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Evaluating the accuracy of morphological traits used in *Anguis* (sub)species differentiation

Tibor SOS

Association for Bird and Nature Protection "Milvus Group",
Crinului Str. 22, Tîrgu Mureş, Mureş County, România, E-mail: tibor.sos@gmail.com

Abstract. Qualitative and quantitative data were used in the differentiation of the subspecies of *Anguis fragilis*. Now the same characters could be used for morphological differentiation in the species complex of *A. fragilis*. Earlier studies dealing with this topic failed to investigate high numbers of specimens from one locality. Thus the constancy or the continuity of respective characters has never been investigated in one particular population. As consistent data from one population were available from the Corner of Carpathians area (central part of Romania), this was one of the issues considered in this study. In several recent studies with similar purposes the specimen data were used without segregation by sex. The second goal of this study was to identify the effect of sexual dimorphism on these characters. The studied population belongs to the eastern subspecies, *A. f. colchica* (Nordmann, 1840), as was previously reported for other populations from the area. Recently the form is described as part of the new species *A. colchica* (Nordmann, 1840) as *A. c. incerta*, Krynicki, 1837. The presence or absence of ear openings and the number of longitudinal series of scales shows the highest constancy in investigated specimens. The occurence of a blue-spotted form, the contrast in color between back and flanks and the state of the vertebral line showed differences in sexes. The blue spotted morph appears mostly in larger male lizards. The contrast between back and flanks and the vertebral line in most females remain in juvenile stage, while in large males they usually disappear.

Keywords: *Anguis colchica* complex, species and subspecies differentiation, sexual dimorphism, Romania.

Introduction

The legless lizard, the "slow-worm" has been the object of intensive taxonomic investigations in the countries in which it occurs (see authors cited in Džukić,

1987). As pointed out by Džukić (1987), most authors adopted the view of Wermuth (1950) and Mertens & Wermuth (1960), namely, that the slow-worm is a polytypic species represented across it's vast range by two subspecies: a nominotypical western form – *A. f. fragilis* Linnaeus, 1758 and an eastern form – *A. f. colchica* Nordmann, 1840. These authors also listed a third subspecies, which currently is recognized as a full species, *A. cephallonica* Werner, 1894 (Štepánek 1937, Grillitsch & Cabela 1990). Contact zones between the two species were identified in Northern Greece, Albania (Cabela & Grillitsch 1989), Bulgaria (Beškov 1966), in the former "Yugoslavia" (Džukić 1987), Romania (Stugren et al. 1962), Hungary (Dely 1972, Dely 1974a,b) and Slovakia (Lác 1967). The contact zone probably continues into Poland to north (Dely 1974a,b). In Sweden the nominotypical, in Finland *A. f. colchica* was described, thus another contact area was predicted on the territory of Finland (Voipio 1956, Voipio 1962). The only recent work dealing with the genetic differentiation in the *A. fragilis* complex (Gvoždík et al. 2010) revealed a more complex genetic structure, than expected before. Beside 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. This third clade is considered restricted to the southern Balkans, and partially sympatric with *A. cephallonica*. *A. fragilis* sensu stricto has an estimated occurrence in Western and Central Europe, in north-western Balkans, with possibly isolated populations in the eastern Balkans, and presumably also in western Scandinavia and Italy. *A. colchica* has a believed distribution from the eastern Czech Republic and the Baltic region eastward to northern Iran, presumably also in eastern Scandinavia, and the north-eastern Balkans. Furthermore *A. colchica* is divided in three other subspecies: the Caucasian nominat form, the east-european *A. c. incerta*, Krynicki, 1837 and the caspian *A. c. orientalis* Anderson, 1872.

Differentiation of the "old" subspecies was based mostly on qualitative characters. These characters were developed to differentiate the two "old" subspecies, "*fragilis*" and "*colchica*" (but also for the third one, *A. cephallonica*). Thus these morphological traits could be roughly applied to the new species also, as similarly is considered in Gvoždík et al. (2010). The problem appears when specimens from the *A. graeca* area are in target, as its populations are known to display intermediate or mosaic characters of the "*fragilis*" and "*colchica*" morphotypes (Cabela & Grillitsh 1989, Grillitsch & Cabela 1990, Gvoždík et al. 2010).

