

# 1 Eco-geographical determinants of the evolution of ornamentation in vipers

2  
3 CRISTIAN PIZZIGALLI <sup>1</sup>, FEDERICO BANFI <sup>2</sup>, GENTILE FRANCESCO FICETOLA <sup>3,4</sup>,  
4 MATTIA FALASCHI <sup>3</sup>, MARCO MANGIACOTTI <sup>5</sup>, ROBERTO SACCHI <sup>5</sup>, MARCO A.L.  
5 ZUFFI <sup>6</sup>, STEFANO SCALI <sup>7\*</sup>

6  
7 Citation: Pizzigalli, C., Banfi, F., Ficetola, G.F., Falaschi, M., Mangiacotti, M., Sacchi, R., Zuffi,  
8 M.A.L., Scali, S., 2020. Eco-geographical determinants of the evolution of ornamentation in vipers.  
9 *Biological Journal of the Linnean Society* 130, 345–358.

10  
11 <sup>1</sup> *CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos da Universidade*  
12 *do Porto, Portugal*

13 <sup>2</sup> *Laboratory of Functional Morphology, Department of Biology, University of Antwerp,*  
14 *Universiteitsplein 1, 2610 Wilrijk, Belgium*

15 <sup>3</sup> *Dipartimento di Scienze e Politiche Ambientali, Università degli Studi di Milano, via Celoria, 26,*  
16 *20133 Milano, Italy*

17 <sup>4</sup> *Univ. Grenoble Alpes, CNRS, Univ. Savoie Mont Blanc, LECA, Laboratoire d'Ecologie Alpine, F-*  
18 *38000 Grenoble.*

19 <sup>5</sup> *Dipartimento di Scienze della Terra e dell'Ambiente, Università degli Studi di Pavia, via Ferrata*  
20 *1, 27100 Pavia, Italy*

21 <sup>6</sup> *Museo di Storia Naturale, Università di Pisa, via Roma 79, 56011 Calci (Pisa), Italy*

22 <sup>7</sup> *Museo di Storia Naturale, corso Venezia 55, 20121 Milano, Italy.*

23 \* Corresponding author. E-mail: stefano.scali@comune.milano.it

24  
25  
26 **Abstract.** Multiple hypotheses have been proposed to explain the variation of dorsal patterns  
27 observed in snakes, but no studies yet have tested them over broad taxonomic and geographical  
28 scales. Viperidae offer a powerful model group to test eco-evolutionary processes that lead to  
29 disruptive and cryptic ornaments. We developed a database reporting dorsal ornamentation,  
30 ecological habitus, habitat features and climatic parameters for 257 out of 341 recognized species.  
31 Three patterns of dorsal ornamentation were considered: “zig-zag”, “blotchy”, and “uniform”  
32 patterns. Phylogenetic comparative analyses were based on 11 mitochondrial and nuclear genes.  
33 Forty-eight species presented a zig-zag pattern type, 224 a blotchy type and 32 a uniform pattern  
34 type. Phylogenetic signal was significant for all the patterns. Character phylogenetic reconstruction  
35 analyses suggested an ancestral state for blotchy ornamentation, with multiple independent  
36 evolutions of the other patterns. The blotchy pattern was more frequent in terrestrial species living  
37 in warm climates and sandy habitats, supporting the hypothesis of a disruptive function. The zig-zag  
38 pattern evolved independently in several isolated taxa, particularly in species living in cold climates  
39 and in dense vegetation or water-related habitats, supporting the hypothesis of disruptive and  
40 aposematic functions. Uniform colouration was particularly frequent in arboreal species, supporting  
41 the hypothesis of a cryptic function.

42  
43 **Key-words.** Viperidae; dorsal ornamentation; phylogenetic-supported characterization; blotchy  
44 pattern; zig-zag pattern; uniform pattern; ecological correlates

## INTRODUCTION

46  
47 Colouration and patterns are among the most intriguing phenomena in biology, involving both  
48 plants and animals. Flowering plants, for instance, show a great variability of colouration, often  
49 associated to odours, flavours and attractive chemicals for pollinator insects (Lev-Yadun &  
50 Ne'eman, 2012; Erbar *et al.*, 2017). In animals, colouration can provide information about health  
51 status (Halliday *et al.*, 2014; Trigo & Mota, 2015; Sepp *et al.*, 2018) and reproductive condition  
52 (Pérez i de Lanuza & Font, 2007; Svensson *et al.*, 2008). Furthermore, mimetic colouration,  
53 camouflage and disruptive patterns reduce the detectability of both predators and preys (Morris &  
54 Reader, 2016; De Bona *et al.*, 2015), while conspicuous colouration alerts on toxicity or  
55 unpalatability (aposematism) (Kraemer *et al.*, 2015; Cuthill *et al.*, 2017). Many studies have  
56 evaluated correlations between ornamentations, colourations, ecological variables, and phylogeny  
57 across taxa of terrestrial vertebrates (Poulton, 1890; Cott, 1966; Waage, 1981; Endler, 1990; Krebs,  
58 1994). These studies highlighted that the evolution of colouration and patterns has been driven by  
59 the interplay between aposematism, crypsis, sexual selection, physiological constraints, and social  
60 selection (Cuthill *et al.*, 2017), confirming that colour patterns and colour polymorphism are  
61 associated with speciation dynamics (Arbuckle & Speed, 2015).

62         Vipers (family Viperidae) are an excellent model group to test evolutionary processes  
63 related to disruptive and cryptic patterns. Vipers are widespread and well known from a  
64 morphological, ecological, molecular, and phylogenetic point of view and multiple mechanisms  
65 have been suggested for the evolution of their colouration patterns. First, vipers are a clade of  
66 highly venomous snakes, thus recurrent highly detectable colours or patterns can be examples of  
67 aposematic colouration or of Müllerian mimicry (Wüster *et al.*, 2004; Valkonen *et al.*, 2011a-b;  
68 Santos *et al.*, 2014). Harmful species, however, do not necessarily show bright colours, in order to  
69 reduce the risk of being detected (Sherrat & Betty, 2003; Endler & Mappes, 2004). In fact, some  
70 patterns (*e.g.* the zig-zag dorsal pattern of many vipers) can be examples of Müllerian mimicry that  
71 allow vipers to be identified as dangerous, without increasing their detectability (Wüster *et al.*,  
72 2004; Valkonen *et al.*, 2011a-b). Second, ambush hunting and predation avoidance have been  
73 proposed as drivers of the evolution of cryptic colourations and disruptive patterns (Cott, 1966;  
74 Ruxton *et al.*, 2004; del Marmol *et al.*, 2016). Disruptive patterns can be achieved through  
75 colourations with complex (either regular or irregular) patterns. Such combinations decrease the  
76 detectability of an individual even if the colouration of the body does not perfectly match the  
77 environment (*e.g.* *Bitis nasicornis*, *B. gabonica*) (Stevens & Merilaita, 2009 a-b).

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

87         Vipers present a striking variability of dorsal ornamentations. Some species show a motif  
88 that is regularly repeated several times on the body surface (*e.g.* zig-zag or blotchy pattern) while  
89 others show a uniform colouration. In addition intraspecific variation exists, with some species  
90 exhibiting both zig-zag, blotchy, and uniform patterns (Fig. 1). The efficiency of the different

91 patterns is expected to vary according to the environmental circumstances. A uniform colouration,  
92 for instance, can be more cryptic in canopies with a homogeneous background colouration, while a  
93 disruptive pattern can favour camouflage in ecotones with a mottled background. Eco-geographical  
94 variables provide a broad-scale picture of the habitat variation and species activity conditions,  
95 which in turn, can affect the relative efficiency of different pattern types. However, no analyses  
96 have yet tested the relationships between colour patterns of snakes and eco-geographical variables  
97 over broad taxonomic and geographical scales. In this study, we performed an extensive  
98 bibliographical research to collect distributional, ecological, behavioural, and bioclimatic  
99 information for most of the recognised viper species. We then used exhaustive phylogenetic data  
100 (Alencar *et al.*, 2016) to reconstruct the evolution of different dorsal patterns and to identify the  
101 eco-geographical factors related to the occurrence of dorsal patterns in vipers.  
102

103

## MATERIALS AND METHODS

104 *Data collection.* Data were acquired from both literature and online sources (see Appendix) and  
105 then used to create a database with morphometric, ecological and zoogeographical information  
106 representing all the 341 recognised species of vipers (Uetz & Hošek, 2017). For each taxon, we  
107 collected the following variables.  
108

109

110 *Dorsal patterns.* Dorsal patterns were classified in three main categories: *zig-zag*, *blotchy*, and  
111 *uniform* (Fig. 1). “*Zig-zag*” is a mostly continuous linear motif characterised by a sequence of small  
112 corners, roughly rounded, with variable inclinations. The “*blotchy*” category included species with  
113 regularly repeated motives such as bars, blotches, circles, ovals, and transversal stripes. Lastly,  
114 “*uniform*” indicates patterns that do not show regular motifs. In several cases, one single species  
115 can show multiple dorsal patterns (see results). For instance, *Vipera aspis* displays a high variability  
116 of patterns among subspecies, and the three patterns are present in this species (Fig. 1; Zuffi &  
117 Bonnet, 1999). Therefore, for each species, we recorded the presence / absence of the three distinct  
118 patterns, where every species can have more than one character state. Melanistic individuals were  
119 not considered; due to possible motif changes during species’ ontogenesis (da Silva *et al.*, 2017), we  
120 only considered the features of adults.

