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Spot polymorphism in *Anguis colchica* Nordmann, 1840 (Reptilia: Anguidae): inter-size class variation

Tibor SOS

Milvus Group, Bird and Nature Protection Association,
Crinului Str. Nr. 22, 540343, Târgu-Mureş, Romania. E-mail: tibor.sos@gmail.com

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Abstract. High spot polymorphism and a high frequency of blue spotted morph is reported from an *Anguis colchica* population in Romania (Rupea, Braşov County). Although the blue-spotted morph is mostly exhibited in males, the morph was recorded in females also – feature characteristic to the eastern clades. The blue-spotted morph in females here seems to be size-related. The blue-spotted females are larger, thus older females. The appearance of the blue spots is considered connected to the maturation process in the lizard.

Key words: *Anguis colchica*, spot polymorphism, blue-spotted morph, size-related, Carpathian Corner, Romania.

The description of eastern subspecies, *Anguis fragilis colchica* Nordmann, 1840 (in Demidoff 1840) was based on the presence of the blue spotted morph. Other morphological characters (e.g. the head-shield pattern, the state of ear openings, the longitudinal series of scales at mid-body) completed this description (Štepánek 1937, Wermuth 1950, Sos 2010). The presence of blue-spotted morph was later confirmed also in the nominat form, *A. f. fragilis* Linnaeus, 1758 (Wermuth 1950, Voipio 1962, Dely 1972). Recently the genetic analysis of the genus *Anguis* revealed a complex genetic structure (Gvoždík et al. 2010). Besides the *A. fragilis* and the newly recognized *A. colchica*, a third clade was identified and described as a new species under the name of *A. graeca* Bedriaga 1881. The frequency of the blue-spotted morphotype decreases from eastern to western parts of Europe, thus is more expressed in the eastern clades (Wermuth 1950, Voipio 1962, Dely 1972, 1974a,b). The relative frequency of the morphs remains essentially at the same high level throughout its distribution in the face of different climatic conditions at the opposite ends of its range (Voipio 1962). Earlier, the blue-spotted morph was considered sex-linked, appearing only in males (Dely 1981, Grillitsh & Cabela 1990). Although in the eastern clades the blue spotted morph was recorded in both sexes (Wermuth 1950, Voipio 1962, Grillitsh & Cabela 1990). In females the morph percentage was always smaller than in males (see for comparisons in Sos 2010).

During ecological studies made on *A. f. colchica* during 2001-2004 in Rupea (Braşov County, in the region of Carpathian Corner, in Romania), I collected morphological data also (Sos & Herczeg 2009, Sos, in prep.). The high spot polymorphism, thus the high blue-spotted morph frequency in males and in females got my attention. The study area is a cemetery of about 0.8 ha in the limit of Rupea (N 46°05'11.5'', E 024°59'15.3, average 503 m a.s.l.). *A. colchica* (=*A. c. incerta* Krynicki, 1837; according to Gvoždík et al. 2010) inhabit the mostly undisturbed parts of this cemetery, and are usually found in or near to the bushy patches, using coverboards or other natural covers. To avoid pseudo-replications, the head markings and other characters useful to individual recognition (body and head measurements, tail condition, color, scarring) were noted, as other marking techniques have failed in marking the species for long periods (see reviewed in Riddell 1996). Sex was determined by the presence or absence of the hemipenis (as one defense reaction of *A. fragilis* is the rejection of hemi-penis) and by using the secondary sexual coloration or other clues (e.g. gravid states of females; e.g. Dely 1981). Datas were available only for specimens above 14 cm, thus all the measurements belong to adults, as the subadults are considered to be those less than 12 cm (e.g. Smith 1990). My sample included 72 males and 119 females.

The blue-spotted morph appears in 79%, and respectively, 26% in the studied males and females (Figs 1-3). In males the percentage is close to the percentage reported in literature for the area of *A. colchica*, while in females the percentage is higher (Table 1).

