*, 2008). This has led to the use of an extensive array of comparative methods, from the first anatomical and developmental comparisons using morphological series (e.g. Fürbringer, 1870; Stokely, 1947), to the modern applications of phylogenetic comparative methods (e.g. Bergmann & Morinaga, 2019). The theoretical basis of these changes has also been repeatedly updated, as have the statistical tools to explore them. Furthermore, several studies have considered the developmental, functional, ecological and biogeographical aspects that underlie the evolution of limb-reduced morphologies.

(1) Morphological series: the search for anatomical laws of limb reduction

The reduction of squamate limbs requires a set of structural and body-shape modifications, as well as profound changes in the relationships between body parts. Defining these relationships over the range of variation was the main focus of early studies concerning limb reduction. Early research in this field (Fürbringer, 1870; Cope, 1892; Siebenrock, 1895; Müller, 1900; Duerden, 1924) focused on the comparison of morphological series of different types of limb-reduced lizards, ordering them across a spectrum from fully limbed pentadactyl forms to totally limbless (Fig. 1). This was intended as a way to reconstruct how the reduction of limbs and associated body parts proceeded over the course of evolution, given the absence of a complete palaeontological record with which to trace back the progression of limb degeneration. Using these methods, early studies established the following general principles: (i) reduced structures of digits, limbs, girdles and muscles are homologous to the same structures in fully limbed lizards (Fürbringer, 1870; Cope, 1892; Siebenrock, 1895); (ii) the regression of the limbs starts in the distal elements and progresses proximally (Fürbringer, 1870; Cope, 1892; Siebenrock, 1895); (iii) the muscles inserting onto the limb bones can persist after the total disappearance of their bone attachments (Siebenrock, 1895; Müller, 1900).

An additional principle was established by Krieg (1919) in his study of girdle degeneration in squamates. He indicates asymmetry, and a high level of intra-specific variability, in the development of the pectoral girdles of limb-reduced reptiles as ‘symptoms’ of limb degeneration. Such asymmetry would be linked to the reduced functionality of such elements. Krieg (1919) also observed that reduction of the sternal apparatus was uncoupled from that of the limbs and the scapular arches.

A common pattern that emerged was the attempt to define not only the morphological changes associated with the acquisition of a limb-reduced or limbless body shape, but also the developmental basis of these changes. The first attempts to explain the mechanisms underlying limb reduction from



Fig 1. Illustrated examples of squamates from across the spectrum of limb reduction (not to scale). (A) *Hemiergis decresiensis*, a limb-reduced skink; (B) *Lerista edwardsae*, a limb-reduced skink; (C) *Tetradactylus africanus*, a limb-reduced gerrhosaurid; (D) *Bachia bicolor*, a limb-reduced gymnophthalmid; (E) *Brachymeles elerae*, a limb-reduced skink; (F) *Anguis fragilis*, a limbless anguillid; (G) *Anomalopus verreauxii*, a limb-reduced skink; (H) *Bipes biporus*, a limb-reduced amphisbaenian; (I) *Carlia longipes*, a fully limbed skink with digit reduction; (J) *Lacerta bilineata*, a fully limbed lacertid; (K) *Malayopython reticulatus*, a snake.

a developmental perspective are to be traced to Camp (1923), Essex (1927), Sewertzoff (1931) and Stokely (1947). Camp's (1923) *Classification of the lizards* contains a detailed review of previous literature concerning limb degeneration in lizards. Explicitly referencing the concept of convergent evolution of limb-reduced forms, Camp (1923) maintained that the ancestor of all limb-reduced and limbless lizards must have been fully limbed because of the perceived impossibility of re-evolving complex structures such as limbs once they are lost (i.e. Dollo's Law; Dollo, 1893). He further observed the striking similarity between the degenerative series of limb-reduced squamates and the embryonic series of fully limbed squamates, noting that limb reduction occurs only in groups where the *rectus superficialis* [a ventral muscle associated with locomotion; but see Moody (1983) for a discussion about its anatomical validity] is present.

Essex's (1927) essay on limb-reduced forms introduced the idea of graduality in the evolution of limb reduction. Opposing the hypothesis put forward by previous researchers such as Duerden (1924) that degeneration is the result of well-defined developmental stages (the so-called 'fixism of the germinal changes'), he instead envisioned gradual changes involving the progressive loss of structures in the reverse order to which they were laid down during ontogeny. Moreover, he offered a tentative explanation for the loss of girdles, arguing that it follows the loss of limb muscles that would be a mechanical hindrance to the free use of the muscles used for burrowing.

In both of these works, Essex (1927) and Camp (1923) maintained that limb reduction is made possible by arrested embryonic development. Sewertzoff (1931), finding no evidence of such an arrest, instead described the sequential processes of 'rudimentation' and 'reduction': to the initial

Table 1. Studies on squamate limb reduction discussed in this review, categorised by type of study

References	Category
Fürbringer (1870); Cope (1892); Siebenrock (1895); Müller (1900); Krieg (1919); Camp (1923); Duerden (1924); Essex (1927); Sewertzoff (1931); Stokely (1947)	Morphological series
Gans (1960); Presch (1975); Gans (1975); Lande (1978); Berger-Dell'mour (1985); Greer (1987, 1990, 1991); Choquenot & Greer (1989)	Informed comparisons and evolutionary hypotheses
Raynaud (1972, 1974); Rahmani (1974); Raynaud & van den Elzen (1976); Gasc & Renous (1989); Raynaud <i>et al.</i> (1989)	Development 1: early studies
Shapiro (2002); Shapiro <i>et al.</i> (2007); Hugi <i>et al.</i> (2012)	Development 2: heterochrony
Cohn & Tickle (1999); Shapiro <i>et al.</i> (2003); Sanger & Gibson-Brown (2004); Woltering <i>et al.</i> (2009); Kohlsdorf <i>et al.</i> (2010); Woltering (2012); Head & Polly (2015); Roscito <i>et al.</i> (2014); Leal & Cohn (2016, 2017)	Development 3: <i>Hox</i> genes, SHH and FGF
Wiens & Slingluff (2001); Wiens, Brandley & Reeder (2006); Kohlsdorf & Wagner (2006); Brandley <i>et al.</i> (2008); Skinner <i>et al.</i> (2008); Skinner & Lee (2009); Bergmann & Irschick (2010); Galis <i>et al.</i> (2010); Kohlsdorf <i>et al.</i> (2010); Skinner (2010); Siler & Brown (2011); Miralles <i>et al.</i> (2015); Morinaga & Bergmann (2017); Wagner <i>et al.</i> (2018); Bergmann & Morinaga (2019); Morinaga & Bergmann (2020)	Phylogenetic comparative methods
Gans (1960, 1975, 1986, 1994); Raveshky (1960); Daan & Belterman (1968); Gasc (1974); Leonard (1979); Berger-Dell'mour (1985); Walton <i>et al.</i> (1990); Gans & Fusari (1994); Bergmann & Irschick (2010); Morinaga & Bergmann (2020)	Locomotion and biomechanics
Wiens & Slingluff (2001); Wiens <i>et al.</i> (2006); Brandley <i>et al.</i> (2008); Bergmann & Morinaga (2019); Morinaga & Bergmann (2020)	Ecomorphology and biogeography

FGF, fibroblast growth factor; SHH, sonic hedgehog.

reduction in size of an organ whose expression is selected against, would follow a progressive subtraction of the ontogenetic distal units of that organ (rudimentation) culminating in the total disappearance of its primordia (reduction).

