Pergamon

Zoologica Scripta, Vol. 23, No. 4, pp. 353–360, 1994
Elsevier Science Ltd

0300–3256/94 $7.00+0.00

0300-3256(94)00016-6

# Phylogeny of the vipers of the Caucasus (Reptilia, Viperidae)

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*Accepted* 14 *June* 1994

Nilson, G., Höggren, M., Tuniyev, B. S., Orlov, N. L. & Andrén, C. 1993. Phylogeny of the vipers of the Caucasus (Reptilia, Viperidae).—*Zool. Scr. 23:* 353–360.

Phylogenetic relationships for five taxa of Palearctic vipers (genus *Vipera*) from the Caucasian region were revealed by cladistic analyses of separate and combined morphological and biochemical characters. The different data sets yielded largely congruent cladograms. *Vipera berus* from Sweden was included as an ingroup and *V. aspis* was used for outgroup comparison. For *V. kaznakovi* and *V. dinniki*, three and four different sub-populations, respectively, were treated as independent terminal taxa in the analyses. The most parsimonious cladograms confirmed the systematic positions of these populations, discussed in a recent study, and support the hypothesis that the montane populations of the western main Caucasus comprise one polymorphic species: *V. dinniki*.

Analyses of combined biochemical and morphological data generated two equally parsimonious cladograms (for all ingroups compared), but yielded only one fully resolved topology when ingroups were condensed to the species level: (*berus* ((*renardi* ('*ursinii*'-*eriwanensis*)))(*dinniki*-*kaznakovi*)).

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## Introduction

The Caucasian vipers, the *Vipera kaznakovi* complex, has currently been the subject of much study (Orlov & Tuniev 1986, 1990) in attempts to clarify its taxonomy and phylogeny. Recently the existence of two Caucasian subalpine and partly sympatric species, *Vipera dinniki* and *V. 'ursinii'*, has been demonstrated, but the picture is complicated by a varying degree of polymorphism in several populations (Nilson *et al.* 1994). At lower altitudes in the moist, western parts of the Caucasus these mountain/subalpine taxa are replaced by populations of *V. kaznakovi*. To the north they are replaced by *V. renardi* and to the south by *V.* (*ursinii*) *eriwanensis* in dry steppe habitats. Morphological similarity and divergence among some of these putative species might either be correlated with microhabitat or be a result of their phylogenetic history. The relationship between phenotypic and genotypic evolution remains poorly understood in this species complex.

Allozyme analysis has proven to be a highly useful technique when facing questions of systematics at levels ranging from populations and above within a genus (e.g. Hillis & Moritz 1990), which is the case with this study. Allozyme data can yield estimates of genetic fragmentation within species, but allozymic divergence is not necessarily coupled with morphological evolution. Thereby allozyme and morphological data permit independent assessments of phylogenetic relationships (e.g. Crother *et al.* 1992).

This study of allozyme evolution at different taxonomic levels concerns several populations of the polymorphic and subalpine *V. dinniki* and of the lowland yellow viper *V. kaznakovi* in the Caucasian region. In addition, samples from different populations of subspecies and species within the *V. ursinii* complex and genus *Vipera* in general are included.

All species involved in this study are closely related to taxa elsewhere in Europe and adjacent Asia and which are not included here. Thereby the study merely represents a phylogeny of the vipers occurring in this geographical region, and our main goal is to elucidate the taxonomy of these vipers through the analysis of their phylogeny.

## Material and methods

### *Biochemical data*

Seventy-nine specimens representing different populations of the *Vipera kaznakovi* and *V. ursinii* complexes in the Caucasus, and *V. berus* from Sweden were examined (see Table I for taxa, localities and sample sizes). *Vipera aspis* was used as a taxonomic outgroup. Fresh or frozen

Table I. *Number of specimens used in the genetic analyses and localities for the examined taxa*

1. '*dinniki*2' (DI2): lake Kardyvach—the eastern border of the Park, close to Mt Loyub but a little further down the Mzymta river. Also situated on the southern slope of the main range. 1850 m alt. Seventeen specimens.
2. '*dinniki*3' (DI3): lake Impsi—at 1980 m alt., situated at river Tsahvoa, a tributary to river Little Laba. The locality is mainly on the slopes of range Damhorts at the northern part of the Park, situated on the northern slope of the main range. Seven specimens.
3. *dinniki*4' (DI4): Aishkha-II—in the same mountain system, further to the west, and situated on the southern slope of the main range. Three specimens.
4. '*dinniki*1' (DI1): Fisht/Oshten—The westernmost high mountain area of the main Caucasus range, and of the Park, and characterized by the peaks Mt Fisht (2868 m alt.) and Mt Oshten (2808 m alt.). This region is separated and isolated from the main range (with Mt Chugush 3226 m alt.) by the forested, moist and warm 'Colchis Gate' at low altitude. Seven specimens.
5. '*kaznakovi*1' (KA1): Dagomys, north of Sochi (Russia) is close to the northernmost part of the range for this species. Close to sealevel. Six specimens.
6. '*kaznakovi*3' (KA3): Hopa, prov. Artwin (Turkey) is situated at the southwestern extreme of the species range. Nine specimens. Near sealevel.
7. '*kaznakovi*2' (KA2): Rudorova, is an eastern inland locality at about 900 m altitude and situated on the border between the deciduous forest (lower forest belt) and the coniferous forest (*Abies nordmaniana*) that separates *kaznakovi* and *dinniki*. Four specimens.
8. '*ursinii*' (URS) of Caucasus, taxonomically discussed further by Nilson *et al.* (1994), are from Armkhi, Checheno-Ingushetia on the northern slopes of central Caucasus. 2000 m alt. Seven specimens.
9. *eriwanensis* (ERI): pooled sample originating from two localities in the Kars province of eastern Turkey: Asbua and Cildir, and one locality in Armenia, at Sevan. Around 1000 m alt. Six specimens.
10. *renardi* (REN): Dnjepr river valley of Ukraine. One specimen.
11. *berus* (BER): Uppsala (terra typica), Sweden, included as in group. Eleven specimens.
12. *aspis* (ASP): Switzerland, Maggia valley, representing a mountain population (*V. aspis atra*). Single specimen.

