

Structural and environmental constraints on reduction of paired appendages among vertebrates

LOREDANA MACALUSO^{1*}, GIORGIO CARNEVALE^{1,○}, RAFFAELLO CASU², DANIEL PIETROCOLA¹, ANDREA VILLA^{1,3,○} and MASSIMO DELFINO^{1,4,○}

¹*Dipartimento di Scienze della Terra, Università degli Studi di Torino, Via Valperga Caluso 35, 10125 Torino, Italy*

²*Dipartimento di Fisica, Università degli Studi di Torino, Via Pietro Giuria 1, 10125 Torino, Italy*

³*Bayerische Staatssammlung für Paläontologie und Geologie, Richard-Wagner-Straße 10, 80333 München, Germany*

⁴*Institut Català de Paleontologia Miquel Crusafont, Universitat Autònoma de Barcelona, Edifici Z (ICTA-ICP), Carrer de les Columnes s/n, Campus de la UAB, E-08193 Cerdanyola del Valles, Barcelona, Spain*

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Burrowing habits or complex environments have generally been considered as potential drivers acting on reduction and loss of the appendicular skeleton among vertebrates. Herein, we suggest that this might be the case for lissamphibians and squamates, but that fin loss in fishes is usually prevented by important structural constraints, because pectoral fins are commonly used to control rolling and pitching. We provide an overview of the distribution of paired appendage reduction across vertebrates while examining the ecological affinities of finless and limbless clades. We analysed the correlation between lifestyle and fin or limb loss using the discrete comparative analysis. The resulting Bayesian factors indicate strong evidence of correlation between: (1) pectoral-fin loss and coexistence of anguilliform elongation and burrowing habits or complex habitat in teleost fishes; and (2) limb loss and a burrowing or grass-swimming lifestyle in squamate reptiles and lissamphibians. These correlations suggest that a complex environment or a fossorial habit is a driving force leading to appendage loss. The only style of locomotion that is functional even in the absence of paired appendages is the undulatory one, which is typical of all elongated reptiles and lissamphibians, but certainly less common in teleost fishes.

ADDITIONAL KEYWORDS: amphibians – comparative phylogenetic analyses – eel-like fishes – elongated tetrapods – limb loss – reptiles.

INTRODUCTION

Reduction or loss of the appendicular skeleton has occurred many times in representatives of several fish and tetrapod lineages. Among extant tetrapod classes (Moyle & Cech, 2003; Vaughan *et al.*, 2011; Pough *et al.*, 2015; Morrison *et al.*, 2018), extreme reduction or loss of limbs has occurred in mammals (cetaceans and sirenians), birds (moa and kiwi), squamate reptiles (snakes, amphisbaenians and several groups of lizards) and ‘amphibians’ (the Carboniferous–Permian and the lissamphibian caecilians plus a few groups of salamanders), and the loss of paired fins has been

observed in several groups of teleost fishes. In both mammals and birds, reduction and skeletal loss affect either the forelimbs or the hindlimbs, whereas there are several groups of squamate reptiles, amphibians and teleost fishes that exhibit a complete absence of paired limbs or fins.

Although these groups are phylogenetically distant, they all share some similarities. First, squamate reptiles, lissamphibians and teleosts characterized by a reduction of the appendicular skeleton usually exhibit an elongated body (Gans, 1975; Ward & Mehta, 2010 and references therein). According to Greer (1991), there are 53 squamate lineages that have undergone limb reduction, distributed among Agamidae, Amphisbaenia, Anguillidae, Anniellidae, Chamaleonidae,

*Corresponding author. E-mail: loredana.macaluso@unito.it

Cordylidae, Dibamidae, Diploglossidae, Gekkota, Gerrhosauridae, Helodermatidae, Lanthanotidae, Ophidia, Pygopodidae, Scincidae and Teiidae. The situation is different among lissamphibians because, although different groups exhibit an elongated body, only three lineages exhibit a reduction or loss of appendages. Caecilians (Gymnophiona) are the only lissamphibians characterized by a complete loss of both fore- and hindlimbs. Members of Sirenidae, in contrast, have lost the hindlimbs but retain reduced forelimbs, whereas the only genus included within Amphiumidae, *Amphiuma*, has very short and motionless limbs. Among extinct basal tetrapods, the Carboniferous–Permian Aistopoda are completely limbless, showing a remarkable convergence with the serpentiform body plan of snakes and caecilians (Carroll *et al.*, 1998; Pardo & Mann, 2018).

Moreover, in general, all the elongated squamates and lissamphibians exhibit a similar lateral undulation even if using different types of locomotion (Gans, 1975). Conversely, elongated fishes exhibit carangiform or anguilliform locomotion (Sfakiotakis *et al.*, 1999; Horner & Jayne, 2008; Pfaff *et al.*, 2016). The carangiform pattern is characterized by more-or-less rigid movements of the caudal portion of the body and tail, whereas the anguilliform pattern is characterized by a sinuous wave that moves throughout the body (e.g. eels) or only in its posterior part (e.g. catfishes). The anguilliform movement, which could be associated with the ‘serpent-like’ movement of tetrapods, is typical of at least half of the highly elongated actinopterygians (Ward & Mehta, 2010; Reece & Mehta, 2013). In particular, anguilliform elongation (*sensu* Ward & Mehta, 2010) is typical of *Erpetoichthys calabaricus* among Polypteriformes (Suzuki *et al.*, 2010), some members of the clades Ophidiiformes and Gobionellidae (genus *Luciogobius*; Yamada *et al.*, 2009), and most of the members of the clades Anguilliformes, Lampridiformes, Mastacembelidae and Zoarcales (Ward & Mehta, 2010). Some elongated members of the clades Ateleopodiformes, Liparidae and Siluriformes show a body plan with an enlarged, heavy head, and their anguilliform movement is limited to the posterior part of the body (i.e. not including the pectoral area). The pelvic fins are lost in several morphologically different groups of fishes, in > 90 teleost lineages (Nelson, 1989; Kriwet & Pfaff, 2019), whereas the pectoral fins are lost primarily in elongated fishes with anguilliform locomotion (Mehta *et al.*, 2010).