Sewertzoff (1931) also observed that an increase in the number of presacral vertebrae, and in the elongation of the trunk, predicts the decrease in relative lengths of the limbs. Stokely (1947) came to similar conclusions, adding that from the perspective of functional morphology, the potential for an evolving lineage to react to functional stimuli must depend on some inherent genetic quality (the 'germ plasm') of the lineage itself. This means that an evolutionary pressure from a new environment is not sufficient to bring about a specific adaptation such as limb reduction if the genetic and developmental constraints of that lineage do not allow it. Stokely (1947) also popularised the practice of correcting limb and trunk lengths by size, using skull length because overall skull shape appeared more conservative than snout-vent length.

These initial studies followed the same general methodological approaches and were based on the understanding that each limb-reduced morph represented an evolutionary endpoint and not true intermediate forms or missing links in the acquisition of total limblessness. However, in their search for the unifying principles of appendicular degeneration, they generally failed to account for the possibility of substantially divergent pathways through which it could be achieved. These works also lacked a solid phylogenetic background – at the time, phylogenies of extant organisms were entirely based on morphological traits – and the relevant comparisons were made across small samples of largely unrelated reptile lineages (for instance, the families Anguillidae, Scincidae and Lacertidae) instead of focusing on variation within a single clade. Despite these shortcomings, they paved the way for more comprehensive studies: identification of the relationships between structures and body shape was crucial to define the anatomical boundaries and rules of the snake-like body plan.

(2) Informed comparisons and evolutionary hypotheses

Once the anatomical relationships between changes in body shapes and structures in the limb-reduced body plan were established, grounding these changes in an evolutionary context became the main focus. In order to understand why, how and in response to what selective environments such body shapes evolved, these studies required the incorporation of perspectives from several fields – morphology, ecology, developmental biology and genetics – and tended to focus on informed comparisons (i.e. within groups considered monophyletic) instead of morphological series.

Presch's (1975) study investigating the morphology, development and evolution of limbs, digits and girdles within the gymnophthalmid genus *Bachia* represents an early example of this novel approach, being one of the first to examine patterns in variation of limbs and digits in a single limb-reduced clade as a means to seek an evolutionary explanation. By regressing several morphometric measurements (limb lengths, snout-vent length, number of presacral vertebrae) against each other, and compounding these observations

with in-depth osteological descriptions, Presch (1975) concluded that *Bachia*'s forelimbs had regressed more rapidly than the hindlimbs, but that they stopped reducing after a certain threshold, whereas the hindlimbs never experienced this interruption and therefore underwent proportionally greater reduction. A corresponding pattern was observed in the girdles, putting Presch (1975) in disagreement with Krieg's (1919) view that the limbs and sternal component of the corresponding girdles were reduced independently of one another. Furthermore, by comparing gymnophthalmids with other limb-reduced squamate lineages, Presch (1975) noted the existence of two distinct structural patterns in the reduction of limbs: the reduction and loss of hindlimbs before forelimbs, as observed in *Bachia* and the scincid genus *Anomalopus* (Fig. 1G), or the reduction of forelimbs before hindlimbs, which would represent a more 'general' and widespread pattern among squamates.

Gans's (1975) study regarding the evolution and functional corollaries of tetrapod limblessness represents an invaluable contribution to the understanding of the evolutionary basis of limb reduction. Gans (1975) addressed the morpho-functional correlates of limblessness, categorised by anatomical regions and body-shape variables (general body shape and elongation, size, vertebrae, myology, skin, viscera, limbs, girdles and skulls), and the ecological correlates categorised by ecological variables (environment, diet and feeding mode, reproduction, sensoriality and locomotion). Based on these relationships, Gans (1975) proposed the hypothesis of 'multimodality': not only could selective pressures generate superficially similar outcomes from multiple distinct starting points, but even the pathways to these outcomes could be divergent. However, one constant factor was still acknowledged as unifying for the evolution of all limb-reduced squamate and amphibian forms: body elongation. Gans (1975) hypothesised that body elongation is a characteristic acquired before limb reduction, and is alternatively associated with crevice-dwelling or undulating locomotion, or a combination of these. Either way, limb reduction would follow as a secondary modification (meaning body elongation would be a 'proto-adaptation' to limb reduction). Body elongation, at least in reptiles, would be achieved through an increase in the number of trunk vertebrae, which Gans (1975) links to the increased complexity of the musculature necessary for effective body undulation (in amphibians, body elongation is achieved differently, through the elongation of the vertebrae themselves; see Bergmann & Morinaga, 2019). The acquisition of an elongated, snake-like body would also correspond to a narrowing and elongation of the viscera, here explained as a way to avoid stress to the inner organs during slithering motion. Finally, considering the diversity of modifications that reflect responses to a divergent array of selective pressures following the acquisition of elongated bodies, Gans (1975) established an ecomorphological characterisation of limbless and limb-reduced squamates. He identified four main categories: (i) fossorial forms that transitioned to fossoriality after an initial crevice-dwelling phase; (ii) crevice-dwelling ('sheltering') but still adapted to surface

environments; (iii) sand-swimming; (iv) snakes and snake-like forms, characterised by an increased gape to allow them to swallow large prey.

Another highly significant contribution that elaborates and improves on Gans's (1975) work is represented by Lande's (1978) essay on the evolution of limb loss in tetrapods. Here Lande (1978) goes a step further by providing an extensive developmental and genetic basis for his own theoretical observations. Recognising the inherent advantage of using squamates as a model group because of the abundance of extant intermediate forms, he compared morphometric measurements within several genera of skinks and gymnophthalmids, which led to the following observations: (i) that a sequence of limb reduction and body elongation could be ordered within each genus, implying that phylogeny did not follow only one direction; (ii) that reduction in the limbs starts at a certain threshold of relative limb size, and that this threshold varied across the different groups; and (iii) that all digits were lost when another threshold, again differing among groups, was reached. To corroborate his observations, Lande (1978) investigated the developmental mechanisms of limb reduction, basing his theoretical formulation on observations of morphological series and on previous studies regarding limb reduction in pythons (Raynaud, 1974) and lizards (e.g. Rahmani, 1974; Presch, 1975; Raynaud & van den Elzen, 1976). Regarding the mechanism of distal-to-proximal reduction of limbs, he adopted Raynaud's (1974) view, acknowledging that reduction in the limb buds resulted from the active early onset of cellular necrosis at the level of the apical ectodermal ridge (instead of arrested development in the distal elements of the limbs, an idea supported in the earliest developmental studies). Thus, the distal elements would be reduced first because they are laid down last during development. Lande (1978) invoked the same mechanism to explain the order of digit reduction – proceeding from the margins of the autopodium towards the centre – that he observed in his morphological series: since the last digits to be laid down in morphogenesis were also the most marginal, they would also be the first to be affected by early necrosis.

