

Eco-geographical determinants of the evolution of ornamentation in vipers

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Received 23 December 2019; revised 27 February 2020; accepted for publication 27 February 2020

Multiple hypotheses have been proposed to explain the variation of dorsal patterns observed in snakes, but no studies yet have tested them over broad taxonomic and geographical scales. The Viperidae offer a powerful model group to test eco-evolutionary processes that lead to disruptive and cryptic ornaments. We developed a database reporting dorsal ornamentation, ecological habitus, habitat features and climatic parameters for 257 out of 341 recognized species. Three patterns of dorsal ornamentation were considered: “zig-zag”, “blotchy” and “uniform” patterns. Phylogenetic comparative analyses were based on 11 mitochondrial and nuclear genes. Forty-eight species presented a zig-zag pattern type, 224 a blotchy pattern type and 32 a uniform pattern type. All the patterns showed a strong phylogenetic signal. Character phylogenetic reconstruction analyses suggested an ancestral state for blotchy ornamentation, with multiple independent evolutions of the other patterns. The blotchy pattern was more frequent in terrestrial species living in warm climates and sandy habitats, supporting the hypothesis of a disruptive function. The zig-zag pattern evolved independently in several isolated taxa, particularly in species living in cold climates and in dense vegetation or water-related habitats, supporting the hypothesis of disruptive and aposematic functions. Uniform coloration was particularly frequent in arboreal species, supporting the hypothesis of a cryptic function.

ADDITIONAL KEYWORDS: blotchy pattern – dorsal ornamentation – ecological correlates – phylogenetic-supported characterization – uniform pattern – Viperidae – zig-zag pattern.

INTRODUCTION

Coloration and patterns are among the most intriguing phenomena in biology, involving both plants and animals. Flowering plants, for instance, show a great variability of coloration, often associated with odours, flavours and attractive chemicals for pollinator insects (Lev-Yadun & Ne'eman, 2012; Erbar *et al.*, 2017). In animals, coloration can provide information about health status (Halliday *et al.*, 2014; Trigo & Mota, 2015; Sepp *et al.*, 2018) and reproductive condition

(P rez i de Lanuza & Font, 2007; Svensson *et al.*, 2008). Furthermore, mimetic coloration, camouflage and disruptive patterns reduce the detectability of both predators and prey (De Bona *et al.*, 2015; Morris & Reader, 2016), whereas conspicuous coloration alerts to toxicity or unpalatability (aposematism) (Kraemer *et al.*, 2015; Cuthill *et al.*, 2017). Many studies have evaluated correlations between ornamentations, colorations, ecological variables and phylogeny across taxa of terrestrial vertebrates (Poulton, 1890; Cott, 1966; Waage, 1981; Endler, 1990; Krebs, 1994). These studies highlighted that the evolution of coloration

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and patterns has been driven by the interplay between aposematism, crypsis, sexual selection, physiological constraints and social selection (Cuthill *et al.*, 2017), confirming that colour patterns and colour polymorphism are associated with speciation dynamics (Arbuckle & Speed, 2015).

Vipers (family Viperidae) are an excellent model group to test evolutionary processes related to disruptive and cryptic patterns. Vipers are widespread and well known from a morphological, ecological, molecular and phylogenetic point of view and multiple mechanisms have been suggested for the evolution of their coloration patterns. First, vipers are a clade of highly venomous snakes, thus recurrent highly detectable colours or patterns may be examples of aposematic coloration or of Müllerian mimicry (Wüster *et al.*, 2004; Valkonen *et al.*, 2011a, b; Santos *et al.*, 2014). Harmful species, however, do not necessarily show bright colours in order to reduce the risk of being detected (Sherratt & Betty, 2003; Endler & Mappes, 2004). In fact, some patterns (e.g. the zig-zag dorsal pattern of many vipers) can be examples of Müllerian mimicry that allow vipers to be identified as dangerous, without increasing their detectability (Wüster *et al.*, 2004; Valkonen *et al.*, 2011a, b). In addition, ambush hunting and predation avoidance have been proposed as drivers of the evolution of cryptic colorations and disruptive patterns (Cott, 1966; Ruxton *et al.*, 2004; del Marmol *et al.*, 2016). Disruptive patterns can be achieved through colorations with complex (either regular or irregular) patterns. Such combinations decrease the detectability of an individual even if the coloration of the body does not perfectly match the environment (e.g. *Bitis nasicornis*, *Bitis gabonica*) (Stevens & Merilaita, 2009a, b).

Several studies observed a correlation between viper colour, pattern and behaviour (Jackson *et al.*, 1976; Allen *et al.*, 2013). Many species displaying uniform and/or stripe coloration have limited defensive abilities and high escape capacity (Jackson *et al.*, 1976), because a moving striped object can create either a “barber pole effect” or a “flicker-fusion effect”, giving the perception of a uniform pattern during motion that may confuse a potential predator and increase the escaping probability (Jackson *et al.*, 1976; Lindell & Forsman, 1996; Allen *et al.*, 2013). Conversely, snakes with bright colours and/or blotched designs are usually more inclined to fight (Jackson *et al.*, 1976; Clark, 2006; Allen *et al.*, 2013), even if it is unclear whether the efficiency of a bar-like pattern may serve as an anti-predatory diversion or not (Lindell & Forsman, 1996).

Vipers present a striking variability of dorsal ornamentations. Some species show a motif that is regularly repeated several times on the body surface (e.g. zig-zag or blotchy pattern) whereas others show a uniform coloration. In addition, intraspecific variation

exists, with some species exhibiting all of the zig-zag, blotchy and uniform patterns (Fig. 1). The efficiency of the different patterns is expected to vary according to the environmental circumstances. A uniform coloration, for instance, can be more cryptic in canopies with a homogeneous background coloration, whereas a disruptive pattern can favour camouflage in ecotones with a mottled background. Eco-geographical variables provide a broad-scale picture of the habitat variation and species activity conditions, which in turn, can affect the relative efficiency of different pattern types. However, no analyses have yet tested the relationships between colour patterns of snakes and eco-geographical variables over broad taxonomic and geographical scales. In this study, we performed extensive bibliographical research to collect distributional, ecological, behavioural and bioclimatic information for most of the recognised viper species. We then used exhaustive phylogenetic data (Alencar *et al.*, 2016) to reconstruct the evolution of different dorsal patterns and to identify the eco-geographical factors related to the occurrence of dorsal patterns in vipers.

MATERIALS AND METHODS

DATA COLLECTION

Data were acquired from both the literature and online sources (Supporting Information, Appendix S1) and then used to create a database with morphometric, ecological and zoogeographical information representing all the 341 recognised species of vipers (Uetz & Hošek, 2017). For each taxon, we collected the following variables: dorsal patterns, ecology and habitat, and climatic variables.