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

126 *Climatic variables.* For each species, we calculated average values of mean annual temperature and  
127 total precipitation. Climatic parameters were calculated as the average value through the whole  
128 species range. Ranges were obtained from Roll *et al.* (2017); climatic values were obtained from the  
129 CRU TS v4.01 (updated from Harris *et al.*, 2014). Because no distribution map was available for  
130 *Crotalus ornatus*, we used the centroid of the range as described in the Reptile Database to extract  
131 climatic values (Uetz & Hošek, 2017). The correlation between variables was generally weak (for  
132 all pairwise correlations,  $|r| \leq 0.6$ ), suggesting that collinearity between independent variables did  
133 not bias the results of regression analyses (Dormann *et al.*, 2013). Although the average conditions  
134 across the range may not represent the full conditions experienced by the species, they provide  
135 excellent information on the ecogeographical factors driving the evolution of species, when the  
136 climate of exact localities is not available (*e.g.* Stark & Meiri, 2018).

136

137 *Data analysis.* For phylogenetic comparative analyses, we used the calibrated tree based on 11  
138 mitochondrial and nuclear genes by Alencar *et al.* (2016). The time-tree included all the taxa for  
139 which we obtained pattern and ecological variables, and was pruned to match the list of species  
140 with available data. We used stochastic reconstruction of character states in order to assess the  
141 evolution of dorsal patterns along the phylogeny. Stochastic character mapping is a technique where  
142 possible histories of characters are sampled in proportion to their probability. Starting from the  
143 topology of the Alencar *et al.* (2016) time-tree, we generated 1,000 random simulations of a  
144 stochastic process of the character state, across the branches of the tree. The posterior probability of  
145 stochastically mapped characters was plotted on the phylogeny, to provide a character state  
146 reconstruction via stochasticity mapping (Revell, 2013). For all the resulting trees, we showed  
147 character state probabilities on both nodes and along branches. In large phylogenetic trees, the rate  
148 of trait evolution can differ significantly among lineages (Beaulieu *et al.*, 2013). We therefore used  
149 the Beaulieu *et al.* (2013) approach to compare a time-homogeneous model of trait evolution, with  
150 models assuming two or more hidden rates. For the three considered traits, the time-homogeneous  
151 model always showed lower Akaike's Information Criterion corrected for limited sample size  
152 (AICc) than the models with hidden rates, therefore we assumed homogeneous evolution across the  
153 tree. Stochastic reconstruction of character states was first performed for the three patterns  
154 separately (presence-absence of blotchy, zigzag and uniform patterns). Furthermore, we used a  
155 Markov model (i.e., a model of trait evolution and ancestral states reconstruction for discrete states)  
156 to analyse the three patterns in the same model. We used the `make.simmap` function in `phytools` to  
157 perform stochastic mapping analysis (1,000 replicates; Revell, 2013). For polymorphic species, we  
158 assumed that the multiple states of the pattern have the same prior probability. We then  
159 reconstructed state evolution through the `describe.simmap` in `phytools` (Revell, 2013).

160 We used the  $D$  statistics (Fritz *et al.*, 2010) to measure the phylogenetic signal of dorsal  
161 patterns. The  $D$  statistics is appropriate to measure phylogenetic signal for discrete traits.  $D = 1$   
162 indicates no phylogenetic signal, while  $D$  values close to zero or lower suggest very strong signal  
163 (Fritz *et al.*, 2010). We used 5,000 random permutations to assess whether  $D$  is significantly  
164 different from the values expected under no phylogenetic structure.

165 Subsequently, we used phylogenetic logistic regression to identify the eco-geographical  
166 parameters related to the evolution of dorsal patterns (Ives & Garland Jr., 2009) using the Alencar *et al.*  
167 *et al.* (2016) tree to consider the evolutionary history. We used a model-selection approach, based on  
168 Akaike's Information Criterion corrected for limited sample size (AICc) to identify the combination  
169 of variables best explaining the occurrence of the three dorsal patterns. First, we built regression  
170 models including all the possible combinations of the considered variables and calculated the AICc  
171 of each model. AICc trades-off explanatory power vs. number of predictors; models explaining  
172 more variation with a limited number of variables have the lowest AICc values and are assumed to  
173 be the "best models" (Symonds & Moussalli, 2011). We then calculated Akaike's weight ( $w$ ) of  
174 each model, which infers the likelihood that a model is the best one given a set of candidate models  
175 (Burnham & Anderson, 2002; Symonds & Moussalli, 2011). We also tested the possibility of non-  
176 linear relationships, assessing quadratic terms of continuous variables included in highly supported  
177 models. Finally, we calculated the sum of weight of each variable, as the sum of the Akaike's  
178 weight where each variable appears. The sum of weights is a measure of the relative importance of  
179 variables and can be used when model selection reveals uncertainty in the identification of best  
180 model(s). The significance of variables within the best-AICc models was assessed using likelihood

181 ratio tests. Analyses were performed using the packages ape (Paradis *et al.*, 2004), corHMM  
182 (Beaulieu *et al.*, 2013), phytools (Revell, 2012), maps (Becker & Wilks, 1993), raster (Hijmas &  
183 van Etten, 2012), ggplot2 (Wickham, 2009), caper (Orme, 2013), MuMIn (Bartoń, 2015), and  
184 phylolm (Ho & Ane, 2014) in R 3.3 (R Core Team, 2017).

185

186

## RESULTS

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

197 The zig-zag pattern was present in 48 species. The character mainly occurred in the  
198 *Montivipera-Macrovipera-Daboia-Vipera* clade, in the *Mixcoatlus-Ophriacus* clade and in both the  
199 *Atheris* and *Cerrophidion* genera with a few exceptions (*Vipera transcaucasiana*, *Daboia deserti*,  
200 *D. siamensis*, *Macrovipera schweizeri*, *Montivipera latifi*, *Mo. bornmuelleri*, *Mo. albizona*, *Atheris*  
201 *squamigera* and *Ath. chlorechis*). According to the character state reconstruction analysis, the trait  
202 evolved independently also in several isolated taxa throughout both Viperinae and Crotalinae sub-  
203 families: *Pseudocerastes fieldi*, *Cerastes vipera*, *Echis pyramidum*, *Bothrops pictus*, *Atropoides*  
204 *occitanus*, *Atr. nummifer*, *Trimeresurus gracilis*, *Protobothrops elegans*, *P. sieversorum*, and *P.*  
205 *kaulbaki* (Fig. 3).

206 The uniform pattern was present in 32 species. This pattern type mostly occurred in Asiatic  
207 taxa of the genus *Trimeresurus* (Fig. 4). It also occurred in *Macrovipera schweizeri*, *Atheris*  
208 *ceratophora*, *Ath. chlorechis*, *Causus resimus*, *Tropidolaemus subannulatus*, *Bothriechis guifarroi*,  
209 *Bothriechis lateralis*, *Bothriechis schlegelii*, and *Bothrops bilineatus*. In each of these species, the  
210 character evolved independently (Fig. 4).