Eel-like clariid species represent a unique case among vertebrates, because their paired fins show a very high intraspecific level of morphological variability, preventing their absence from being used as a diagnostic feature at the species level (Devaere *et al.*, 2004). As in other vertebrates (O’Reilly *et al.*, 1997), the loss of fins in clariids was regarded as being

related to a highly specialized fossorial mode of life by Devaere *et al.* (2004), although this hypothesis has not yet been tested.

More generally, the idea that the presence of appendages might represent an impediment for burrowing for both terrestrial and aquatic animals is widely accepted. Recently, Da Silva *et al.* (2018) demonstrated that fossoriality has been the evolutionary driver leading to the origin and development of the snake body plan. Extinct relatives of extant limbless forms (e.g. amphisbaenians and caecilians) indicate that fossoriality evolved before the limb loss, because they have cranial adaptations for burrowing but maintain variably developed appendages (Evans & Sigogneau-Russell, 2001; Jenkins *et al.*, 2007; Tañanda, 2016). Nevertheless, fossoriality is not the only evolutionary driver for limb loss in reptiles, and limbless squamates are traditionally divided into short-tailed burrowers or long-tailed surface dwellers, moving through loose sand or vegetation (Evans, 1998; Wiens *et al.*, 2006).

As far as fishes are concerned, it is known that a number of fishes exhibiting an eel-like body morphology are either crevice dwellers or burrowers (tail or head first; De Schepper *et al.*, 2007a, b; Herrel *et al.*, 2011). Nevertheless, previous studies mainly focused on the correlation between elongation and habitat, or trophic adaptations (Mehta *et al.*, 2010; Ward & Mehta, 2010; Claverie & Wainwright, 2014), but did not find any apparent connection. Mehta *et al.* (2010), in particular, stated that although it is generally true that terrestrial vertebrates evolved an elongated, limb-reduced body plan as an adaptation for the burrowing lifestyle, little is known about how much the elongate body form might be adapted for aquatic habits.

Herein, we suggest that a distinction can be drawn in fishes between anguilliform and stiffer body elongation (*sensu* Ward & Mehta, 2010) when studying correlations between habitat and body plan, because the constraints owing to the problem of balance during swimming connected with these two body forms are different. Moreover, we attempt to address the question: once a fish has evolved an eel-like elongation, does the habitat have an influence on the loss of paired fins? Is there a common trend in appendage loss in teleosts and in tetrapods? Herein, we argue that the limbless body plan in vertebrates is affected by similar environmental constraints provided by the habitat or lifestyle, and we try to summarize how widespread this pattern (i.e. dense, complex environment acting on appendage reduction and loss) is.

GENETIC AND DEVELOPMENTAL CONTROL OF APPENDAGE LOSS

The development of appendages is polygenic, involving genes with pleiotropic effects (Lande, 1978; Hall, 2008).

The genes involved in the development of limbs and paired fins also function in other developing systems, such as jaws or genitals (Rosa-Molinar & Burke, 2002). This is the reason why genes associated with limb buds are generally not lost, even in limbless forms (Bejder & Hall, 2002).

The developmental mechanism of the formation of paired appendages is deeply conserved among gnathostomes (Dahn *et al.*, 2007; Letelier *et al.*, 2018), and it involves two signalling centres located in the fin/limb bud. The first of them is the apical ectodermal ridge, which helps to maintain the second one, the zone of polarizing activity, the cells of which express the Sonic hedgehog (*Shh*) gene, associated with the development of the fins or limbs (Cohn, 2001; Bejder & Hall, 2002; Thewissen *et al.*, 2006). At the same time, Hox genes control the position of both girdles and appendages along the body. In particular, the anterior expression boundaries of *HoxC-6* and *HoxC-8–10* coincide with the localization of fore- and hindlimb buds, respectively (Bejder & Hall, 2002).

Reduction and loss of appendages can occur owing to regression of different phases in the conserved genetic pathway for appendage development. Tanaka *et al.* (2005), for example, reported that pelvic-fin loss can be achieved through different mechanisms in pufferfishes and sticklebacks. In the first case, the reduction is attributable to an altered expression of the gene *Hoxd9a* in the lateral mesoderm, whereas in the second case *Pitx1*, a gene responsible for appendage initiation, fails to be expressed (Shapiro *et al.*, 2004). In pythons, limb development is arrested in two different ways. Forelimb buds are not developed at all, because of the widespread expression of *HoxC-6* and *HoxC-8* genes throughout the lateral plate mesoderm, meaning that no boundary conditions are established for forelimbs to form and therefore there is no pectoral limb initiation (Cohn & Tickle, 1999; Cohn, 2001; Bejder & Hall, 2002). In contrast, hindlimb buds are formed, but they have a very smooth ectodermal jacket, forming a small apical ectodermal ridge, which causes a precocious interruption of the growth (Cohn, 2001). Serpentine lizards and urodeles have different levels of limb reduction that can vary considerably depending on the species (Greer, 1991). Nevertheless, in general, the formation of their limb buds starts and then regresses, or the expression of *Shh* can have a shortened duration, leading to loss of some digits or of a larger part of the limbs (Raynaud, 1990; Shapiro *et al.*, 2003; Hinchliffe, 2004; Tanaka *et al.*, 2005). As far as cetaceans are concerned, pelvic limb buds begin to form but fail to develop fully, in a similar way to the python hindlimbs (Bejder & Hall, 2002; Tanaka *et al.*, 2005). Nevertheless, it is likely that the mechanism is slightly different from that of snakes, because the dolphin *Stenella attenuata* shows a normal apical

ectodermal ridge during development, but the absence of *Hand2*, one of the upstream regulators of *Shh* transcription, causes a perturbed initial establishment of the zone of polarizing activity and the consequent absence of *Shh* expression (Thewissen *et al.*, 2006).

