A MULTIVARIATE APPROACH TO THE SYSTEMATICS OF ITALIAN RAT SNAKES OF THE *ELAPHE LONGISSIMA* COMPLEX (REPTILIA, COLUBRIDAE): REVALIDATION OF CAMERANO'S *CALLOPELTIS LONGISSIMUS* VAR. *LINEATA*

PETER LENK[1] AND WOLFGANG WÜSTER[2]

[1]*Hessisches Landesmuseum Darmstadt, Darmstadt, Germany*

[2]*School of Biological Sciences, University of Wales, Bangor, LL57 2UW, Wales, UK*

We used multivariate analysis to study patterns of geographic variation in morphology in the Aesculapian snake, *Elaphe longissima*, in Italy and other parts of its range, in order to evaluate the status of the southern Italian form, hitherto known as *E. l. romana*. Although that taxon was previously regarded as weakly differentiated, a recent study based on blood proteins showed a high level of differentiation, similar to that observed between full species of European *Elaphe*. Fourteen characters relating to external morphology were recorded from 104 adult specimens of *Elaphe longissima* from 52 localities, and subjected to multivariate analysis. The results show that southern Italian specimens are clearly distinct from those of other populations. The morphological divergence is coupled with a parapatric distribution pattern and a sharp transition at the contact zone in central Italy. We found the distribution limits of the two taxa to be situated about 100 km further south than previously suggested. Parapatry among morphologically clearly distinct forms, and differences in blood plasma suggesting considerable evolutionary divergence, provide reasons for considering the southern Italian form as a separate evolutionary species from *E. longissima*. The name *Coluber romanus* Suckow, 1798 is not available for the southern Italian species, for which the oldest available name is *Callopeltis longissimus* var. *lineata* Camerano, 1891, in the combination *Elaphe lineata*.

Key words: *Elaphe*, multivariate analysis, taxonomy, morphology, zoogeography

INTRODUCTION

The Aesculapian snake, *Elaphe longissima* (LAURENTI, 1768) (Type locality (restr.): Vienna, Austria) is the most widespread rat snake in Europe. It occurs in the Mediterranean and submediterranean regions, from northern Spain to the Middle East. Due to its wide distribution, it has been an intensively studied species (see Böhme, 1993). However, basic aspects of its intraspecific classification and pattern of geographic variation remain uncertain or poorly defined. This applies particularly to the Italian populations, which are the subject of this study.

A recent study of blood plasma proteins in European *Elaphe* species (Lenk & Joger 1994) noted that specimens from Sicily currently assigned to *Elaphe longissima romana* (Suckow, 1798) are strikingly distinct from other European *E. longissima* populations, possibly at a higher taxonomic level than hitherto believed.

Mertens & Müller (1928) first recognized the distinct nature of the southern Italian taxon, and applied the name *Coluber romanus* Suckow, 1798 to this form. They regarded it as a subspecies of *Elaphe longissima* and restricted the range from 'warmer parts of Europe, Spain, France, Italy, and especially surroundings of Rome' (Suckow, 1798) to 'central and southern Italy, Sicily' with the type locality 'surroundings of Rome', but without any explicit analysis.

Capocaccia (1964) conducted the first substantial study of the subdivision of *E. longissima* in Italy. Her description of *romana* (grey belly; the shape of the temporal blotches, which are united with the posterior submandibular blotches; a high number of ventral scales; and a rather light coloration) deviates considerably from Suckow's, which was chiefly based on the occurrence of longitudinal stripes on the posterior part of the trunk. Further doubts on the accuracy of the type locality and the availability of the name *romana* Suckow (1798) for the southern Italian form arose from Cattaneo's (1975) description of a melanistic population of *E. l. longissima* from the Roman littoral.

The prevailing uncertainty regarding the status of the southern Italian Aesculapian snake gave us reason to initiate the following study. Here, we consider some newly defined characters, as well as those used by Capocaccia (1964), in order to re-analyse the distinctness of both forms in Italy and additional localities in the Balkans by means of multivariate analysis of morphological characters. Multivariate techniques, and especially ordination methods such as principal components analysis (PCA), have been shown to be useful tools in revealing patterns of geographic variation in the generalized phenotype which are often obscured when characters are analysed individually (e.g. Thorpe, 1976, 1980; Wüster, Otsuka, Thorpe & Malhotra, 1992, Wüster, Warrell, Cox, Jintakune & Nabhitabhata, 1997).

Correspondence: P. Lenk, Hessisches Landesmuseum Darmstadt, Zoologische Abteilung, Friedensplatz, D-64283 Darmstadt, Germany. *E-mail*: Peter.Lenk@hmrag.com

MATERIALS AND METHODS

Twenty-eight characters of external morphology and colour pattern were recorded from 104 adult specimens from the Italian Peninsula and other parts of the species range. A special effort was made to include specimens from the Balkans, some of which share characters with southern Italian populations *sensu* Capocaccia (1964) (see above).

In addition to the 104 specimens mentioned above, eight juvenile specimens were examined, but not included in the further analyses, as ontogenetic variation in some colour pattern characters would have affected the results of the analysis. Furthermore, one adult specimen (LPPC 313 from Udine / Northern Italy) was excluded from further analyses due to highly aberrant scalation characters.

In order to identify characters useful for multivariate analysis, a two-way analysis of variance (BMDP 2V - Dixon, 1991) was performed to determine which characters show significant geographic variation between localities, sexual dimorphism, or both. For this purpose, specimens were grouped into six geographic test groups on the basis of collecting gaps, potential physiographic barriers, and past taxonomic subdivisions. Only characters showing significant geographical variation were subjected to multivariate analysis. Most of them were qualitative, and coded in binary form (0,1). Progressive states (e.g. absent, inconspicuous, conspicuous) were coded accordingly (e.g. 1, 2, 3).