This work gave rise to the first hypotheses of the genetics of variation in the digit formulae and their relationships to limb proportions in limb-reduced lizards. Assuming (based on previous studies examining the genetic mechanisms of polydactyly in tetrapods: see Wright, 1934*a,b*, 1935) that the existence of various digit morphs was under polygenic control, he observed that in two species of the genus *Bachia* (Fig. 1D) there appeared to be a specific relationship between digit morphs and relative limb proportions. This correlation was identified as a case of pleiotropy, in that digit number is partially determined by the relative amount of tissue available during limb bud development. This was under the assumption that, to the extent that the two factors are genetically independent, digit number would be influenced by the functional constraints arising from reduced limbs and undulatory locomotion due to stabilising selection. Lande (1978) also used his argument for pleiotropy to make the case for re-expression of lost limb and digit structures, arguing that since

genes that code for limbs and digits tend to have pleiotropic effects on other adaptive structures, those genes would be preserved through evolution in a partially inactivated form even if the limbs were lost: he concluded that the re-expression of lost limb and digit structures, although relatively unlikely, cannot be excluded *a priori*.

These works inaugurated a new era in the study of squamate limb reduction, incorporating elements from different investigative approaches, namely comparative anatomy, functional morphology, developmental biology and genetics, and they set a precedent for providing a comprehensive theoretical basis to this complex evolutionary problem.

An important study of comparative osteological and myological aspects of three species of the African cordylid genus *Tetradactylus* (Fig. 1C) was carried out by Berger-Dell'mour (1985). Relevant comparisons across a cline of progressive limb reduction were used as a basis to propose a series of evolutionary hypotheses. Locomotory tests on live animals allowed the author to conclude that the first step in the transition from lizard to snake-like body shapes would be explained by a change in locomotion, clarifying that it would precede body elongation and limb reduction. Thus, for the genus *Tetradactylus*, the evolution of progressively more limb-reduced and body-elongated forms is explained by functional adaptation to moving faster through high grasses (grass-swimming); only once the functionality of the limbs was completely lost, would rudimentation represent a way to save precious building material during ontogeny. He also notes that the processes of limb reduction are associated with marked asymmetry in the elements of limbs, girdles and sacral vertebrae (corroborating the observations of Krieg, 1919) and that, with the onset of body elongation, the vertebral spine tends to become less regionalised and the position of the girdles relative to it tends to vary inter- and intra-specifically (thus refuting van Bemmelen's (1952) hypothesis that the position of the girdles is fixed and the increase of presacral vertebral numbers is due to the splitting of embryonic somites). Ontogenetically, according to Berger-Dell'mour (1985), these 'anatomical anomalies' are explained by shifts in embryological development, and not by well-defined genetic changes or a set of mutations that would allow for reduction of limbs in coordination with the increase in number of presacral vertebrae. In fact, Berger-Dell'mour (1985) finds no evidence of a genetic linkage between body elongation and limb reduction: intraspecifically, the studied specimens with more 'derived' characteristics also possessed some 'primitive' characters (e.g. the most limb-reduced specimens of *Tetradactylus seps* also had the lowest vertebral counts of the series). This conclusion is at odds with some other views, such as those of Raynaud (e.g. Raynaud, 1974) and Lande (1978), that saw a precise relationship between reduction and degree of elongation at the genetic level.

Greer's (1987, 1990, 1991, 1992; Choquenot & Greer, 1989) work on Australian limb-reduced reptiles was instrumental in bringing new perspectives, by providing an updated definition of what constitutes limb reduction based

on osteology. In a series of studies regarding the osteology of the scincid genus *Lerista* (Fig. 1B), Greer (1987, 1990) established a relationship between the progressive loss of phalanges and an increase in the number of presacral vertebrae. Greer (1991) sought to provide a classification of the types of limb reduction based on limb osteology of the relevant taxa, to reconstruct the reduction that happened through the loss of limb bones and provide insights about the common ancestors of these lineages based on these bone configurations. Identifying a squamate as limb-reduced if it had lost one or more limb bones (namely, the phalanges in the digits) compared to the ancestral squamate condition, Greer (1991) was able to quantify the extent of limb reduction in the group, noting it was present in at least 53 squamate lineages, in which reduction had evolved a total of 62 times; across these lineages, there existed a substantial amount of variability in the reduction of forelimbs and hindlimbs. He also observed that the great majority of taxa had lost a small number of bones (one or two phalanges), whereas a minority had experienced substantial reduction of more elements. According to this rather broad definition of limb reduction, many lineages (e.g. the scincid genus *Carlia*: Fig. 1I) that had experienced loss of limb bones did not display a tendency towards body elongation. However, these taxa exhibited only moderate bone losses, whereas the most dramatically reduced lineages (e.g. several taxa within Scincidae and Anguillidae) tended towards body elongation. Commenting on the current distribution of phalangeal loss across squamates, Greer (1991) considered the possibility of large 'saltations' in phalangeal numbers in contrast with the relatively more frequent gradual losses of one or two phalanges. If instead one follows the traditional idea that reduction proceeds through small incremental steps determined by truncations in development (see Shubin & Alberch, 1986), he reasoned, one would expect to see more extant intermediate forms than we observe today.

The studies summarised above show that the integration of many aspects of research in a multidisciplinary perspective allowed improvements in appreciation of factors influencing the evolution of snake-like body shapes. However, the lack of a solid phylogenetic framework still represented a hindrance to a full understanding of the dynamics of these transitions.

(3) The developmental and genetic basis of limb reduction and body elongation

(a) Early studies

Whereas early authors such as Essex (1927) and Stokely (1947) offered tentative insights into the developmental machinery involved in limb reduction, these mechanisms remained largely unexplored. The 'developmental' school of thought emerging in the second half of the 20th century considered the evolutionary degeneration of limbs as proceeding through well-defined stages, more or less localised truncations in development that reflected corresponding ontogenetic stages in the embryo (Alberch & Gale, 1985;

Shubin & Alberch, 1986), and was focused on defining the specific developmental mechanisms and genetic factors associated with each of these stages.

The work of Raynaud and collaborators (e.g. Raynaud, 1972, 1974; Raynaud & van den Elzen, 1976; Raynaud *et al.*, 1989) on the ontogeny of squamate embryos represents the first important effort in defining the developmental mechanisms and stages through which limbs were reduced. Methodologically, these investigations were made possible by a combination of anatomical observations on developmental series of limb-reduced lizards and snakes (e.g. *Anguis fragilis*, *Malayopython reticulatus*: Fig. 1F, K), and experimental approaches to inhibit limb formation in embryos of normal-limbed lizards (*Lacerta bilineata*: Raynaud, 2003; Fig. 1J). The innovative aspect of these studies and those of other contemporary authors (e.g. Rahmani, 1974) is the recognition of the early onset of necrosis (cell death) of the apical ectodermal ridge (AER, a structure on the apex of the limb bud responsible for distal growth: see Gilbert, 2000) as the mechanism of limb reduction during ontogeny. The number of somites from which the limb buds originate was hypothesised to be the main determinant of this mechanism.

A hypothesis on the role of the somites in degeneration of the limb bud was provided by Gasc & Renous (1989). The mechanism is described as depending on two distinct oscillating systems that influence one another during development: the somitogenic system (from which the generation of the somites and the degree of body elongation is determined) and the limb-generating system (which generates the limb buds from the ventral extensions of specific somites). A facilitator emitted by the somitogenic system helps the diffusion of the inhibitor of the limb-generating system, which inhibits the development of the limb buds late in ontogeny. Regulation of this facilitator would influence the differential development of forelimbs and hindlimbs, as well as the reduction in the number of cervical vertebrae that can be observed in many limb-reduced reptiles. These mechanisms are envisioned as being genetically linked to the appearance of dominant alleles that modify the rhythm of receptivity of the cells within somitomeric units. Evolutionarily speaking, the reason why intermediate fossil forms are mostly absent is here seen as due to the extreme speed of the process of limb reduction and correlated body-shape reorganisations, which would in turn stem from the need to re-establish genetic stability after the rupturing of the cohesion of the initial genotype in response to environmental stimuli (Mayr, 1982).

