

Morphometric differences between extant and extinct Italian populations of the adder, *Vipera berus* (Linnaeus, 1758)

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Vipera berus (Linnaeus, 1758) is the terrestrial snake showing the widest distribution in the world, occurring from Great Britain, France and Northern Italy to the Sakhalin Island and North Korea (Nilson, 1980; Saint Girons, 1980; Nilson et al., 1994; Nilson & Andrén, 1997a). However, adders do not occur uniformly over their distribution area, but are scattered in several isolated populations (Nilson & Andrén, 1997a). Frequently, ecological traits of borderline and isolated populations differ from those living in the core area of the distribution range of the species, and might be subjected to higher risks of stochastic extinction and higher differentiation rates (Mayr, 1970). For example, meadow vipers (*Vipera ursinii*) show a highly fragmented distribution, many of isolated groups being different subspecies (Nilson & Andrén, 1997b, 2001).

In Italy *Vipera berus* inhabits the eastern and central Alps, occurring in most of the Po plain until the nineteenth century (Fig. 1, Bruno, 1992; Societas Herpetologica Italica, 1996). The extinction of the Po plain populations might be caused by land reclamation occurred between the end of 18th century and 1930-1940. The progressive heating occurred over the same period (Ghezzi & Riva, 1989; Camuffo, 1990) might have contributed to the extinction of the Po plain adders, favoring more thermophilic species, such as *V. aspis* (Saint Girons, 1975).

Po plain adders inhabited only wetlands and marshlands (Ninni, 1879; Camerano, 1888), while alpine adders regularly frequent dry and rocky habitats, generally above 1000 asl (Nilson & Andrén, 1997a; Lapini et al., 1999; Caldonazzi et al., 2002). Moreover, any historical or contemporary record of this species in the pre-alpine area is known (Fig. 1) (Massalongo, 1854, 1859; De Betta, 1857, 1874, 1880; Lessona, 1877, 1879; Bruno, 1992; Societas Herpetologica Italica, 1996), probably because both climatic regime (Tommaselli et al., 1973) and habitat features of this area do not favour adders. Therefore, Po plain populations might have been geographically isolated from the alpine ones. The isolation of the Po plain population from the main distribution range of the species leads to hypothesize that Po plain adders might belong to a different form or subspecies.

In this work we investigated this hypothesis, looking for differences in morphometric features between Po plain and alpine adders.

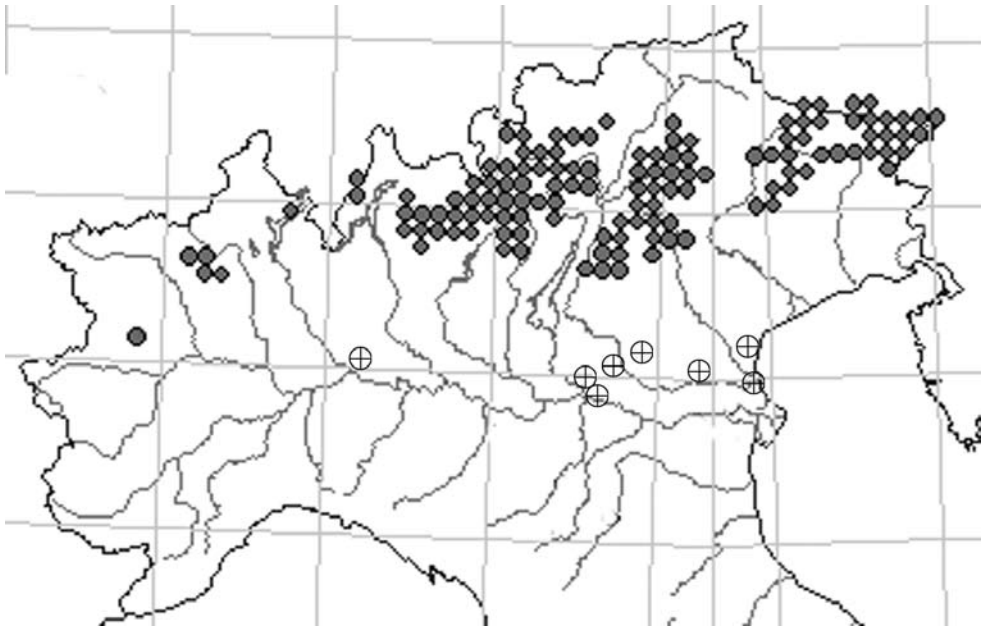


Fig. 1. Distribution of extant (●) and extinct (⊕) *Vipera berus* in Italy; (Bruno, 1992; Societas Herpetologica Italica 1996, modified by the authors).