Characters found to be invariable, unrecordable in a large percentage of specimens, or incompatible with the assumptions of PCA were not included in further analyses. However, some of these characters do convey considerable information and will be discussed below.

PCA was used to reveal the pattern of morphological variation in the complex, with the specific aim of testing whether the southern Italian populations and the remaining populations form two homogeneous groups that are distinct from each other. PCA was run on a computer program written by R. G. Davies (Imperial College London) and modified by R. S. Thorpe (University of Wales, Bangor). All variables were standardized to zero mean and unit standard deviation. To rule out confusion due to sexual dimorphism, both sexes were analysed separately. The analyses were run on the data from individual specimens, to avoid *a priori* assumptions of taxon membership. For all runs, the eigenvector coefficients of each character for the first principal component were extracted to identify the characters that contribute most to the observed pattern of variation.

RESULTS

The results of the ANOVA reveal that 13 characters show significant geographical variation among males and 14 among females (Table 1). These characters were subjected to an initial PCA (PCA 1) as described above.

TABLE 1. Characters used in the PCA. Characters 7-10 were recorded as follows: Starting with the last ventral we moved 10, 20, 30, etc. scales forwards and transversely counted the dorsal scale rows at each site. The relative positions of the segments where the changes occured were assessed.

1. No of ventrals (Arnold & Burton, 1979).
2. No of pairs of subcaudal scales.
3. Ventral colour: % ventral scale position of the transition from bright to dark ventrals.
4. Dorsal background colour: micro-speckling on undamaged dorsal scales at posterior trunk predominantly (see. Fig 5) due to absent epidermal pigmentation.
5. Presence of longitudinal stripes at midbody.
6. Temporal blotches fused with posterior mandibular blotch at least on one side.
7. % ventral scale position of reduction from 19 to 17 dorsal scale rows.
8. % ventral scale position of reduction from 21 to 19 dorsal scale rows.
9. % ventral scale position of reduction from 23 to 21 dorsal scale rows.
10. % ventral scale position of addition from 21 to 23 dorsal scale rows.
11. Bright blotches, bright blotches with greyish spots on each scale, or absent nuchal blotches, (at least above the elongation of the mouth slit).
12. Keeling of dorsals of posterior body absent (0), present in less than 50% of scales (1), present in more than 50% (2).
13. White dashes at midbody absent, present in less than 10 % of scales, present in more than 10%.
14. Presence of greyish marbling on the bright venter.

In the males, this analysis resulted in two well-defined geographical groups separated along the first principal component: one group comprises specimens from southern Italy, and the other comprises specimens from northern Italy and the remainder of the range of the *E. longissima* complex (Fig. 1). In females, however, the resolution was less clear, as the first principal component scores of some specimens from the Balkans lay within the range of southern Italian specimens. Reassessment of these Balkan specimens showed them to be melanistic individuals.

Some characters (3 and 11, Table 1) recorded from all specimens were affected by melanism, so that character states recorded as identical in melanistic Balkans specimens and non-melanistic southern Italian specimens may not have been homologous. Consequently, the PCA was repeated, with the exclusion of the suspect characters. This re-analysis (PCA 2) resulted in a good resolution in the females, as both geographical groups were separated along the first principal component (Fig. 2). In the males, a similar pattern was exhibited, as the first PC separates southern Italian specimens from the others. However, two non-melanistic individuals,
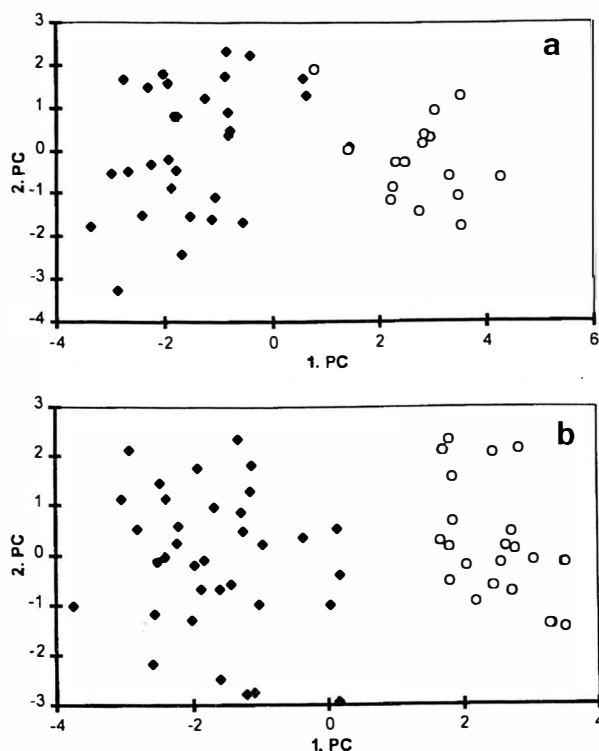
FIG. 1. PCA 1 - scatter diagram of the component scores (normalized vectors) of the first and second PCA factors, of females (a) and males (b), extracted from all characters. Squares indicate *E. l. longissima* individuals, circles indicate individuals of the southern Italian form. The first two principal components account, respectively, for 37.97% and 13.24% of the total variance in males, and 36.88% and 12.93% in females.
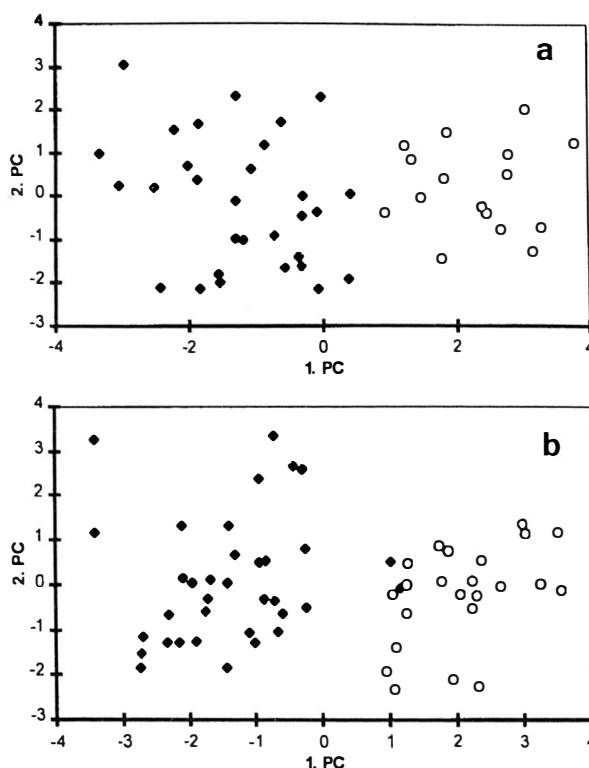
FIG. 2. PCA 2 - scatter diagram of the component scores (normalized vectors) of the first and second PCA factors, of females (a) and males (b), extracted from all but characters 3 and 11. Squares indicate *E. l. longissima* individuals, circles indicate individuals of the southern Italian form. The first two principal components account, respectively, for 34.27 % and 15.06 % of the total variance in males and 31.38 % and 14.96 % in females.