211 The stochastic reconstruction of the three pattern states in the same model confirmed the  
212 blotchy pattern as the ancestral state, followed by multiple transitions (Fig. 5). The model suggested  
213 that the uniform pattern evolved from the blotchy pattern 6 times, while the zigzag pattern evolved  
214 from the blotchy pattern 5 times. Furthermore, several reversals occurred, particularly from the  
215 uniform to the blotchy pattern (14 transitions), while reversals from zigzag to blotchy were rare (2  
216 transitions; Fig. 5).

217

218

## ECO-GEOGRAPHICAL DETERMINANTS OF DORSAL PATTERNS

219

220

### BLOTCHY PATTERN

221 The best-AIC phylogenetic regression model suggested that the blotchy pattern was related to  
222 climate, species habitus and habitat (Table 1a). Blotches were particularly frequent in ground-living  
223 species ( $\chi^2_1 = 26.0$ ,  $P < 0.0001$ ) (Fig. 6d), in species living in sandy areas ( $\chi^2_1 = 11.5$ ,  $P = 0.0007$ )  
224 (Fig. 6e) and in warm climates ( $\chi^2_1 = 7.5$ ,  $P = 0.006$ ) (Fig. 6f). An alternative model, with very  
225 similar AICc value, included annual precipitation instead of sandy habitat and confirmed the high

226 frequency of this pattern in species living in arid areas ( $\chi^2_1 = 12.1, P = 0.0005$ ). The ground-living  
227 habitus and the association with warm climate were the variables with the highest relative  
228 importance (Table 2).

229

230

#### ZIG-ZAG PATTERN

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

237

238

#### UNIFORM PATTERN

239 According to the best AICc model, the uniform colouration was particularly frequent in arboreal  
240 species ( $\chi^2_1 = 28.3, P < 0.0001$ ) (Fig. 6g and Table 1c). None of the remaining variables was  
241 included in models with AICc weight  $> 0.01$ , indicating the arboreal habitus as the most important  
242 variable to explain the occurrence of this pattern (Table 2).

243

244

#### DISCUSSION

245 This study represents the first global scale characterisation of dorsal ornamentations for the family  
246 Viperidae. Our analyses clearly show that multiple factors can jointly determine the evolution of a  
247 certain pattern, with a major role of both climatic conditions and lifestyle. We observed a strong  
248 phylogenetic signal, with highly conserved basal character (blotchy) from which different patterns  
249 arose independently multiple times in different areas of the world. In most of the cases, the  
250 evolution of the new patterns corresponded to a loss of the ancestral state (*e.g.* the green concolour  
251 pattern in most of the *Trimeresurus* species and the zig-zag pattern in *Vipera*). However, there are  
252 also multiple cases of intraspecific variability (species in which a new pattern arose, but the blotchy  
253 remains, *e.g.* *V. aspis* see Zuffi & Bonnet, 1999). Such complex evolution of colour patterns along  
254 the phylogeny has been observed in multiple snake lineages. For instance, coral snakes (*Micrurus*),  
255 include distinct phylogenetic lineages that can be also distinguished by their dorsal patterns  
256 (Slowinski, 1995; Gutberlet & Harvey, 2004; Marques *et al.*, 2013; Jowers *et al.*, 2019): the  
257 monadal pattern (one black ring between two white or yellow annuli separated by red annuli), the  
258 triadal pattern (three black rings), and a bicolour colouration. Jowers *et al.* (2019) confirmed that  
259 there is a phylogeographic explanation behind the evolution of the two predominant colour patterns  
260 in these coral snakes, where from a basal triadal pattern the monadal form evolved in the Middle  
261 Miocene and more recently also with a bicolour colouration.

262

263

#### DRIVERS OF THE EVOLUTION OF BLOTCHY PATTERNS

264 The evolution of different patterns in vipers was strongly related to habitat and habitus (Fig. 6d).  
265 That is, each colouration is characteristic of species living in specific climatic conditions, habitats or  
266 lifestyle. The blotchy ornamentation appears to be frequent in ground-dwelling species living at  
267 low-latitudes in environments with warm and arid climates (Fig. 6e-f). Avoiding the detection by  
268 visual hunting predators is essential for ground-dwelling species. Previous studies already assumed  
269 the disruptive function of the blotchy pattern, which mimics the dark shadows of the litter-free sand  
270 beneath the vegetation (Sherbrooke, 2002). These properties of the blotchy pattern result to be a

271 particularly effective antipredator strategy of defence in ground-dwelling species (Brodie, 1992-  
272 1993) because allow them to confuse the outlines of their bodies with the substratum (e.g. *Bitis*  
273 *gabonica*), with shrubs and grass stems (e.g. *Vipera aspis*) or disguise by blending their dorsal  
274 pattern with the shadow created by bushes (Cott, 1966). Moreover, blotchy patterns create a “barber  
275 pole effect” when fleeing throughout the bushy and herbaceous vegetation (Jackson *et al.*, 1976;  
276 Lindell & Forsman, 1996). Shadows-like colouration has useful camouflage property in sandy  
277 habitats (Serventy, 1971). Our results clearly show that blotchy colouration patterns are more  
278 frequent in species inhabiting sandy and arid environments, where most of the species move  
279 between the patches of vegetation (seeking for shade in the warmest hour of the day) and the open  
280 areas to thermoregulate or hunt.

281

#### 282 DRIVERS OF THE EVOLUTION OF ZIG-ZAG PATTERNS

283 Our phylogenetic reconstruction suggests that zig-zag colouration pattern evolved multiple times  
284 (approx. 23 times) in vipers, mostly from ancestor with a blotchy pattern (Fig. 2 and Fig. 3). The  
285 zig-zag pattern presents a very strong phylogenetic signal and is more common in species living in  
286 cold climates (Fig. 6c). In these areas, more time is needed for thermoregulation and consequently  
287 snakes are more exposed to predation. The zig-zag pattern has been proposed to have a disruptive  
288 effect (from afar) but can also represent a case of Müllerian mimicry (Valkonen *et al.*, 2011b). The  
289 hypothesis of Müllerian mimicry is supported by the strong phylogenetic signal. The zig-zag pattern  
290 is particularly clustered within the phylogeny (Fig. 3), and closely related species which often live  
291 in nearby geographical regions (Warren *et al.*, 2014). For instance, all the species of the genus  
292 *Vipera* display this colour pattern and are mostly distributed in the same geographic region, Europe.  
293 This has probably allowed potential predators to learn from more than one species-model that  
294 animals showing zig-zag colouration patterns are most likely a danger. Further support to this  
295 hypothesis is the presence of several cases of Batesian mimicry from innocuous species emulating  
296 this colouration pattern, e.g. the Viperine Water Snake, *Natrix maura*, which is harmless and  
297 belongs to the *Colubridae* family (Santos *et al.*, 2017).

298

#### 299 DRIVERS OF THE EVOLUTION OF UNIFORM PATTERNS

300 Repeated evolutions have also been observed for the uniform dorsal pattern, which evolved from  
301 both blotchy and zig-zag patterns (Fig. 2 and Fig. 3). The uniform colouration has proved to be  
302 particularly frequent in Asian clades and specifically in species with arboreal habits (Fig. 6g) for  
303 hunting, thermoregulation, roosting or all of them. Accordingly, many species with uniform pattern  
304 exhibit a green colouration, which likely improves crypsis in canopy. Cases of uniform colouration  
305 (or concolour form) have also been reported in individuals within the genus *Vipera* (e.g. former *V.*  
306 *aspis atra*, *V. aspis aspis* and *V. berus bosniensis*), especially at high altitudes in rocky and open  
307 areas (Colombo & Di Nicola, 2012; Tessa, 2016; Nikolić & Simović, 2017). In this genus  
308 individuals with uniform pattern display a greyish colouration, which can have the same cryptic  
309 function of the green colouration in arboreal or semi-arboreal Asian Pit-vipers. However, our model  
310 does not completely explain the evolution of all the uniform colouration patterns. In some species,  
311 individuals often are uniformly dark (melanism), and multiple hypotheses have been proposed to  
312 explain these colourations, such as thermoregulation (Kettlewell, 1973; Kingsolver & Wiernasz,  
313 1991; Trullas *et al.*, 2007), crypticism (Kettlewell, 1973; Endler, 1984), aposematism (Turner,  
314 1977), protection from ultraviolet radiation (Gunn, 1998) and sexual selection (Wiernasz, 1989), but  
315 further investigation is needed to corroborate or confute these assumptions.