We collected data for 28 extant and 28 extinct adders from Italian natural history museums ($n = 50$, 89.2% of specimens, see Appendix 1 for details), and literature ($n = 6$, 10.8% of specimens, Camerano 1888; Boulenger 1896). All the extinct adders from the Po plain preserved in Italian museums were considered.

Since adders are sexually dimorphic (Saint Girons, 1978; Scali & Gentilli, 1998), males and females were analysed separately: the Po plain group included 15 males and 13 females, while the alpine one included 12 males and 16 females.

In order to describe head morphology, for each specimen we considered (see Scali & Gentilli, 1998 for details): i) the number of postocular scales, ii) the number of front dorsal head scales, iii) the number of subocular scale rows, iv) head length and width (mm), v) the length and width of the right parietal scale (mm), and vi) length and width of the frontal scale (mm). Since head width correlated with head length (males: $r_s = 0.45$, $n = 22$, $P = 0.034$; females: $r_s = 0.52$, $n = 27$, $P = 0.006$), we replaced the head width by the correspondent unstandardized residuals versus head length. Since the length and width of frontal and parietal scales were intercorrelated (r_s ranging from 0.48 to 0.88 for males and from 0.42 to 0.69 for females), we used a principal component analysis (PCA) to reduce them to a “head scale size” component. For both sexes, PCA extracted only one component (eigenvalue > 1), which accounted for 77.2% and 59.5% of the cumulative variance for males and females respectively, and was positively related to each one of the head scale measures (all variables entered PCA with component loadings higher than 0.75 for males

and 0.63 for females). The scores of this “head scale size” component for each adder were used to examine if the size of head scales differed between extinct and extant adders.

In order to describe body morphology, we considered body length (from the head tip to the vent, mm) and the number of ventral scales. Since these variables were highly correlated both in males ($r_s = 0.60$, $n = 21$, $P = 0.004$) and females ($r_s = 0.37$, $n = 27$, $P = 0.05$), we used a principal component analysis to reduce them to a single “body morphology” component. For both males and females, PCA extracted only one component (eigenvalue >1), which accounted for 69.2% and 71.5% of the cumulative variance for males and females respectively, and was positively related both to the body length and to the number of ventral scales (the two variables entered PCA with component loading higher than 0.83 for both sexes). The scores of this “body morphology” component for each adder were used to examine if the body size differed between extinct and extant adders.

Finally, tail features were measured for each specimen using the unstandardized residuals of the tail length versus body length, and the number of subcaudal scales. Since these variables were highly correlated (males: $r_s = 0.46$, $n = 20$, $P = 0.039$; females: $r_s = 0.82$, $n = 27$, $P < 0.001$), we used a principal component analysis to reduce them to a single “tail morphology” component. For both males and females, PCA extracted only one component (eigenvalue >1), which accounted for 71.3% and 94.2% of the cumulative variance for males and females respectively, and was positively related either to the residuals of tail length as to the number of subcaudal scales (the two variables entered PCA with component loadings higher than 0.84 for males and 0.97 for females). The scores of this “tail morphology” component for each adder were used to investigate if tail features differed between extinct and extant adders.

We could not collect all measures from each specimens because their non optimal conservation, so sample sizes varied in different analyses (Tab. 1).

Correlation Analysis (Spearman’s rho coefficient) was used to investigate relationships among variables. We used the Bonferroni correction for multiple tests to adjust the observed significance level to the number of comparisons (Rice, 1989): in a set of K simultaneous comparisons, a given test was considered to be statistically significant at 0.05 level only if it was significant also at the $0.05/K$ level. We used Discriminant Function Analysis (DFA, stepwise forward method) using all morphometric measures as independent variables and collecting area (Alps vs Po plain) as classifying factor, in order to determine i) if Po plain (extinct) and alpine (extant) adders differed on the basis of their morphological features, and ii) what morphological features, if any, accounted for such a difference. All analyses were performed using SPSS rel. 6.1.2 software package (1996).