DORSAL PATTERNS

Dorsal patterns were classified into three main categories: zig-zag, blotchy and uniform (Fig. 1). “Zig-zag” is a mostly continuous linear motif characterised by a sequence of small corners, roughly rounded, with variable inclinations. The “blotchy” category included species with regularly repeated motifs such as bars, blotches, circles, ovals and transversal stripes. Lastly, “uniform” indicates patterns that do not show regular motifs. In several cases, one single species can show multiple dorsal patterns (see *Results*). For instance, *Vipera aspis* displays a high variability of patterns among subspecies, and the three patterns are present in this species (Fig. 1; Zuffi & Bonnet, 1999). Therefore, for each species, we recorded the presence/absence of the three distinct patterns, where every species can have more than one character state. Melanistic individuals were not considered; due to possible motif

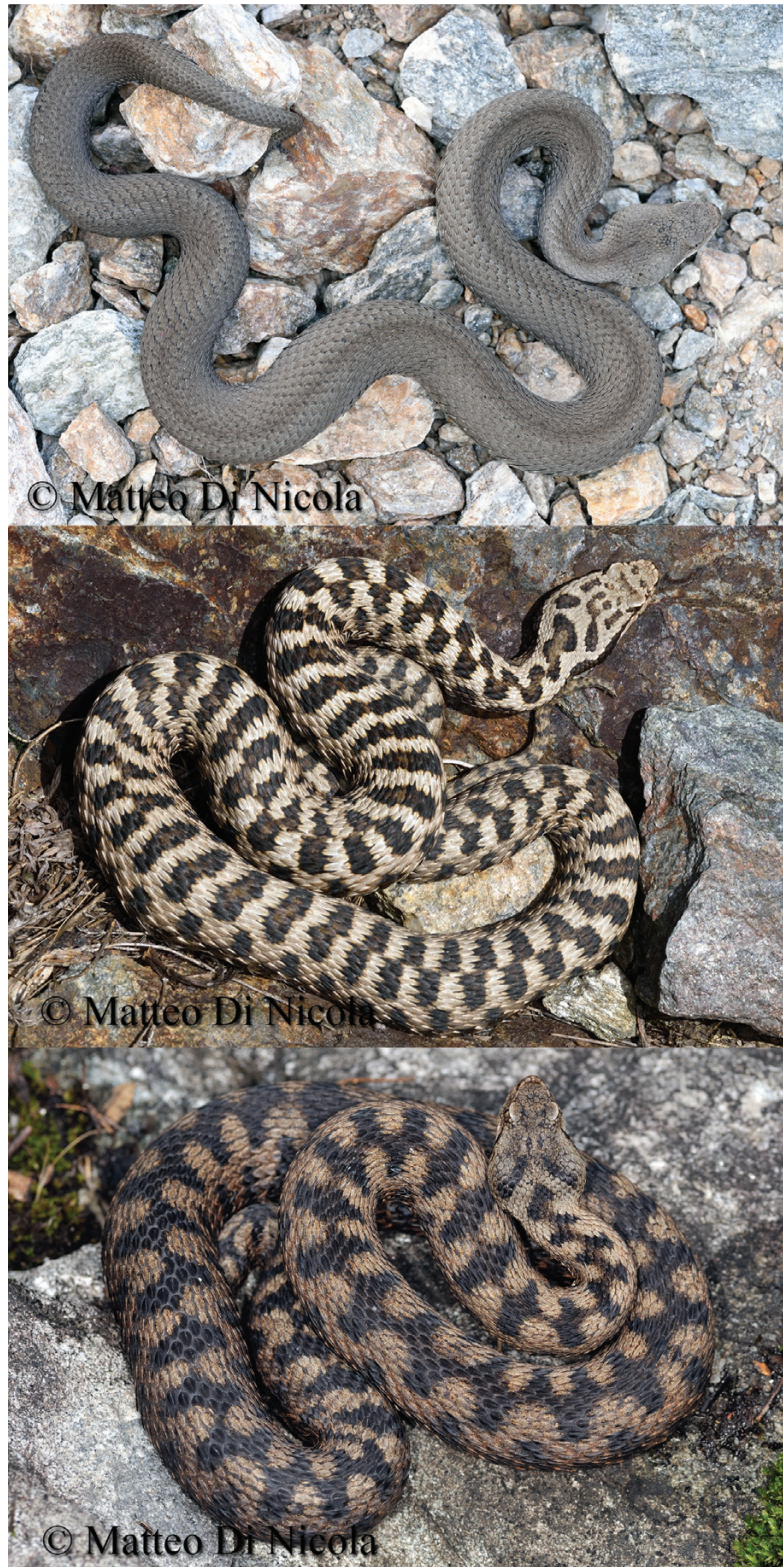


Figure 1. Example of intraspecific polymorphism in *V. aspis*, on the top a concolor individual displaying uniform pattern coloration, in the middle an example of blotchy pattern (bars in this case) and on the bottom a zig-zag pattern-like coloration. Credit to Matteo Di Nicola (<http://www.matteodinicola.it/>).

changes during species' ontogenesis (da Silva *et al.*, 2017), we only considered the features of adults.

ECOLOGY AND HABITAT

The ecological habitus of each species was coded as a semi-quantitative variable (strictly ground living: 0; semi-arboreal: 0.5; arboreal: 1). Furthermore, we identified the habitat typologies where each species can be present [dummy variables: sandy areas, rocky areas, open vegetated areas (e.g. grasslands, croplands, meadows, etc.), forests, water-associated (i.e. living in riparian or moist areas)].

CLIMATIC VARIABLES

For each species, we calculated average values of mean annual temperature and total precipitation. Climatic parameters were calculated as the average value through the whole species range. Ranges were obtained from Roll *et al.* (2017); climatic values were obtained from the CRU TS v.4.01 (updated from Harris *et al.*, 2014). Because no distribution map was available for *Crotalus ornatus*, we used the centroid of the range as described in the Reptile Database to extract climatic values (Uetz & Hošek, 2017). The correlation between variables was generally weak (for all pairwise correlations, $|r| \leq 0.6$), suggesting that collinearity between independent variables did not bias the results of the regression analyses (Dormann *et al.*, 2013). Although the average conditions across the range may not represent the full conditions experienced by the species, they provide excellent information on the ecogeographical factors driving the evolution of species, when the climate of exact localities is not available (e.g. Stark & Meiri, 2018).