The head-shield pattern, i.e., the size and the position of prefrontale related to the other neighboring horny plates, the state of ear openings, the longitudinal series of scales at mid-body and the presence of the blue spots are the most known and used qualitative characters (e.g. Wermuth 1950). According to previous works, in the "*fragilis*" morphotype the prefrontals are in contact, definitively separating the frontal from the internasal (e.g. Štepánek 1937, Voipio 1962, Lác 1967). In the southern part of its distribution, the prefrontals may contact only at a point (Lác 1967) or may exhibit no contact (e.g. Wermuth 1950, Voipio 1962, Lác 1967). At least one ear opening is closed (e.g. Wermuth 1950, Voipio 1962). Midbody scale rows are mostly 24-26, however there is higher number in the contact zone (e.g. Wermuth 1950, Dely 1966). Specimens with blue spots are less common than in the eastern subspecies (Wermuth 1950, Voipio 1962, Dely 1972), and such spots are common mostly in males (Grillitsh & Cabela 1990). In the "*colchica*" morphotype ther is no broad contact between the prefrontals (Štepánek 1937, Voipio 1962, Lác 1967), but specimens exhibiting a transitional condition in which the prefrontals touchat least at on point commonly appear (Wermuth 1950. Fuhn & Vancea 1961, Lác 1967). Specimens of this forms usually have distinct ear opennings (e.g. Wermuth 1950, Fuhn & Vancea 1961, Voipio 1962) and midbody scale rows vary between 26-30 (e.g. Štepánek 1937, Wermuth 1950, Fuhn & Vancea 1961, Dely 1966). The blue spots appear more frequently than in the nominate form, and also appear in females (Wermuth 1950, Voipio 1962, Grillitsh & Cabela 1990).

The contrast between the back and flank stripes was another characters used in subspecies differentiation (Grillitsch & Cabela 1990), however this feature was not used in the context of sexual differences. Sexual dimorphism in color and patterns was described quite early in *Anguis* sp., although some authors denied the existence of sex-linked color differences (e.g. Petzold 1971). Ray (1693) described an obvious contrast between the back stripe and flanks in females, which disappear in males. This observation was subsequently discussed later in a very low number of works (see works cited in Dely 1981), however it was noted by Wermuth (1950). The great variation in color and pattern, which was the starting point for the description of so many forms (e.g. Schreiber 1875), probably, obscured the recognition of this feature. The juvenile color in both subspecies and sexes is similar. A lighter back stripe with different tones (mostly brown, but occasionaly reddish, yellowish, etc.) usually divided by two closely positioned vertebral lines is bordered by darker flanks which continue in onto similarly colored or darker belly.

The juvenile color and pattern changes differently over ontogeny in each sexes. According to Wermuth (1950) several tendencies characterise ontogenetic color change in *Anguis*, i.e., a shift from a darker flank to a lighter one and from a sharpe back stripe to the total absence of a stripe. This may be summarised together as a change from a higher contrast between back and flanks to an absence of contrast as described by Ray (1693). Similarly, another character used by Grillitsch & Cabela (1990) independent to sex, is the status of the vertebral line, which similarly shows gender specific differentiation (Wermuth 1950).

Distribution patterns were derived from subspecific allocations based on the above described characters, which were mostly checked on several specimens from particular populations. All these studies failed to investigate a large number of specimens from a single locality, as the biological material (usually museum specimens) available to authors never exceeded more than a few exemplars from any one location whether the study involved the entire distribution area of species (e.g. Wermuth 1950, Voipio 1962, Dely 1972, Gely 1974a,b, Muster & Bosch 1982) or the territory of particular countries (e.g. Stugren et al. 1962, Beškov 1966, Lác 1967, Džukić 1987, Cabela & Grillitsch 1989, Grillitsch & Cabela 1990). Thus the constancy or the continuity of the qualitative and quantitative characters used in subspecies differentiation has never been investigated in one particular population. Furthermore, until now it has not been possible to meaningfully evaluate the the validity of these characters.