316

317

#### LIMITATIONS

318

319

320

321

322

323

324

325

326

327

328

329

330

331

332

333

334

335

336

337

338

339

340

341

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, but additional processes are 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 limited information on sexual dimorphism in patterns in vipers, still, this hypothesis requires future attention. Another process that we did not consider is the changes in colouration during the ontogenesis. Unfortunately, detailed information on the colouration of juveniles and sub-adults is only available for a subset of species. Nevertheless, the mortality of juveniles is not consistently higher than the one 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, but improving the completeness of information could allow analysing intraspecific variability. Finally, our analysis at 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 colourations is probably different. Future analyses could consider the fine-scale variation within the different patterns.

342

#### CONCLUSIONS

343

344

345

346

347

348

349

350

351

352

353

354

355

356

357

358

Our study revealed the complexity of factors determining the evolution of colour patterns in vipers, suggesting that multiple processes, ranging from 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 colourations, 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 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 evolution of morphological, behavioural and ecological traits (Ficetola *et al.*, 2018; Kaliontzopoulou *et al.*, 2018).

#### ACKNOWLEDGMENTS



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

REFERENCES

- 362
- 363 **Alencar LRV, Quental TB, Graziotin FG, Alfaro ML, Martins M, Venzon M, Zaher H. 2016.**  
 364 Diversification in vipers: Phylogenetic relationships, time of divergence and shifts in  
 365 speciation rates. *Molecular Phylogenetics and Evolution* **105**: 50-62.
- 366 **Allen WL, Baddeley R, Scott-Samuel NE, Cuthill IC. 2013.** The evolution and function of  
 367 pattern diversity in snakes. *Behavioral Ecology* **24**: 1237-1250.
- 368 **Arbuckle K, Speed MP. 2015.** Antipredator defenses predict diversification rates. *Proceedings of*  
 369 *National Academy of Sciences U.S.A.* **112**: 13597-13602.
- 370 **Barton K. 2015.** *MuMIn: Multi-model inference. R package version 1.15.1.* [http://CRAN.R-](http://CRAN.R-project.org/package=MuMIn)  
 371 [project.org/package=MuMIn](http://CRAN.R-project.org/package=MuMIn).
- 372 **Beaulieu JM, O'Meara BC, Donoghue MJ. 2013.** Identifying Hidden Rate Changes in the  
 373 Evolution of a Binary Morphological Character: The Evolution of Plant Habit in Campanulid  
 374 Angiosperms. *Systematic Biology* **62**: 725-737.
- 375 **Becker RA, Wilks AR. 1993.** Maps in S. *AT&T Bell Laboratories Statistics Research Report*  
 376 [93.2].
- 377 **Brodie ED III. 1992.** Correlational selection for color pattern and antipredator behavior in the  
 378 garter snake *Thamnophis ordinoides*. *Evolution* **46(5)**: 1284-1298.
- 379 **Brodie ED. III. 1993.** Consistency of individual differences in anti-predator behaviour and colour  
 380 pattern in the garter snake, *Thamnophis ordinoides*. *Animal Behaviour* **45(5)**: 851-861.
- 381 **Burnham KP, Anderson DR. 2002.** *Model selection and multimodel inference: a practical*  
 382 *information-theoretic approach*. New York: Springer Verlag.
- 383 **Clark RW. 2006.** Fixed videography to study predation behavior of an ambush foraging snake,  
 384 *Crotalus horridus*. *Copeia* **2006**: 181-187.
- 385 **Colombo M, Di Nicola M. 2012.** Quando la vipera non ha lo zigzag. *Piemonte Parchi* **221**: 26-27.
- 386 **Cott HB. 1966.** *Adaptive Coloration in Animals*. London: Methuen & Co. Ltd.
- 387 **Cuthill IC, Allen WL, Arbuckle K, Caspers B, Chaplin G, Hauber ME, Hill GE, Jablonski**  
 388 **NG, Jiggins CD, Kelber A, Mappes J, Marshall J, Merrill R, Osorio D, Prum R, Roberts**  
 389 **NW, Roulin A, Rowland HM, Sherratt TN, Skellhorn J, Speed MP, Stevens M, Stoddard**  
 390 **MC, Stuart-Fox D, Talas L, Tibbetts E, Caro T. 2017.** The biology of color. *Science* **357**:  
 391 eaan0221.
- 392 **da Silva FM, de Oliveira LS, de Souza Nascimento LR, Machado FA, da Costa Prudente AL.**  
 393 **2017.** Sexual dimorphism and ontogenetic changes of Amazonian pit vipers (*Bothrops atrox*).  
 394 *Zoologischer Anzeiger-A Journal of Comparative Zoology* **271**: 15-24.
- 395 **del Marmol GM, Mozaffari O, Gállego J. 2016.** *Pseudocerastes urarachnoides*: the ambush  
 396 specialist. *Boletín de la Asociación Herpetológica Española* **27**: 36-42.
- 397 **De Bona S, Valkonen JK, López-Sepulcre A, Mappes J. 2015.** Predator mimicry, not  
 398 conspicuousness, explains the efficacy of butterfly eyespots. *Proceedings of the Royal Society*  
 399 *B* **282**: 20150202.
- 400 **Dormann CF, Elith J, Bacher S, Buchmann C, Carl G, Carre G, Marquez JRG, Gruber B,**  
 401 **Lafourcade B, Leitao PJ, Munkemuller T, McClean C, Osborne PE, Reineking B,**  
 402 **Schroder B, Skidmore AK, Zurell D, Lautenbach S. 2013.** Collinearity: a review of  
 403 methods to deal with it and a simulation study evaluating their performance. *Ecography* **36**:  
 404 27-46.
- 405 **Endler J. 1984.** Progressive background matching in moths, and a quantitative measure of crypsis.

- 406 *Biological Journal of the Linnean Society* **22**: 187-231.
- 407 **Endler JA. 1990.** On the measurement and classification of colour in studies of animal colour  
408 patterns. *Biological Journal of the Linnean Society* **41**: 315-352.
- 409 **Endler JA, Mappes J. 2004.** Predator mixes and the conspicuousness of aposematic signals. *The*  
410 *American Naturalist* **163**: 532-547.
- 411 **Erbar C, Heiler A, Leins P. 2017.** Nectaries in fly-deceptive pitcher-trap blossoms of *Aristolochia*.  
412 *Flora* **232**: 128-141.
- 413 **Ficetola GF, Lunghi E, Canedoli C, Padoa-Schioppa E, Pennati R, Manenti R. 2018.**  
414 Differences between microhabitat and broad-scale patterns of niche evolution in terrestrial  
415 salamanders. *Scientific Reports* **8**: 10575.
- 416 **Fritz SA, Purvis A. 2010.** Selectivity in Mammalian Extinction Risk and Threat Types: a New  
417 Measure of Phylogenetic Signal Strength in Binary Traits. *Conservation Biology* **24**: 1042-  
418 1051.
- 419 **Gunn A. 1998.** The determination of larval phase coloration in the African armyworm, *Spodoptera*  
420 *exempta* and its consequences for thermoregulation and protection from UV light. *Entomologia*  
421 *experimentalis et applicata* **86(2)**: 125-133.
- 422 **Gutberlet RL, Harvey MB. 2004.** The evolution of New World venomous snakes. In: Campbell  
423 JA Lamar WW, eds. *The venomous reptiles of the Western Hemisphere*. Ithaca, NY: Cornell  
424 University Press: 634-682.
- 425 **Halliday WD, Paterson JE, Patterson LD, Cooke SJ, Blouin-Demers G. 2014.** Testosterone,  
426 body size, and sexual signals predict parasite load in Yarrow's spiny lizards (*Sceloporus*  
427 *jarrovi*). *Canadian Journal of Zoology* **92**: 1075-1082.
- 428 **Harris I, Jones PD, Osborn TJ, Lister DH. 2014.** Updated high-resolution grids of monthly  
429 climatic observations – the CRU TS3.10 Dataset. *International Journal of Climatology* **34**:  
430 623-642.
- 431 **Hijmans RJ, van Etten J. 2012.** Raster: Geographic analysis and modelling with raster data. R  
432 package version 2.0-12. Available from: <http://CRAN.R-project.org/package=raster>.
- 433 **Ho LST, Ane C. 2014.** A linear-time algorithm for Gaussian and non-Gaussian trait evolution  
434 models. *Systematic Biology* **63(3)**: 397-408.
- 435 **Ives AR, Garland T Jr. 2010.** Phylogenetic logistic regression for binary dependent variables.  
436 *Systematic Biology* **59(1)**: 9-26.
- 437 **Jackson JF, Ingram WT III, Campbell HW. 1976.** The dorsal pigmentation pattern of snakes as  
438 an antipredator strategy: a multivariate approach. *The American Naturalist* **110**: 1029-1053.
- 439 **Jowers MJ, Garcia Mudarra JL, Charles SP, Murphy JC. 2019.** Phylogeography of West  
440 Indies Coral snakes (*Micrurus*): Island colonisation and banding patterns. *Zoologica Scripta*  
441 **2019**: 1-14.
- 442 **Kaliontzopoulou A, Pinho C, Martínez-Freiría F. 2018.** Where does diversity come from?  
443 Linking geographical patterns of morphological, genetic, and environmental variation in wall  
444 lizards. *BMC evolutionary biology* **18(1)**: 124.
- 445 **Kettlewell HBD. 1973.** *The Evolution of Melanism: The Study of a Recurring Necessity, With*  
446 *Special Reference to Industrial Melanism in the Lepidoptera*. Oxford: Clarendon Press. [isEP]
- 447 **Kingsolver JG, Wiernasz DC. 1991.** Seasonal polyphenism in wing melanin pattern and  
448 thermoregulatory adaptation in *Pieris* butterflies. *The American Naturalist* **137(6)**: 816-830.
- 449 **Kraemer AC, Serb JM, Adams DC. 2015.** Batesian mimics influence the evolution of