Thus, fin and limb formation has highly conserved genetic and ontogenetic pathways among gnathostomes, but the developmental causes of appendage loss can be very diverse within and among different groups (Hall, 2008).

MATERIAL AND METHODS

We compiled a database (Supporting Information, Appendices S1 and S2) that includes 125 species of teleost fishes, 74 species of lissamphibians and 151 species of squamate reptiles. For the taxon sampling of teleost fishes, we selected all the groups characterized by pectoral fin loss: Anguilliformes, Clariidae, Gobionellidae (strong reduction of the pectoral fin in the genus *Luciogobius*) and Trichomycteridae. The groups of Zoarcales and Mastacembelidae have some finless members, but we did not include them in our analysis because their ecology and phylogenetic relationships are poorly known.

We combined different phylogenetic trees that contain the group included in the study (either the most recent or the most complete phylogenetic analyses) and, in particular: Santini *et al.* (2013) for Anguilliformes; Baskin (1973), Datovo & Bockmann (2010) and DoNascimento (2015) for Trichomycteridae; Wright (2017) for Clariidae; and Yamada *et al.* (2009) and Thacker (2013) for Gobionellidae. We sampled some of the taxa contained in these phylogenies, with several of them being used as outgroups because they do not show any pectoral fin loss. Particular attention was paid to groups with finless members included in these phylogenies, among which at least one representative species for every family has been selected, but including all the taxa with complete information about fins and ecological habits that were close to the node where pectoral fins were lost. In fact, for studies concerning causal relationships of specific traits, the most important taxa to sample are the ones near to the node where the trait we want to study (e.g. fin or limb loss) first occurred and got fixed. Any potential evolutionary innovation that originated after the first occurrence and fixation of the trait should not be considered as a potential evolutionary driver, and it is therefore not essential to insert many derived taxa in the sample (Macaluso & Tschopp, 2018).

The same procedure was followed to compile the database of lissamphibians (complete sample of basal Gymnophiona, Sirenidae and Amphiumidae), starting from the phylogeny published by Pyron & Wiens

(2011), and squamate reptiles (complete sample of basal limbless Agamidae, Amphisbaenia, Anguidae, Anniellidae, Dibamidae, Diploglossidae, Ophidia, Pygopodidae and Scincidae), using the phylogenies of Reeder *et al.* (2015), Da Silva *et al.* (2018) and Pyron *et al.* (2013).

The taxon sampling was, of course, limited by the information available for the taxa, and we chose species for which the following is known: (1) phylogenetic position; (2) presence or absence of appendages; and (3) behaviour or ecology. Our limited knowledge of these data is particularly relevant in the case of extinct taxa, because of the difficulty in reconstructing their life habits. Moreover, it is not common to find a complete articulated skeleton of terrestrial animals that can tell us whether appendages were present or not in a certain taxon (for example, see the case of the stem-group caecilian described by Evans & Sigogneau-Russell, 2001). For these reasons, the sample of extinct taxa is limited in the present study to relatives of extant taxa representing their stem, whenever information about them is available.

We collected information about elongation of the body, presence or absence of the appendages, and lifestyle (for references, see Supporting Information, Appendices S1–S3). Elongation has been scored only for fishes, which display different types of elongation (anguilliform and stiffer body; Ward & Metha, 2010; Maxwell & Wilson, 2013), and it is therefore important to consider this character in the analysis. In particular, fishes are considered in the present study as being elongated if their length is more than five times the maximal body depth.

The considered cases of fin loss in fishes are restricted to those taxa without pectoral fins, because the pelvic fins are absent in a huge number of groups owing to different factors (e.g. Nelson, 1989). *Luciogobius* has been scored as lacking its pectoral fins because it shows an extreme pectoral fin reduction compared with its close relatives (Hyun-Geun & Seung-Ho, 2014). Squamate reptiles and lissamphibians were scored as lacking their limbs (1) if both pairs of appendages were absent and as intermediate (01) if only one pair of appendages has been lost. The only exceptions to this rule are snakes with underdeveloped hindlimbs (e.g. pythons, *Pachyrhachis*, *Haasiophis*), the genus *Dibamus*, in which the presence of hindlimbs is a sexual dimorphic character (vestigial hindlimbs present only in males; Koppetsch *et al.*, 2019), and *Amphiuma*, which have been scored as limbless although they possess small vestigial hindlimbs useless for locomotion.

Lifestyle has been divided into ‘fossorial’ or ‘not fossorial’. Fishes are scored as 1 concerning ‘fossoriality’ (i.e. column ‘fossoriality’ in Supporting Information, Appendix S1) when they either have burrowing habits

or are crevice dwellers, whereas squamate reptiles and lissamphibians were scored as ‘fossorial’ (i.e. one in column ‘fossoriality’ in Supporting Information, Appendix S2) when they are either burrowers or grass swimmers. Although burrowing and grass-swimming result in different constraints acting on the whole body, the lateral sides of the body (and, consequently, the appendages) of burrowing and grass-swimming animals are constrained in a similar way, by the substrate in burrowers and crevice dwellers and by the grass in grass swimmers. Additional information and references about lifestyle, phylogenetic position and presence/absence of appendages are presented in the Supporting Information (Appendices S1–S3).