This school of thought had the merit of being the first to describe potential mechanisms that gave rise to limb-reduced forms during ontogeny, and considered these developmental processes as a basis for evolutionary explanations. Some authors (e.g. Greer, 1991) would later criticise this perspective, arguing that the development of reduced limbs cannot solely be traced back to a progression of gradual stages, and if present, these stages are not necessarily reflected by the evolutionary processes of limb degeneration.

(b) Heterochrony

Another important aspect of the developmental perspective considers that changes in morphology are modulated through shifts in the timing of chondrogenesis, osteogenesis and myogenesis during development. This heterochronic hypothesis (Alberch *et al.*, 1979) has gained much traction in the study of limb-reduced squamates because of its potential to link developmental processes with biological structures, and to explain the existence of intermediate limb-reduced forms. While multiple studies (Bininda-Emonds *et al.*, 2007; Richardson *et al.*, 2009; Čerňanský *et al.*, 2019) have highlighted heterochronic shifts in the differential development of forelimbs, hindlimbs and vertebrae across tetrapods, no definitive answer has been reached in regard to the adaptive value of such shifts, or their role in limb reduction and loss.

The role of heterochrony in squamate digit loss was tested by Shapiro (2002) and Shapiro, Shubin & Downs (2007). They compared ontogenetic series of species of the scincid genus *Hemierris* (Fig. 1A), which displays graded intra- and inter-specific reduction in the number of digits, noting abrupt shifts, or heterochronic truncations, in ontogenetic timing during the generation of bones, muscles and cartilage for each morph. They concluded that heterochronic truncations could explain the formation of incomplete digits and phalangeal loss, but could not be considered the mechanism for the formation of fewer complete digits. However, in a more recent study (Hugi *et al.*, 2012), instances of heterochronic shifts have been recorded in developmental series of limb-reduced reptiles. Notably, the onset of ossification in the bones of limbs, girdles, skulls and vertebrae of these reptiles was found to be comparatively earlier and more rapid than in fully limbed taxa, which in turn corresponds to changes in the areas of muscle insertion and functionality of these structures. These changes were interpreted as being associated with a shift from limbed locomotion to lateral undulation and head-first burrowing, which in fact characterises most limb-reduced and limbless lizards as adaptations to sub-surface habitats such as sand or leaf litter.

(c) Hox genes, SHH and FGF

The discovery of the role of homeobox (*Hox*) and related genes in specifying the axial patterns of organismal body plans (Shubin & Alberch, 1986; Coates, 1994; Coates & Cohn, 1999) marked a turning point in understanding of the interaction between genetic and developmental mechanisms giving rise to appendicular degeneration. Cohn & Tickle's (1999) investigation of the patterns of genetic expression intervening in python morphogenesis found that axial de-regionalisation observed in limbless squamates was explained by the expansion of *Hox* gene domains along the body axis; loss of limbs would instead be mediated by changes in two signalling pathways involved in the formation of the limb buds. The initiation of limb bud development through expression of a fibroblast growth factor (FGF;

specifically FGF8) would be arrested by the de-activation of signalling pathways of the AER and of the zone of polarising activity (ZPA, the region of the bud that determines the polarity of limb outgrowth); this pathway is also linked to the expression of the *Shh* gene, which encodes the sonic hedgehog (SHH) protein produced at the level of the ZPA (Hinchliffe, 2002; Litingtung *et al.*, 2002; Welscher *et al.*, 2002). The authors observed that this protein can still be produced by the buds of limbless reptiles but is inhibited during ontogenesis due to absence or regression of the AER.

These observations were limited to fully limbless squamates, but forms with intermediate degeneration presented a different picture, complicated by the presence of variably reduced girdles, limbs and digits. Shapiro, Hanken & Rosenthal (2003) described some of these mechanisms for the skink genus *Hemiergus*. The number of digits in adult lizards were found to be controlled by the duration of SHH protein expression in the early stages of limb bud development, whereas a halt in SHH expression caused an arrest in the proliferation of the limb bud itself. Successive studies (Sanz-Ezquerro & Tickle, 2003; Stricker & Mundlos, 2011) also found that the differential expression of FGF8 was linked to the development of shorter digits and loss of phalanges, while others (Chiang *et al.*, 2001; Ros *et al.*, 2003) observed that the expression of SHH was not essential to the formation of the limb buds.

The role of SHH and FGF signalling pathways in determining the developmental patterns of limbs and digits is still subject to debate. Studies on limb-reduced gymnophthalmids (Roscito, Nunes & Rodrigues, 2014) underscore the prospect that the mechanisms regulating moderate limb and digit degeneration may not be universal for all squamates, while still recognising SHH and FGF and their interaction with the AER as the main factors involved in dramatic reductions. Others [Young *et al.* (2009), for the scincid genus *Chalcides*] highlight the possibility of shifts in digit identity as a consequence of digit reduction and modulation of the SHH pathway.

Regarding the role of *Hox* genes, Sanger & Gibson-Brown (2004) questioned the idea that body elongation and limb reduction were genetically determined by the same pathways, observing that *Hox* genes were only involved in specifying the identity of axial regions (essentially, whether a region produces limb buds or not), not the rates of somitogenesis that would in turn determine body elongation. However, this and other studies (Kohlsdorf *et al.*, 2008; Woltering *et al.*, 2009; Woltering, 2012; Head & Polly, 2015; Leal & Cohn, 2016, 2017) still maintain that some *Hox* genes may be directly involved in limb morphogenesis (and not limited only to axial regionalisation) (Fig. 2); in particular, the patterns of expression of the genes *Hoxa-13* and *Hoxd-13* and related proteins were found to be of fundamental importance in limb reduction because of their roles in autopodial specification (Leal & Cohn, 2016).

These lines of research have been instrumental in gaining a more thorough understanding of the interactions between genetic and developmental mechanisms giving rise to limb-

reduced and limbless body shapes. However, framing of these mechanisms within the bigger picture of evolutionary transitions is still fundamentally lacking. With the exception of some studies (e.g. Hugi *et al.*, 2012) these analyses are typically based on the study of a single taxon or low numbers of closely related taxa (e.g. Shapiro, 2002; Shapiro *et al.*, 2007) and are rarely, if ever, compared across large-scale phylogenetic frameworks. While recognising the methodological and practical challenges associated with this kind of analysis, piecing together these patterns of developmental and genetic variation may grant a deeper insight into the evolutionary pathways of limb reduction in squamates.