450 conspicuousness in an aposematic salamander. *Journal of Evolutionary Biology* **28**: 1016-  
451 1023.

452 **Krebs CJ. 1994.** Ecology: the experimental analysis of distribution and abundance. *Evolution and*  
453 *ecology* **57**: 17-26.

454 **Lev-Yadun S, Ne'eman G. 2012.** Does bee or wasp mimicry by orchid flowers also deter  
455 herbivores? *Arthropod-Plant Interactions* **6**: 327-332.

456 **Lindell LE, Forsman A. 1996.** Sexual dichromatism in snakes: support for the flicker-fusion  
457 hypothesis. *Canadian Journal of Zoology* **74**: 2254-2256.

458 **Marques OAV, Pizzatto L, Santos SMA. 2013.** Reproductive strategies of New World Coral  
459 Snakes, Genus *Micrurus*. *Herpetologica* **69**: 58-66.

460 **Martínez-Freiría F, i de Lanuza GP, Pimenta AA, Pinto T, Santos X. 2017.** Aposematism and  
461 crypsis are not enough to explain dorsal polymorphism in the Iberian adder. *Acta*  
462 *oecologica* **85**: 165-173.

463 **Merilaita S. 2006.** Frequency-dependent predation and maintenance of prey polymorphism.  
464 *Journal of Evolutionary Biology* **19**: 2022-2030.

465 **Morris RL, Reader T. 2016.** Do crab spiders perceive Batesian mimicry in hoverflies?  
466 *Behavioural Ecology* **27**: 920-931.

467 **Münkemüller T, Lavergne S, Bzeznik B, Dray S, Jombart T, Schiffers K, Thuiller W. 2012.**  
468 How to measure and test phylogenetic signal. *Methods in Ecology and Evolution* **3**: 743-756.

469 **Nikolić S, Simović A. 2017.** First report on a trichromatic lowland *Vipera berus bosniensis*  
470 population in Serbia. *Herpetological Conservation and Biology* **12(2)**: 394-401.

471 **Orme D, Freckleton RP, Thomas GH, Petzoldt T, Fritz S, Isaac N, Pearse W. 2013.** *caper*:  
472 *Comparative Analyses of Phylogenetics and Evolution in R*. R package version 0.5.2.  
473 <http://CRAN.R-project.org/package=caper>.

474 **Pagel M. 1999.** Inferring the historical patterns of biological evolution. *Nature* **401(6756)**: 877.

475 **Paradis E, Claude J, Strimmer K. 2004.** APE: analyses of phylogenetics and evolution in R  
476 language. *Bioinformatics* **20**: 289-290.

477 **Pérez I de Lanuza G, Font E. 2007.** Ultraviolet reflectance of male nuptial colouration in sand  
478 lizards (*Lacerta agilis*) from the Pyrenees. *Amphibia-Reptilia* **28(3)**: 438-443.

479 **Pike DA, Pizzatto L, Pike BA, Shine R. 2008.** Estimating survival rates of uncatchable animals:  
480 the myth of high juvenile mortality in reptiles. *Ecology*, **89(3)**, 607-611.

481 **Poulton EB. 1890.** *The colours of animals: their meaning and use, especially considered in the*  
482 *case of insects*. New York: D. Appleton and Company.

483 **R Core Team. 2017.** *R: A language and environment for statistical computing*. Vienna: R  
484 Foundation for Statistical Computing.

485 **Revell LJ. 2012.** phytools: An R package for phylogenetic comparative biology (and other things).  
486 *Methods in Ecology and Evolution* **3**: 217-223.

487 **Revell LJ. 2013.** Two new graphical methods for mapping trait evolution on phylogenies. *Methods*  
488 *in Ecology and Evolution* **4**: 754-759.

489 **Roll U, Feldman A, Novosolov M, Allison A, Bauer AM, Bernard R, Böhm M, Castro-Herrera**  
490 **F, Chirio L, Collen B, Colli GR, Dabool L, Das I, Doan TM, Grismer LL, Hoogmoed M,**  
491 **Itescu Y, Kraus F, LeBreton M, Lewin A, Martins M, Maza E, Meirte D, Nagy ZT, de C.**  
492 **Nogueira C, Pauwels OSG, Pincheira-Donoso D, Powney GD, Sindaco R, Tallowin O,**  
493 **Torres-Carvajal O, Trape J-F, Enav Vidan E, Uetz P, Wagner P, Wang Y, Orme CDL,**  
494 **Grenyer R, Meiri S. 2017.** The global distribution of tetrapods reveals a need for targeted

495 reptile conservation. *Nature ecology and evolution* **1**: 1677-1682.

496 **Ruxton GD, Sherratt TN, Speed MP, Speed MP, Speed M. 2004.** *Avoiding attack: the*  
497 *evolutionary ecology of crypsis, warning signals and mimicry.* Oxford: Oxford University  
498 Press.

499 **Santos X, Vidal-García M, Brito JC, Fahd S, Llorente GA, Martínez-Freiría F, Sillero N.**  
500 **2014.** Phylogeographic and environmental correlates support the cryptic function of the zigzag  
501 pattern in a European viper. *Evolutionary ecology* **28**: 611-626.

502 **Santos X, Azor JS, Cortés S, Rodríguez E, Larios J, Pleguezuelos JM. 2017.** Ecological  
503 significance of dorsal polymorphism in a Batesian mimic snake. *Current zoology* **64(6)**: 745-  
504 753.

505 **Sepp T, McGraw KJ, Kaasik A, Giraudeau M. 2018.** A review of urban impacts on avian life-  
506 history evolution: Does city living lead to slower pace of life? *Global Change Biology* **24**:  
507 1452-1469.

508 **Serventy DL. 1971.** Biology of desert birds. In: Farner DS, King JR, Parkes KC, eds. *Avian*  
509 *Biology, Vol. 1.* New York: Academic Press: 287-339.

510 **Sherbrooke WC. 2002.** Do vertebral-line patterns in two horned lizards (*Phrynosoma spp.*) mimic  
511 plant-stem shadows and stem litter? *Journal of Arid Environments* **50(1)**: 109-120.

512 **Sherratt TN, Beatty CD. 2003.** The evolution of warning signals as reliable indicators of prey  
513 defence. *American Naturalist* **162**: 377-389.

514 **Slowinski JB. 1995.** A phylogenetic analysis of the new world coral snakes (Elapidae:  
515 *Leptomicrurus*, *Micruroides* and *Micrurus*) based on the allozymic and morphological  
516 characters. *Journal of Herpetology* **29**: 325-338.

517 **Stark G, Meiri S. 2018.** Cold and dark captivity: Drivers of amphibian longevity. *Global Ecology*  
518 *and Biogeography* **27**: 1384-1397.