After collecting these data, we compiled a composite phylogenetic tree in Mesquite (Maddison & Maddison, 2018), reporting all the included taxa, based on the phylogenies listed above. We performed the discrete comparative analysis available in the software BayesTraits v.3.0 (Meade & Pagel, 2017). Discrete comparative analysis is used to test whether two binary traits are correlated, and its significance is established by comparing the likelihoods (derived using Markov chain Monte Carlo) of two models, one assuming that the traits evolved independently and the other assuming that their evolution is correlated. Two binary traits can be described by four possible states, written as ‘0,0’, ‘0,1’, ‘1,0’ and ‘1,1’. The independent model assumes that the two traits evolve independently, e.g. the transition from zero to one in the first trait is independent from the state of the second trait, whereas the dependent model assumes that the traits are correlated and the rate of change in one trait is dependent on the state of the other. The test was performed by structuring an input database as an Excel file of a table at two entrances (see Supporting Information, Appendices S1 and S2): species in the rows and characters in the columns. Concerning fishes, the two binary characters are pectoral fin loss and a character that is scored as 1 only if the taxon is both elongated (as defined above) with anguilliform locomotion and either fossorial or a crevice dweller. Given that all the fossorial (or grass-swimming) lissamphibians and squamates have an extremely elongated body, elongation was not considered as an essential character in their case, and the two binary characters are therefore limb loss and fossoriality or grass-swimming. Given that arbitrary branch lengths are commonly used and well supported in the literature using comparative methods (Grizante *et al.*, 2012, and references therein), we performed the statistical analyses using an arbitrary branch length of 1.0, and all branches were scaled to 0.1, as suggested for the software BayesTraits v.3.0 (Pagel & Meade,

2006; Meade & Pagel, 2017). As is usual in this type of analysis, we set all the priors to an exponential with a mean of ten and use the stepping stone sampler with 100 stones and 1000 iterations per stone to estimate the marginal likelihood (see the manual for users of BayesTraits v.3.0). We performed two different analyses, one for teleost fishes and a separate one for lissamphibians and squamate reptiles, to make it easier to manipulate the large trees in Mesquite. The phylogenetic trees we built are reported in Figures 1 and 2. It is worth noting that in the discrete analysis of BayesTraits

v.3.0 it is not necessary to infer the ancestral state of the characters, and thus the coloured branches in the figures are merely graphical representations. Here, we represent the character states with different colours, relating to the Supporting Information (Appendices S1 and S2). In particular, names depicted in red indicate species scored with 1 for appendage loss, whereas light blue species are the ones scored as 01. The colour of the branches refers to the second character, intended as the co-occurrence of eel-like elongation and burrowing behaviour (or crevice or sea-grass inhabitants) for