(4) The history of limbs and digits: phylogenetic comparative methods and ancestral reconstructions

The rise to prominence of phylogenetic comparative methods (Felsenstein, 1985), and the widespread use of phylogenies obtained from genome sequencing efforts, represented a new way to investigate and reconstruct the evolution of limb-reduced squamates. Recent studies making use of these methods (Wiens & Slingluff, 2001; Kohlsdorf & Wagner, 2006; Wiens *et al.*, 2006; Brandley *et al.*, 2008; Skinner, Lee & Hutchinson, 2008; Skinner & Lee, 2009; Skinner, 2010) have brought together contrasting aspects of the question of limb reduction and changes related to it, attempting to reconstruct the evolutionary history of characters to explain observed patterns in extant taxa.

A substantial number of these studies have focused on single lineages [Wiens & Slingluff (2001) for anguids; Skinner *et al.* (2008), Skinner & Lee (2009), Skinner (2010) and Morinaga & Bergmann (2017) for the scincid genus *Lerista*; Kohlsdorf & Wagner (2006) and Kohlsdorf *et al.* (2010) for the gymnophthalmid genus *Bachia*] and often on reconstructing the history of single characters such as digits. The evolution of digit morphs has been the subject of ongoing debate regarding the reversibility of digit loss and its implications for Dollo's law (Gould, 1970), which states that reversals of complex characters should be impossible or at least constrained by development. Some reconstructions of the evolutionary history of digits propose that reversions – the re-expression of lost digits – might have happened multiple times in some groups (Kohlsdorf & Wagner, 2006; Brandley *et al.*, 2008; Kohlsdorf *et al.*, 2010; Wagner *et al.*, 2018); these conclusions have been contested by studies like Galis, Arntzen & Lande (2010) on the grounds of the invalidity of the assumption of character independence of the digits, which would automatically exclude the possibility of pleiotropic effects in the case of digit re-evolution. Other studies (Skinner *et al.*, 2008; Skinner & Lee, 2009; Skinner, 2010) chose a more cautious approach, noting that while for some clades (e.g. *Lerista*) most evolutionary models yield only very low probabilities of digit reversions, they do not imply that such reversions are impossible.

In the broader context of body-shape evolution, several analytical efforts (Brandley *et al.*, 2008; Siler & Brown, 2011; Morinaga & Bergmann, 2017) have found significant

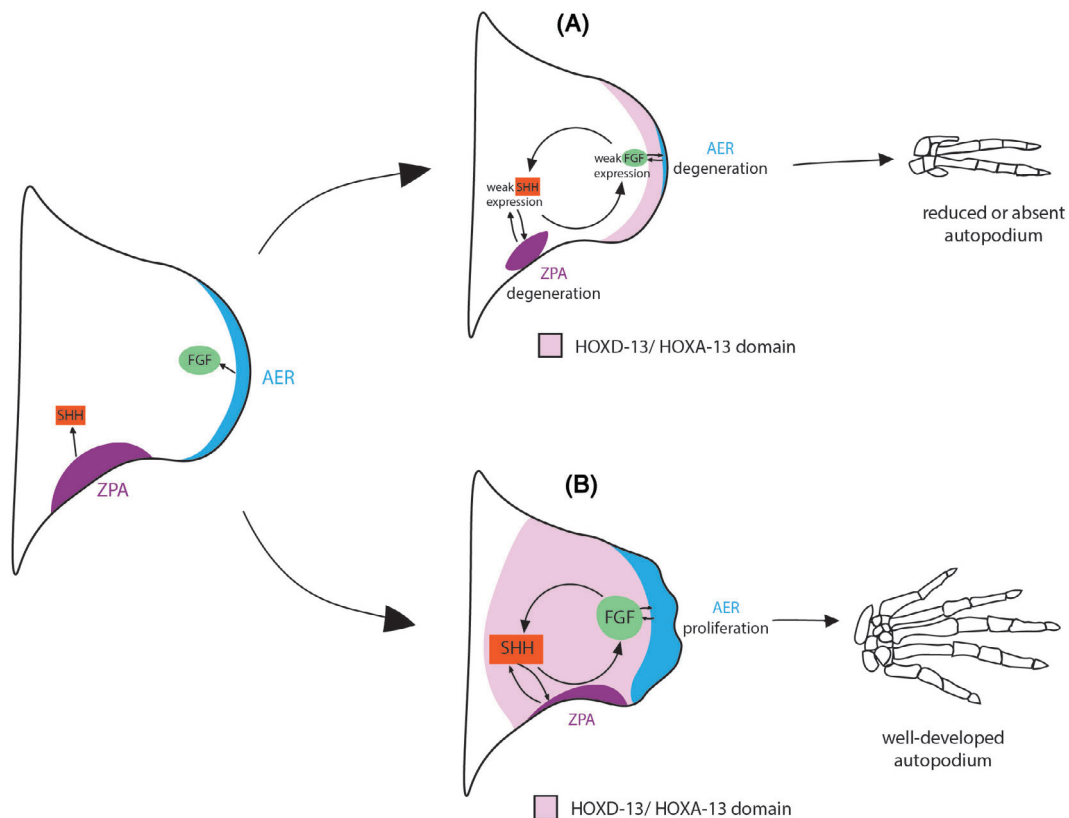


Fig 2. Schematisation of the general mechanism of autopodial specification in limb-reduced (A) and limbed (B) tetrapods. The zone of polarising activity (ZPA) produces sonic hedgehog (SHH) protein, and the apical ectodermal ridge (AER) produces fibroblast growth factor (FGF). In limb-reduced forms (A), a weak expression of autopodial *Hox* genes and related proteins (e.g. HOXD-13 and HOXA-13) determines weak production of SHH and FGF and the degeneration of both zones. In a normal-limbed tetrapod (B), these systems are reinforced in a positive feedback loop which stimulates the growth of the AER. Adapted from Hinchliffe (2002) and Leal & Cohn (2016, 2017).

correlations between body elongation, reduction in limb length, and digit loss, elaborating on ideas previously highlighted by Presch (1975), Gans (1975), Lande (1978) and Greer (1991). Regardless of the adaptive and genetic factors that may determine why these changes are correlated (e.g. pleiotropy: see Lande, 1978), the relationships among them have been shown to vary across clades, often displaying threshold patterns or following logistic curves. Some of these studies (Brandley *et al.*, 2008; Skinner *et al.*, 2008; Miralles *et al.*, 2015) have also considered the rates and tempo of character evolution across different branches of phylogenetic trees, in order to estimate the minimum time necessary to evolve limblessness from a limbed pentadactyl state, and to assess the evolutionary stability of intermediate morphs through time. These analyses yielded relatively short evolutionary times for the acquisition of limbless body shapes – between 16 and 30 million years (My) for squamates (Brandley *et al.*, 2008), or less than 11.3 My for *Lerista*, with the loss of a single digit estimated to take as little as 3.6 My (Skinner *et al.*, 2008). Notably, some intermediate morphs were found to have retained their present characteristics for relatively long timespans (9–36 My: Brandley *et al.*, 2008), evidence that these

forms may represent evolutionarily stable points in themselves, whose supposedly transitional morphologies may actually be functionally adaptive.

Contemporary approaches have also sought to investigate whether the evolutionary pathways that gave rise to convergent body shapes in limb-reduced forms are convergent or divergent. Pathways here refer to the complex combinations of changes that would lead to the acquisition of limb-reduced morphologies. Interestingly, recent studies (Morinaga & Bergmann, 2017; Bergmann & Morinaga, 2019) found significant support for divergence in the pathways for acquisition of convergent limb-reduced body forms across squamates, even between closely related species, meaning that different lineages have evolved through different arrays of structural changes. The common process across these taxa was found to be the increase in presacral vertebrae numbers to achieve trunk elongation.