Head, body, and tail variables were not intercorrelated after Bonferroni correction ($K = 28$; $P < 0.00178$ for relationships to be significant).

Discriminant function analysis for males generated a high significant discriminant function (19.9, $P < 0.0001$, $\lambda = 0.26$), which correctly classified 90% of specimens. The stepwise procedure extracted only two variables: the number of postocular scales and the tail morphology. The males of Po plain adders had more postocular and subcaudal scales and longer tails than alpine ones (Tab. 1).

Similarly, DFA for females generated a highly significant discriminant function (27.6, $P < 0.0001$, $\lambda = 0.15$), which correctly classified 95.6% of specimens. The stepwise procedure extracted three variables: the number of postocular scales, the tail morphology, and the

Table 1. Morphometric measures of Po plain and alpine adders.

| | Males | | | | | | | | | | | | Females | | | | | | | | | | | | |
|------------------------------|----------|-------|-------|-------|-------|----|-------|-------|-------|-------|----|-------|----------|-------|-------|----|-------|-------|-------|-------|----|-------|-------|-------|-------|
| | Po plain | | | | | | Alps | | | | | | Po plain | | | | | | Alps | | | | | | |
| | n | Min | Max | Mean | SD | n | Min | Max | Mean | SD | n | Min | Max | Mean | SD | n | Min | Max | Mean | SD | n | Min | Max | Mean | SD |
| Head morphology | | | | | | | | | | | | | | | | | | | | | | | | | |
| Post ocular scales (n) | 11 | 3 | 5 | 3.7 | 0.8 | 12 | 2 | 4 | 2.8 | 0.6 | 9 | 3 | 5 | 3.9 | 0.8 | 15 | 2 | 4 | 3.2 | 0.6 | 15 | 2 | 4 | 3.2 | 0.6 |
| Front dorsal head scales (n) | 8 | 7 | 13 | 10.5 | 2.2 | 12 | 6 | 22 | 11.6 | 5.2 | 11 | 11 | 30 | 17.6 | 5.6 | 15 | 5 | 27 | 14.5 | 5.0 | 15 | 5 | 27 | 14.5 | 5.0 |
| Subocular rows (n) | 11 | 1 | 2 | 1.2 | 0.4 | 12 | 1 | 2 | 1.1 | 0.3 | 13 | 1 | 2 | 1.6 | 0.5 | 16 | 1 | 2 | 1.2 | 0.4 | 16 | 1 | 2 | 1.2 | 0.4 |
| Head width (mm) | 10 | 10.7 | 15.9 | 13.6 | 1.74 | 12 | 9.2 | 15.3 | 12.8 | 1.78 | 13 | 9.2 | 19.0 | 14.0 | 2.9 | 14 | 11.8 | 18.2 | 14.9 | 1.91 | 14 | 11.8 | 18.2 | 14.9 | 1.91 |
| Head length (mm) | 10 | 19.3 | 25.8 | 22.5 | 1.91 | 12 | 16.2 | 29.2 | 21.5 | 3.58 | 13 | 15.3 | 34.6 | 24.1 | 4.8 | 14 | 18.1 | 28.0 | 23.2 | 2.44 | 14 | 18.1 | 28.0 | 23.2 | 2.44 |
| Parietal scale length (mm) | 10 | 3.5 | 6.2 | 4.8 | 0.84 | 12 | 3.0 | 4.8 | 3.9 | 0.62 | 10 | 2.6 | 5.2 | 3.9 | 0.8 | 13 | 2.7 | 5.5 | 4.4 | 0.84 | 13 | 2.7 | 5.5 | 4.4 | 0.84 |
| Parietal scale width (mm) | 10 | 2.2 | 3.3 | 2.6 | 0.38 | 12 | 1.6 | 2.9 | 2.2 | 0.40 | 10 | 1.6 | 3.0 | 2.2 | 0.4 | 13 | 1.7 | 3.3 | 2.5 | 0.47 | 13 | 1.7 | 3.3 | 2.5 | 0.47 |
| Frontal scale length (mm) | 10 | 3.7 | 5.7 | 4.6 | 0.70 | 12 | 2.4 | 4.6 | 3.6 | 0.71 | 8 | 2.7 | 4.5 | 3.7 | 0.5 | 15 | 2.7 | 5.7 | 4.6 | 0.79 | 15 | 2.7 | 5.7 | 4.6 | 0.79 |
| Frontal scale width (mm) | 10 | 2.6 | 3.7 | 3.3 | 0.36 | 12 | 1.8 | 4.6 | 3.0 | 0.78 | 8 | 2.3 | 3.7 | 2.9 | 0.4 | 15 | 2.7 | 3.6 | 3.1 | 0.29 | 15 | 2.7 | 3.6 | 3.1 | 0.29 |
| Body morphology | | | | | | | | | | | | | | | | | | | | | | | | | |
| Body length (mm) | 10 | 311.4 | 536.0 | 437.0 | 67.27 | 12 | 166.8 | 412.6 | 326.1 | 85.23 | 13 | 173.7 | 690.0 | 420.9 | 133.8 | 15 | 306.9 | 532.0 | 436.1 | 56.33 | 15 | 306.9 | 532.0 | 436.1 | 56.33 |
| Ventral scales (n) | 15 | 136 | 148 | 142.1 | 3.72 | 12 | 136 | 149 | 141.0 | 3.63 | 13 | 140 | 154 | 145.9 | 4.6 | 16 | 138 | 155 | 146.4 | 5.20 | 16 | 138 | 155 | 146.4 | 5.20 |
| Tail morphology | | | | | | | | | | | | | | | | | | | | | | | | | |
| Tail length (mm) | 10 | 44.0 | 92.0 | 76.3 | 15.78 | 11 | 30.0 | 74.0 | 56.0 | 15.31 | 13 | 28.0 | 100.0 | 56.6 | 20.4 | 16 | 34.0 | 58.0 | 49.3 | 7.07 | 16 | 34.0 | 58.0 | 49.3 | 7.07 |
| Subcaudal scales (n) | 14 | 35 | 47 | 41.5 | 3.50 | 11 | 33 | 41 | 36.8 | 2.66 | 12 | 29 | 42 | 33.8 | 3.7 | 16 | 18 | 31 | 26.8 | 3.49 | 16 | 18 | 31 | 26.8 | 3.49 |