DATA ANALYSIS

For phylogenetic comparative analyses, we used the calibrated tree based on 11 mitochondrial and nuclear genes by Alencar *et al.* (2016). The time-tree included all the taxa for which we obtained pattern and ecological variables, and was pruned to match the list of species with available data. We used stochastic reconstruction of character states in order to assess the evolution of dorsal patterns along the phylogeny. Stochastic character mapping is a technique where possible histories of characters are sampled in proportion to their probability. Starting from the topology of the Alencar *et al.* (2016) time-tree, we generated 1000 random simulations of a stochastic process of the character state, across the branches of the tree. The posterior probability of stochastically mapped characters was plotted on the phylogeny, to provide a character state reconstruction via stochasticity

mapping (Revell, 2013). For all the resulting trees, we showed character state probabilities on both nodes and along branches. In large phylogenetic trees, the rate of trait evolution can differ significantly among lineages (Beaulieu *et al.*, 2013). We therefore used the Beaulieu *et al.* (2013) approach to compare a time-homogeneous model of trait evolution, with models assuming two or more hidden rates. For the three considered traits, the time-homogeneous model always showed lower Akaike's Information Criterion corrected (AICc) for limited sample size than the models with hidden rates, therefore we assumed homogeneous evolution across the tree. Stochastic reconstruction of character states was first performed for the three patterns separately (presence-absence of blotchy, zig-zag and uniform patterns). Furthermore, we used a Markov model (i.e. a model of trait evolution and ancestral states reconstruction for discrete states) to analyse the three patterns in the same model. We used the `make.simmap` function in `phytools` to perform stochastic mapping analysis (1000 replicates; Revell, 2013). For polymorphic species, we assumed that the multiple states of the pattern have the same prior probability. We then reconstructed state evolution through the `describe.simmap` in `phytools` (Revell, 2013).

We used D statistics (Fritz *et al.*, 2010) to measure the phylogenetic signal of dorsal patterns. D statistics is appropriate to measure phylogenetic signal for discrete traits. The value $D = 1$ indicates no phylogenetic signal, whereas D values close to zero or lower suggest very strong signal (Fritz *et al.*, 2010). We used 5000 random permutations to assess whether D is significantly different from the values expected under no phylogenetic structure.

Subsequently, we used phylogenetic logistic regression to identify the eco-geographical parameters related to the evolution of dorsal patterns (Ives & Garland, 2010) using the Alencar *et al.* (2016) tree to consider the evolutionary history. We used a model-selection approach, based on AICc to identify the combination of variables best explaining the occurrence of the three dorsal patterns. First, we built regression models including all the possible combinations of the considered variables and calculated the AICc of each model. AICc trades off explanatory power vs. number of predictors; models explaining more variation with a limited number of variables have the lowest AICc values and are assumed to be the "best models" (Symonds & Moussalli, 2011). We then calculated Akaike's weight (w) of each model, which infers the likelihood that a model is the best one given a set of candidate models (Burnham & Anderson, 2002; Symonds & Moussalli, 2011). We also tested the possibility of non-linear relationships, assessing quadratic terms of continuous variables included in highly supported models. Finally, we calculated the sum

of weight of each variable, as the sum of the Akaike's w where each variable appears. The sum of weights is a measure of the relative importance of variables and can be used when model selection reveals uncertainty in the identification of best model(s). The significance of variables within the best AICc models was assessed using likelihood ratio tests. Analyses were performed using the packages *ape* (Paradis *et al.*, 2004), *corHMM* (Beaulieu *et al.*, 2013), *phytools* (Revell, 2012), *maps* (Brownrigg, 2018), *raster* (Hijmas & van Etten, 2012), *ggplot2* (Wickham, 2016), *caper* (Orme, 2013), *MuMIn* (Bartoń, 2015) and *phylolm* (Ho & Ane, 2014) in R v.3.3 (R Core Team, 2017).

RESULTS

Overall, we obtained complete information for 257 species. Forty-five species presented more than one dorsal pattern (e.g. in several instances the blotchy and zig-zag pattern occurred in individuals of the same species). All the patterns showed a strong phylogenetic signal, with a particularly strong signal for the zig-zag and blotchy ornamentalions (zig-zag: $D = -0.20$; blotchy: $D = 0.02$; uniform: $D = 0.28$); in all cases, the D values indicated a phylogenetic signal stronger than expected from random phylogenetic structure (all $P < 0.0001$). The blotchy pattern was the most widespread pattern among vipers, being recorded in 224 species. The *Trimeresurus* and *Vipera* genera showed mainly the uniform coloration and the zig-zag ornamentation type, respectively. Character phylogenetic reconstruction analyses suggested an ancestral state for blotchy ornamentation with multiple independent evolution of both of the other two types of dorsal patterns (Figs. 2–4).

The zig-zag pattern was present in 48 species. The character mainly occurred in the *Montivipera-Macrovipera-Daboia-Vipera* clade, in the *Mixcoatlus-Ophryacus* clade and in both the *Atheris* and *Cerrophidion* genera with a few exceptions (*Vipera transcaucasiana*, *Daboia deserti*, *Daboia siamensis*, *Macrovipera schweizeri*, *Montivipera latifi*, *Montivipera bornmuelleri*, *Montivipera albizona*, *Atheris squamigera* and *Atheris chlorechis*). According to the character state reconstruction analysis, the trait evolved independently also in several isolated taxa throughout both the Viperinae and Crotalinae sub-families: *Pseudocerastes fieldi*, *Cerastes vipera*, *Echis pyramidum*, *Bothrops pictus*, *Atropoides occiduus*, *Atropoides nummifer*, *Trimeresurus gracilis*, *Protobothrops elegans*, *Protobothrops sieversorum* and *Protobothrops kaulbacki* (Fig. 3).

The uniform pattern was present in 32 species. This pattern type mostly occurred in Asiatic taxa of the genus *Trimeresurus* (Fig. 4). It also occurred in *Macrovipera*

schweizeri, *Atheris ceratophora*, *Atheris chlorechis*, *Causus resimus*, *Tropidolaemus subannulatus*, *Bothriechis guifarroi*, *Bothriechis lateralis*, *Bothriechis schlegelii* and *Bothrops bilineatus*. In each of these species, the character evolved independently (Fig. 4).

The stochastic reconstruction of the three pattern states in the same model confirmed the blotchy pattern as the ancestral state, followed by multiple transitions (Fig. 5). The model suggested that the uniform pattern evolved from the blotchy pattern six times, whereas the zig-zag pattern evolved from the blotchy pattern five times. Furthermore, several reversals occurred, particularly from the uniform to the blotchy pattern (14 transitions), whereas reversals from zig-zag to blotchy were rare (two transitions; Fig. 5).