In both sexes, blue-, brown-, and white-spotted specimens were encountered. Many specimens were spotted with two or even three different colored spots and sexes differ in freq-

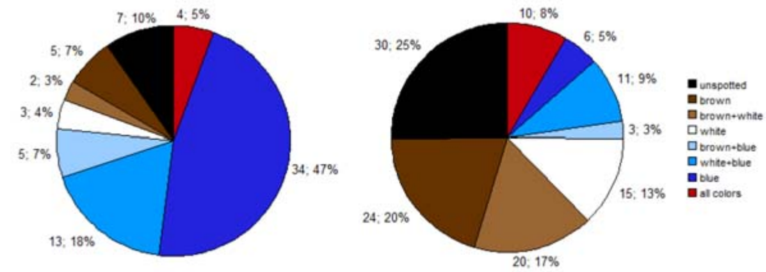

Figure 1. Distribution of different color spotted specimens in males (left) and females (right).

Figure 2. The blue-spotted morph in two females of *Anguis colchica* from Rupea (Rupea (Braşov County, Romania).

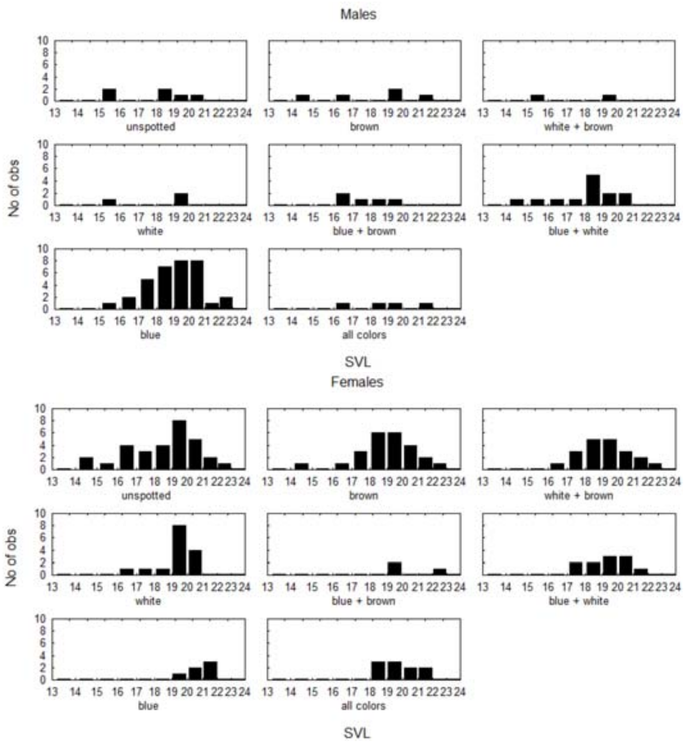

Figure 3. The distribution of different color spotted morphs in male and female size-classes (cm).

Table 1. The presence of blue-spotted specimens in *Anguis fragilis* complex (N = sample size, + = number and % = percentage of specimens with blue spots, ? - only the percentage is presented in the study). In Sos (2010) data of adults is counted from 12 cm SVL.

Zone/Country	all		male		female		Source
	N	+/%	N	+/%	N	+/%	
Nederland	138	22/15.9	?	22/41.5	?	0	Musters & In den Bosch, 1982
Sweden	101	0	?	0	?	0	Voipio 1962
W Hungary	28	4/14.2	6	4/6.6	?	0	Dely 1972
Rupea (Romania)	201	89/44.2	78	57/73.0	123	32/26.0	Sos 2010
E Hungary	25	18/90.0	15	15/100	10	3/30.0	Dely 1972
Slovakia	101	?	?	?/80.3	?	?/2.0	Lác 1967
Finland	61	20/32.8	29	19/65.5	32	1/3.1	Voipio 1962

Table 2. Descriptive statistics of morphometric characters used in this study. Valid cases (N), means (x), standard deviations (S.D.) and minimum – maximum ranges (min – max) are shown. For abbreviations see text.

Character	N	x ± S.D.	Min-Max
males	72	18.64 ± 1.82	14.70-22.80
SVL unspotted + brown	11	18.10 ± 2.17	14.90-21.30
SVL white	4	17.57 ± 2.47	15.10-19.70
SVL blue	57	18.81± 1.69	14.70-22.80
females	119	19.31 ± 1.63	14.40-22.70
unspotted + brown			
SVL	54	18.90 ± 1.88	14.40-22.70
L.pil.	46	1.34 ± 0.59	1.22-1.50
Lt.pil.	49	0.63 ± 0.04	0.48-0.72
Alt.par.	51	0.43 ± 0.03	0.33-0.51
white			
SVL	34	19.36 ± 1.34	16.70-22.10
L.pil.	31	1.34 ± 0.06	1.24-1.48
Lt.pil.	32	0.62 ± 0.04	0.50-0.69
Alt.par.	32	0.42 ± 0.03	0.34-0.50
blue			
SVL	31	20.00 ± 1.18	17.60-22.30
L.pil.	21	1.33 ± 0.06	1.25-1.49
Lt.pil.	30	0.63 ± 0.56	0.56-0.72
Alt.par.	30	0.42 ± 0.03	0.36-0.47