Phylogenetic approaches offer a great opportunity to elucidate the evolutionary history of morphological changes such as limb reduction, and currently represent an essential tool in any morphological analysis involving multiple taxa. However, several factors argue for caution in the exclusive

use of these methods as a reliable source of historical information. The low accuracy of ancestral reconstructions (due to the high uncertainty that characterises phylogenies themselves and its increase earlier in time; Wiens, 2009), influence of the model of character evolution, and lack of resolution in cases of transitions happening over relatively short evolutionary timescales (Skinner & Lee, 2010; King & Lee, 2015) mean that these approaches are still a growing area of research.

(5) Locomotion and biomechanics

Our consideration of the evolution of body shapes and structure would be incomplete without associating body shape with potential functions. In the context of limb-reduced squamates, this means translating the variation from fully limbed to limbless forms into functional diversity. Gans (1962, 1975, 1994) hypothesised that the evolutionary degeneration of limbs in reptiles could be due to functional constraints in the acquisition of an elongated body shape: because limbs could represent an obstacle to undulating locomotion and movement through crevices, their loss may be explained by the necessity to eliminate any encumbrance to free movement and to make space for the musculature used for such a locomotory pattern. These conclusions have been validated by recent studies (e.g. Da Silva *et al.*, 2018; Camaiti *et al.*, 2019; Macaluso *et al.*, 2019) linking the evolution of limb loss to the adaptation to complex three-dimensional environments such as underground burrows and crevices. Whereas these studies have been instrumental in incorporating ecological and functional constraints as main factors in the acquisition of serpentiform body shapes, they have been disproportionately focused on their implications with regard to the evolution of limbless squamates instead of intermediate limb-reduced body shapes.

Functional studies of limb-reduced and limbless reptiles (e.g. Gans, 1960, 1962, 1986, 1994; Gasc, 1974; Leonard, 1979; Berger-Dell'mour, 1985; Dial, Gatten & Kamel, 1987; Gans & Gasc, 1990; Gasc & Gans, 1990; Walton, Jayne & Bennet, 1990; Gans, Morgan & Allen, 1992; Gans & Fusari, 1994; Renous, Hoffling & Gasc, 1998; Benesch & Withers, 2002; Bergmann & Irschick, 2010; Hohl *et al.*, 2017; Morinaga & Bergmann, 2020) have often based their observations on locomotory assays. These are used as a tool to analyse and compare how animals move relative to the substrate, dissecting this movement into several analytical components such as speed, and in the case of limb-reduced lizards, amplitude and wavelength relative to body undulation [for mathematical models of body undulation and lateral bending in squamates, see Raveshky (1960), Daan & Belterman (1968) and Navas *et al.* (2004)].

One of the most important works making use of these techniques is Leonard's (1979) thesis in which the author offers a comparative analysis of locomotion across a spectrum of limb reduction from fully limbed to limbless morphs. Integrating these observations with in-depth morphometric regressions and osteological and myological comparisons across taxa,

Leonard (1979) was able to formulate a comprehensive locomotory model for limb-reduced and limbless skinks. This was accomplished through the definition of the functional relationships between axial and appendicular components in locomotion. While in fully limbed species undulations of the axial skeleton are mostly subordinate to the movement generated by the limbs, with the progression of limb reduction and body elongation this relationship is gradually inverted, and the lateral undulations of the axial skeleton take over as the main means of propulsion. Similar conclusions were reached by Renous *et al.* (1998). This idea is also supported by Gans (1975), stating that the evolution of snake-like body shapes is due to locomotory changes associated with shifts to a subterranean or crevice-dwelling lifestyle, where the limbs would represent a hindrance to free movement. Leonard (1979) also observed that the morphometric relationship between decreasing limb lengths and increasing numbers of presacral vertebrae were not constant, sometimes even varying intraspecifically, indicating a loose relationship between body elongation and limb reduction; this is hypothesised to be due to the disruption of the functional link between limbs and the body axis that happens when the former cede their locomotory duties to the latter. These findings were supported by the author's anatomical observations, showing that with the onset of limb reduction the muscles and bones of the ribs and vertebrae were reinforced as an adaptation to withstand mechanical stress due to undulating locomotion, instead of the fulcrum of the limbs and girdles that would be reinforced in normal-limbed squamates.

Gans & Fusari (1994) also examined and experimentally tested the functionality of reduced limbs in a comparative framework. By conducting locomotory assays on different substrates for three variably limb-reduced lizards of the genus *Lerista*, the authors established that varying degrees of limb reduction and body elongation corresponded to divergent locomotory modes and performance, in a spectrum from full employment of the limbs for locomotion without recurring to body undulation (typical of fully limbed species), to partial use of the limbs alternating with body undulation, to full body undulation with rare or non-existent use of the limbs (typical of limbless or severely limb-reduced species) (Fig. 3). The locomotory differences exhibited by the three *Lerista* species were linked to adaptations to the substrate conditions of the microhabitats in which these species live.

As mentioned previously, ancestral character reconstructions have deepened our understanding regarding the evolution of limb reduction (Brandley *et al.*, 2008; Skinner *et al.*, 2008), challenging the idea that intermediate forms are bound to become limbless over the course of evolution, and showing that morphs that retain limbs are relatively evolutionarily stable (persisting for between 9 and 36 My: Brandley *et al.*, 2008) after initial events of rapid diversification. Given that the selective pressures that prompted the reduction of limbs in the past may not be active at present, at least three hypotheses can be formulated: (i) that there is no incentive to lose these structures even when functionally useless because they have virtually no impact on fitness (they have

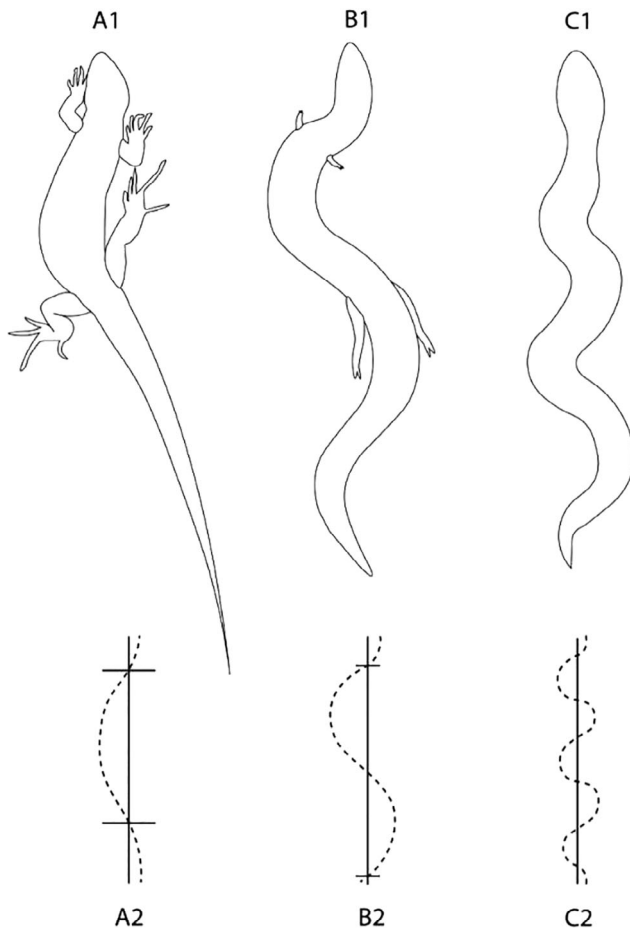


Fig 3. Transition from limbed to limbless locomotion in squamates. In the top half, from left to right, a normal-limbed lizard (A1), a limb-reduced lizard (B1), and a limbless lizard (C1). In the bottom half, from left to right, sketches representing the changes that happen in the transition towards limbless locomotion, from fully limbed lizards (A2), to limb-reduced (B2), to limbless forms (C2). Solid lines represent the general body shapes of these forms, with intergirdle length being represented by the distance between the perpendicular lines. Dashed curves represent the extent of lateral undulation of the axial skeleton, which increases as the number of presacral vertebrae, and body elongation, increase. An increase in the reduction of the limbs and body elongation corresponds with an increased reliance on the axial system and on body undulation. Adapted from Gans (1986).