ECO-GEOGRAPHICAL DETERMINANTS OF DORSAL PATTERNS

BLOTCHY PATTERN

The best AICc phylogenetic regression model suggested that the blotchy pattern was related to climate, species habitus and habitat (Table 1a). Blotches were particularly frequent in ground-living species ($\chi^2_1 = 26.0$, $P < 0.0001$) (Fig. 6b), in species living in sandy areas ($\chi^2_1 = 11.5$, $P = 0.0007$) (Fig. 6c) and in warm climates ($\chi^2_1 = 7.5$, $P = 0.006$) (Fig. 6d). An alternative model, with very similar AICc values, included annual precipitation instead of sandy habitat and confirmed the high frequency of this pattern in species living in arid areas ($\chi^2_1 = 12.1$, $P = 0.0005$). Ecological habitus and association with mean temperature were the variables with the highest relative importance (Table 2).

ZIG-ZAG PATTERN

The best AICc model suggested that the zig-zag pattern was particularly frequent in species living in cold climates ($\chi^2_1 = 15.2$, $P < 0.0001$) (Fig. 6a). The zig-zag pattern tended to be more frequent in species living in water-related habitats ($\chi^2_1 = 2.5$, $P = 0.12$) and was slightly less frequent in species living in open habitats ($\chi^2_1 = 3.75$, $P = 0.053$); however, these variables were not significant at the 5% level. The mean temperature was the variable with the highest relative importance to explain the occurrence of this pattern (Table 2).

UNIFORM PATTERN

According to the best AICc model, uniform coloration was particularly frequent in arboreal species ($\chi^2_1 = 28.3$, $P < 0.0001$) (Fig. 6e and Table 1c). None of the remaining variables were included in models with AICc weight > 0.01 , indicating ecological habitus as

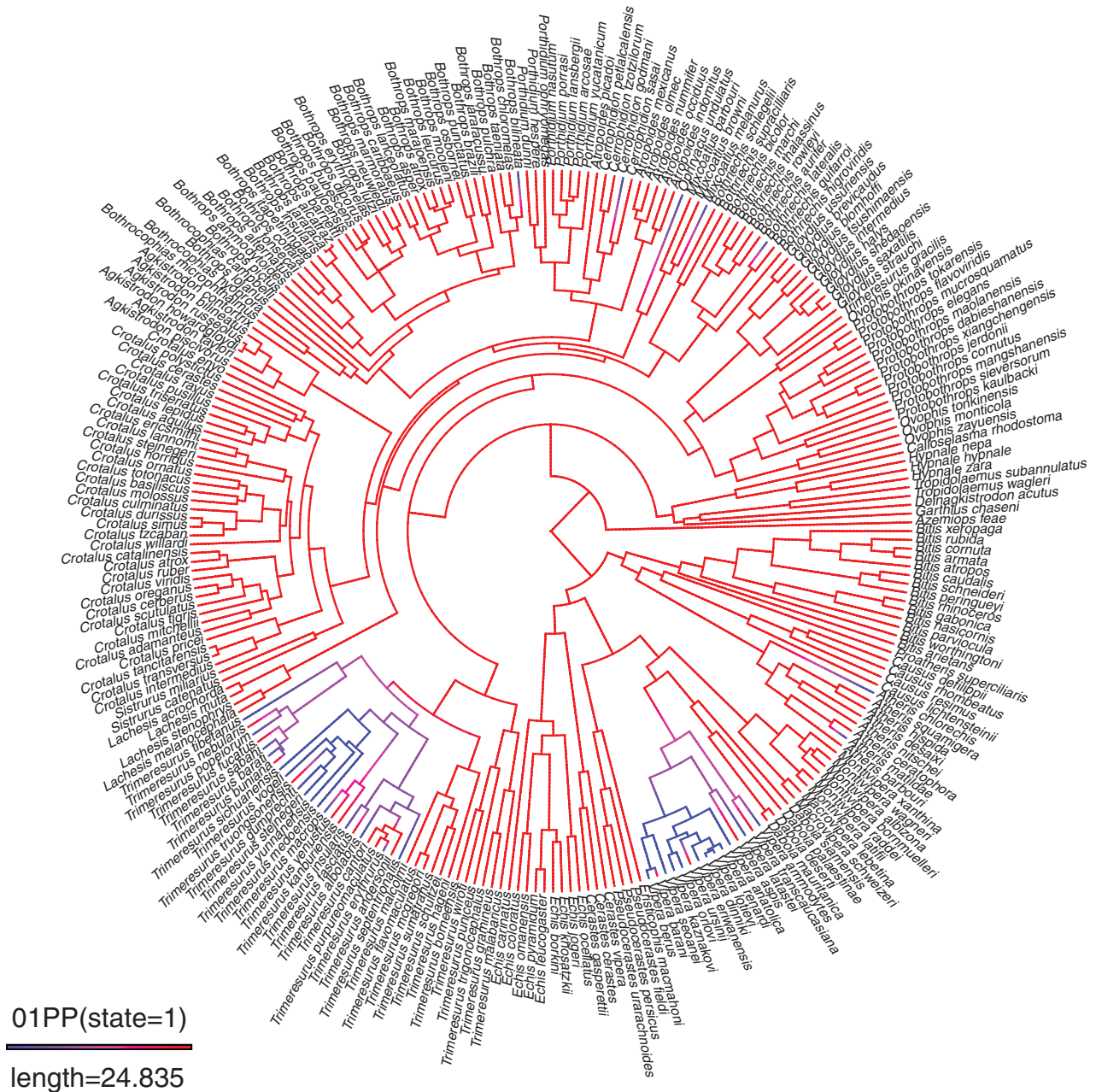


Figure 2. Ancestral character state reconstruction of the blotchy pattern along the branches of the phylogenetic tree of the Viperidae. Red indicates a high posterior probability of the occurrence of the blotchy pattern within a clade, blue indicates low probability of occurrence, and pink indicate uncertainty. The phylogeny is from [Alencar et al. \(2016\)](#).

the most important variable to explain the occurrence of this pattern ([Table 2](#)).