uency of different color of spots (Figs 1-3). The blue-spotting is considered more frequent in larger and older specimens (e.g. Capula et al. 1997 – but see Musters and Bosch 1982 which report a size-free appearance of blue spotted individuals, although based on small sample size). On the basis of my observations, the young males display whitish or lighter blue spots. I consider that the blue coloration develops implicitly after a "whitish" phase of spots. To test the hypothesis that the "unspotted-whitish-blue" spot phases are correlated with age, thus with sizes, I analyzed the data of the female group. The high frequency of blue-spotted morph and the unequal distribution of different color spotted groups in males made their dataset useless (Table 2). In the female dataset each former group with blue spotted specimens was pooled in the blue-spotted group. The remaining groups were separated in two groups: one with white-spotted specimens and another with unspotted and the brown-spotted specimens (Table 2). Beside the SVL, snout-vent length (measured from the tip of the snout to the vent) the next three head measures were also considered: L.pil., pileus length (from the tip of the snout to the dorsal edge of the occipital scale), Lt.pil., pileus width (between the farthest edge of the parietale scales) and Alt.par., parietale height (from the edge of mouth to the top of the head; table 2). I compared the groups using a factorial ANOVA and the Tukey HSD post-hoc test. Formerly each dataset was checked for normality with the Shapiro-Wilk W test. ANCOVA was used with SVL as covariance to test for differences in the three head characters excluding the effect of SVL.

The female "color" groups exhibited significant difference in their SVL ($F_{2.116}$= 4.76, p = 0.01). Post-hoc comparation revealed that only the blue-spotted morph differed significantly from the first group of unspotted and the brown-spotted group (p = 0.007; Fig. 4, Table 2), while the other groups did not (unspotted and brown-spotted vs. white-spotted: p = 0,382, white-spotted vs. blue-spotted: p = 0.239). Although the groups show slightly different size-classes (Fig. 4). The ANCOVA and the post-hoc comparations did not reveal any differences in the three head morphological characters (L.pil.: $F_{2.94}$= 2.27, p = 0.10; Lt.pil: $F_{2.107}$= 0.96, p = 0.38; Alt.par: $F_{2.109}$= 2.50, p = 0.08). The uniformity of head size in females at different SVL has already been reported in the species (e.g. Sos & Herczeg 2010). At similar growth rates the body size of females have a positive allometry as regards head size. Females may grow larger because young females allocate more of their available

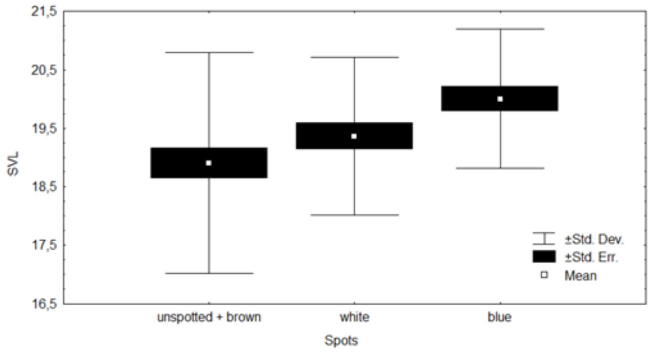

Figure 4. The differences between the mean of SVL between the three pooled groups in female *Anguis colchica*. For explanation see text.

energy to egg production (e.g. Anderson & Vitt 1990).