become non-adaptive); (ii) that the limbs retain some functionality and may now be actively selected for; (iii) that, independent of their functionality, there may be a threshold over which the pathway to limb loss would be all but assured.

Recent studies (Bergmann & Irschick, 2010; Morinaga & Bergmann, 2020) have tested these hypotheses by associating body-shape evolution with locomotory patterns. Interestingly, Morinaga & Bergmann (2020) highlight the lack of a trade-off between burrowing and surface locomotion, which would explain the relatively long persistence and abundance

of intermediate morphs. Furthermore, even species with highly reduced limbs were shown to use them in assisting both types of locomotion, showing that maintaining such structures may be adaptive. Intermediate morphs were also linked to larger body sizes compared to limbless species, which may represent further evidence of the adaptive value of such features, due to differences in diet and microhabitat.

Many questions remain over the locomotion and biomechanics of limb-reduced forms. For instance, whereas the works cited above are mostly concerned with the causal relationship between undulatory locomotion and the evolution of snake-like body shape, and how different locomotory patterns explain the existing variations of limb-reduced morphs, there has been little investigation regarding how anatomical variation in the structures involved in locomotion translate into locomotory patterns. Moreover, only a few studies (e.g. Morinaga & Bergmann, 2020) have considered the relationships between locomotory mode and body shape in light of the different substrate compositions in which the animals live and move, and which may have substantial implications regarding the functionality of limbs and associated structures.

(6) Ecomorphology and biogeography

Studies investigating the evolution of limb-reduced squamates often consider the adaptive context that prompted these changes in body shape. Limb reduction in squamates has been shown to be the result of divergent evolutionary pathways that can lead to similar endpoints (Bergmann & Morinaga, 2019), reflecting the adoption of multiple distinct lifestyles in a wide range of environments. In the framework of ecological factors underlying the evolution of elongated, limb-reduced body shapes, several studies (e.g. Camp, 1923; Gans, 1975; Wiens & Slingluff, 2001; Wiens *et al.*, 2006; Brandley *et al.*, 2008) have established the existence of two ecomorphs of limb-reduced reptiles: the burrowing ecomorph and the grass-swimming ecomorph. The first reflects an adaptation to life underground and is characterised by short tails and elongated trunks, whereas the second is typical of surface-dwelling forms, characterised by long tails (Fig. 4). The application of phylogenetic comparative methods (Wiens & Slingluff, 2001; Wiens *et al.*, 2006; Brandley *et al.*, 2008; Bergmann & Morinaga, 2019) made it possible to establish that these ecomorphs reflect distinct trends in the evolution of limb-reduced squamates.

Among these studies, Wiens *et al.* (2006) is of particular interest because it highlights the importance of large-scale ecological factors in the repeated evolution of snake-like body shapes in squamates. Biogeographic isolation and competition were identified to be opposing influences in the evolution of limb-reduced squamate lineages. Many lineages of limb-reduced squamates may have evolved, reasoned the authors, because each was able to occupy available niche space in a new environment. Geographical isolation was linked to the limited ability of lizards to disperse once they evolved limb reduction. The observation that lineages sharing the same

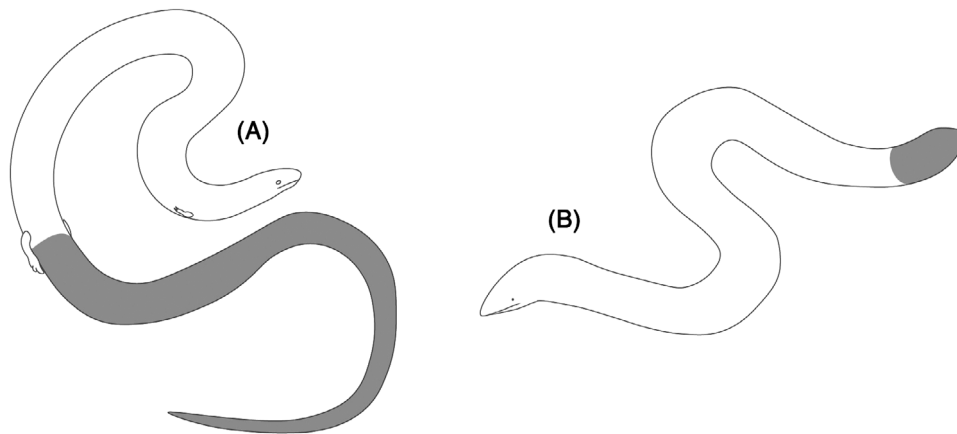


Fig 4. The two main ecomorphs of limb-reduced lizards. (A) Long-tailed, grass-swimming ecomorph; (B) short-tailed, burrowing ecomorph. Tail (defined as posterior to the cloaca) highlighted in grey. Adapted from Brandley *et al.* (2008).

ecomorph rarely if ever evolve in sympatry prompted Wiens *et al.* (2006) to recognise that competition would instead work against multiple origins, by preventing newly arrived taxa from occupying niche space that has already been filled.

Further studies linking ecology and morphology at a finer scale are still needed. At present, we have a general grasp of the ecomorphology of limb-reduced squamates, which has allowed them to be categorised as having surface or subsurface lifestyles (e.g. Wiens & Slingluff, 2001; Wiens *et al.*, 2006). However, the fine-grain relationships between each limb-reduced morphology and the specific conditions in which they live – whether soil composition and compaction, vegetation cover or microhabitat partitioning – remain virtually unexplored. A more precise delineation of these relationships might prove fundamental in defining the selective pressures that shape the evolution of snake-like lizards.

III. REMARKS AND FUTURE DIRECTIONS

Based on our surveys of the historical and scientific approaches to limb reduction, we highlight and discuss the most pressing questions to be addressed for future growth of this field of study. Although the field is continually evolving to incorporate new techniques and constantly exploring new directions, much remains to be done, especially in integrating the existing knowledge into a comprehensive synthesis. Thus, in addition to critical analysis of each of the approaches focused on limb reduction, we provide an informed list of the directions in which this field should develop.