The *V. dinniki* localities (1–4) are all situated in the National Park: Western Caucasus Biosphere Reserve. The *kaznakovi* samples (5–7) originate from three different corners of its known range. The *ursinii* (s.lat.) samples (8–10) originate from three different regions and recognized taxa within this complex. The outgroup consists of one taxon.

Table II. *Enzymes and electrophoretic conditions of the polymorphic loci scored in this study. Nomenclature and commission numbers follow the International Union of Biochemistry, Nomenclature Committee (1984). Abbreviations for tissue sources are: L = liver and M = skeletal muscle*

| Enzyme | Commission number | Locus | Tissue | Buffer system | Reference |
|---|---|---|---|---|---|
| Alcohol dehydrogenase | 1.1.1.1 | Adh-1 | L | B | 1 |
| Esterase | 3.1.1.1 | Est-1 | M | A | 1 |
| Glucose-6-phosphate isomerase | 5.3.1.9 | Gpi-1 | M,L | B | 5 |
| Hexokinase | 2.7.1.1 | Hk-1 | L | B | 1,3,4 |
| Isocitrate dehydrogenase | 1.1.1.42 | Idh-1 | L | B | 1 |
| | | Idh-2 | L | B | |
| L-Lactate dehydrogenase | 1.1.1.27 | Ldh-2 | M,L | B | 3 |
| Phosphoglucomutase | 5.4.2.2 | Pgm-2 | L | B | 1 |
| Superoxide dismutase | 1.15.1.1 | Sod-1 | L | A | 1,2 |

(1) Harris and Hopkinson (1976)
(2) Johnson *et al.* (1970)
(3) Shaw and Prasad (1970)
(4) Murphy *et al.* (1990)
(5) De Lorenzo and Ruddle (1969)
(A) Tris-citrate/lithium hydroxide, boric acid, pH 8.0 10 V/cm, 4 h (Ridgway *et al.* 1970)
(B) *N*-(3-amino-propyl)morpholine/citrate, pH 6.1 10 V/cm, 6 h (Clayton and Tretiak, 1972)

tissues (−75°C) from liver and skeletal muscle were homogenized in distilled water. The extracts were centrifuged for 10 min at 10,000 rpm and 4°C and the supernatants were then stored at −75°C until used. Standard horizontal starch gel electrophoresis was carried out, as described by Harris & Hopkinson (1976) and Murphy *et al.* (1990). Enzymes, tissues, modified electrophoretic conditions and staining references are listed in Table II. Twenty-seven presumptive gene loci from 14 enzyme systems and one general protein were scored for inter- and intraspecific variation. Thirteen of the loci assayed were polymorphic and allelic products (allozymes) for each locus were designated in order of increasing anodal mobility (Table III).

*Morphological data*

More than 200 preserved or live specimens of vipers from the Caucasus and adjacent regions have been used in this study (see Nilson *et al.* 1994) for analysis of phylogenetically informative characters. Additionally large series of *Vipera berus* and *V. aspis*, as well as several other taxa within the genus from different localities of their ranges, were used in outgroup comparison. Twenty-two morphological characters of scalation and colour-pattern were used (Table IV) and coded into discrete states for phylogenetic analyses (Table V).

*Character coding and phylogenetic analyses*

Allozymes and morphological characters were qualitatively coded into discrete states for analysis in the two computer programs PAUP (Phylogenetic Analysis Using Parsimony, version 3.0) (Swofford 1991) and MacClade (Analysis of phylogeny and character evolution, version 3.0) (Maddison & Maddison, 1992). For allozymes, the locus was considered the character, with alleles as the character states. This procedure is more in agreement with the assumption of independence among characters than the method of using the allele as a character and its presence/absence as a binary state (e.g. Mickevich & Mitter 1981; Buth 1984; Swofford & Olsen 1990). With a few exceptions the molecular multistate characters were coded as unordered (Fitch 1971). For certain characters with comparatively high numbers of states (e.g. Sod-1, N = 6) and where allelic variation was partially shared between terminal taxa, ordering of the states was applied using the step matrix option of MacClade, with transformation series from the outgroup allelic states e.g. 100 <> 100/105 <> 105. This arrangement allows allelic combinations to be included in the analysis as potentially informative states (but see Mabee & Humphries (1993) for a comprehensive review of coding polymorphic data in phylogenetic analysis). It should be pointed out that the above procedure does not treat differences in relative mobility of electro-