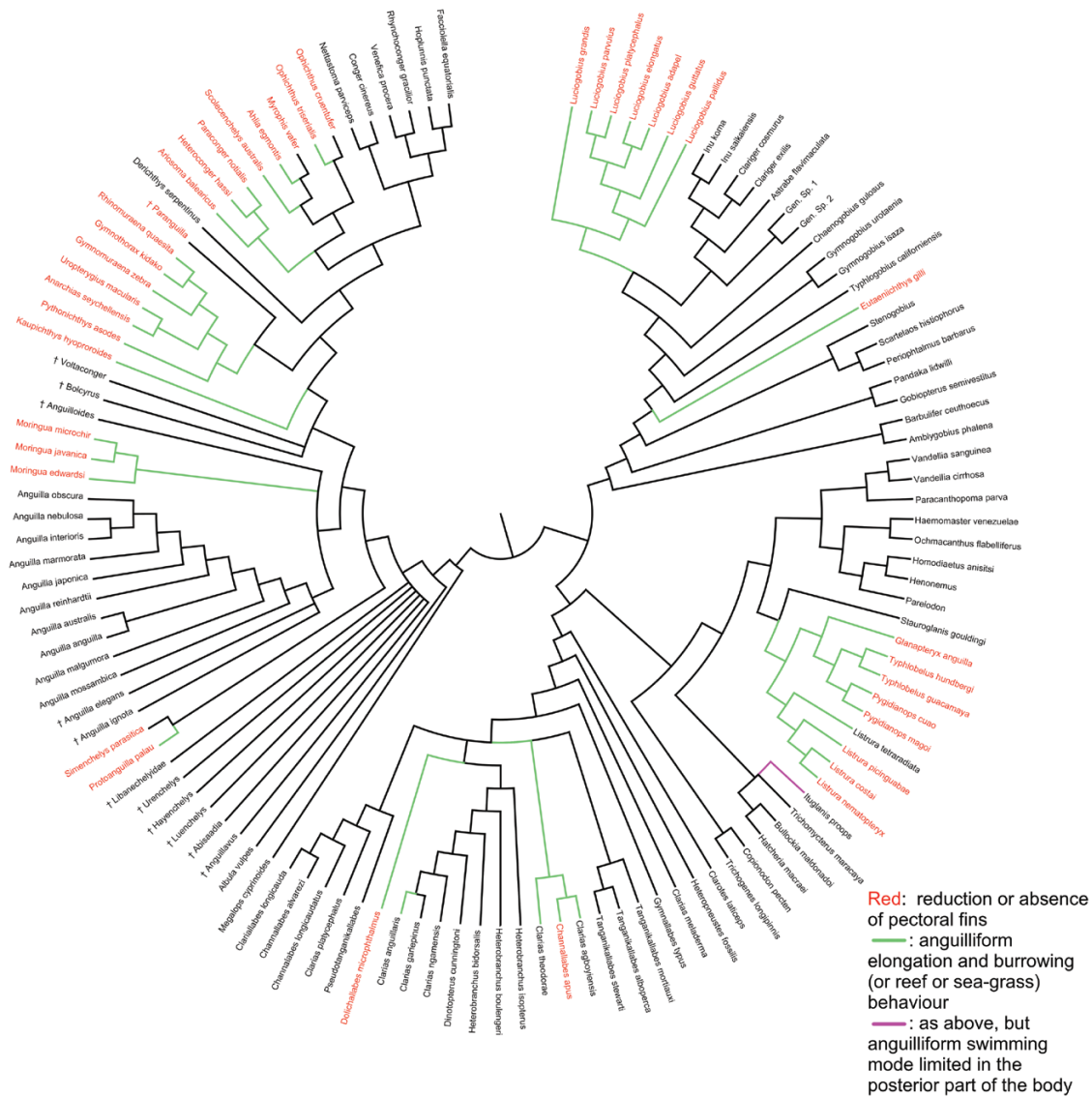


Figure 1. Composite phylogenetic tree of teleost fishes used for the analysis. See Material and Methods section for the references concerning phylogenetic position and character state reconstruction. The coloured branches in the figure are graphical representations and do not represent any ancestral state reconstruction, because it is not a necessary step for the discrete comparative analysis using BayesTraits v.3.0.

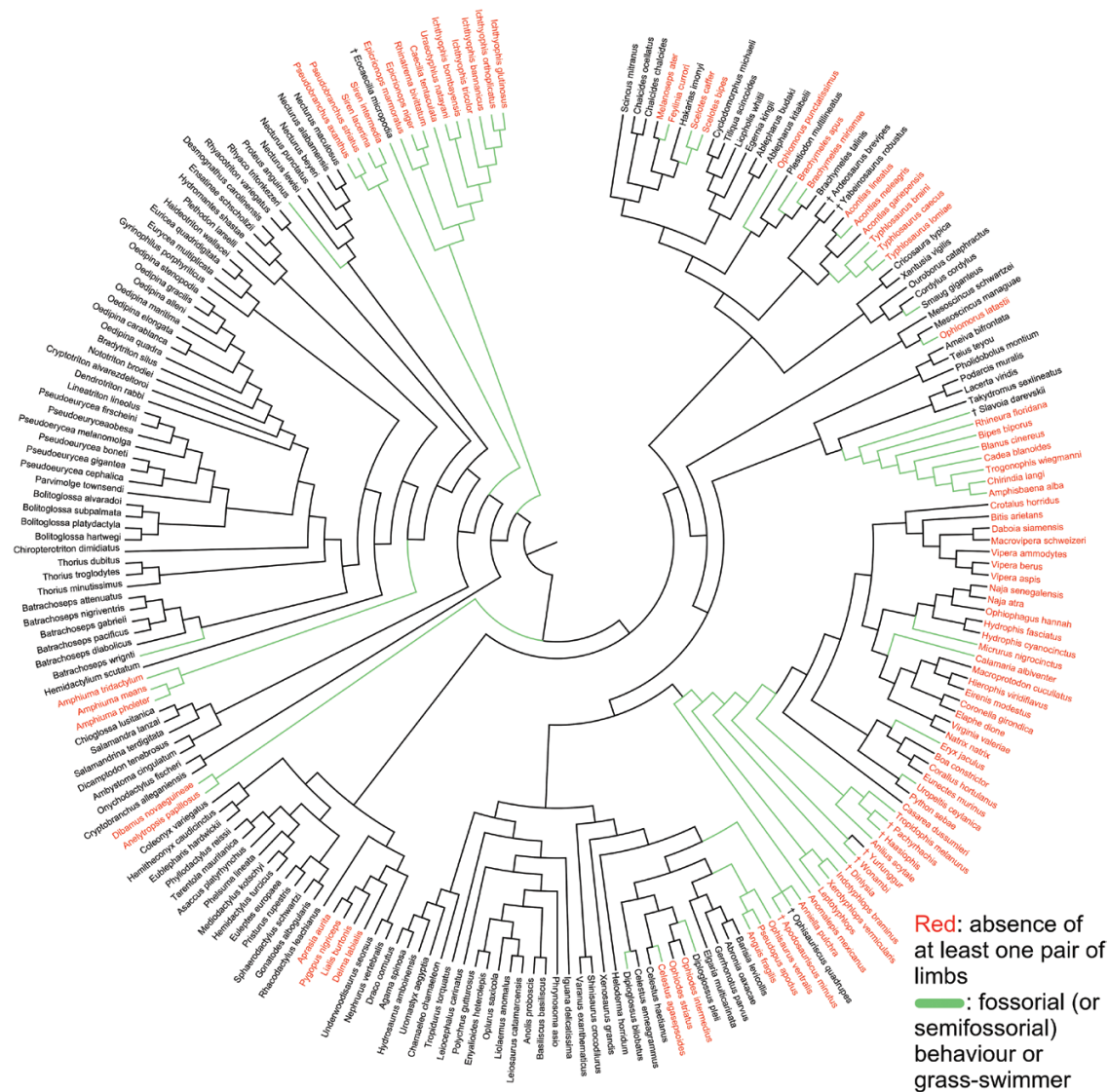


Figure 2. Composite phylogenetic tree of lissamphibians and squamates used for the analysis. See Material and Methods section for the references concerning phylogenetic position and character state reconstruction. The coloured branches in the figure are graphical representations and do not represent any ancestral state reconstruction, because it is not a necessary step for the discrete comparative analysis using BayesTraits v.3.0.

teleost fishes and burrowing or grass-swimming habits for squamate reptiles and lissamphibians.