Firstly, our review underscores the need to expand our knowledge of changes in body shapes and structures beyond external morphological evaluations. While external morphology is generally the main focus of most modern studies of limb reduction, osteology and myology still remain unstudied for the large majority of taxa, and are rarely used in comparative studies: our knowledge of limb-reduced forms is literally and figuratively skin-deep. As past and recent studies that focused on internal morphology

(e.g. Fürbringer, 1870; Stokely, 1947; Greer, 1991; Westphal *et al.*, 2019) have shown, the osteology and myology of lizards are far superior tools for investigation of patterns of limb reduction compared to characterisations purely based on external characters. For instance, species that appear externally convergent in body shape might prove quite different in their internal anatomies. Furthermore, even for limbless or severely limb-reduced species, reduced or vestigial structures of limb bones and girdles can inform on how limbs and associated parts have regressed through evolution (see Miralles *et al.*, 2015; Camaiti *et al.*, 2019). These internal patterns, primarily represented by differential degrees of development of modules of limbs and girdles, can prove an invaluable tool to reconstruct the evolutionary pathways that led to independent events of body-shape transformation. Thus, improving how morphological variation is quantified represents a key to a more thorough understanding of the patterns of limb reduction that exist in squamates. The relatively recent emergence of new technological advances such as computed tomography (CT) scanning and DiceCT (diffusible iodine-based contrast-enhanced computed tomography) – allowing non-invasive examination respectively of hard and soft internal anatomical structures (Kleinteich *et al.*, 2008; Metscher, 2009*a,b*; Gignac *et al.*, 2016) – and of comparative analytical tools such as geometric morphometrics represents an opportunity to fill these gaps.

Secondly, in the context of the modern applications of geometric morphometrics, the patterns of morphological integration between body parts of limb-reduced reptiles have not been the subject of sufficient attention. The changes that lizard body plans undergo while evolving limb reduction are not limited to the structures of limbs and digits. As in all vertebrates, the musculoskeletal systems of lizards are characterised by varying degrees of integration and modularity among their parts both at the anatomical and functional level (Klingenberg, 2013; Watanabe *et al.*, 2019). Ecological, functional and developmental factors can influence the extent to which different body parts are integrated. Interestingly, similar selective pressures have been shown to generate convergent patterns of integration across multiple groups

(Watanabe *et al.*, 2019). Recent studies (Sanger *et al.*, 2012; Hipsley & Müller, 2017; Kazi & Hipsley, 2018; Urošević, Ljubisavljević & Ivanović, 2019; Watanabe *et al.*, 2019) have highlighted how different selective pressures from diet and environment can result in divergent rates of evolution among and within skull bones in reptiles. In the case of limb-reduced reptiles, such variations in skull modularity can also be expected to be deeply correlated with ecology. For example, the labyrinth of the inner ear has been shown to be a strong indicator of a fossorial lifestyle (Yi & Norell, 2015), and examining its patterns of integration across different limb-reduced species may represent a way reliably to quantify their ecomorphological variation. These approaches can also be applied under the lens of evo-devo, and might prove instrumental in linking the known developmental mechanisms that determine the emergence of limb-reduced forms to their ecomorphological and evolutionary contexts.

Thirdly, while a significant proportion of what is known about limb-reduced lizards relates to the evolution of their body shapes and factors related to this, little to no attention has been given to other important questions, such as correlated changes in their internal organs and the physiological implications of these changes. Body-elongated reptiles like snakes are known to possess elongated and asymmetrical organs due to the need to fit them into an increasingly tubular body cavity (Gans, 1975; van Soldt *et al.*, 2015; Lambertz, Arenz & Grommes, 2018). How this relates to the degree of limb reduction and body elongation in limb-reduced forms is unknown except for some Australian reptiles (Greer, 1989). The physiological characterisation of these lizards in relation to normal-limbed lizards has also rarely been considered (Pough, 1971; Withers, 1981; Brownlie & Loveridge, 1983), and might provide insights into the adaptations to underground environments that are typical of most limb-reduced lizards (Brandley *et al.*, 2008). Such a lifestyle is known to bring about a series of secondary modifications that allow lizards to overcome the challenges of subsurface environments. For example, an important factor to consider is the conditions of relative oxygen scarcity typical of underground environments, which are likely to affect the metabolism of burrowing organisms (Vihar *et al.*, 2015).

In summary, we find the need for a more holistic consideration of the study of squamate limb reduction, and in general of body-shape transformations. Incorporating previously highlighted perspectives and approaches could represent a way to obtain a better understanding of the numerous levels of change that compound the evolution of snake-like reptiles.

IV. CONCLUSIONS

- (1) Having evolved multiple times independently across unrelated lineages, squamate limb reduction represents a model system to deepen our understanding of macroevolutionary transitions and morphological transformations. Historically, various perspectives

and analytical approaches have investigated these body-shape modifications (Table 1).

- (2) The earliest studies of squamate limb reduction investigated anatomical patterns using morphological series, and were instrumental in elucidating changes in relationships between body parts as limb reduction progressed, such as elongation of the body and reduction of the girdles. However, these studies suffered from the lack of a phylogenetic basis.
- (3) Later comparative studies considered the patterns of limb reduction in single clades, and used them as a basis for hypotheses on the evolution of the snake-like body shape in squamates, including the integration of a developmental and genetic perspective. According to these works, such a body plan originated as an adaptation to specific terrestrial environments such as crevices and burrows, and is linked to a locomotory strategy of body undulation. Less consensus has been reached on the genetic basis and developmental mechanisms of body elongation and limb reduction.
- (4) Studies of the developmental and genetic basis of limb reduction and correlated changes have focused on several aspects of the ontogenetic mechanisms that cause limb degeneration. While the earliest studies established the importance of necrosis of the apical region of the limb buds in determining the regression of limbs during ontogeny, modern efforts have included the role of *Hox* and *Shh* genes in the specification of reduced body structures. Heterochronic shifts in the timing of ossification and chondrification of body structures have also been linked to limb-reduced morphologies.
- (5) Phylogenetic comparative methods have provided reliable tools to reconstruct the evolutionary history of limb-reduced lineages. While phylogenetic uncertainty represents a hindrance to the accuracy of these reconstructions, especially over long timescales, these approaches have allowed the testing of evolutionary hypotheses and the development of models of evolution to explain present-day patterns.
- (6) Investigations of the link between the evolution of snake-like squamates and the functional adaptations of their body shapes have mostly focused on the implications of limb reduction on locomotion. As limb reduction and body elongation become more accentuated, the appendicular skeleton cedes its locomotory role to the axial skeleton. Locomotory tests have also revealed that intermediate morphs that still retain reduced limbs are well adapted to their environments and are not necessarily constrained to become limbless. However, the lack of understanding of the biomechanical aspects of reduced structures (such as the limbs) and how this translates to locomotory variation still represents a gap that needs to be addressed.
- (7) Studies of the relationships between morphology and ecology have revealed that limb-reduced lizards can evolve in at least two distinct ways, each of which

reflects a particular selective environment and necessitates specific morphological adaptations: a burrowing ecomorph and a surface-dwelling ecomorph (Fig. 4). The role of larger-scale biogeographic factors in the radiation of these reptiles has also been tentatively explored.

- (8) Limb-reduced squamates hold enormous potential for investigations of macroevolutionary transitions. However, the ways in which they are currently studied still leave several gaps, especially in understanding of their patterns of internal morphological variation. Moreover, further investigations are needed of the ecomorphological, functional, and physiological aspects of limb reduction. Future efforts should focus on bringing together these aspects for a more holistic understanding of morphological transformations.

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