Table III. *Allele frequencies of polymorphic loci (see Table II for locus abbreviations). For taxon abbreviations, sample size and localities, see Table I*

| Locus | Allele | KA1 | KA2 | KA3 | DI1 | DI2 | DI3 | DI4 | URS | ERI | REN | BER | ASP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Adh-1 | −100 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | — | — |
| | −110 | — | — | — | — | — | — | — | — | — | — | 1.00 | 1.00 |
| Aat-1 | −100 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | — |
| | −110 | — | — | — | — | — | — | — | — | — | — | — | 1.00 |
| Aat-2 | 100 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | — |
| | 150 | — | — | — | — | — | — | — | — | — | — | — | 1.00 |
| Est-1 | 100 | 1.00 | 1.00 | — | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | — |
| | 80 | — | — | 1.00 | — | — | — | — | — | — | — | — | — |
| | 105 | — | — | — | — | — | — | — | — | — | — | — | 1.00 |
| Est-2 | 100 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | — |
| | 105 | — | — | — | — | — | — | — | — | — | — | — | 1.00 |
| Gp-1 | 100 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | — |
| | 95 | — | — | — | — | — | — | — | — | — | — | — | 1.00 |
| Gpi-1 | −100 | 0.75 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | — | — | — | — | — |
| | −50 | 0.25 | — | — | — | — | — | — | 1.00 | 1.00 | — | — | — |
| | −75 | — | — | — | — | — | — | — | — | — | 1.00 | 1.00 | 1.00 |
| Hk-1 | −100 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | — | — |
| | −120 | — | — | — | — | — | — | — | — | — | — | 1.00 | 1.00 |
| Idh-1 | −100 | 1.00 | 1.00 | 1.00 | 0.93 | 0.86 | 0.83 | 0.97 | 1.00 | 1.00 | 1.00 | — | — |
| | −10 | — | — | — | 0.07 | 0.14 | 0.17 | 0.03 | — | — | — | — | — |
| | −130 | — | — | — | — | — | — | — | — | — | — | 1.00 | 1.00 |
| Idh-2 | 100 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | — | 1.00 | 1.00 | 1.00 |
| | 130 | — | — | — | — | — | — | — | — | 0.10 | — | — | — |
| | 180 | — | — | — | — | — | — | — | — | 0.90 | — | — | — |
| Ldh-2 | 100 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.77 | — |
| | 120 | — | — | — | — | — | — | — | — | — | — | 0.23 | — |
| Pgm-2 | 100 | 1.00 | 1.00 | 1.00 | 0.71 | 1.00 | 1.00 | 0.88 | 1.00 | 1.00 | 1.00 | — | — |
| | 103 | — | — | — | — | — | — | — | — | — | — | 1.00 | 1.00 |
| | 105 | — | — | — | 0.29 | — | — | 0.12 | — | — | — | — | — |
| Sod-1 | 100 | — | — | — | 0.71 | 0.21 | 1.00 | 0.71 | — | — | — | — | 1.00 |
| | 33 | 0.50 | 0.12 | 0.39 | 0.29 | 0.79 | — | 0.29 | 1.00 | 1.00 | — | — | — |
| | 40 | — | — | — | — | — | — | — | — | — | 1.00 | — | — |
| | 95 | 0.50 | 0.88 | 0.61 | — | — | — | — | — | — | — | — | — |
| | 103 | — | — | — | — | — | — | — | — | — | — | 1.00 | — |

morphs as evolutionary directionality, following Patton & Avise (1983) who rejected attempts to simply interpret net charge differences of allelic products as phylogenetically informative transformation series. No further *a priori* assumptions of polarity or weighting of the molecular data set were made.

The 22 morphological characters were coded into ordered binary states. The branch-and-bound algorithm in PAUP was employed to obtain the most parsimonious cladogram(s). A 1000-replicate bootstrap analysis (Felsenstein 1985; Sanderson 1989) was conducted to estimate confidence limits for each node, presumably indicating topological accuracy of the best-fit tree(s) although its validity and utility have been subject to considerable controversy in recent theoretical systematic literature (see Felsenstein & Kishino 1993; Hillis & Bull 1993; Jones *et al.* 1993).

Awaiting a more robust resampling technique to be developed, we have still chosen to use bootstrapping as a measure of relative confidence for positions of terminal taxa in a given tree. Skewed tree length distribution provides a measure of systematic information within a data set, with strength of phylogenetic 'signal' thought to correlate with the degree of negative (left-tailed) skewness among sampled trees (Fitch 1979; Hillis & Huelsenbeck 1992; but see Källersjö *et al.* 1992, for an alternative test). In order to assess the phylogenetic structure in our data, the random trees option in MacClade was used to draw 10,000 trees from all possible bifurcating tree topologies of the combined data set.

Morphological data was used as in Table IV. Details about methods of counting are presented in Nilson & Andrén (1986).

*Comments on the morphology and polarity of morphological characters (Table IV)*

(1) Low numbers of dorsal scale rows have currently been looked upon as primitive states of characters in *Vipera* (e.g. Kramer 1961; Saint Girons 1978). But numbers below those generally occurring in related groups of snakes are also considered as apomorphic states, e.g. Marx & Rabb (1970, 1972). The degree of dorsal scale reduction varies extensively between different populations. The midbody scale row number has consistently been used as an important character. In *renardi* dorsal scale reduction from 21 to 19 dorsal scale rows is usually situated on the last third of the body, which results in 21 midbody scale rows. In '*ursinii*' the reduction from 21 to 19 scale rows is more anterior on the body, which in turn results in a lower midbody scale row number (20.6). In the geographically disjunct but morphologically similar *Vipera dinniki* populations the reduction is meanwhile more posterior (X = 21.8 in the Fisht population). A more anterior scale row reduction might be derived.

(2) Nine dorsal head shields is the normal state within Colubroidea. Species or groups with fragmented shields as well as taxa with secondarily united shields are meanwhile considered as derived (Marx & Rabb 1970).