DISCUSSION

This study represents the first global scale characterisation of dorsal ornamentations for the family Viperidae. Our analyses clearly show that

multiple factors can jointly determine the evolution of a certain pattern, predominantly climatic conditions and lifestyle. We observed a strong phylogenetic signal, with highly conserved basal character (blotchy) from which different patterns arose independently multiple times in different areas of the world. In most of the cases, the evolution of the new patterns corresponded to a loss of the ancestral state (e.g. the green concolor pattern in most of the *Trimeresurus* species and the

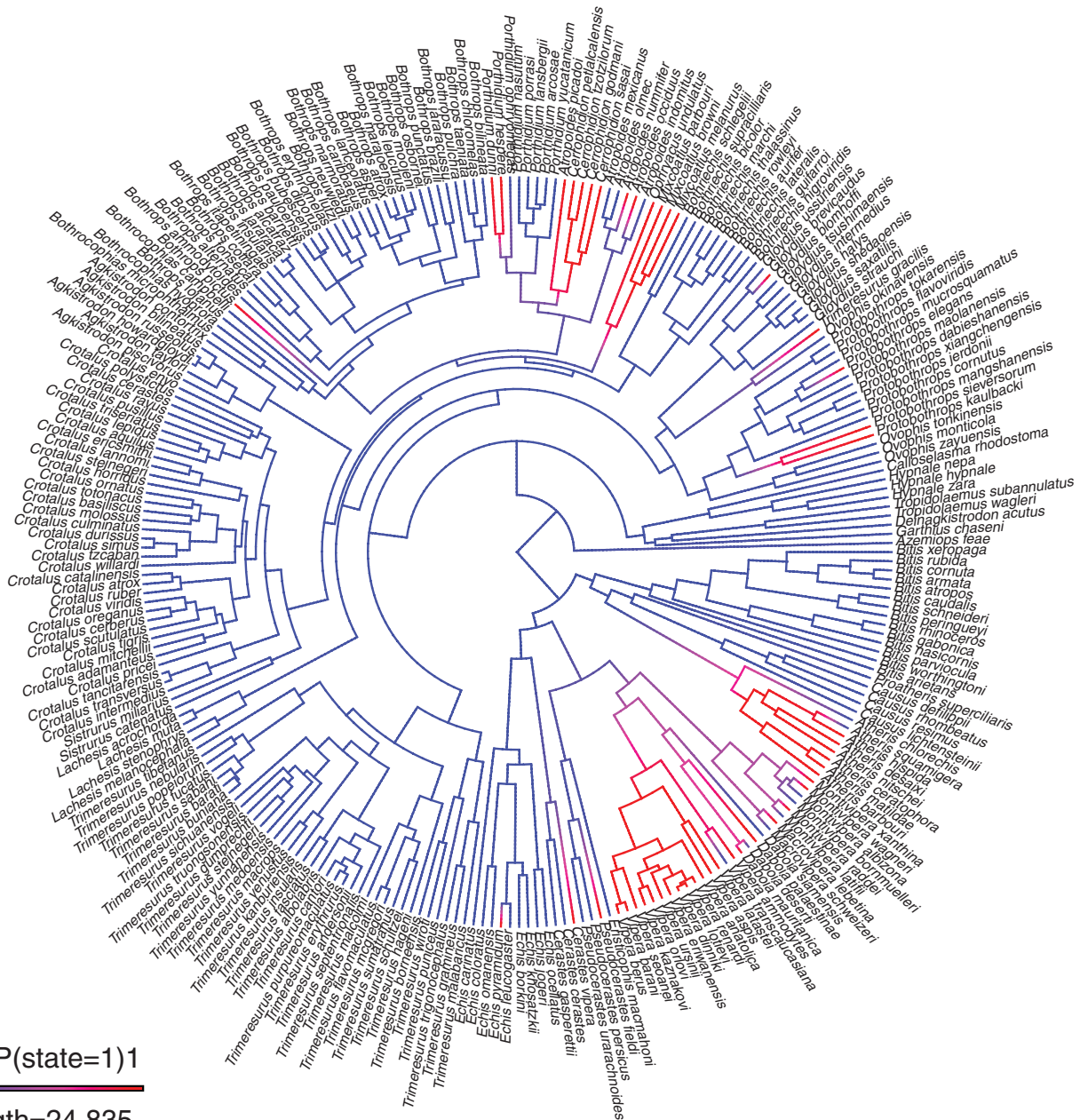


Figure 3. Ancestral character state reconstruction of the zig-zag pattern along the branches of the phylogenetic tree of the Viperidae. Red indicates a high posterior probability of the occurrence of the blotchy pattern within a clade, blue indicates low probability of occurrence, and intermediate colours indicate incertitude.

zig-zag pattern in *Vipera*). However, there are also multiple cases of intraspecific variability (species in which a new pattern arose, but the blotchy remains, e.g. *V. aspis* see Zuffi & Bonnet (1999)). Such complex evolution of colour patterns along the phylogeny has been observed in multiple snake lineages. For instance, coral snakes (*Micrurus*), include distinct phylogenetic lineages that can be also distinguished

by their dorsal patterns (Slowinski, 1995; Gutberlet & Harvey, 2004; Marques *et al.*, 2013; Jowers *et al.*, 2019): the monadal pattern (one black ring between two white or yellow annuli separated by red annuli), the triadal pattern (three black rings), and a bicolor coloration. Jowers *et al.* (2019) confirmed that there is a phylogeographic explanation behind the evolution of the two predominant colour patterns in these