In parallel with an ecological study, I had the opportunity to collect data related to the subspecific identification of specimens of one population of slow worm population in the Corner of Romanian Carpathians (Rupea, Braşov County), which according to Stugren *et al.* (1962) belongs to the eastern subspecies, "*A. f. colchica*" (now in the range of *A. c. incerta*). Two hundred thirty-one specimens were examined, which exceeds the number of specimens checked for an entire country or several countries pooled in most previous studies related to subspecies identification (e.g. those cited in Dely 1972, Dely 1974a,b, Cabela & Grillitsch 1989). The goals of this study were to verify the constancy or continuity, and thus the validity, of characters used in subspecies identification in a particular population and to evaluate the degree of sexual dimorphism in these characters.

Materials and methods

Data used were collected during 2001-2004 from specimens inhabiting a 0.8 ha territory (part of a cemetery in Rupea, Braşov County, Romania, Fig. 1), located in the south-western part of Transylvania, at about 503 m a. s. l. Slow worms inhabit the relatively undisturbed parts of the cemetery, and are usually found in or near bushy patches under coverboards and other natural cover (Sos, in prep.). Head markings and measurement details, tail condition, color, scarring and any other clues useful in identifying different individuals were recorded in order to avoid pseudoreplication over time, as marking techniques have been ineffective over long periods in this species

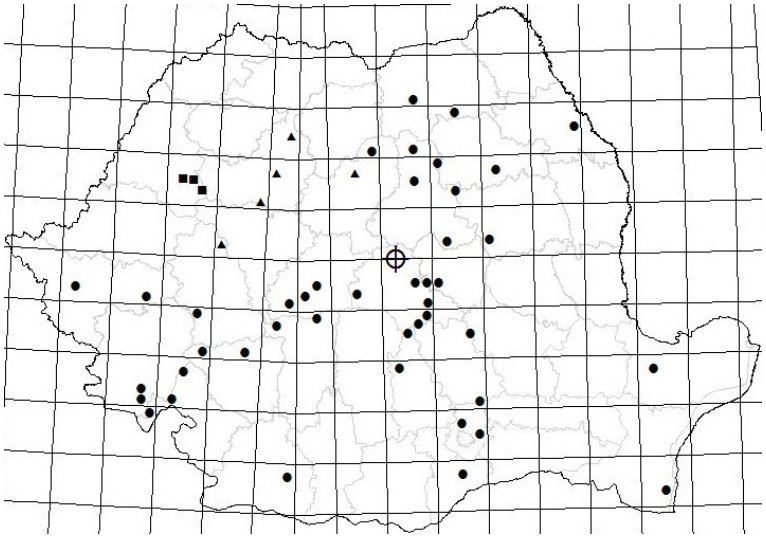

Figure 1. The distribution of *Anguis fragilis* complex in Romania. Square: *A. f. fragilis*(?), circle: "*A. f. colchica*" (=*A. c. incerta*), triangle: transitional forms; after Stugren *et al.*, 1962, Dely, 1972, Dely 1974b, own data). In target the study area.

(see Riddell, 1996). Ninety-eight male and 133 female specimens were examined, although sample sizes for some features were lower due to incomplete data. The presence or absence of the hemipenis (eversion of the hemipenis is a defensive behavior of *Anguis* sp.) complemented by secondary sexual coloration or other clues (e.g., gravid state of females) was used to identify the sex of the lizards. Only specimens with a

snout-vent length (SVL) greater than 100 mm were considered. Lizards with low SVLs are difficult to sex, but the yearly recapture of specimens insured their allocation to sex as they grew. Thus, no lizards were sacrificed for this purpose, although Voipio (1962) and Dely (1972, 1974a,b) sexed doubtful exemplars via dissection. Specimens of unidentified sex were excluded from the study. Qualitative characters are listed and summarized in Table 1. The definitions of comparative qualitative characters and their abbreviations are listed in Table 2. The SVL were recorded with a ruler, and with calipers, respectively, with a precision of 0.01 mm. I calculated the average and standard error for each character. To test the hypothesis that the samples compared belong to the same groups I used the nonparametric Mann-Whitney U-test comparing two independent groups and a Kruskall-Wallis ANOVA comparing multiple independent groups.

Table 1. Scores of the qualitative characters examined.