one from Castelfusano (Rome) and one from the Balkans, grouped with the southern Italian form, which was not the case in the PCA using all characters.

A third analysis (PCA 3) was run on all specimens from the Italian Peninsula and Sicily. As none of the Italian specimens was melanistic, homologies of character states in characters 3 and 11 were unproblematic, and consequently, all characters were included. In both sexes, this resulted in a clear separation of northern and southern specimens along the first principal component (Fig. 3).

Loadings of eigenvectors reveal that, in both sexes, numbers of ventrals, dorsal colour, nuchal blotches, and positions of dorsal scale row reductions appear to be the most important characters in distinguishing the two forms (Table 2). However, the remaining characters also contribute more or less strongly to the separation of the two groups.

In order to relate the separation of the two morphological groups to their distribution, the first principal component scores of all specimens were plotted against their geographical position along a latitudinal transect along the Italian Peninsula. Fig. 4 shows a striking morphological shift between northern and southern Italian specimens, with no indication of clinal variation. Specimens of the nominate form can be found south to

around 41 ° N, but are replaced by the southern Italian form to the south of that latitude.

ADDITIONAL CHARACTERS

Although the frequency of specimens with dark longitudinal stripes is not related to locality, the shape of the stripes, when present, is highly diagnostic. In specimens from southern Italy, the stripes are narrower than the light spaces between them. They are set off rather clearly by the light background colour on the posterior body. In specimens from the remainder of the range, the stripes are broader than the spaces between them. It is clear that the two morphs follow different genetic programs.

DISCUSSION

Our data suggest a marked differentiation between northern and southern Italian specimens of the *Elaphe longissima* complex. This is evident from the PCA plots, which clearly separate the two forms along the first principal component. The characters listed as diagnostic for the southern Italian form by Capocaccia (1964) can also be found in occasional specimens from other parts of Europe. If only Capocaccia's characters are used, there is a risk of misidentifying some specimens from northern Italy as belonging to the southern

TABLE 2. Normalized eigenvector coefficients of the first principal components of PCA 1 (all characters all specimens), PCA 2 (all characters, exclusively 3 and 11, all specimens), and PCA 3 (all characters, Italian specimens only) in all females and males.

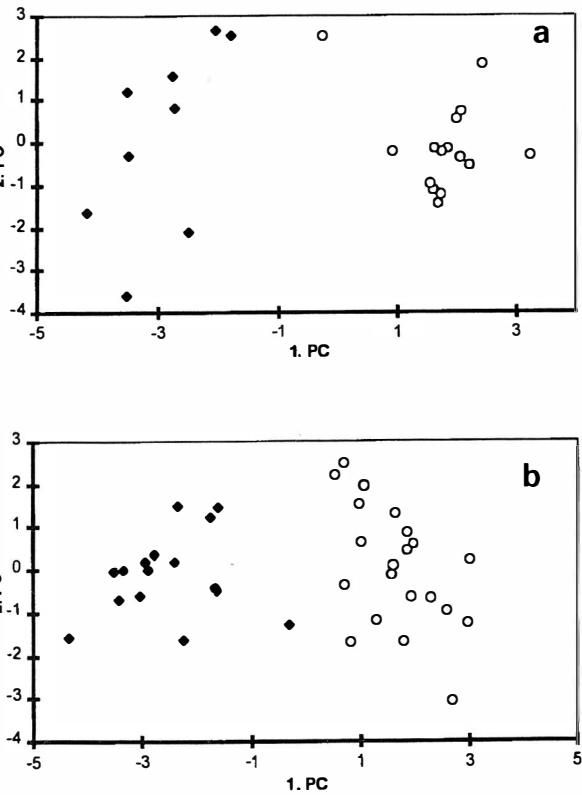| Characters	PCA 1 female	PCA 1 male	PCA 2 female	PCA 2 male	PCA 3 female	PCA 3 male
1 No of ventrals	0.294	0.318	0.332	0.335	0.319	0.245
2 No of subcaudals	-0.051	-0.221	-0.080	-0.247	-0.078	-0.155
3 Ventral transition bright/dark	0.389	0.358	-	-	0.375	0.394
4 Dorsal colour	0.393	0.333	0.447	0.397	0.377	0.399
5 Presence of stripes	0.006	-0.155	0.022	-0.202	0.010	0.022
6 Temporal blotches	0.202	0.265	0.227	0.289	0.250	0.174
7 Pos. red. 19-17	-0.245	-0.296	-0.297	-0.354	-0.280	-0.261
8 Pos. red. 21-19	-0.387	-0.337	-0.459	-0.412	-0.367	-0.404
9 Pos. red. 23-21	-0.302	-0.254	-0.390	-0.333	-0.292	-0.330
10 Pos. add. 21-23	0.168	0.169	0.195	0.246	0.166	0.051
11 Bright nuchal blotches	0.393	0.391	-	-	0.367	0.395
12 Keeling	-0.073	-0.226	-0.105	-0.242	-0.035	-0.119
13 White dashes	-0.171	-0.133	-0.260	-0.142	-0.156	-0.240
14 Marbling	-0.214	-	-0.251	-	-0.244	-
Percentage of total variance	36.88	37.97	31.38	34.27	45.45	44.56