(3) The gold edged iris seen in live specimens of *Vipera kaznakovi* and *V. dinniki* is unique within *Vipera*. In other species, like *V. berus*, this colour pattern of iris is either lacking or sometimes expressed as a weaker light edge of the pupil.

(4) In *Vipera berus*, as well as, in almost every taxon of Palearctic vipers, the dorsal pattern is separated from the head pattern. The fusion of these two patterns in *V. kaznakovi* and *V. dinniki* is therefore probably a unique event within *Vipera*.

(5) A higher number of supralabials is in accordance with a series of other derived character states in the *ursinii* scalation pattern and can be considered apomorphic (fulfils the third criterion for derivativeness '=correlation of derived states' of Marx & Rabb 1972). *Vipera ursinii* has in general eight supralabials while the *renardi* group has nine. Is eight a plesiomorphy and nine derived, as generally believed, or is the reverse true? Eight to nine is the normal state in Viperidae and in Colubroidea in general. Increasing numbers of supralabials in *Vipera* is an apomorphic state (Nilson & Andrén 1986) and we believe that a much reduced number must also be considered as an apomorphic state in opposite direction.

(6) Low numbers of ventrals have currently been looked upon as primitive states of characters in *Vipera* (e.g. Kramer 1961; Saint Girons 1978). Low numbers below those generally occurring in related groups of snakes are also considered as apomorphic states, e.g. Marx & Rabb (1970, 1972).

(7) Typical for the genus as a whole is a dorsal pattern of zig-zag windings, dorsal blotches or transverse bands. The bronze morph seen in some of the investigated populations is considered as derived, although it can be suspected to be a homoplasy. Outside the group studied here it frequently occurs in *Vipera latifii* and *Vipera seoanei seoanei* (Nilson & Andrén 1986; Bea *et al.* 1984) and has been observed as a very rare event in e.g. *V. aspis* and *V. berus* (own observations).

(8) However, fragmentation of certain scales can be a secondary effect of temperature or stress in certain species (e.g. Fox *et al.* 1961) and

Table IV. *Polarity of morphological characters in the vipers of Caucasus*

| No. | Character | Coding | |
|---|---|---|---|
| | | 0 | 1 |
| 1 | Midbody scale rows | Many | Few |
| 2 | Intercanthals and intersupraoculars | Few | Numerous |
| 3 | Iris gold-edged in life | No | Yes |
| 4 | Neck-head pattern united | No | Yes |
| 5 | Supralabials | 9 or less | Usually more |
| 6 | Number of ventrals | Large number | Low number |
| 7 | Dorsal pattern | Zig-zag type | Incl. bronze |
| 8 | Parietals | Few, symetr. | Fragmented |
| 9 | Frontal | Large, regular plate | Divided irregular plate |
| 10 | Colour-pattern | Mainly monomorphic | Mainly polymorphic |
| 11 | Sublabials | Usually less than 11 | Usually more |
| 12 | Scale rows on neck | Many | Few |
| 13 | Rostral shape | Squarish | Raised rectangular |
| 14 | Apicals | Two | One |
| 15 | Circumoculars | Few | More |
| 16 | Loreals | Few | Many |
| 17 | Head shape | Normal | Broader |
| 18 | Preocular enlarged | Short | Reaches nasal |
| 19 | Ventral ground colour | Black | White |
| 20 | Canthus shape | Rounded | Sharp, raised |
| 21 | Crown spot | Absent | Present |
| 22 | Number of blotches | Few | Many |

care is necessary when selecting characters and states that are appropriate to measure phylogenetic history. Marx & Rabb (*op. cit.*) state that *V. berus* has the most plesiomorphic state by having large paired parietals. *Vipera ursinii* is considered more derived in this character as the parietals were fragmented into smaller scales in Marx & Rabb's (*op. cit.*) material of *ursinii*.

(9) An increasing degree of fragmentation of the large dorsal head shields is possibly a derived state, as is an irregularly divided frontal.

(10) Some populations show a remarkable polymorphism in colour-pattern characteristics. Hypotheses for this have been discussed in our previous study (Nilson *et al.* 1994) and the polymorphism has been considered to be of young origin in this case. For this reason, the polymorphism is thought to be derived.

(11) A similar argument given for the supralabials can be applied to the sublabials. Low numbers of sublabials have currently been looked upon as primitive states of characters in *Vipera* (e.g. Kramer 1961; Saint Girons 1978). Low numbers below those generally occurring in related groups of snakes are also considered as apomorphic states, e.g. Marx & Rabb (1970, 1972). For this group we consider an increasing number as derived.

(12) All the examined populations have 21 dorsal scale rows on the neck (one head length posterior to head). On the contrary all the European mountain populations of *V. ursinii* have 19 dorsal scale rows on the neck, and in these snakes the reduction from 21 to 19 scale rows takes place immediately behind the head. The Middle East subalpine populations of *ursinii* s.l. in Iran and Armenia normally have 21 dorsal midbody scale rows as the scale reduction also in these populations is behind the midbody. The equally subalpine *dinniki* populations again often exhibit a lower dorsal scale count on the neck. On the contrary, the lowland *kaznakovi* shows higher values (e.g. X = 22.1 at Dagomys and 23 at Rudorova). In subalpine populations there seems to be selection for a reduction of dorsal scale rows as the zone of reduction is relatively forward on the body in such small specimens. Perhaps this is in concordance with size reduction as many of these mountain taxa consist of small specimens. A reduced number of scale rows seems to be derived.