Character	Character class	Code
type of pileus (position of prefrontals)	in contact (type A)	1
	contact in one point (type B)	2
	no contact (type C)	3
ear opening	missing	0
	present	1
dots	missing	0
	brown	1
	white	2
	blue	3
dorso-lateral contrast	sharp	0
	transitional	1
	pale or missing	2
vertebral line	distinctly continuous	0
	discontinuous	1
	pale or missing	2

Results

The specimens of studied populations did not show a clear pattern in percentage of pileus type, related to the relation between the prefrontals. In both sexes the B

pileus type is represented by a smaller percentage of specimens than the other types (Table 2). The differences between percentages of type A and C are similar in both sexes and there is no significant statistical difference in distribution of pileus types between sexes ($p > 0.1$). With no exception, all specimens had ear openings (Table 3). There was great consistency in the number of longitudinal series of scales at mid-body, with 91.1% of specimens displaying 28 rows of scales (Table 5). Based on an earlier study (Sos, in prep.) the sexes differed significantly in regard to spot color. In males blue spots occurred in 73% of specimens, while in females this value was 26%, yielding a pooled frequency of 44%, independent of sex.

Females displayed a high variance in the different color of spots and larger specimens (greater SVL) showed a tendency to be spotted (brown and white) or blue-spotted animals rather than unspotted. The sexes also differs significantly respect to contrasting dorsal and lateral color ($p < 0.0001$). Females usually retain the juvenile contrast between the back stripe and flanks through ontogeny (Table 2). In males there is a significant difference between the SVL of specimens with sharp contrast to those in which contrast is weak or absent ($p < 0.05$; 1: n = 26, x = 17.8 ± 0.60/2: n = 22, x = 18.3 ± 0.44/3: n = 30, x = 19.2 ± 0.28). I found also significant differences in vertebral line pattern between sexes ($p < 0.01$). The comparison of different groups of males shows that males with larger bodies have a pale vertebral line or lack the line all together ($p < 0.1$; 1: n = 27, x = 17.2 ± 0.60/ 2: n = 18, x = 18.3 ± 0.44/ 3: n = 33, x = 18.8 ± 0.31; for abbreviations see Table 1). In females, specimens with a distinctly conti- nuous vertebral line were most numerous, followed by specimens with a discontinuous vertebral line (Table 2).

Discussion

The Romanian slow-worm was described by Kirițescu (1930) and Călinescu (1931) as *A. f. fragilis*, but this was later corrected to *A. f. colchica* by Fuhn (1960) and Fuhn & Vancea (1961). Although the blue-spotted variation (var. *Erix*) it was earlier cited from Romania (e.g. from Cluj and Sibiu by Bieltz 1888; from the Carpathians by Călinescu 1931; from the forelands of the Carpathians, in Oltenia by Kirițescu 1930), the presence of such variation does not confirm the presence of *A. f. colchica*, as the blue-spotted variation also appears in the nominate form (e.g. Wermuth 1950). According to Stugren et al. (1962) in Romania both subspecies occur.

Table 2. Phenotypical variability of 5 qualitative characters between the 2 sexes in *A. c. incerta* from Rupea, Romania (abbrev.: *min.* and *max.* - minimum and mximum score, *1st* and *3rd* - 1st and 3rd quartile, *med.* - median).

Characters	N	0 (N/%)	1 (N/%)	2 (N/%)	3 (N/%)	min.	1st	med.	3rd	max
type of pileus			*A type*	*B type*	*C type*					
all	231		106/45.88	40/17.31	85/36.79	1	1	2	3	3
male	98		44/44.89	18/18.36	36/36.73	1	1	2	3	3
female	133		62/46.61	22/16.54	49/36.84	1	1	2	3	3
ear opening		*absent*	*present*							
all	228	1/0.43	227/99.56			0	1	1	1	1
male	97	0/0	97/100			1	1	1	1	1
female	131	1/0.76	130/99.23			0	1	1	1	1
dots		*absent*	*brown*	*white*	*blue*					
all	201	39/19.40	30/14.92	43/21.39	89/44.27	0	1	2	3	3
male	78	8/10.25	5/6.41	8/10.25	57/73.07	0	2	3	3	3
female	123	31/25.20	25/20.32	35/28.45	32/26.01	0	0	2	3	3
dorso-lat. contrast		*sharp*	*transitional*	*pale/absent*						
all	201	146/72.63	25/12.43	30/14.92		0	0	0	1	3
male	78	26/33.33	22/28.20	30/38.46		0	1	1	3	3
female	123	120/97.56	3/2.43	0/0		0	0	0	0	1
vertebral line		*distinctly, continuous*	*dis-continuous*	*pale/absent*						
all	201	97/48.25	62/30.84	42/20.89		0	0	1	2	3
male	78	27/34.61	22/28.20	29/37.17		0	0	1	2	3
female	123	70/56.91	40/32.52	13/10.56		0	0	0	2	2