FIG. 3. PCA 3 - scatter diagram of the component scores (normalized vectors) of the first and second PCA factor of Italian females (a) and males (b) extracted from all characters. Squares indicate *E. l. longissima* individuals, circles indicate individuals of the southern Italian form. The first two principal components account, respectively, for 44.56% and 11.38% of the total variance in males and 45.45% and 15.73% in females.
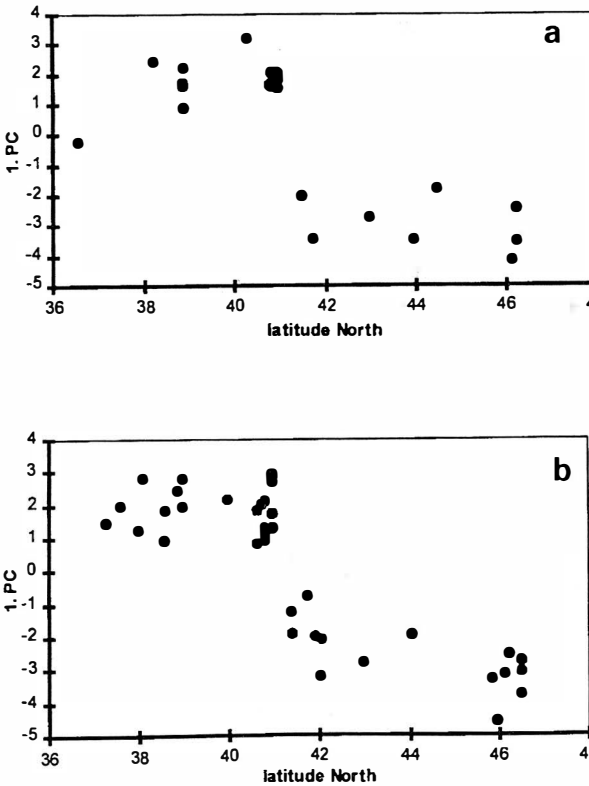
FIG. 4. Scatter diagram of the 1st. PC scores of PCA 3 of females (a) and males (b) on a transect running along the Apennine Peninsula. Sardinia excluded.

Italian form (but not vice versa). This is especially true for specimens from the province of Lazio. However, if Capocaccia's characters are redefined and simultaneously analysed with additional characters, a clear pattern is obtained that separates two geographically distinct groups of snakes. This is also true for those individuals of the nominate form that show superficial similarities with the southern Italian form.

A problem arises with non-homologous character states in the case of melanistic specimens. In particular, melanistic specimens from the Balkans exhibit convergent similarities with the southern Italian form in some characters. However, if characters affected by melanism are left out, the remaining characters clearly assign them to the nominate form, although the overall differentiation is somewhat reduced (Fig. 2). When melanistic specimens are excluded, maximum resolution is gained when all characters are used, as is shown for the Italian samples (Fig. 3). These analyses demonstrate a clear differentiation, with no overlap between northern and southern specimens along the first principal component. Variation within each group is due to individual differences, which can be of considerable magnitude, as indicated by outliers in both directions (Fig. 3). These rare specimens are responsible for the lack of any absolute diagnostic character for the two forms.

Some non-melanistic specimens from the southern part of the Balkans also show superficial similarities with the southern Italian form (e.g. grey venter, high ventral scale counts). However, our PCA results indicate that these can be unambiguously assigned to the nominate form (Fig. 2).

To test for the occurrence of intergrades, first principal component scores of Italian specimens were plotted against their origin along a latitudinal transect along the Italian Peninsula (Fig. 4). This approach showed no evidence of intergradation between the two taxa near their contact zone. Furthermore, variation within the two forms is not associated with geographical latitude. Thus, considerable variation exists within each group, but is clearly distinct from the variation representing the evolutionary signal of the two independent evolutionary lineages.