(13) The squarish rostral plate is a normal pattern within *Vipera* while the raised rectangular one is a more rare state. For the '*Rhinaspis*' group within *Vipera* (*V. aspis, V. ammodytes, V. latastii, V. monticola*) it must be considered as derived, and this may also be true in our study group of taxa.

(14) A unique character for *V. ursinii* is its single apical plate, and a discussion of whether or not *V. ursinii* is a 'primitive' taxon cannot be undertaken without evaluating its degree of derivativeness. An outgroup comparison with Colubridae or with any other viperid shows that a single apical plate is a unique character which only rarely occurs in other (mainly tropical) colubroid species. The normal state among colubrids is two apical shields in contact with the rostral, as is also the case in *V. berus*, and this state is considered here as the most original state within *Vipera*. A single apical is hence derived.

(15) A similar argumentation as was given for the supra- and sublabials can be applied for the circumoculars. An increased number is considered as derived.

(16) As is the situation with most of the other head scalation characteristics, a high number of loreals is considered as derived in this group of vipers, albeit a number below a typical number could as well be considered as apomorphic. For example, in some European alpine taxa of *V. ursinii* (in manuscript), a reduced number of loreals compared with *V. berus* and other taxa might be considered as derived.

(17) *Vipera kaznakovi* is unique in having a pronounced broader head. This differs from all other examined taxa as well as from the outgroup.

(18) The large upper preocular is in contact with the nasal, and it also could be looked upon as a derived state. This is a unique character for *V. ursinii*, comparable with the single apical plate. In other head scalation characteristics, high numbers have normally been considered as derived states, but in these two cases of head scalation we believe that the rare state of a single shield is an apomorphy. See also the discussion under 'apicals' (point 14).

(19) There is a general pattern of lowland populations having black bellies while subalpine ones are whitish in the *ursinii* complex. There are white-bellied mountain *ursinii* populations from China in the east to France in the west, and most of these have their closest relatives in adjacent lowland black-bellied *ursinii* populations. We therefore believe that pale belly colouration has arisen on several occasions and, albeit being a homoplasy, we consider it as derived.

(20) A sharp canthus rostralis due to a concave upper snout is a unique event and considered as a derived trait. It is absent in *V. berus*.

(21) The crown spot is a unique event for the *ursinii* complex.

(22) A high fragmentation of the dorsal pattern; high number of blotches, transverse bands or zig-zag windings (i.e. around 70 or more) is believed to be derived.

## Results

Of 27 presumptive protein loci scored, 13 were found to be polymorphic. Analysis of biochemical data produced 13 equally parsimonious cladograms, each with a consistency index (CI; Kluge & Farris 1969) of 0.95 (autapomorphies excluded) and a length of 24 steps. Analysis of morphological characters gave three equally parsimonious cladograms (CI = 0.51, 45 steps). Consensus trees of biochemical and morphological cladograms are shown in Fig. 1. The consensus trees illustrated should merely be looked upon as a comparison at large of topologies based on two data sets, since several soft polytomies arise when

Table V. *Combined character matrix of 13 biochemical (multistate) characters (1–13) and 22 morphological (binary) characters (14–35) used in phylogenetic analyses of the Caucasus vipers. The order of allozymes corresponds to Table III and of morphological data to Table IV*

| Samples | Allozymes | Morphology |
|---|---|---|
| *kaznakovi*1 | BADACAAAAAAAA | 0011010100110011100100 |
| *kaznakovi*2 | BADADAAAAAAAA | 0111110101110011000101 |
| *kaznakovi*3 | AADADAAAAAAAA | 0111010110100010100100 |
| *dinniki*1 | BABBDBAAAAAAA | 0011010001010000000000 |
| *dinniki*2 | BABBDBAAAAAAA | 0011011001000000000001 |
| *dinniki*3 | BABADBAAAAAAA | 0011011101100000000001 |
| *dinniki*4 | BAAADBAAAAAAA | 0111010001100100000001 |
| *"ursinii"* | BACAAAAAAAAAA | 1000001000000100011111 |
| *renardi* | BAEABAAAAAAAA | 0000000100100100010110 |
| *eriwanensis* | BACAAABAAAAAA | 0000100000001100001011 |
| *berus* | BAFCBCABAAABB | 0100000000100000000001 |
| *aspis* | CBACBCACBBBBB | 0100000111101001100101 |