Table 3. The distribution of different head-shield patterns in *Anguis fragilis* complex (n/%).

zone/country	A type	B type	C type	source
		"fragilis"		
W and S-W Europe	63/80.7	11/14.1	2/2.6	Wermuth 1950*
Nederland	91/65.9	44/31.9	3/2.2	Musters & In den Bosch 1982
Sweden	79/78.3	15/14.8	1/1	Voipio 1962
Central Europe	210/64.5	74/22.8	7/2.2	Wermuth 1950*
W Hungary	15/65.2	8/34.7	0/0	Dely 1972
W "Yugoslavia"	119/62	56/29.2	17/8.8	Džukić 1987
Bulgaria	?/77.4	?/22.6	0/0	Beškov 1966
		***"colchica", "graeca"* (and mixed populations)**		
S-E Europe	28 /39.5	25 /35.2	14 /19.7	Wermuth 1950*
"Yugoslavia"	18/41.9	15/34.9	10/23.2	Džukić 1987
S-E "Yugoslavia"	8/17.8	4/8.9	33/73.3	Džukić 1987
		"colchica"		
Bulgaria	0/0	?/25	?/75	Beškov 1966
Rupea (Romania)	**106/45.8**	**40/17.3**	**85/36.7**	**present study**
Slovakia	?/23.7	?/14.0	?/62.3	Lác 1967
Finland	18/29.5	20/32.8	21/34.4	Voipio 1962
Asia Minor + Persia	6/17.2	11/31.4	18/51.4	Wermuth 1950*

*Cited in Voipio 1962.

Moreover, the existence of a form representing a lineage older than the split between the recognized subspecies (in the Apuseni Mts. = Bihor Mts.) has been suggested. This form is considered to be a vestige from before the (last?) Ice Age, which in postglacial times came into contact with the typical form. Thus, the resulting form shows the characteristics of the typical subspecies. In later works (e.g. Lác 1967, Petzold 1971), these results are used in similarly way as results of Mertens (1952). The presence of *A. f. fragilis* is considered in term of introgression in the eastern form. In the rest of the country, except an unclearly delimited contact zone in the midle of theTransylvanian Basin, the eastern subspecies, *A. f. colchica* was identified, which probably avanced in the area in postglacial times (Fig. 1). The result of this process is the presence of *„colchica"* characteristics in the

populations from the Bihor Mountains. The presence of *A. f. colchica* from Romania (e.g. Retezat Mountains, Carpathian Corner) is also confirmed by Dely (1972, 1974b). Recently the form is considered part of *A. c. incerta*, Krynicki, 1837 (Gvoždík et al. 2010).

Table 4. The status of ear opening in *Anguis fragilis* complex (–: missing, (+): transitional, +: visible).

zone/country	–/%	(+)/%	+/%	source
		"fragilis"		
W and S-W Europe	77/98.7	1/1.3	0/0	Wermuth 1950*
Nederland	138/100	0/0	0/0	Musters & In den Bosch 1982
Sweden	101/100	0/0	0/0	Voipio 1962
Central Europe	339/98	7/2	0/0	Wermuth 1950*
W Hungary	23/85.2	4/14.8	0/0	Dely 1972
W "Yugoslavia"*	123/66.1	59/31.7	4/2.2	Džukić 1987
Bulgaria	120 /100	0/0	0/0	Beškov 1966
		***"colchica", "graeca"* (and mixed populations)**		
S-E Europe	37/51.4	9/12.5	26/36.1	Wermuth 1950*
"Yugoslavia"*	18/42.8	20/47.6	4/9.6	Džukić 1987
S-E "Yugoslavia"*	0/0	2/4.76	40/95.3	Džukić 1987
		"colchica"		
Bulgaria	0/0	0/0	120 /100	Beškov 1966
Rupea (Romania)	**0/0**	**1/0.4**	**227/99.6**	**present study**
E Hungary	0/0	0/0	25/100	Dely 1972
Slovakia	?/31.6	?/18.8	?/31.6	Lác 1967
Finland	15/26.8	8/14.3	33/58.9	Voipio 1962
N-Turkey	0/0	0/0	12/100	Baran 1977
Asia Minor + Persia	10/27.8	10/27.8	16/44.4	Wermuth 1950*

Data pooled from 5 categories to 3.