519 **Stevens M, Merilaita S. 2009a.** Animal camouflage: Current issues and new perspectives.  
520 *Philosophical Transactions of the Royal Society B: Biological Sciences* **364**: 423-427.

521 **Stevens M, Merilaita S. 2009b.** Defining disruptive coloration and distinguishing its functions.  
522 *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **364**: 481-  
523 488.

524 **Svensson PA, Pélabon C, Blount JD, Forsgren E, Bjerkeng B, Amundsen T. 2008.** Temporal  
525 variability in a multicomponent trait: nuptial coloration of female two-spotted gobies.  
526 *Behavioral Ecology* **20**: 346-353.

527 **Symonds MRE, Moussalli A. 2011.** A brief guide to model selection, multimodel inference and  
528 model averaging in behavioural ecology using Akaike's information criterion. *Behavioral*  
529 *Ecology and Sociobiology* **65**: 13-21.

530 **Tessa G. 2016.** Preliminary data on distribution of a rare dorsal pattern in *Vipera aspis aspis*  
531 (Ophidia: Viperidae) in the Gran Paradiso National Park. In: Menegon M., Rodriguez-Prieto  
532 A, Deflorian MC, eds. *Atti XI Congresso Nazionale della Societas Herpetologica Italica,*  
533 *Trento 22-25 settembre 2016.* Trento: Ianieri Edizioni, 325-328.

534 **Trigo S, Mota PG. 2015.** What is the value of a yellow patch? Assessing the signalling role of  
535 yellow colouration in the European serin. *Behavioral Ecology and Sociobiology* **69**: 481-490.

536 **Trullas SC, van Wyk JH, Spotila JR. 2007.** Thermal melanism in ectotherms. *Journal of Thermal*  
537 *Biology* **32(5)**: 235-245.

538 **Turner JRG. 1977.** Butterfly mimicry: the genetical evolution of an adaptation. *Evolutionary*  
539 *Biology* **10**: 163-206.

540 **Uetz P, Hošek J. 2017.** The reptile database. Available from: <http://reptile-database.org/>.

541 **Valkonen JK, Nokelainen O, Mappes J. 2011a.** Antipredatory function of head shape for vipers  
542 and their mimics. *PLoS ONE* **6(7)**: e22272.

543 **Valkonen JK, Niskanen M, Björklund M, Mappes J. 2011b.** Disruption or aposematism?  
544 Significance of dorsal zigzag pattern of European vipers. *Evolutionary Ecology* **25**: 1047-  
545 1063.

546 **Waage J. 1981.** How the zebra got its stripes-biting flies as selective agents in the evolution of  
547 zebra coloration. *Journal of the Entomological Society of Southern Africa* **44**: 351–358.

548 **Warren DL, Cardillo M, Rosauer DF, Bolnick DI. 2014.** Mistaking geography for biology:  
549 inferring processes from species distributions. *Trends in Ecology & Evolution* **29**: 572-580.

550 **Wickham H. 2009.** Elegant graphics for data analysis (ggplot2).

551 **Wiernasz DC. 1989.** Female choice and sexual selection of male wing melanin pattern in *Pieris*  
552 *occidentalis* (Lepidoptera). *Evolution* **43**: 1672-1682.

553 **Wüster W, Allum CSE, Bjargardóttir IB, Bailey KL, Dawson KJ, Guenioui J., Pollard CP.**  
554 **2004.** Do aposematism and Batesian mimicry require bright colours? A test, using European  
555 viper markings. *Proceedings of the Royal Society B: Biological Sciences* **271**: 2495–2499.

556 **Zuffi MAL, Bonnet X. 1999.** Italian subspecies of the asp viper, *Vipera aspis*: patterns of  
557 variability and distribution. *Italian Journal Zoology* **66**: 87-95.

558

559 **Table 1.** Candidate mixed models explaining variation in the occurrence of dorsal patterns among  
 560 vipers. Results of phylogenetic logistic regression models. Models are ranked on the basis of  
 561 corrected Akaike's information criterion (AICc); only models with Akaike's weight > 0.01 are  
 562 reported.  $\Delta$ AICc: AICc difference with the best model;  $w$ : Akaike's weight

563

564

Rank	Independent variables	AICc	$\Delta$ AICc	$w$
a) Dependent: occurrence of blotchy pattern				
1	Arboreal habitus (-), mean temperature (+), sandy habitat (+)	134.04	0.00	0.48
2	Arboreal habitus (-), mean temperature (+), precipitation (-)	134.67	0.63	0.35
3	Arboreal habitus (-), forest habitat (-)	137.09	3.06	0.11
4	Arboreal 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	Arboreal habitus (+)	140.79	0	>0.99

565

566

567

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

571

Independent variables	Dependent variables:		
	Blotchy pattern	Zig-zag pattern	Uniform pattern
Arboreal habitus	<b>1.00</b>	0.01	<b>1.00</b>
Habitat:			
Sandy	0.48	0.04	<0.01
Rocks	<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
Climate:			
Mean temperature	<b>0.84</b>	<b>0.86</b>	<0.01
Annual precipitation	0.35	<0.01	<0.01