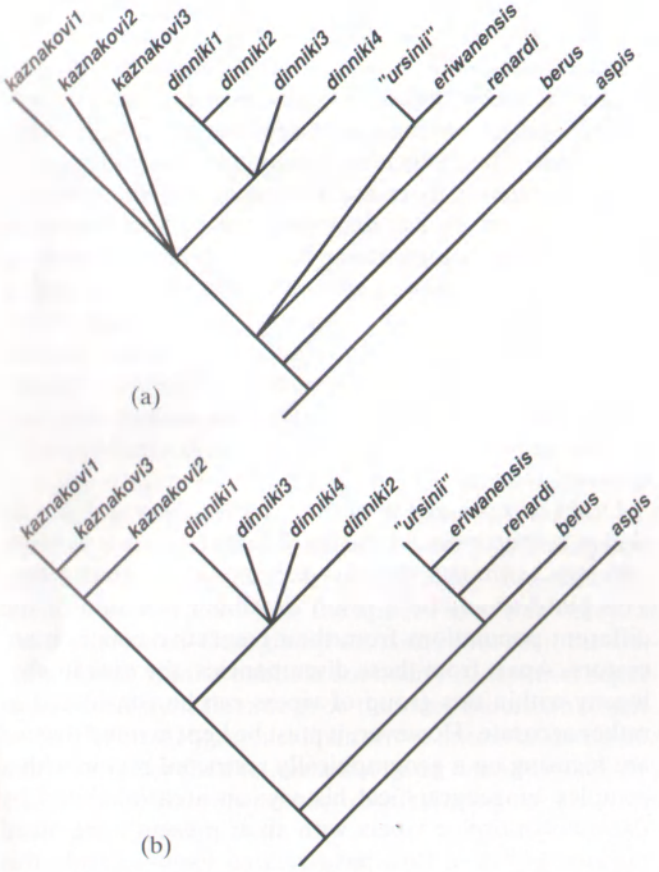


*Fig. 1.—a.* Strict consensus tree condensed from 13 equally parsimonious cladograms for the Caucasian vipers, based on biochemical characters (see Table III). *Vipera aspis* is used as outgroup.—*b.* Strict consensus tree condensed from 13 equally parsimonious cladograms for the Caucasian vipers, based on morphology (see Table IV). *Vipera aspis* is used as outgroup.

different trees are condensed to a consensus. The analyses yielded largely congruent cladograms. The differences between the consensus trees, in resolution and within-clade positions of the *kaznakovi* and *dinniki* clades, respectively, lack significance in this context, since both consensus trees support a monophyletic origin for the two taxa, although few synapomorphies for *kaznakovi* were provided by the biochemical data. Moreover, the biochemical analysis identifies *berus* as a monophyletic group, while the morphological characters cluster *berus* with '*ursinii*'-*eriwanensis*-*renardi*.

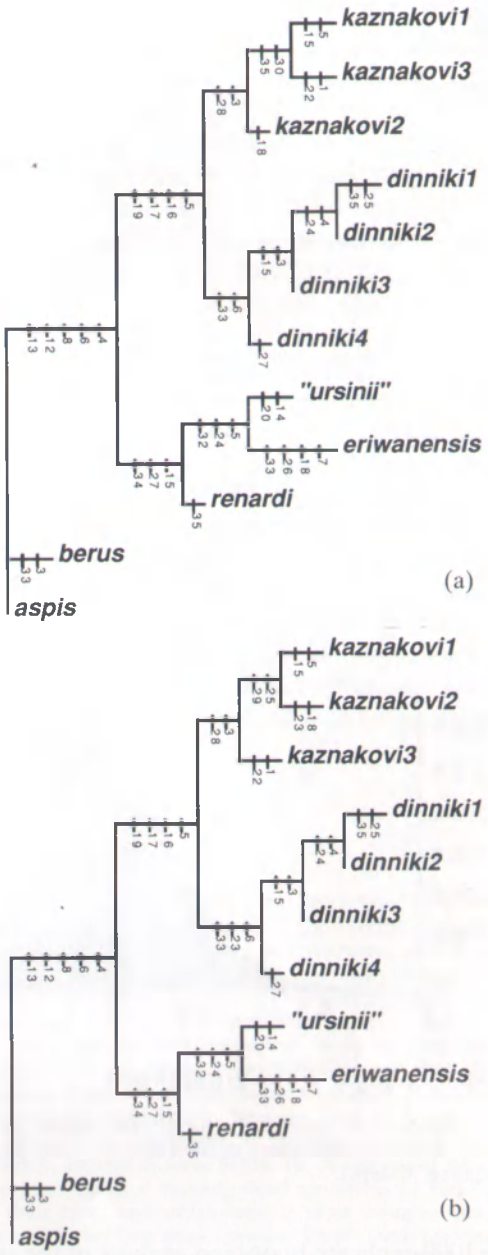


*Fig. 2.—a.* One of the two equally parsimonious phylograms, based on combined data, showing unambiguous character changes (CI; 0.60, tree-length 70 steps). See Table VI for individual consistency index for each of the 35 characters involved.—*b.* The second of two equally parsimonious phylograms, based on combined data, showing unambiguous character changes (CI; 0.60, tree-length 70 steps). See Table VII for individual consistency index for each of the 35 characters involved.

Analysis of combined allozyme and morphology data produced two equally parsimonious and fully dichotomous trees, each 70 steps in length and with a CI of 0.60 (autapomorphies excluded). Phylograms (relative branch lengths outlined) with unambiguous character changes and histograms showing CI values for each individual character are shown in Fig. 2 and Tables VI and VII. The character-specific consistency indices suggest certain morphological apomorphies to be weak, while some should probably be considered as homoplasies. Resampling of 10,000 random dichotomous trees from the combined data set generated a markedly skewed tree-length distribution (Fig. 3), with a prolonged left tail indicating significant phylogenetic information in the data (the two most parsimonious trees required 70 steps).