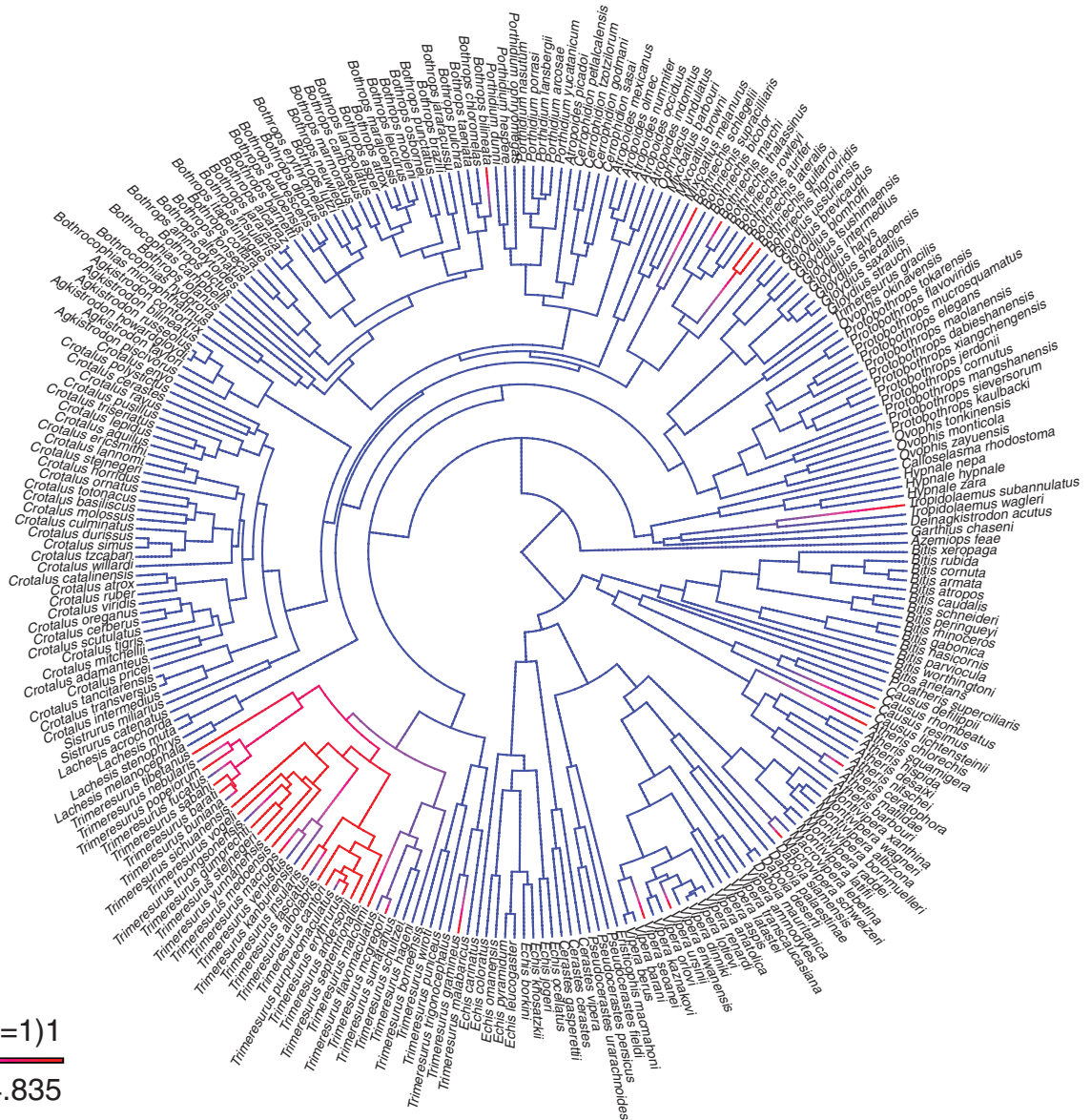


Figure 4. Ancestral character state reconstruction of the uniform pattern along the branches of the phylogenetic tree of the Viperidae. Red indicates a high posterior probability of the occurrence of the blotchy pattern within a clade, blue indicates low probability of occurrence, and intermediate colours indicate incertitude.

coral snakes, where from a basal triadal pattern the monadal form evolved in the Middle Miocene and more recently also with a bicolour coloration.

DRIVERS OF THE EVOLUTION OF BLOTCHY PATTERNS

The evolution of different patterns in vipers was strongly related to habitat and habitus. That is, each coloration is characteristic of species living in specific climatic conditions, habitats or lifestyle. The blotchy ornamentation appears to be frequent in ground-dwelling species living at low latitudes in environments with warm and arid climates (Fig. 6c-d).

Avoiding detection by visual hunting predators is essential for ground-dwelling species. Previous studies already assumed the disruptive function of the blotchy pattern, which mimics the dark shadows of the litter-free sand beneath the vegetation (Sherbrooke, 2002). These properties of the blotchy pattern result in being a particularly effective anti-predator strategy of defence in ground-dwelling species (Brodie, 1992, 1993) because it allows them to confuse the outlines of their bodies with the substratum (e.g. *B. gabonica*), with shrubs and grass stems (e.g. *V. aspis*) or disguise them by blending their dorsal pattern with the shadow created by bushes (Cott, 1966). Moreover,

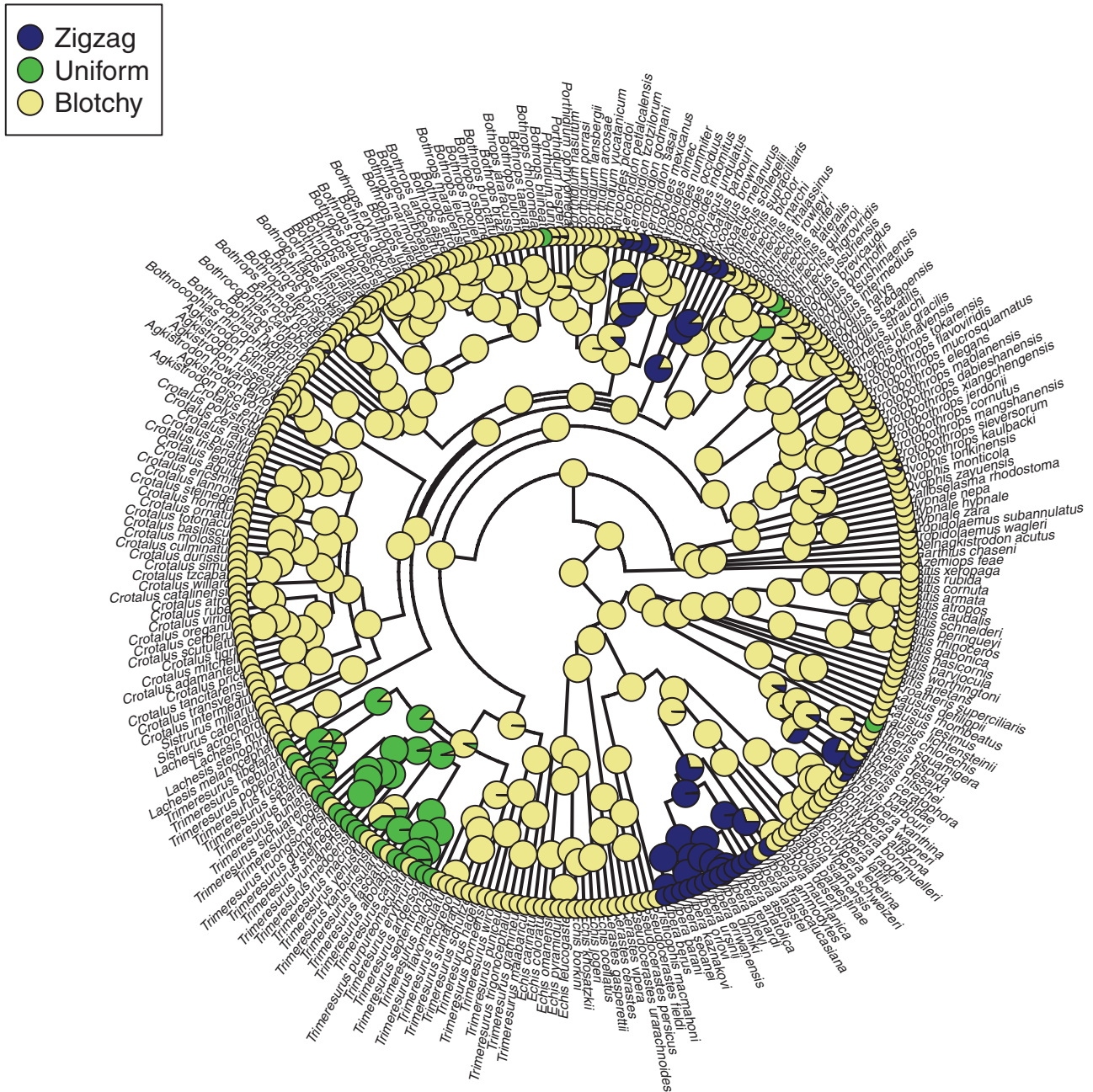


Figure 5. Ancestral character state reconstruction for the three patterns. Pies indicate the probability of the occurrence of each pattern within a clade.