The contact zone between the two forms is located further south than hitherto believed, at least in western Italy. Whereas Capocaccia (1964) assigned all populations from the coastal regions of Lazio to the southern Italian form, all our specimens from the surroundings of Rome and Circeo (50 km S-E of Rome) are clearly assignable to the nominate form, as noted by Cattaneo (1975). This is also the case with additional material, such as published illustrations (Bruno & Maugeri, 1990) and juvenile snakes not included in the analysis (MRSN R1317, from Cocullo, province Aquila, and BM 1914.4108, from Lake Albano).The contact zone identified in this study is situated more than 100 km south of that mentioned by Capocaccia (1964).

The transition between the two forms is abrupt, and occurs over a distance of less than 100 km between Rosello (Abruzzo - northern form) and Benevento (Campania - southern form; see Fig. 7). Although our comparatively coarse sampling scale does not allow the determination of the exact position and nature of the presumed contact zone, our data suggest that a sharp transition is far more likely than a clinal variation over hundreds of kilometres. Furthermore, Capocaccia (1964) mentioned specimens from the Matese region that clearly belong to the northern Italian form. As Capocaccia's description of the nominate form is reliable, the contact zone appears to lie within the 50 km separating the Matese mountains and Benevento.

STATUS OF THE SOUTHERN ITALIAN FORM

Our morphological results, coupled with the molecular results of Lenk & Joger (1994), strongly support the hypothesis that the southern Italian form represents a separate taxon from the nominate form. The southern Italian form and the nominate form represent two clearly distinct, but reasonably homogeneous, entities.

Morphological differences on their own do not necessarily indicate that two distinct groups of populations represent separate evolutionary lineages: morphological variation may simply represent an adaptation to some ecological parameter, and thus reflect selection pressure due to current ecological conditions rather than separate ancestry (e.g. Thorpe, Brown, Malhotra & Wüster, 1991, Thorpe, Brown, Day, Malhotra, McGregor & Wüster, 1994, Thorpe, Malhotra, Black, Daltry & Wüster, 1995). However, this is unlikely to be the case in *Elaphe longissima*: parallel patterns of variation in plasma proteins suggest a separate history for the southern Italian form and the nominate form. In addition, the nominate form is relatively homogeneous across a very large range, which spans a wide variety of climatic and environmental conditions, and the contact zone between the southern Italian and nominate forms does not correspond to any sharp environmental transition.

There remains the problem of the status of the southern form, and, in particular, the question of whether it represents a full species or a subspecies of *E. longissima*. Our data provided no evidence for sympatry or hybridization between the two forms. There is no physiographic barrier likely to separate the two forms, and a distribution gap that divides both ranges into two allopatric entities is unlikely. If a hybrid zone exists, our results suggest that it is relatively narrow, with a maximum width less than 100 km, and probably less than 50 km. The distributions of the two species can thus be described as approximately parapatric.

Parapatric contact and hybrid zones typically occur between closely related taxa, such as sister species or subspecies of one species. Although hybridization may occur in nature in those cases, any selection against hybrids results in reduced fitness of the offspring (Barton
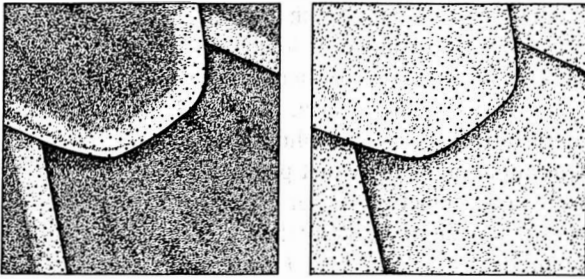
FIG. 5. Character states 'dark dorsals' and 'bright dorsals' are defined on the basis of whether fine speckling is visible on the entire scale (right) or largely invisible due to strong epidermal pigmentation (left).

& Hewitt, 1989). Thus selection acts against the free diffusion of genes between both taxa and helps maintain distinctness and independent evolution in different lineages.

We assume a similar scenario for the two Italian taxa. If no genetic break existed, a greater degree of morphological intergradation should be observed. Taking into account that, on grounds of zoogeographic and climatic constraints, both taxa are likely to have occurred together in Italy for at least several thousand years (or far longer), the parapatric distribution suggests a considerable extent of genetic incompatibility. This is consistent with the results of Lenk & Joger (1994): the level of divergence in plasma protein patterns between the two forms is similar to that typically found between uncontested full *Elaphe* species.

Based on this evidence, the southern Italian and the nominate form appear to be distinct lineages on different evolutionary trajectories. In recent years, the general trend has been to recognize such lineages as separate evolutionary or phylogenetic species (Cracraft, 1989; Frost & Hillis, 1990; Wiley, 1981). In view of our results, we regard the southern Italian form as a separate evolutionary species, distinct from *E.*

longissima. Alternatively, some may prefer the superspecies/semispecies terminology of Mayr (1969), which may also be applicable in this case: in this, the southern Italian form would be a semi-species within the *E. longissima* superspecies.

Further studies should be focused on the exact determination of the contact zone in middle Italy, and could reveal interesting insights into the extent and maintenance of stable contact zones. Therefore surveys should be intensified in the corresponding regions, preferably in conjunction with a population genetic study.

NOMENCLATURE

The question of the correct name for the southern Italian species is complex, particularly due to two major problems associated with the name *romana* traditionally used for it. First, the restricted type locality of *romana* ('vicinity of Rome' - Mertens & Müller, 1928) lies within the range of the nominate form.

Second, Suckow's description of *romana* (1798) which is nearly identical with that of Lacépède's Aesculapian snake (Lacépède, 1789), is incompatible with the appearance of the southern Italian species or any related taxon. He mentioned a reddish brown body colour, a dark longitudinal stripe at each side, darkening to black along its ventral edge, black margins on white scales next to the ventrals forming a lateral line of white triangles, head rather thick, 239 (175 ventrals and 64 pairs of subcaudals) black and white spotted scales, and a length of 3.5 - 4.5 feet (105-150 cm). In the absence of a type specimen to confirm the identity of Suckow's intended taxon, the name *Coluber romanus* Suckow, 1789 must be regarded as unavailable for the southern Italian species.