The head-shield pattern and a visible ear opening, and, respectively, blue-spotting have been described as correlated characters (e.g. Wermuth 1950, Voipio

1962, Dely 1972, Dely 1974a,b, Džukić 1987). The occurrence of the blue-spotted morph and the visible ear opening are correlated, the proportion of the visible ear opening being considerably greater among the blue-spotted individuals than among the normal ones (e.g. Voipio 1962), while the presence of a vertebral line is negatively associated with blue-spotting (Nordmann, 1840; Stugren et al. 1962). This theory is supported by samples from the periphery of the range (in west for the western form and east for the eastern form) where the relationship between the characteristics are nearly perfectly established (e.g. Voipio 1962, Beškov 1966). Otherwise, according to these cited authors, these characters show high variability and are less correlated in the contact zones (Wermuth 1950, Voipio 1962, Dely 1972, Dely 1974a,b), although animals with mixed characters may occur in populations from the "pure" subspecies area. These phenotypes are considered having atavistic origins or purely displayed by abnormal specimens (Dely 1974a,b).

Table 5. Number of longitudinal series of scales in *Anguis fragilis* complex (n/%).

zone/locality	N	22	23	24	25	26	27	28	29	30	31	sources
						"fragilis"						
Netherlands	222	1 0.7	9 6.5	82 59.4	18 13.0	28 20.3						Musters & In den Bosch 1982
W „Yugoslavia"	182		2 1.1	59 32.4	47 25.8	63 34.6	7 3.9	4 2.2				Džukić 1987
						***"colchica", "graeca"* (and mixed populations)**						
contact zone in "Yugoslavia"	35			1 2.9	8 22.9	20 57.1	4 11.4	2 5.7				Džukić 1987
S-E "Yugoslavia"	66			3 7.0	25 2.0	15 34.9	7 16.3	10 23.3	3 7.0	2 4.6	1 2.3	Džukić 1987
						"colchica"						
Rupea (Romania)	**223**					**5 2.2**		**203 91.1**		**15 6.7**		**present study**
N Turkey	12					11 91.6	1 8.4					Baran 1977

Regarding head-shield pattern the Rupea population did not show constancy. The pileus type of the nominate form (A type) appears in the highest percentage,

but is followed closely by the eastern C type (Table 2). These close values of different pileus types are similar within populations from the contact zone and in those adjacent to contact zones (Table 3). A high constancy is showed in the ear opening condition. The ear opening, which is characteristic of eastern form (Table 4), is present in all specimens except one (Table 2). The high percentage of specimens with 28 rows scales around mid-body shows that in this feature the population belongs to the *"colchica"* morphotype (Table 5). The percentage of blue-spotted males from the population of Rupea is similar to those in other eastern populations (Sos, in prep.; Table 6). However, the high percentage of the blue-spotted females is novel. All qualitative data pooled together (except the head-shield pattern) highlight the *"colchica"* morphotype origine of the studied population. These data correspond with the view of Stugren *et al.* (1962; Fig. 1) briefly described above.

Accordind to Dely (1972) the most reliable characters in subspecies differentiation are the presence or absence of ear openings, followed by the number of longitudinal series of scales, the head-shield pattern, and the presence or absence of blue spots. This is the rank of consistency in the characters of the studied population too, although these characters are not constant from juveniles to adult lizards. In juveniles inspected by Dely (1972, 1974a,b), the longitudinal series of scales were similar with parental characteristics, but no ear openings were found, although these appear in subadults, and there was great variation in head shield pattern. The presence of ear openings in juvenile *A. f. colchica* is described by Musters & Bosch (1982), but only in one example. This is consistent with the known variability and instability of development of ear opening is in this species (Hochstetter 1951). The above listed quantitative characters, except the blue-spotting, show no sexual dimorphism. Voipio (1950) described some tendency for the readily visible ear opening to be relatively commoner among males, but I was unable to test this as only the presence of the ear was used in this study. It is probable that the larger head of males is correlated with the presence of the ear opening.