blotchy patterns create a “barber pole effect” when fleeing throughout the bushy and herbaceous vegetation (Jackson *et al.*, 1976; Lindell & Forsman, 1996). Shadow-like coloration has useful camouflage properties in sandy habitats (Serventy, 1971). Our results clearly show that blotchy coloration patterns are more frequent in species inhabiting sandy and arid environments, where most of the species move between patches of vegetation (seeking shade in the warmest

hours of the day) and open areas (to thermoregulate or hunt).

DRIVERS OF THE EVOLUTION OF ZIG-ZAG PATTERNS

Our phylogenetic reconstruction suggests that the zig-zag coloration pattern evolved multiple times (approximately 23 times) in vipers, mostly from an ancestor with a blotchy pattern (Figs 2-3). The zig-zag

Table 1. Candidate mixed models explaining variation in the occurrence of dorsal patterns among vipers. Results of phylogenetic logistic regression models. Models are ranked on the basis of corrected Akaike's information criterion (AICc); only models with Akaike's weight > 0.01 are reported.

Rank	Independent variables	AICc	Δ AICc*	w^\dagger
a) Dependent: occurrence of blotchy pattern				
1	Ecological habitus (-), mean temperature (+), sandy habitat (+)	134.04	0.00	0.48
2	Ecological habitus (-), mean temperature (+), precipitation (-)	134.67	0.63	0.35
3	Ecological habitus (-), forest habitat (-)	137.09	3.06	0.11
4	Ecological habitus (-)	138.41	4.38	0.05
b) Dependent: occurrence of zig-zag pattern				
1	Mean temperature (-), water habitat (+), open habitat (-)	169.26	0.00	0.45
3	Mean temperature (-)	169.43	0.18	0.41
4	Water habitat (+), sandy habitat (+)	175.39	6.13	0.02
5	Rock habitat (+), forest habitat (-)	175.58	6.32	0.02
6	Precipitation (-), sandy habitat (+)	175.64	6.39	0.02
c) Dependent: occurrence of uniform pattern				
3	Ecological habitus (+)	140.79	0.00	> 0.99

* Δ AICc = AICc difference with the best model.

$^\dagger w$ = Akaike's weight

pattern presents a very strong phylogenetic signal and is more common in species living in cold climates (Fig. 6c). In these areas, more time is needed for thermoregulation and consequently snakes are more exposed to predation. The zig-zag pattern has been proposed to have a disruptive effect (from afar) but can also represent a case of Müllerian mimicry (Valkonen *et al.*, 2011b). The hypothesis of Müllerian mimicry is supported by a strong phylogenetic signal. The zig-zag pattern is particularly clustered within the phylogeny (Fig. 3), and in closely related species which often live in nearby geographical regions (Warren *et al.*, 2014). For instance, all the species of the genus *Vipera* display this colour pattern and are mostly distributed in the same geographic region, Europe. This has probably allowed potential predators to learn from more than one species-model that animals showing zig-zag coloration patterns are most likely a danger. Further support to this hypothesis is the presence of several cases of Batesian mimicry from innocuous species emulating this coloration pattern, e.g. the viperine water snake, *Natrix maura*, which is harmless and belongs to the Colubridae family (Santos *et al.*, 2017).

DRIVERS OF THE EVOLUTION OF UNIFORM PATTERNS

Repeated evolutions have also been observed for the uniform dorsal pattern, which evolved from both blotchy and zig-zag patterns (Figs 2-3). Uniform coloration has proved to be particularly frequent in Asian clades and specifically in species with arboreal habits (Fig. 6a) for hunting, thermoregulation, roosting or all of three activities combined. Accordingly, many species with uniform pattern exhibit a green coloration, which

likely improves crypsis in the canopy. Cases of uniform coloration (or concolor form) have also been reported in individuals within the genus *Vipera* (e.g. former *Viper aspis atra*, *Viper aspis aspis* and *Viper berus bosniensis*), especially at high altitudes in rocky and open areas (Colombo & Di Nicola, 2012; Tessa, 2016; Nikolić & Simović, 2017). In this genus, individuals with uniform pattern display a greyish coloration, which can have the same cryptic function of the green coloration in arboreal or semi-arboreal Asian pit-vipers. However, our model does not completely explain the evolution of all the uniform coloration patterns. In some species, individuals often are uniformly dark (melanism), and multiple hypotheses have been proposed to explain these colorations, such as thermoregulation (Kettlewell, 1973; Kingsolver & Wiernasz, 1991; Trullas *et al.*, 2007), crypticity (Kettlewell, 1973; Endler, 1984), aposematism (Turner, 1977), protection from UV radiation (Gunn, 1998) and sexual selection (Wiernasz, 1989); however, further investigation is needed to corroborate or confute these assumptions.

LIMITATIONS

Our study provides one of the most complete evaluations of colour evolution in snakes, still it cannot be regarded as exhaustive since we have not explored all possible drivers for colour patterns. First, we adopted a macroevolutionary perspective and focused on eco-geographical drivers; however, additional processes were certainly at work. For instance, sexual selection is one of the most frequent drivers of colour patterns (Cuthill *et al.*, 2017). In our study, we did not consider sexual selection as a factor because of the