The earliest description that agrees with our findings is that of Camerano (1891), who studied a specimen from Naples and named it *Callopeltis longissimus* var. *lineata*. He described it as having a grey belly, and four
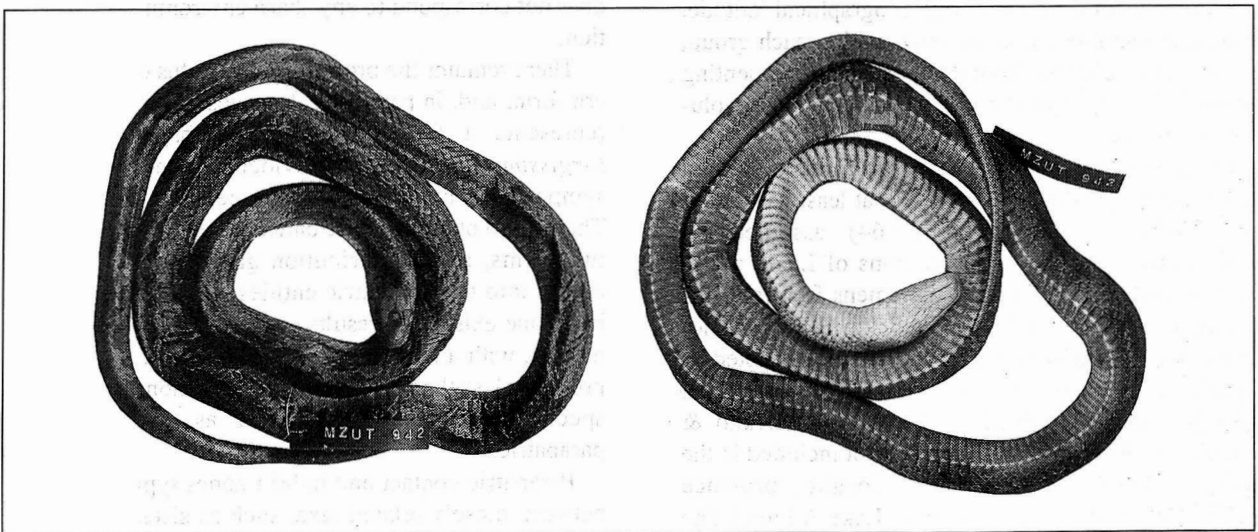

FIG. 6. Dorsal and ventral side of the holotype of *Elaphe lineata* (Camerano, 1891) MZUT 942 (original number MZUT 434) from the type locality Naples.

TABLE 3. The quantitative description of characters in the two species. For explanation of characters see also Table 1.

Males	*E. longissima* (n = 35)				*E. lineata* (n = 23)			
Characters (no.)	mean	SD	max.	min.	mean	SD	max.	min.
length head-trunk [cm]	87.44	12.39	111.00	75.00	81.76	18.22	105.00	55.00
length tail [cm]	22.87	3.24	28.00	15.00	20.91	6.03	34.00	10.00
ventrals (1)	225.77	4.01	236.00	214.00	232.13	3.44	238.00	225.00
subcaudals (2)	82.91	3.35	94.00	78.00	79.30	2.60	82.00	72.00
pos. red. 19-17 (7)	82.67	8.74	69.00	100.00	91.68	4.20	86.85	100.00
pos. red. 21-19 (8)	62.16	2.83	56.14	68.47	67.09	2.77	61.38	71.21
pos. red. 23-21 (9)	55.59	2.37	50.90	60.53	58.03	2.33	53.19	61.54
pos. red. 21 -23 (10)	25.33	5.08	17.03	42.99	22.60	4.71	16.67	35.62
Females	*E. longissima* (n = 31)				*E. lineata* (n = 16)			
Characters (no.)	mean	SD	max.	min.	mean	SD	max.	min.
length head-trunk [cm]	73.52	11.23	90.00	48.00	70.28	13.30	83.50	49.00
length tail [cm]	16.69	2.62	22.00	12.00	16.25	3.60	20.00	10.00
ventrals (1)	225.06	4.54	235.00	214.00	231.88	2.58	235.00	225.00
subcaudals (2)	72.52	3.41	79.00	65.00	71.81	4.12	80.00	61.00
pos. red. 19-17 (7)	93.96	7.99	69.16	100.00	99.47	2.14	91.46	100.00
pos. red. 21-19 (8)	64.61	3.47	59.83	74.03	73.87	4.57	65.82	82.98
pos. red. 23-21 (9)	57.66	4.25	38.33	63.20	60.98	2.76	56.90	65.67
pos. red. 21 -23 (10)	20.97	2.64	16.30	30.74	18.86	2.61	14.16	23.41

dark brown, longitudinal stripes running along the back and the flanks from the head to the tail. This description identifies his specimen as belonging to the southern Italian species. Although Camerano did not explicitly designate a type specimen, we assume that his type is identical with MZUT 942 (formerly MZUT 434) of the Museo di Zoologia e Anatomia Comparata Torino, which originates from Naples and has a note 'det. Camerano'. We also examined a second specimen mentioned in the same publication (Camerano, 1891), from Serra San Bruno (Calabria) (MZUT 935) in that collection. Consequently, *Callopeltis longissimus* var. *lineata* Camerano, 1891, in the combination *Elaphe*

lineata, is the oldest available name for the southern Italian form, and MZUT 942 represents its holotype.