The occurrence of the blue spots in both sexes (but only rarely in females) shows that this character is not sex-limited (Wermuth 1950, Voipio 1962, Capula et al. 1997, present study). However, in the western populations the spotted morph is considered to occur in males exclusively (Dely 1981, Grillitsh & Cabela 1990). Thus sex probably exerts an influence upon its expression (Voipio 1962, Capula et al. 1998). In some lizards the blue color on the ventral body part is a sexually dimorphic trait that is more pronounced in males. Quinn and Hews (2003) described how elevated testosterone levels induce both dermal melanization and blue abdominal skin in Phrynosomatid lizards. Exogenous testosterone not only enhanced the blue color of male abdominal skin, but also produced male-like blue abdomens in females. The case seems to be similar in *A. fragilis*, as bluish abdomens could be found here (Schreiber 1875, Wermuth 1950, Dely 1981, Jablonski & Meduna, 2010), but only in males in the studied *A. colchica* population (Sos, in prep.).

I found that the appearance of spots with different colors in females is related to the SVL, which is correlated to the age. The SVL of females is growing from unspotted and brown-spotted exemplars through white-spotted specimens to the bluish ones. Thus the appearance of the blue-spotted morph could be considered connected to the process of maturation in *A. colchica* (Capula et al. 1998).

One of the hypotheses to explain the appearance of the blue spots is the pigment loss of skin surface of lizards. The result of pigment loss in scales could result in a skin surface which reflects the blue wavelengths with greater intensity owing to lack of any special cell components (i.e. pigments). However, this tendency must not be interpreted as a general feature. A similar observation in cases of injured scales was made by Wermuth (1950). In addition to the "sky-blue" spots, Wermuth described an opaque blue-spotting type, which was considered due to results of injuries of some scales, when the epidermis of these scales was injured deeply, thus a pathologic cause was hypothesized. This hypothesis could be a starting point in studies of the color morph, through biochemical and biophysical studies of *Anguis* skin structure.

The ecological significance of blue-spotted morph is still not understood. According to Voipio (1962) the blue spotting is unconnected with the mating period and consequently is not an epigamic character, while no correlations between intensity of color and season were found. However, according to Capula et al. (1998) the blue-spotted coloration in the males of an alpine *A. f. fragilis* population, when displayed, is especially bright during the mating season (Capula et al. 1997), suggesting that dorsal pattern could play a role in sexual selection or in sexual competition. The significance of the morph in females has never been investigated. The intra- and intersexual communication value of blue-spotted morph in and out of the breeding season could be an interesting matter for further studies.

The occurrence of blue spotted morph could be limited naturally, thus the frequency of blue-spotted morph in different populations and even in sex groups could change under the pressure of natural selection. A predation experiment with model lizards demonstrated that the blue-spotted individuals suffered higher risk of predation than normal colored ones in an alpine *A. f. fragilis* population (Capula et al. 1997). Comparing the data of surface occurrence of alpine specimens with my data from the Rupea's hilly population, a higher percentage of open occurrence could be observed in the alpine population, while in my research almost all specimens were found in or under different shelters (Sos, in prep.). Probably due to the suitable thermal environment under shelter from the hilly zones, they stay mostly covered. Thus the predation risk of blue spotted individuals could be much lower than in open space and it's frequency could be higher in both sexes.

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References

Anderson, R.A., Vitt, L.J. (1990): Sexual selection versus alternative causes of sexual dimorphism in teiid lizards. Oecologia 84: 145-157.

Bagnara, J.T., Fernandez, P.J., Fujii, R. (2007): On the blue colouration of vertebrates. Pigment Cell Research 20: 14-26.

Bagnara, J.T., Frost, S.K., Matsumoto, J. (1978): On the development of pigment patterns in amphibians. American Zoologist 18: 301-312.

Bagnara, T.T., Taylor, J.D., Hadley, M.E. (1968): The dermal chromatophore unit. Journal of Cell Biology 38: 67-69.

Baran, I. (1977): Türkize'de Anguidae familyasi türlerinin taksonomisi. Ege Üniversitesi su ürünleri Fakültesi Su ürünleri Dergisi, seri B, cilt I 2: 145-153.

Beškov, W. (1966): Untersuchungen über Systematik und Verbreitung der Blindschleiche (*Anguis fragilis*) in Bulgarien. Bulletin de l'Institut de Zoologie et Musee Sofia 21: 185-201.

Capula, M., Anibaldi, C., Filippi, E., Luiselli, L. (1998): Sexual combats, matings, and reproductive phenology in an alpine population of the slow-worm, *Anguis fragilis*. Herpetological Natural History 6(1): 33-39.