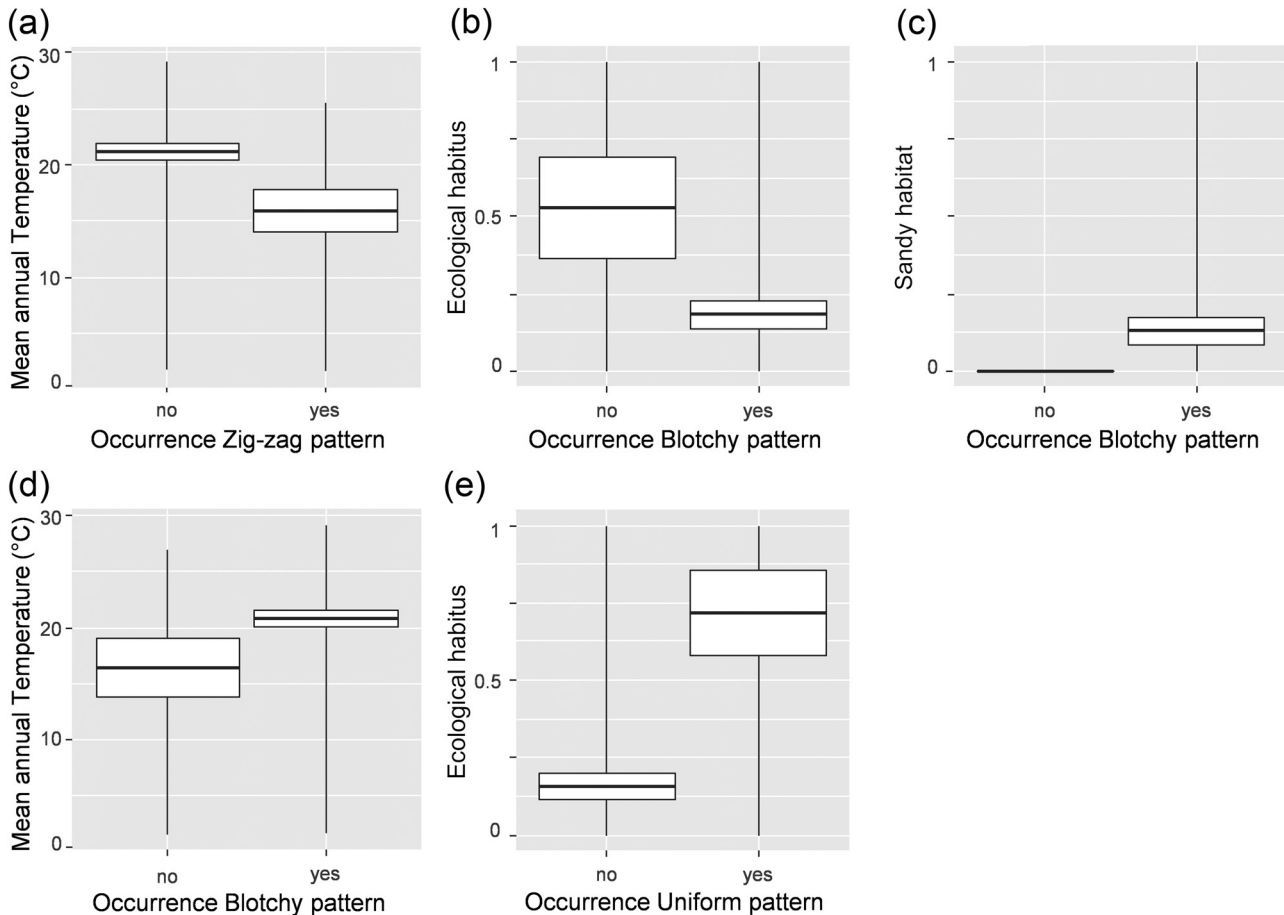


Figure 6. Occurrence of dorsal patterns in relation to the variables, predicted on the basis of the best phylogenetic logistic regression models. Boxplots indicate: the occurrence of the zig-zag pattern in relation to (a) mean annual temperature; the occurrence of blotchy pattern predicted by (b) ecological habitus, (c) occurrence in sandy habitat and (d) mean annual temperature; the occurrence of uniform pattern type predicted by (e) ecological habitus. The categorical variables ecological habitus (b) and (e) and occurrence in sandy habitat (c), express the probability that a species has a given pattern based on the habitat where it lives.

limited information on sexual dimorphism in patterns in vipers; however, this hypothesis requires future attention. Another process that we did not consider is the change in coloration during the ontogenesis. Unfortunately, detailed information on the coloration of juveniles and sub-adults is only available for a subset of species. Nevertheless, the mortality of juveniles is not consistently higher than that of adults (Pike *et al.*, 2008), suggesting that our conclusions are not biased by difference in mortality among age classes. Additional hypotheses that can be tested in the future include the role of fine-scale interactions between individuals and their micro-habitat (relating colour patterns to vegetation cover), diet and hunting strategies.

Our results could be partially affected by the uneven distribution of information. First, some taxa are less known, for instance because they live in inaccessible/poorly studied areas. For these taxa, it is possible that intraspecific variation exists (i.e. more than one single

pattern). The possible bias determined by incomplete information hampered the analysis of intraspecific variability; however, improving the completeness of information could allow analysing intraspecific variability. Finally, our analysis at a broad phylogenetic scale used a coarse definition of patterns. For instance, the “blotchy” patterns group includes a broad range of patterns (blotches, transversal lines, ellipses, bars, etc.). Furthermore, both green, grey and black vipers are “uniform”, but the role of these colorations is probably different. Future analyses could consider the fine-scale variation within the different patterns.

CONCLUSIONS

Our study revealed the complexity of factors determining the evolution of colour patterns in vipers, suggesting that multiple processes, ranging from

Table 2. Relative importance of ecological habitus, habitat and climatic conditions in determining the blotchy, zig-zag and uniform patterns. Importance was measured as the sum of AICc weights of models where a variable was included. Bold numbers indicate importance > 0.5.

Independent variables	Dependent variables:		
	Blotchy pattern	Zig-zag pattern	Uniform pattern
Ecological habitus	1.00	0.01	1.00
Ground-living habitus			
Sandy	0.48	0.04	< 0.01
Rocky	< 0.01	0.02	< 0.01
Open	< 0.01	0.47	< 0.01
Forest	< 0.01	< 0.01	< 0.01
Water-related	< 0.01	0.48	< 0.01
<i>Climate:</i>			
Mean temperature	0.84	0.86	< 0.01
Annual precipitation	0.35	< 0.01	< 0.01

cryptic to Müllerian mimicry and thermoregulation, can have a different strength in different areas of the globe, depending on geography and climate. Broad-scale analyses are a powerful approach to identify overall patterns, still they need to be complemented by focused studies testing the functional effects of colorations, and the evolutionary forces at play on species. For instance, experimental tests can be used to verify the effectiveness of the different patterns for mimicry (Martínez-Freiría *et al.*, 2017), and image analyses can allow testing of relationships between colour quality, shape of dark ornamentation of the dorsal surface and environmental factors such as UV radiation. The integration of analyses at multiple scales can allow more accurate inference on colour evolution, helping to obtain robust generalizations that can lead us to better understand the complex determinants of the evolution of morphological, behavioural and ecological traits (Ficetola *et al.*, 2018; Kaliontzopoulou *et al.*, 2018).

ACKNOWLEDGMENTS

We thank two anonymous reviewers for constructive comments on previous drafts of the manuscript. We are grateful to Matteo di Nicola who provided the pictures of *Vipera aspis* and helped in the design of the image.

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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. Sources of data used in the study.