ELAPHE LINEATA (CAMERANO, 1891) NEW COMB.

Holotype. MZUT 942 (original number: MZUT 434; Fig. 6).

Terra typica. Naples.

Diagnosis. A European rat snake that differs from the most similar species, *E. longissima*, in usually having a light dorsal colour, grey ventral colour, often four dark longitudinal stripes that are narrower than the spaces between them (broader in *longissima*), in high average counts of ventral scales, slightly smaller size than

TABLE 4. The frequency [%] of qualitative character states in both species.

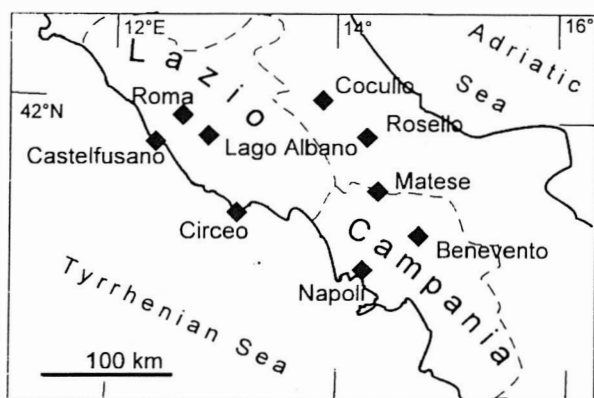| Character states (no.)	*E. longissima*		*E. lineata*	
	females (n = 31)	males (n = 35)	females (n = 16)	males (n = 23)
bright nuchal blotches (11)	89	85	5	0
keeling (12)	16	60	8	37
white dashes (13)	66	65	40	50
venter with marbling (14)	35	0	-	-
ventral transition bright/dark (3)	13	18	94	100
bright dorsal background (4)	0	20	100	91
presence of stripes (5)	32	17	38	48
temporal blotches (6)	39	26	94	91

FIG. 7. Locations of *E. longissima* and *E. lineata* in central Italy mentioned in the text (solid squares).

Elaphe longissima, and in not having bright nuchal blotches (see Table 3 and 4).

Elaphe lineata attains at least 102 cm total body length in females, and 139 cm in males. It is likely that *E. lineata* may considerably exceed this length, as is known *for E. longissima* (Böhme, 1993; Schulz, 1995).

Except for one individual from Modica, Sicily (MZUT 940), nuchal blotches are always absent in *E. lineata*. In *E. longissima*, the blotches (yellow to orange in males, ivory to yellow in females) are usually apparent, except in strongly melanistic individuals. This character is not subject to ontogenetic variation: both juvenile snakes and adults *of E. longissima* exhibit nuchal blotches, whereas juveniles and adults of *E. lineata* do not.

The background dorsal body colour is usually rather light, showing a fine speckling on the scales (Fig. 5). The dorsum often carries four dark longitudinal stripes, which are narrower than the spaces between them on the posterior part of the body. The white dashes on individual dorsal scales, known from *E. longissima,* are less developed in *E. lineata* and usually restricted to the dark stripes. In *E. longissima*, the dorsal ground colour is comparatively dark, especially on the posterior part of the body (Fig. 5). Striped specimens are also frequent in *E. longissima*.

The ventral colour of *E. lineata* is yellowish under the head and neck, and usually uniformly grey under the trunk and tail. Light spots on the lateral edges of the ventrals mark a line that is running along the whole trunk. Only one female from Modica, Sicily, mentioned above, exhibits a totally light venter. In contrast, the ventral colour of *E. longissima* is uniformly yellow, especially in males after ecdysis. The ventral colour of females is pale yellow. Some specimens (particularly females) exhibit grey spots or marbling on the posterior part of the venter, but light colours always predominate on each scale. Predominantly dark ventral scales are very rare. Some specimens from the Caucasus region lack yellow colours but show merged dark and light marbling on their venter in equal proportions on each scale. In melanistic specimens from the Balkans, the posterior two-thirds of the venter is covered by dark

grey pigmentation. However, a few small bright spots of the size of a dorsal scale usually remain visible.

In Capocaccia (1964), the shape of the dark temporal and mandibular blotches is used for discriminating between the two forms. In our specimens from southern Italy, the temporal blotches are usually connected with the posterior submandibular blotches, whereas in *E. longissima* these blotches are usually separated. However, we found that a notable number of *E. longissima* specimens depart from that rule (Table 4).

The number of ventrals in *E. lineata* (mean=232) exceeds that of *E. longissima* (mean=225; see Table 4). The dorsal scale row reduction from 21 to 19 rows is displaced caudally in *E. lineata* compared with *E. longissima*. In males the reduction is located at a ventral scale position corresponding to 67% (mean) of the trunk in *E. lineata* and 62% (mean) in the nominate form. In females, the corresponding values are 73 % (mean) in the southern Italian form and 65 % (mean) in the nominate form.

Finally, living *E. lineata* have a reddish iris (Schulz, 1995), whereas the iris of *E. longissima* is brown or greyish in life. In our personal observations, a red iris colour was never seen in living specimens of the nominate form, ($n \gg 200$), but present in all *E. lineata* ($n=20$).

Juvenile specimens of *E. lineata* can be recognised by the lack of bright nuchal blotches, the reddish iris, and often a marked dark pattern on the pileus. Dark spots are always present on the dorsal parts of the trunk in hatchlings but often disappear during early youth. On the neck, they are fused into several larger spots.