RESULTS

The analysis resulted in two values of the marginal likelihood, one for the dependent model and one for the independent model (Table 1). Both are described on a logarithmic scale. To test whether the traits are correlated or not, we calculate a logarithmic Bayes factor (BF) between the dependent and independent models. The calculation for logarithmic Bayes factors

is as follows: $\log BF = 2 \times (\log \text{marginal likelihood-dependent model} - \log \text{marginal-likelihood independent model})$. The log BF of 41.467358 in one case and 44.253558 in the other suggest that there is strong evidence for correlated evolution, because ‘strong evidence’ of correlation is considered when log BF has values higher than five (Gilks et al., 1996).

DISCUSSION

Environmental conditions may represent relevant evolutionary drivers leading to the emergence of

Table 1. Values of the logarithm of marginal likelihood of the dependent and independent models and Bayes factor obtained using BayesTraits v.3.0

	Log marginal likelihood (dependent model)	Log marginal likelihood (independent model)	Log Bayes factor
Teleost fishes	-70.555348	-91.289027	41.467358
Lissamphibians and reptiles	-137.900575	-160.027354	44.253558

Both in teleost fishes and in lissamphibians and reptiles, the logarithmic values of the Bayes factor indicate strong evidence of correlation.

new body morphologies within clades. Nevertheless, very different environmental conditions may provide similar constraints on organisms living and moving in them, thereby leading to the development of convergent morphologies. Burrowing (or interstitial) animals, grass swimmers and marine crevice dwellers represent an example of this, because the presence of appendages is not favoured in the environment where they live.

SQUAMATES AND LISSAMPHIBIANS

Previous hypotheses about squamate reptiles and lissamphibians developing a limbless body plan as an adaptative response to burrowing or grass-swimming (Evans, 1998; Wiens *et al.*, 2006; Da Silva *et al.*, 2018) are confirmed by our results. Basal scolecophidian snakes and more derived fossil taxa (e.g. *Dinilyisia*, *Wonambi*) are fossorial (or semifossorial, as in the case of *Yurlunggur*; Palci *et al.*, 2018), and several lines of evidence support the hypothesis that the fossoriality of basal snakes is plesiomorphic (Miralles *et al.*, 2018). Moreover, comparative geometric morphometric studies on skulls have demonstrated that lizards could not have transitioned to snakes by any other evolutionary path than through fossoriality (Da Silva *et al.*, 2018).

The phylogenetic relationships of the stem lineage of Ophidia are still highly controversial, and it is therefore difficult to understand the ecology of the most basal fossil snakes. For example, the lifestyle of the Cretaceous snake *Coniophis* has been reconstructed as fossorial (Longrich *et al.*, 2012), but its phylogenetic position is not resolved. In fact, Longrich *et al.* (2012) considered it to be the most basal stem ophidian, but Caldwell *et al.* (2015) placed this taxon in a more derived position. The situation is similar regarding most of the stem taxa. In general, caution is warranted when using single fossil snakes to make broad extrapolations about early snake biology (Palci *et al.*, 2018). It is also still unresolved whether the body-first or the head-first hypothesis is the more likely, with different evidence sustaining either the former or the latter (Longrich *et al.*, 2012; Caldwell *et al.*, 2015; Da Silva *et al.*, 2018).

It is important, nevertheless, to remark that our results suggest a general evolutionary trend connecting fossoriality (and, more broadly, complex habitats) with limb loss, but this does not mean that the same evolutionary force acted in every single group that evolved a reduction or loss of the limbs, because different constraints can act in different groups (Macaluso & Tschopp, 2018). There are, in fact, few groups of squamates that have evolved a limbless body that are generalist surface dwellers. Two remarkable exceptions are, for example, the skinks and the pygopodids. Skinks evolved limblessness independently in several lineages, even within a single genus (e.g. *Lerista*; Skinner *et al.*, 2008; Fig. 3H), many of which are burrowers, whereas some others are also more generalist surface dwellers (Wiens *et al.*, 2006; Camaiti *et al.*, 2019). Pygopodidae is the only family of gekkotans that has members devoid of limbs. They are generally surface dwellers, although the most basal forms live in the litter (Dorrough & Ash, 1999; Wall & Shine, 2013), a lifestyle that can be regarded as fossorial. The fact that limb loss is so common within squamates is not surprising, because elongated reptiles are characterized by undulatory locomotion and appendages that primarily help to carry the body forward (Sfakiotakis *et al.*, 1999; Grillner, 2011), and limbs can therefore be lost without a relevant impact on their fitness.

Within Amphisbaenia (Fig. 3I), the stem taxon *Slavoia darevskii* apparently proves that fossoriality evolved before the limb loss, because it has clear cranial adaptations for burrowing, but also limbs (Taľanda, 2016). A different phylogenetic analysis placed *Cryptolacerta hassiaca* on the stem of this group (Müller *et al.*, 2011), but the situation is substantially unchanged, because it shows somewhat reduced limbs and cranial adaptations for burrowing. The same situation is found in the clade Gymnophiona (Fig. 3E), in which the fossil taxon *Eocaecilia micropodia* possesses fossorial adaptations and small limbs (Jenkins *et al.*, 2007). Salamanders of the family Sirenidae (Fig. 3G) lack hindlimbs and show some digital reduction of the forelimbs, and Amphiumidae (Fig. 3F) have very small and motionless limbs, but there is no information on

the appendicular skeleton of stem forms referred to these groups (Lande, 1978).