As recognized earlier, the blue morph is confined to the eastern form, *A. f. colchica*, in which it appear in both males and females, although at a lower frequency in the latter; in the nominate form it appears rarely and was reported only from males (Wermuth 1950, Voipio 1962, Dely 1972, Dely 1974a,b - Table 6.). Thus, in subspecies identification only the frequency of the morph in a population

(thus requiring a large samples) has indicator value.

Table 6. The presence of blue-spotted specimens in *Anguis fragilis* complex.

zone/country	all (N, +/%)		male (N, +/%)		female (N, +/%)		source
			"fragilis"				
W and S-W Europe	78	2/2.6	-	-	-	-	Wermuth, 1950**
Nederland	138	22/15.9	?	22/41.5	?	0	Musters & In den Bosch, 1982
Sweden	101	0	?	0	?	0	Voipio, 1962
Central Europe	346	18/5.2	-	-	-	-	Wermuth, 1950**
W Hungary	28	4/14.2	6	4/6.6	?	0	Dely, 1972
W "Yugoslavia"	76	23/30.3	-	-	-	-	Džukić, 1987
Bulgaria*	?	?/9.8	-	-	-	-	Beškov, 1966
			***"colchica", "graeca"* (and mixed populations)**				
S-E Europe	72	20/27.8	-	-	-	-	Wermuth, 1950**
"Yugoslavia"	23	17/74.0	-	-	-	-	Džukić, 1987
S-E "Yugoslavia"	20	16/80	-	-	-	-	Džukić, 1987
			"colchica"				
Bulgaria*	?	?/66.7	-	-	-	-	Beškov, 1966
Rupea (Romania)	**201**	**89/44.2**	**78**	**57/73.0**	**123**	**32/26.0**	**present study**
E Hungary	25	18/90.0	15	15/100.0	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
Asia Minor + Persia	36	5/13.9	-	-	-	-	Wermuth, 1950**

* The two samples pooled: N = 120; ** Cited in Voipio 1962.

The dorso-lateral contrast is different in sexes, as was reported earlier (e.g. Ray 1693, Wermuth 1950, Dely 1981). According to Wermuth (1950) the contrast was exhibited in 95% of females, but only 35% of males. In Rupea females the

percentage is similar (about 98%). As in my study three categories exist regarding to the contrast, the males exhibit almost similar percentage in the three categories (Table 2). The SVL of the three groups are statistically significantly different, as the SVL increases from males with sharp contrast through those with an intermediate condition and the largest males exhibit little or no contrast. In my opinion the loss of contrast between back and flanks could serve in a nuptial context. The vertebral line also changes during growth in both sexes. Wermuth (1950) found that 80% of females had a vertebral line, while about 25% of males show it. In Rupea's females a similar frequency is found; about 89% of females have continuous or discontinuous but distinct vertebral line. In Rupea males a vertebral line is expressed in about 62% of the specimens. Although the frequency in the three categories studied is almost similar (Table 2). Males with distinctly a continuous vertebral line are shorter than those in the second and third groups. A similar tendency was found by Muster & Bosch (1982). They concluded that specimens with vertebral line are mostly young specimens. Thus, the use of these two characters in subspecies differentiation is inadequate.

In conclusion, on basis of a study on the eastern form, "*colchica*" (= *A. c. incerta*) some qualitative characters used for the differentiation of (sub)species in the *A. fragilis* complex show no sexual dimorphism, while others do, and thus are inadequate for such purposes. The state of ear openings, the number of longitudinal series of scales, and the head-shield pattern do not display sexual dimorphism. The first two characters show a high constancy in samples, while the third one is variable. The occurrence of a blue-spotted morph, the contrast between back and flanks and the state of the vertebral line shows differences in sexes. The blue-spotted morph appears mostly in larger male lizards. In most females the contrast between back and flanks and the vertebral line retain the juvenile condition, while in large males they usually disappear.

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