While many characters showed geographic variation, and in particular differentiation between *E. longissima* and *E. lineata*, other characters were chiefly affected by sexual dimorphism, with parallel trends in both species. This was manifested in the relative length of the tail, the number of subcaudals, number of dorsal scale rows, ventral colour (see above) and the extent of keeling (see Tables 3 and 4).

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APPENDIX

The specimens of this study were provided by the following collections:

MTKD Staatliches Museum für Tierkunde, Dresden, MZUF Museo Zoologico de La Specola, Firenze, MZUT Museo Zoologico dell' Università di Torino, MRSN Museo Regionale di Scienze Naturali, Torino, SMF Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt, ZIN Zoological Institute, Russian Academy of Sciences, St. Petersburg, ZFMK Zoologisches Museum und Forschungsinstitut Alexander Koenig, Bonn, BM Museum Natural History. London, LLPC Luiselli, Luca, private collection, LPPC Lenk, Peter, private collection, EONAR Exhibition Oasi Naturale WWF Abetina di Rosello

Elaphe longissima
Adult specimens: *Albania* -Illyria (BM 1920.I.20.466) *Bosnia* -Herzegovina, Capoljina (BM 96.9.5.10) -Travnik (BM 93.II.13.I) *Bulgaria* -?

(MTKD 6177, MTKD 3350, MTKD 15656) -Bistrica (MTKD 28560) -Sandanski-Bistrica, Liljanova (MTKD 25440, MTKD 28285) -Pirin mountains Bansko (MTKD 25491) -Baile Herculane (MTKD 28808, MTKD 29108, MTKD 29107, MTKD 26622, MTKD 28807) -Arkutino (MTKD 27316, MTKD 19535, MTKD 25493, MTKD 19386) -Harmanli (MTKD 6802, MTKD 7114, MTKD 4699, MTKD 4135, MTKD 4698) -Primorsko (MTKD 8855) *Georgia*-Abchasia, Pizunda (ZIN 17070, ZIN 11847, ZIN 17068, ZIN 17069) -Novyy Afon (ZIN 12964) *Greece* -Vernon, Vitsi (MZUF 30862) -Mt. Olympus (BM 1931.12.15.2) *Italy* -Trentino, Caldaro (SMF 70693) - Trentino, Primolano (SMF 70478) -Trentino, Jenesien (SMF 18431, SMF 18432) -Friuli, Udine (LPPC 313) - Lombardia, Mori (MTKD 44259) -Liguria, Albenga/ Allassio (ZFMK 23111) -Liguria, Laigueglia (ZFMK 58155) -Emilia-Romagna, Bologna (RR.1964.1558) - Toscana, Viterbo, Farnese (MZUF 15647) -Umbria, Perugia, Mt. S. Maria Tiberina (MZUF 7960) -Umbria, Perugia, Collazone (MZUF 26601) -Lazio, 10 km south of Roma (LLPC), -Lazio, Monte Circeo, Commune della Marsione (2 x LLPC) -Lazio, Roma, Via Ada (LLPC) -Lazio, Castelfusano (LLPC) -Lazio, 15 km north of Veio (LLPC) -Abruzzo, Pacentro (LPPC 312) - Abruzzo, Rosello (EONAR) *Yugoslavia* -? (MTKD 3146) *Romania*-?(MTKD 32629, MTKD 32626, MTKD 32628, MTKD 32627) *Russia* -Sochi (ZIN 15303, ZIN 11608) *Switzerland* -Ticino, Mte. Brè, Locarno (SMF 44131) -Ticino, Arcegno (SMF 23989) -Ticino, Maggia (SMF 52979, SMF 59280, SMF 48243)

Juveniles: *Italy* Lazio, Lago Albano (BM 1974.4108), -Aquila, Cocullo (MRSN R1317), Toscana, Campagnatico (MZUF 55992)

Elaphe lineata

Adult specimens: *Italy* -Campania, Benevento, Pannarano (MRSN 1362) -Campania, Napoli (ZFMK 5950, ZFMK 23112, ZFMK 5949, ZFMK 5948, MZUT 942) -Campania, Roccarainola (MZUF33017, MZUF 33019, MZUF33005, MZUF33002, MZUF 33018, MZUF 33007, MZUF 32991, MZUF 33016, LPPC295) Campania, Vesuvio (MZUF 33020, MZUF 33021) - Campania, Sarno, Pianta Marina (MZUF32992, MZUF32993) -Campania, Salerno, Laurino (MRSN 1269) -Campania, Salerno Giffoni Valle Piana (MRSN 1422) -Basilicata, Potenza, Viggiano (MZUF 31666) - Basilicata, Potenza, Cazzavella (MZUF 18733) -Calabria, Jozzo, Cardinale (MZUF 31206, MZUF 30859,MZUF 30698, MZUF 31960, MZUF 30699, MZUF30529) -(MZUF 30087) -Calabria, Mellara (MZUF 22498) -Calabria, Delianuova (MZUF 35175) - Calabria, Serra San Bruno (MZUT 935) -Sardegna, Santu Lussurgiu (SMF 65083) -Sicilia, Modica (MZUT 940) -Sicilia, Dioppo Monreale (ZFMK 16557) - Sicilia, Acireale (SMF36390) -Sicilia, Agrigento (SMF 47100) -Sicilia, Palermo (SMF 54226) -S-Italy (ZFMK 52264)

Juveniles: *Italy* Calabria, Novalba di Cardinale (MZUF 30087), -Campania, Avella (MZUF 32995), - Campania, Roccarainola (MZUF 33003, MZUF 33004), Calabria, Delianuova (MZUF 31176).