Studies of developmental genetics clearly indicate that there are multiple ways to produce a limbless body plan (Kohlsdorf *et al.*, 2008). In a similar way, it is likely that fin or limb loss in different, unrelated groups might originate via different evolutionary drivers. In any case, our analysis suggests that there is a general correlation between burrowing or grass-swimming habits and limb loss.

TELEOSTS

As far as fishes are concerned, the situation is more complex because of their style of locomotion. Fishes generally use the caudal fin as a propulsor, whereas the paired fins are used to control lateral movement and to prevent rolling and pitching, although there are also fishes that use oscillatory or undulatory movements of the paired fins for generation of thrust (for an extensive review on fish swimming mode, see Sfakiotakis *et al.*, 1999). Short-bodied fishes with an ostraciiform swimming mode and elongated fishes with carangiform locomotion use their pectoral (and pelvic) fins to control their body, and therefore they simply cannot lose their fins, even if this change would be advantageous in their environment. On the contrary, the anguilliform swimming mode may permit the loss of paired fins without a remarkable effect on locomotion capability. In this case, in fact, the paired fins are not useful to prevent rolling or pitching, even if they can help locomotion (Sfakiotakis *et al.*, 1999). This is also clearly demonstrated by cetaceans and sirenians, the only mammal clades with representatives characterized by elongated bodies, which lose a pair of appendages. During the course of their evolutionary history, cetaceans and sirenians have lost pelvic fins, in a similar way to numerous fish clades (e.g. Nelson, 1989; Bejder & Hall, 2002). Their locomotion is undulatory, but different from that of eel-like fishes, because waves are produced in a vertical plane, which do not prevent from the rolling and pitching problems. This is surely coupled with the fact that they did not lose their pectoral fins, which have acquired a stabilizing and steering function, not generating any propulsive movements (Bejder & Hall, 2002).

Our results show that fin loss in fishes is restricted to taxa characterized by an eel-like morphology of the body and most probably related to the burrowing lifestyle or to cryptic life in reef ecosystems. This is clearly evidenced by the consistently eel-like morphology of finless taxa that are characterized by burrowing or crevice-dwelling habits (e.g. Muraenidae; Fig. 3A). The most diverse clade of eel-like fishes is the elopomorph order Anguilliformes, in which

pelvic fins are generally absent, but pectoral fins are present in some groups. Although the interpretation of the ecological preference of extinct fishes is not always easy, it is reasonable to hypothesize that basal anguilliforms (e.g. *Anguillavus* or *Luenchelys*; Belouze, 2002; Belouze *et al.*, 2003a, b) were in some ways reef associated, because the Cretaceous Plattenkalk deposits in which they have been found originated on the outer part of the Lebanese carbonate platform, which was mostly occupied by oyster and rudist mounds and patch reefs (Hemleben & Swinburne, 1991). Members of the extant families Protanguillidae and Synaphobranchidae, regarded as the most basal lineages of crown Anguilliformes (Santini *et al.*, 2013), have small pectoral fins and are characterized by a variety of ecological adaptations. For example, protoanguillids live in submarine caves (Johnson *et al.*, 2011), and the most basal synaphobranchid, *Simenchelys parasitica*, developed a peculiar parasitic lifestyle (e.g. Jaquet, 1920). The main anguilliform group of real burrowers is the Moringuidae, whose members are, in general, burrowers (head or tail first) or crevice dwellers with a marked reduction of paired fins (Castle, 1986; Allen & Steene, 1988; De Schepper *et al.*, 2005). The extant species of the genus *Anguilla* are demersal and do not show a clear reduction of the paired fins, a pattern also shared with three extinct species, *Anguilla ignota*, *Anguilla multiradiata* and *Anguilla elegans* (Winckler, 1861; Micklich, 1985; Riede, 2004; Gaudant *et al.*, 2018). Some lineages within the family Congridae (i.e. the clade composed by *Ariosoma*, *Heteroconger* and *Paraconger*; see Santini *et al.*, 2013) include burrowing fishes devoid of paired fins (Smith, 1981; Riede, 2004; Bacchet *et al.*, 2006). Pectoral fins are also lost in certain crevice-dwelling species belonging to the family Muraenidae (Robins *et al.*, 1991; Chen *et al.*, 1994; Lieske & Myers, 1994; McCosker, 2010; Reece *et al.*, 2010).

A unique case is the benthic gobionellid genus *Luciogobius*, because it is probably the only fish taxon adapted to an interstitial life in gravel beaches (Yamada *et al.*, 2009). The main adaptation consists of an anguilliform elongation of the body that confers sufficient agility for it to move in a three-dimensional complex habitat, similar to that characteristic of terrestrial and aquatic burrowers (Gans, 1975; Yamada *et al.*, 2009). Interestingly, in parallel with the elongation of the body, interstitial species of *Luciogobius* (Fig. 3C) underwent fin reduction, whereas elongated species of Gobionellidae, which are not interstitial but have a benthic or nektonic lifestyle (e.g. genera *Inu* or *Clariger*), exhibit completely developed paired fins. A similar condition is also characteristic of those species of the Gobionellidae that live in reefs or on muddy substrates but are not eel shaped (e.g. *Periphtalmus barbarous*, *Scartelaos histiophorus*).