572

573

574

575

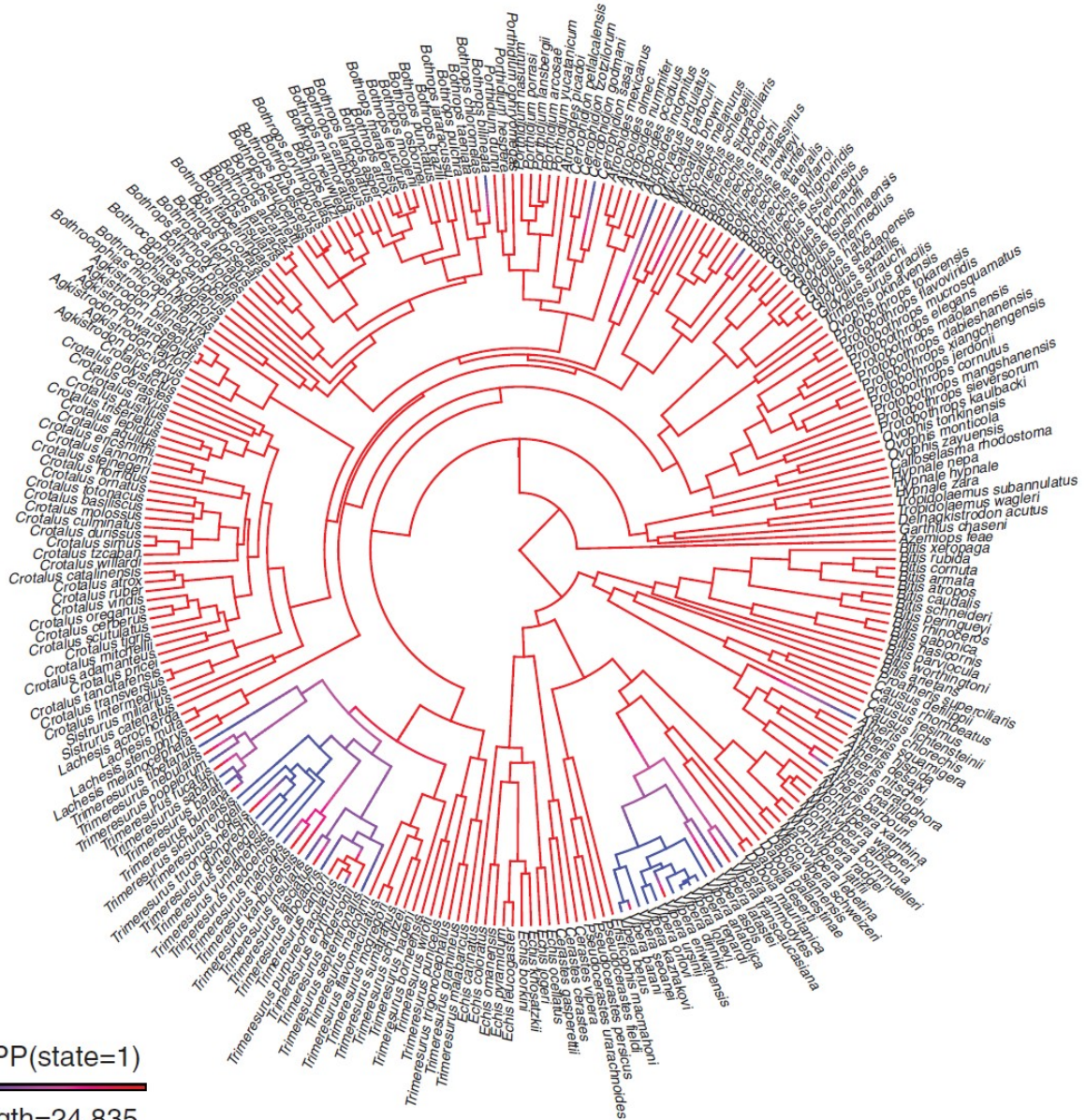
576





577

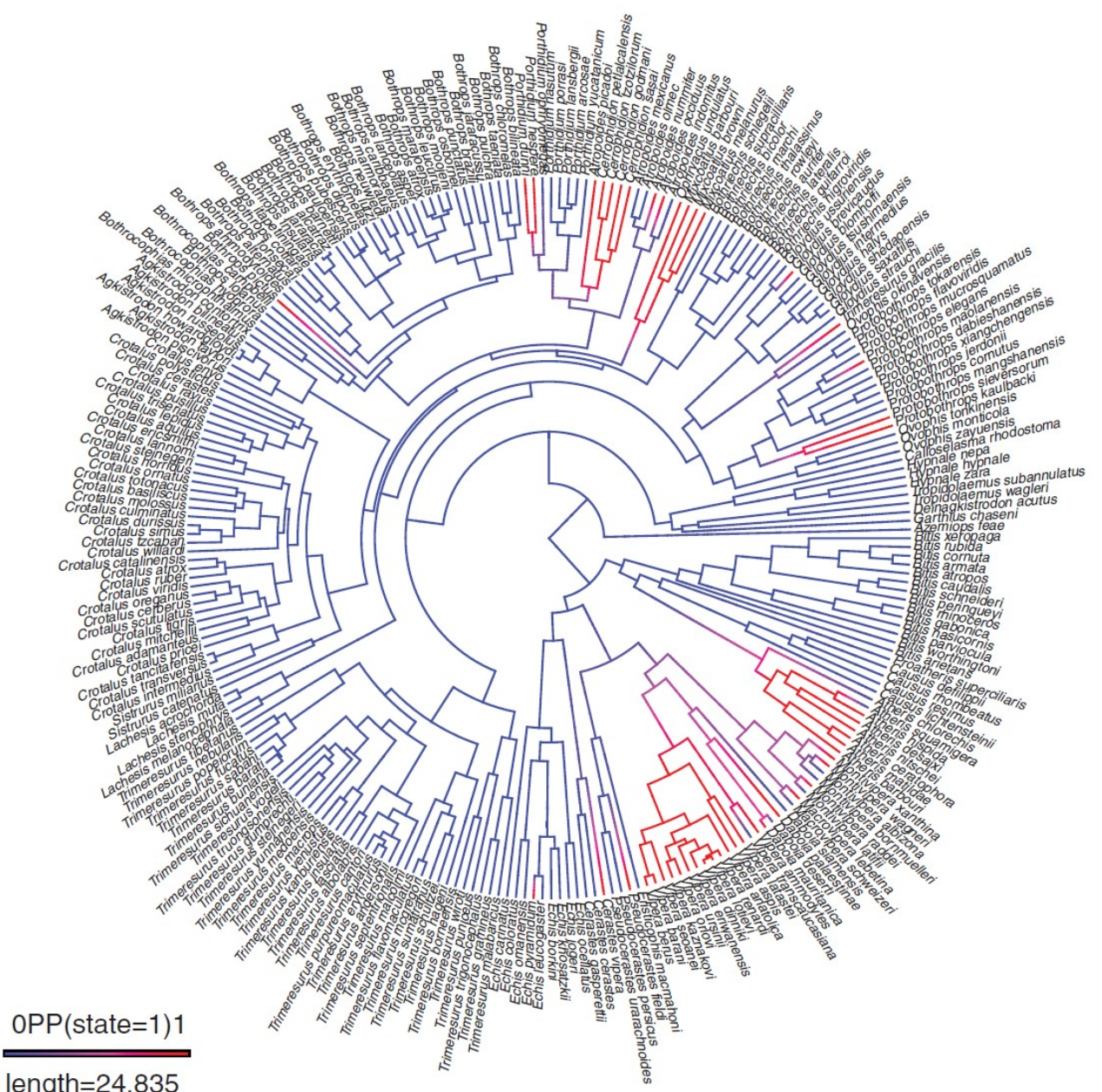
578 **Figure 1.** Example of intraspecific polymorphism in *V. aspis*, on the top a concolor individual  
579 displaying A) uniform coloration, in the middle an example of B) blotchy pattern (bars in this case)  
580 and on the bottom a C) zig-zag pattern-like coloration. Credit to Matteo Di Nicola  
581 (<http://www.matteodinicola.it/>).  
582



583  
584

585 **Figure 2.** Ancestral character state reconstruction of the blotchy pattern along the branches of the  
586 phylogenetic tree of Viperidae. Red indicates a high posterior probability of the occurrence of the  
587 blotchy pattern within a clade, blue indicates low probability of occurrence, and intermediate  
588 colours indicate uncertainty. Phylogeny is from Alencar *et al.* (2016).