Table VI. *Individual consistency index for each of the 35 characters used in the first of the two equally parsimonious phylograms (Fig. 2a)*

| CI | Characters |
|---|---|
| 1.00 | 1–4, 6–14, 16, 17, 19, 28, 32, 34 |
| 0.75 | 5 |
| 0.50 | 18, 22, 24, 26, 27, 30, 31 |
| 0.33 | 15, 20, 23, 25, 29, 33, 35 |
| 0.25 | 21 |

Table VII. *Individual consistency index for each of the 35 characters used in the second of the two equally parsimonious phylograms (Fig. 2b)*

| CI | Characters |
|---|---|
| 1.00 | 1–4, 6–14, 16, 17, 19, 28, 32, 34 |
| 0.75 | 5 |
| 0.50 | 18, 22, 24–27, 29, 31 |
| 0.33 | 15, 20, 23, 30, 33 |
| 0.25 | 21, 35 |

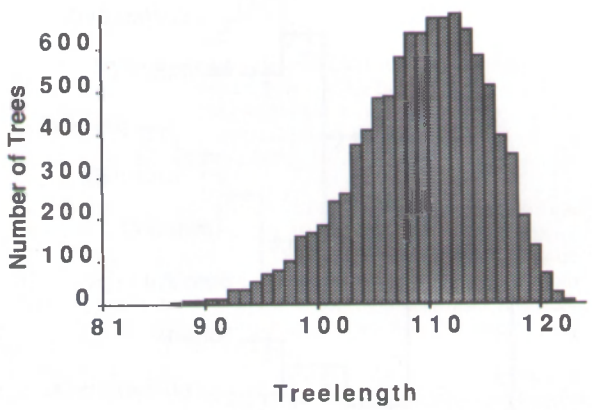


*Fig. 3.*—Histogram of tree-lengths for 10,000 random dichotomous trees samples from the combined data set (the two most parsimonious trees required 70 steps).

The 1000-replicate bootstrap analysis of the combined data, based on the branch-and-bound search algorithm in PAUP, gave weak support for the positions within the *kaznakovi* and *dinniki* clades (confidence limits of 62 and 73%, respectively). However, this outcome may simply reflect the fact that the characters chosen do not resolve infra-specific variation as well as lower taxonomic levels. The bootstrap puts a confidence limit of 93% for the *berus* position, while the '*ursinii*'-*eriwanensis*-*renardi* clade was supported by 87% of the generated trees. Felsenstein (1985) recommended accepting as well supported only those groups that occur in >95% of the trees, hence we interpret the confidence limits presented here as reflecting certain contradictions in the data sets, as discussed above.

In both the morphology and the molecular cases the *ursinii* group falls out separately from *kaznakovi* and *dinniki*, and the '*ursinii*'-*eriwanensis* taxa together form a clade separated from *renardi*.

As a whole the morphology based cladograms agreed well with the allozyme based analyses. Thus relationships among the 11 (12 when *V. aspis* was included) populations and taxa were concordant irrespective of which of the two kinds of data the analyses were based on. They all demonstrated that the *kaznakovi* group, the *dinniki* group and the mountain *ursinii* group form a clade each distinct from each other, and that *renardi* is distinctly by itself.

## Discussion

In the present study, the phylograms based on external morphology and on allozymes show a similar pattern. Different data sets related to the same group of animals can make the drawing of conclusions of phylogenetic relationships more complex (Shaffer *et al.* 1991) but in this study the different data sets support the same phylogeny. The probability of an incorrect cladogram due to homoplasy affecting all data sets in a similar way must be considered small. It is reasonable to assume that the results point to the same historical sequence of character changes, and thereby a similar phylogenetic hypothesis.

Despite these results, details in the molecular and morphological phylogenetic information is not totally congruent. The molecular data point at an earlier separation of *renardi* from the remaining *ursinii* groups, a separation not clearly demonstrated by the cladogram based on morphology. Being parapatric in northern Caucasus (albeit at different altitudes) the Caucasian '*ursinii*' and *renardi* obviously have separate evolutionary histories. In both cladograms, and consequently in the two most parsimonious phylograms based on combined morphological and biochemical data the Caucasian '*ursinii*' and Armenian *eriwanensis* are sister taxa with *renardi* as more distantly related.

Other exceptions are within the *kaznakovi* and *dinniki* clades, respectively, where the different data sets produce different, although equally parsimonious, conclusions. This pattern may be a result of similar isolation of the different populations from their respective common ancestors. Apart from these discrepancies, the overall phylogeny within this group of vipers can be considered as rather accurate. However, it must be kept in mind that we are focusing on a geographically restricted region with a complex biogeographical history; an area inhabited by these polymorphic vipers with an at present unresolved taxonomy. These taxa have related forms outside this region and therefore our phylogeny can be used as an instrument for helping to resolve their taxonomy based on the evolutionary species concept (Frost & Hillis 1990; Frost *et al.* 1992). Identification of monophyletic 'clades' may not be derived by phenetic methods (although by coincidence relationships based on clustering by overall character similarity may identify the pattern of evolutionary history). Earlier phylogenetic hypotheses (e.g. Kramer 1961; Saint Girons 1980) are not based on cladistic methods and their results are not directly comparable with the present study.

The three *kaznakovi* samples cluster together in all trees, as do the four *dinniki* populations, thereby demonstrating two unique evolutionary lineages and supporting the present taxonomic division of '*Vipera kaznakovi*' s.l. in a lowland *V. kaznakovi* s.str. and an alpine *V. dinniki*. Although slightly differentiated genetically (Table III)

and in external morphology (Nilson *et al.* 1994) the Hopa population of *kaznakovi* shares an evolutionary history with the other two northern *kaznakovi* samples (at Dagomys & Rudorova), and cannot be further resolved here within the clade (a potential northern subspecies of *kaznakovi*, '*Vipera k. tigrina*' (*sensu* Kramer 1961), is synonymous with *Vipera dinniki*). The type specimen of Tzarevsky's *tigrina* is a *V. dinniki* (Orlov & Tuniyev 1990).