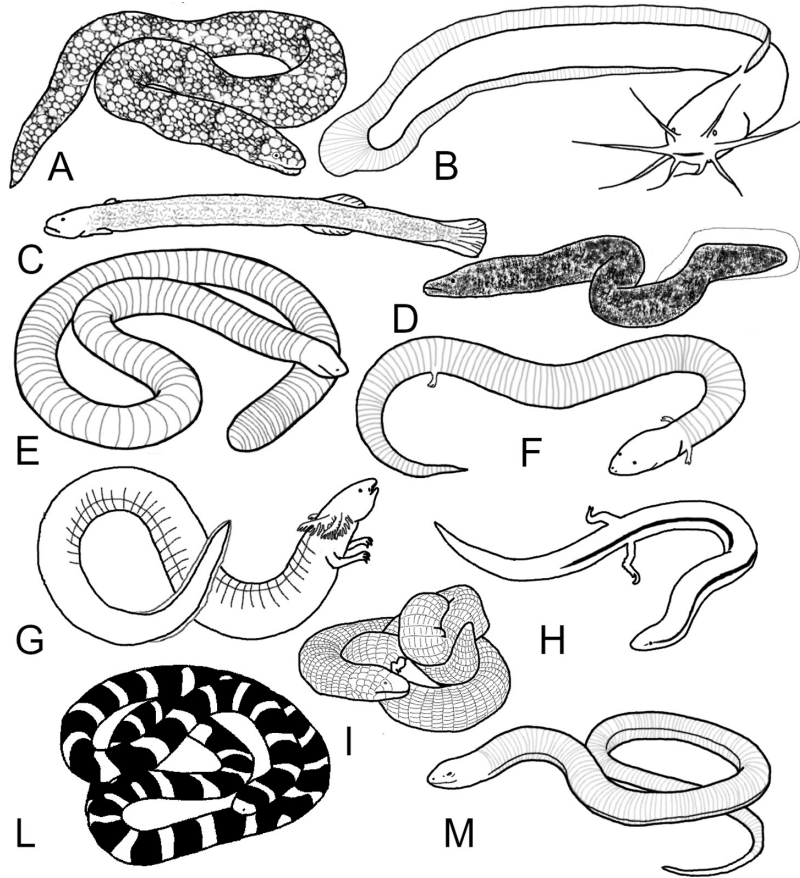


Figure 3. Examples of elongated vertebrates with fin or limb reduction or loss. A, *Anarchias seychellensis*, crevice dweller (fish). B, *Channalabes apus*, living among tree roots (fish). C, *Luciogobius elongatus*, interstitial (fish). D, *Mastacembelus apectoralis*, living among sea vegetation (fish). E, *Caecilia volceni*, burrower (amphibian). F, *Amphiuma means*, burrower (amphibian). G, *Siren lacertina*, burrower (amphibian). H, *Lerista bipes*, surface dweller (reptile). I, *Blanus cinereus*, burrower (reptile). J, *Anilius scytale*, burrower (reptile). K, *Pseudopus apodus*, grass swimmer (reptile).

Within the Neotropical catfish family Trichomycteridae, the Glanapteryginae are interstitial fishes living in complex habitats, and they are mostly eel shaped with a clear reduction of paired fins, which are usually very thin, becoming filiform and without any function in maintenance of equilibrium (De Pinna, 1988; Schaefer *et al.*, 2005; Villa-Verde & Costa, 2006). Catfishes belonging to Clariidae are generally elongated, with an anguilliform swimming mode that is often limited to the posterior part of the body, also extending to the anterior part in some species. All the species devoid of paired fins live in complex or highly vegetated habitats, thereby confirming the hypothesis of fin reduction driven by environmental complexity (Fig. 3B; for references, see Supporting Information, Appendix S1 and S3).

Finally, the Mastacembelidae represent another very peculiar case, because they are eel-shaped fishes, also called spiny eels because of the long series of dorsal-fin spines (Vreven, 2005). It is worth mentioning them

because of the particular case of the only two species of this group [*Mastacembelus apectoralis* (Fig. 3D) and its sister taxon, *Mastacembelus micropectus*] that exhibit a considerable reduction in pectoral-fin size, which is considered to be related to the highly structured environments in which they live (Brown *et al.*, 2011). Their phylogenetic relationships are poorly known, and thus they have not been sampled in our comparative analysis.

Our study reveals the existence of a correlation between the reduction and/or loss of pectoral fins and the coexistence of an eel-like body morphology, which makes the use of pectoral fins to prevent rolling and pitching unnecessary, and of an environmental constraint as a result of burrowing and dwelling in crevices or seagrass. In fact, despite being different habitats, these all have a similar effect on the appendages of fishes, because paired fins may hamper free movement in these constrained environments. A similar correlation between burrowing or

grass-swimming habits and limb loss is also found in squamate reptiles and lissamphibians, but the structural constraints are much less important in these groups, because the locomotion is less constrained by appendages than in fishes, owing to the absence of balance problems.

FUTURE PERSPECTIVES

A relationship between long-tailed patterns and surface dwelling and, conversely, between precaudally elongated morphotypes and burrowers has been proposed for squamates reptiles (Bellairs & Underwood, 1951; Evans, 1998), although this hypothesis has never been proved with rigorous analyses. An interesting future perspective is to expand this hypothesis to anguilliform fishes. In fact, crevice dwellers and seagrass dwellers could somehow be associated with terrestrial surface dwellers. Mehta *et al.* (2010) reported that the elongation in muraenids (i.e. crevice dwellers) results from the addition of caudal rather than precaudal vertebrae to their axial skeleton, whereas elongation of the body in ophichthids and congrid (i.e. burrowers) is achieved by adding a similar number of vertebrae to their precaudal and caudal regions; however, additional studies on this subject, including a larger taxon sampling and much more comparative information, would be desirable.

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SUPPORTING INFORMATION

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

Appendix S1. Database of teleost fishes. Grey columns are the two columns used for the discrete analysis.

Appendix S2. Database of squamate reptiles and lissamphibians. Grey columns are the two columns used for the discrete analysis.

Appendix S3. References cited in Appendix S1 and S2.