589

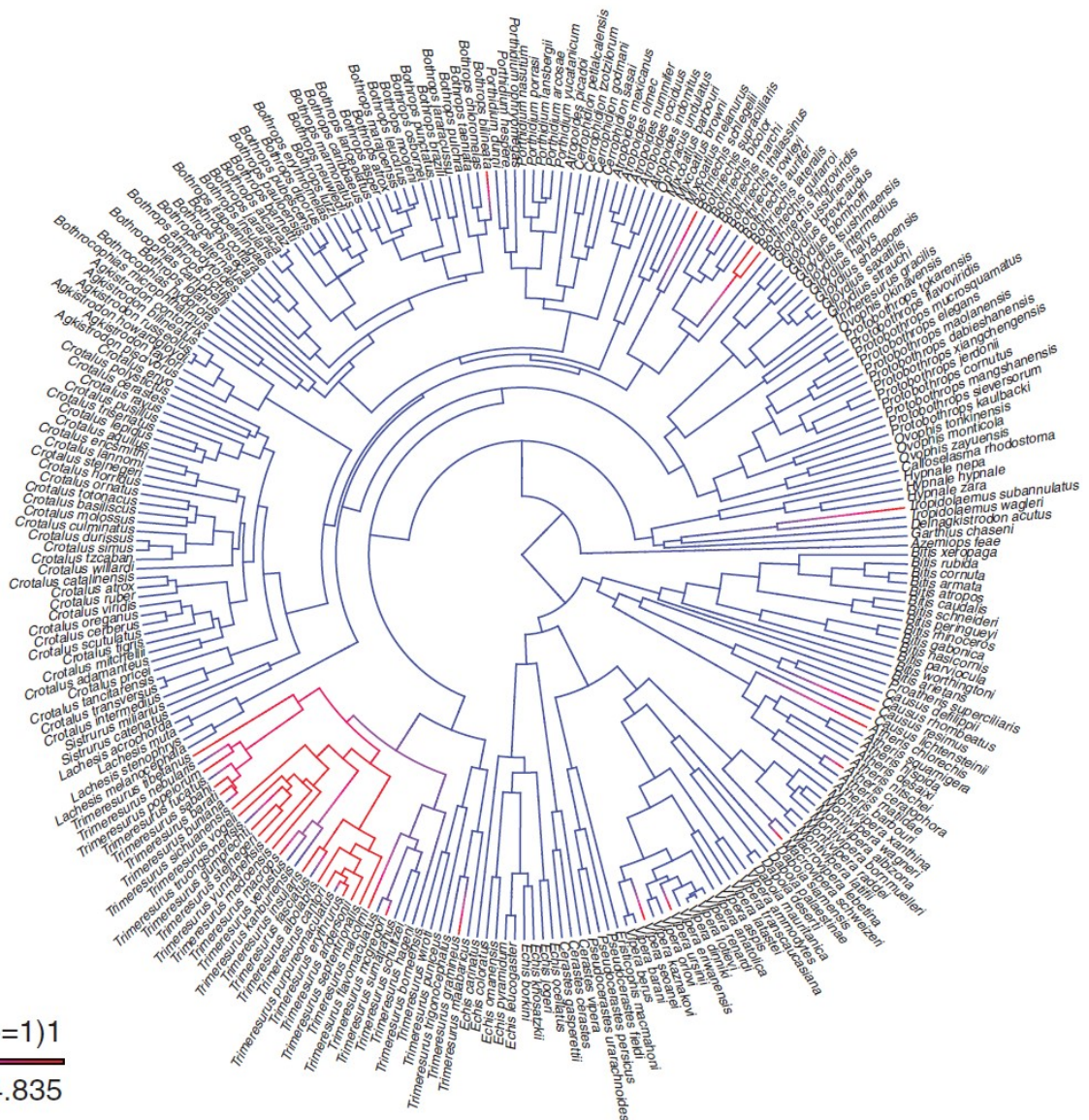


590

591

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

596

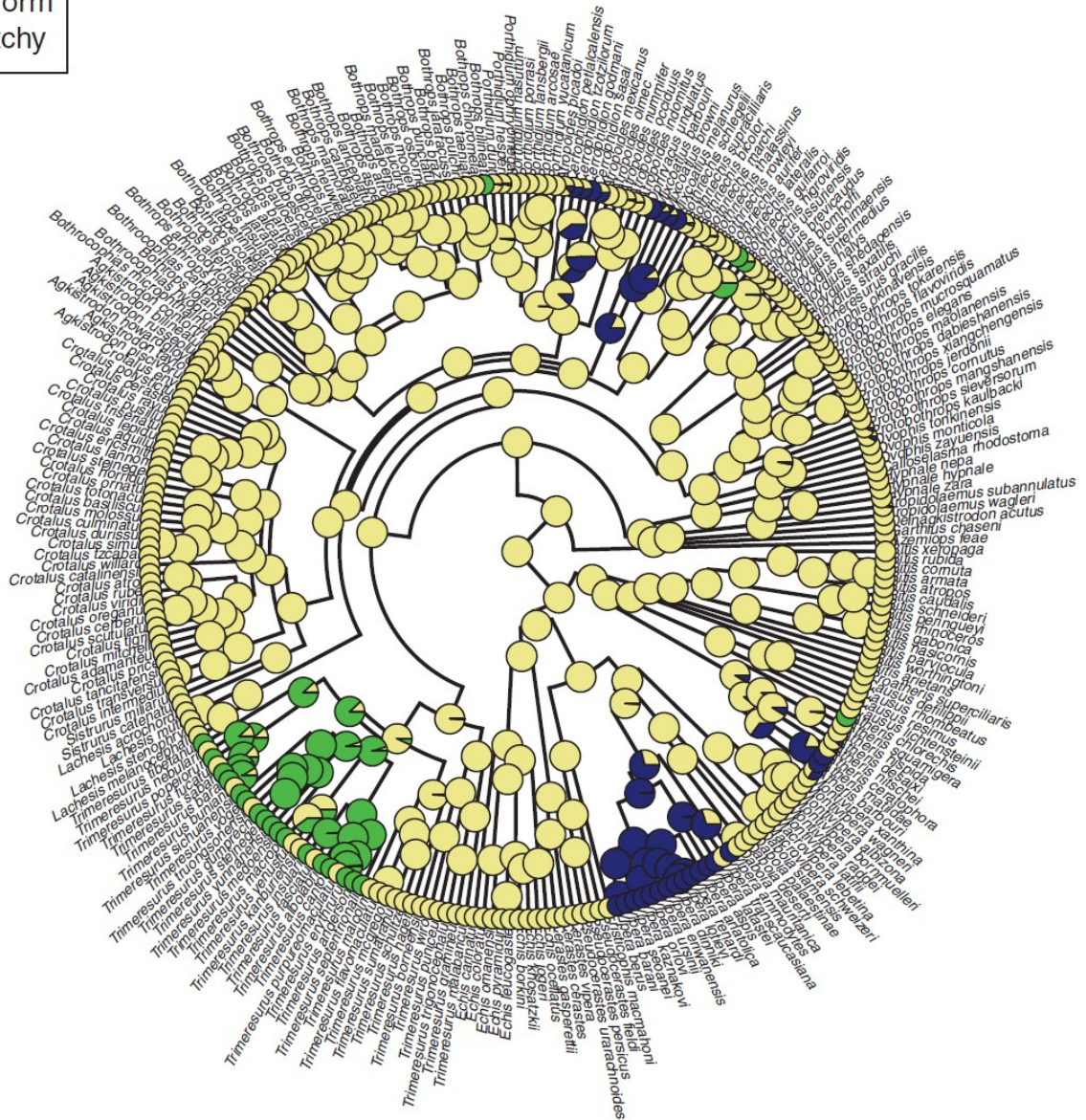


597

598

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

603

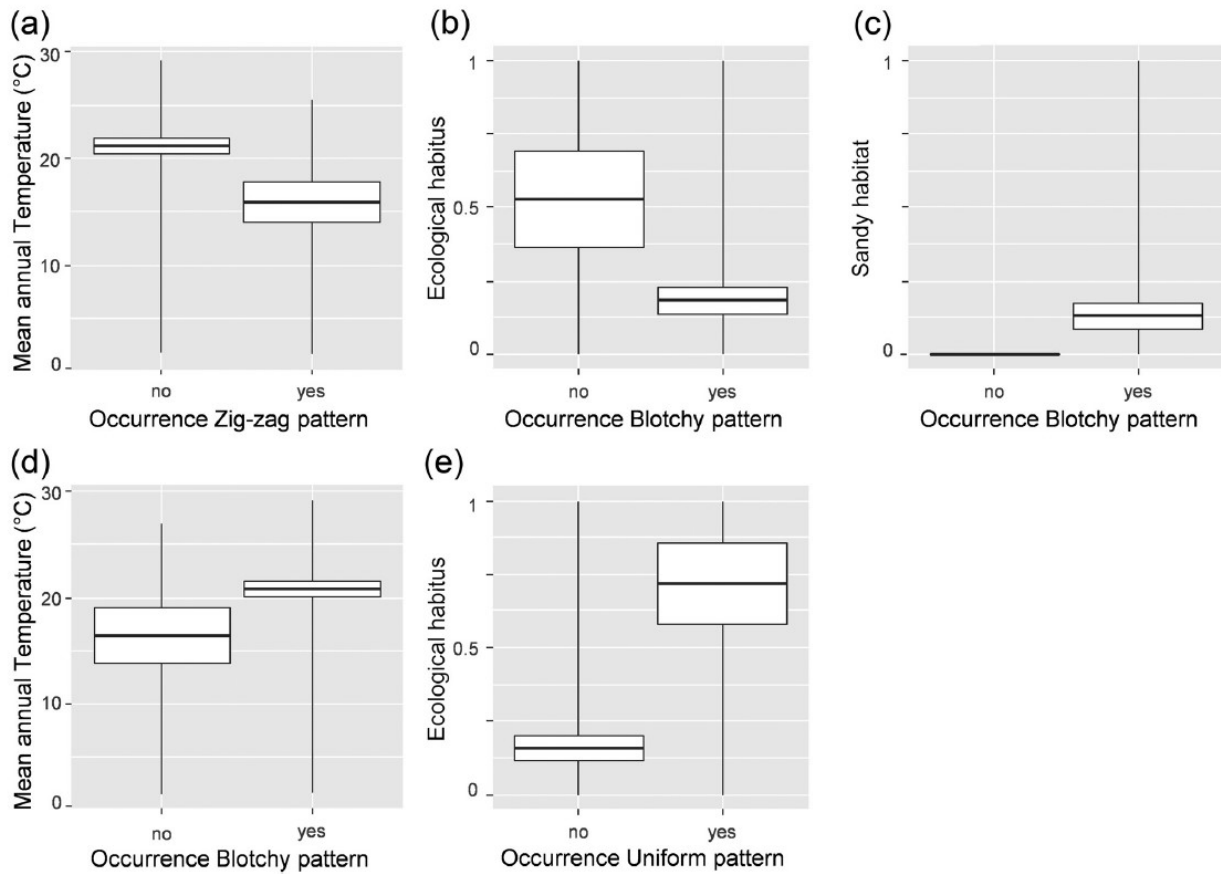


604

605

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

608



609

610

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

618