Capula, M., Luiselli, L., Capanna, E. (1997): The blue-spotted morph of the slow-worm, *Anguis fragilis*: colour polymorphism and predation risks. Italian Journal of Zoology 64: 147-153.

Dely, O.Gy. (1972): Adatok a kárpátmedencei törékenygyík (*Anguis fragilis* Linnaeus) rendszertanához és elterjedéséhez. Vertebrata Hungarica 13: 39-79.

Dely, O.Gy. (1974a): A törékeny gyík (*Anguis fragilis* Linnaeus) rendszertani és elterjedési problémái. Állattani Közlemények 61: 17-26.

Dely, O.Gy. (1974b): Über die Unterarten der Blindschleiche, *Anguis fragilis*. Vertebrata Hungarica 15: 11-37.

Dely, O.Gy. (1981): *Anguis fragilis* Linnaeus 1758 – Blindschleiche. pp. 241-258. In: Böhme, W. (ed.): Handbuch der Reptilien und Amphibien Europas, Band 1/3, Echsen (Sauria). Aula-Verlag, Wiesbaden.

Demidoff, A. de. Voyage (1840): Voyage dans la Russe Méridionalle and la Crimée. Paris, Ernest Bourdin et Cie, Paris, pp.1-621.

Džukić, G. (1987): Taxonomical and biogeographical characteristics of the slow-worm (*Anguis fragilis*) in Jugoslavia and on the Balkan Peninsula. Scopolia 12: 1-47.

Grillitsch, H., Cabela, A. (1990): Zum systematischen Status der Blindschleichen der Peloponnes und der südlichen Ionischen Inseln (Griechenland). Herpetozoa 2(3/4): 131-153.

Gvoždík, V., Jandzik, D., Lymberakis, P., Jablonski, D., Moravec, J. (2010): Slow worm, *Anguis fragilis* (Reptilia: Anguidae) as a species complex: Genetic structure reveals deep divergences. Molecular Phylogenetics and Evolution 55(2): 460-72.

Jablonski, D., Meduna, P. (2010): Blue colour of the ventral body part of Eastern Slow Worm *Anguis colchica* (Nordmann, 1840). Herpetology Notes 3: 295-296.

Lác, J. (1967): K szstematike slepúcha lámavého (*Anguis fragilis* L.) a jeho rozsirenie na slovensku. Biológia (Bratislava) 22(12): 908-921.

Muster, C.J.M., In den Bosch, H.A.J. (1982): Einige Bemerkungen zu den Unterarten von *Anguis fragilis* L., mit berucksichtigung niederlandisher exemplare (Reptilia: Sauria: Anguidae). Salamandra 18: 196-204.

Riddell, A. (1996): Monitoring slow-worms and common lizards, with special reference to refugia occupancy and individual identification. pp. 46-60. In: Foster, J., Gent, T. (eds), Reptile survey methods. English Nature, London.

Schreiber, E. (1875): Herpetologia europaea. Friedrich Vieweg und Sohn, Braunschweig.

Smith, N.D. (1990): The Ecology of the Slow-Worm (*Anguis fragilis* L.) in Southern England. Unpublished M.Ph. Thesis, University of Southampton.

Sos, T. (2010): Evaluating the accuracy of morphological traits used in *Anguis*(sub)species differentiation. Herpetologica Romanica 4:29-44.

Sos, T., Herczeg, G. (2009): Sexual size dimorphism in eastern slow-worm (*Anguis fragilis colchica* Nordmann, 1840, Reptilia: Anguidae). Russian Journal of Herpetology 16: 304 - 310.

Štepánek, O. (1937): *Anguis fragilis peloponnesiacus* n. ssp. Zoologischer Anzeiger, Leipzig 118: 107-110.

Quinn, S., Hews, D.K. (2003): Positive relationship between abdominal colouration and dermal melanin density in Phrynosomatid lizards. Copeia 4: 858-864.

Voipio, P. (1962): Multiple phaneromorphism in the European Slow-worm (*Anguis fragilis*) and the distributional and evolutionary history of the species. Annales Zoologici Societatis Zoologicae Botanicae Fennicae Vanamo 22(2): 1-20.

Wermuth, H. (1950): Variationsstatistische Untersuchung der Rassen- und Geslechtmerkmale bei der Blindschleiche (*Anguis fragilis* Linné). Deutsche Zoologische Zeitschrift 1(2): 81-121.