number of subocular scale rows. The females of Po plain adders had more postocular scales, more subocular scale rows, more subcaudal scales, and tails longer than alpine ones (Tab. 1).

In conclusion, our results showed morphological differences between Po plain and alpine adders, supporting our hypothesis that Po plain and alpine populations of this species might be considered two different ecotypes or subspecies. However, our morphological analyses have to be considered only a preliminary step, and genetic analyses are needed to better clarify the systematic position of Po plain adders. Further analyses would also check for relationships between Po plain adders and *V. b. bosniensis*, since their distribution range might overlap in the coastal wetlands of North Adriatic.

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Appendix 1. List of specimens.

| Museum | Collection code |
|--|--|
| Museo Civico di Storia Naturale (Milano) | 1052; 1529; 1530; 1531; 1532; 1533; 1534; 1535; 1536; 1578A; 1578B; 1591; 1592; 1623; 3286; 3579; 3749; 3758; 3760 |
| Museo Civico di Storia Naturale (Morbegno) | 3; 4; 26; 29; 41; 65; 67; 1f Unknown number |
| Museo Regionale di Scienze Naturali (Torino) | R283; R522 |
| Museo Civico di Storia Naturale (Verona) | 866; 867; 868 (2 specimens); 869 (2 specimens); 870; 872; 873a; 873b; 873c; 873d; 887 (2 specimens); 1330 |
| Museo Zoologico "G. Scarpa" (Treviso) | 2mm Unknown numbers |
| Museo Zoologico dell'Università (Firenze) | 12062; 12101-02 |
| Museo Zoologico dell'Università (Pavia) | 230/476; 230/477 |