The major groups, *kaznakovi, dinniki* and *ursinii*, have been treated as distinct groups of taxa in the recent literature, although attempts to include all or parts of these groups into one species or another have been made at different times (see Orlov & Tuniyev 1986, 1990 for a historical review). It has not always been obvious that the populations concerned have separate evolutionary history. Here, the cladistic analyses based on allozyme data are unanimous, with a *kaznakovi, a dinniki* and a *ursinii* s.l. clade. The cladistic analyses based on morphological data show the same pattern, both reflecting and verifying separated evolutionary history for these three groups.

The clades have a mainly allopatric distribution. The *kaznakovi* clade occupies humid lowland habitats, the *dinniki* clade occupies moist subalpine and alpine habitats, and the *ursinii* clade occupies dry meadow areas (*renardi* in lowland areas, *eriwanensis* and '*ursinii*' in alpine/subalpine habitats). However, a certain degree of sympatry between the clades has been demonstrated (Nilson *et al.* 1994) as the Caucasian '*ursinii*' and *V. dinniki* are sympatric (but not necessarily syntopic) at several locations in central and eastern Caucasus. In fact all three clades show potential sympatry and parapatry. In the Mzymta river valley, the Rudorova population of *Vipera kaznakovi* is distributed at higher elevations and reaches a similar altitude as the lowest *V. dinniki* populations (albeit separated by the winding mid-mountain coniferous forest belt). In the upper Psou river valley, at Aibga (Russian–Georgian (Abshasian) border), the *kaznakovi* and *dinniki* habitats are nearly parapatric, separated by different elevations on the steep mountain slopes. A similar zoogeographical pattern of potential parapatry seems to exist for the *kaznakovi* and *ursinii* groups (*V. darevskii* and *eriwanensis*, respectively) in Armenia (Orlov & Tuniyev 1990).

As shown elsewhere (Joger *et al.* 1992; Nilson *et al.* 1993) immunological methods indicate that the *ursinii* complex consists of a number of full (sibling) evolutionary species rather than subspecies. *Vipera* (*u.*) *eriwanensis* is a sister taxon to *renardi* and not to the European mountain populations in the *ursinii* phylogeny. Consequently also *eriwanensis* and the Caucasian '*ursinii*' must be separated from *ursinii* s.str. The present phylogenetic analyses support a hypothesis that *renardi* as well as *eriwanensis* and Caucasian '*ursinii*' must be looked upon as separate evolutionary taxa.

Hypotheses concerning the dispersal history of the taxa in the *kaznakovi* and *ursinii* species groups have earlier been expressed (Orlov & Tuniyev 1986, 1990). As shown elsewhere (Herrmann *et al.* 1987, 1992) the two groups have different evolutionary history and consequently different dispersal history. Our phylogenetic hypothesis can be used for elucidating further the historical biogeography of these vipers. The *ursinii* group are represented by dry habitat taxa while the taxa of the *kaznakovi* group are adapted to humid habitats (e.g. Kramer 1961; Orlov & Tuniyev 1990). A further three of the five species (*'ursinii', dinniki, eriwanensis*) are restricted to alpine and subalpine habitats while the remaining two taxa (*renardi* and *kaznakovi*) are restricted to lowlands and foothill areas. The sympatry of the genetically well separated *dinniki* and '*ursinii*' is probably of rather recent origin, and the very similar colour pattern in some specimens (Nilson *et al.* 1994) can be considered as a convergent adaptation (possibly Müllerian mimicry). The Caucasian '*ursinii*' seems to be most closely related to the Armenian *eriwanensis*, and the dispersal of this taxon into Caucasus may have been from the south. However, *eriwanensis* is genetically distinct from the Caucasian '*ursinii*' as it exhibits unique apomorphic alleles (Idh-2) which are lacking in the Caucasian '*ursinii*'. It is reasonable to regard them as sister taxa in a southern branch of the *ursinii* complex. At the northern foothills of the Caucasus, '*ursinii*' is replaced by *V. renardi*, which has its range at the northern lowlands, and most certainly has had its distribution center in these regions prior to the Pleistocene (Nilson & Andrén 1994).

## Acknowledgements

Support for this project was received from the (former) Soviet Academy of Sciences for fieldwork in 1989, and from the Swedish Natural Sciences Research Council (to GN and CA). Morphological studies were performed at the Department of Zoology, University of Göteborg, and additional material was made available from the Zoological Institute, St Petersburg; Caucasian State Biosphere Reserve, Sochi; Göteborg Natural History Museum; Dr Volker Mahnert, Museum d'Histoire Naturelle, Geneve; Carl Edelstam, Sven Kullander and Erik Åhlander, Naturhistoriska Riksmuseet, Stockholm; Dipl.-Biol. F.J. Obst, Staatliches Museum für Naturkunde, Dresden; and Dr Greg Schneider, Museum of Zoology, University of Michigan, Ann Arbor. Goran Dursej, Zürich, assisted in some fieldwork. A number of persons have during the years donated recently dead specimens of non-Caucasian vipers in their care, and which were a most valuable addition for comparison with the Caucasian samples. To all these persons we are much obliged. Licences for bringing scientific specimens from the Caucasus to our lab. were kindly granted from the USSR Goskomprirooda, (No. 668, 13.07.1990) and the Ministry of Agriculture (certificate No. 237, 1990). A special thank you must be given to Ilya S. Darevsky and Natalia B. Ananjeva from the St Petersburg Zoological Institute of the Russian Academy of Sciences who assisted us in scientific as well as in bureaucratic issues. Robert Paxton is acknowledged for linguistic help.

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