This is the final accepted version of the article (DOI: 10.1163/15685381-00003031). The final published version can be found at http://booksandjournals.brillonline.com/content/journals/10.1163/15685381-00003031

Full title: Species distribution modelling leads to the discovery of new populations of one of the least known European snakes, *Vipera ursinii graeca* in Albania

Short title: New populations of *Vipera ursinii graeca* in Albania

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Article

Nr. of Words: 7187

Abstract:

Vipera ursinii graeca is a restricted-range, endemic snake of the Pindos mountain range in the southwestern Balkans. The subspecies was previously reported from eight localities in Greece and one locality in southern Albania. We used species distribution modelling based on climate data from known localities in Greece, to estimate the potential distribution of the subspecies. The model predicted suitable areas for eleven mountains in southern Albania, which we visited during ten field expeditions in four years. Based on 78 live individuals and 33 shed skins, we validated the presence of the snake on eight of the eleven mountains. Six populations (Dhëmbel, Llofiz, Griba, Shendelli, Tomorr and Trebeshinë Mountains) are reported here for the first time. Morphological measurements undoubtedly supported that all individuals found at these new localities belonged to *V. u. graeca*. Genetic analysis of mitochondrial DNA sequences also confirmed the identity of the snakes as *V. u. graeca* and a low number of identified haplotypes suggests low genetic variability among populations despite significant spatial isolation. All localities were subalpine-alpine calcareous meadows above 1600 m. These high mountain habitats are separated by deep valleys and are threatened by overgrazing, soil erosion, and a potential increase in the elevation of the tree line due to climate change. Our surveys increased the number of known populations by 60% and the known geographical range of the subspecies by approximately 30%. Our study serves as a baseline for further ecological research and for conservation measures for one of the least known European viperid snakes.

Keywords: alpine grassland, detection probability, Species Distribution Modelling, MaxEnt, threatened species

Introduction

An increasing number of species are threatened by habitat changes (Brooks and Kennedy, 2004) and those with restricted distributions face an elevated risk of extinction (Malcolm et al., 2006). Mountain species are especially vulnerable in this regard, as their distribution is often not only restricted, but also highly fragmented and isolated (Ehrich et al., 2007), therefore, precise information on their distribution is fundamental for the effective preservation of their populations (Johnson and Gillingham, 2005).

Taxa in the *Vipera ursinii* species complex (*Acridophaga* subgenus, Reuss, 1927, hereafter referred to as meadow vipers) are especially vulnerable, because most have a highly fragmented distribution, ranging from Eastern France to Western China (Nilson and Andrén, 2001). Members of the *V. ursinii* complex are among the most endangered viperid species in Europe (Nilson and Andrén, 2001). *V. ursinii* is a species listed in CITES (Appendix I), in the Bern Convention (Appendix II) and in the European Union's Habitat Directive (Appendix II and IV), and also protected by national legislation in most of its distribution range, except in Albania, Bosnia-Herzegovina and Montenegro. Some taxa are classified in the IUCN Red List of Threatened Species as vulnerable (*V. ursinii* including all subspecies), endangered (*V. u. rakosiensis*) or critically endangered (*V. u. moldavica*).

Meadow vipers live in highly fragmented lowland steppe or subalpine-alpine grasslands. Much of their typical lowland steppe habitats have been lost due to human activity (e.g. crop production, grazing) in the 20th century and, as a consequence, meadow vipers have gone extinct in the lowland plains of Austria, Bulgaria and possibly Moldova (Nilson and Andrén, 2001). Subalpine-alpine meadow habitats are less affected by habitat alteration, even though many are used for intensive grazing by sheep, cattle and goats. The remaining European populations survive in small, isolated habitat patches that are often suboptimal for long-term persistence of viable populations (Filippi and Luiselli, 2004; Nilson and Andrén, 2001; Újvári et al., 2002; Edgar and Bird, 2005).

Meadow vipers typically exhibit a low level of morphological variability between taxa, thus, proper identification of specimens may be difficult, especially in the field (Nilson and Andrén, 2001). The phylogeny of meadow vipers has not been fully resolved (Gvoždik et al., 2012; Ferchaud et al., 2012; Zinenko et al., 2015), due to limited knowledge on certain taxa and inadequate sampling of taxa or geographical regions. In the last two decades, some of the formerly recognised subspecies of the complex were elevated to full species status based on morphological, allozyme or immunological analyses (*V. eriwanensis*: Nilson et al., 1995; *V. renardi*: Kotenko et al., 1999, Nilson & Andrén, 2001; *V. anatolica* and *V. ebneri*: Nilson & Andrén, 2001).

The Greek meadow viper (*Vipera ursinii graeca* Nilson and Andrén, 1988) is among the rarest and least-known taxon among the meadow vipers. *V. u. graeca* represents a distinctly divergent evolutionary lineage basal to all other clades of *V. ursinii* (Ferchaud et al., 2012; Zinenko et al., 2015), and therefore, a possible candidate for full species status. All the more so because other, less divergent taxa have been recognized as full species within the complex. *V. u. graeca* is considered endemic to the Pindos mountain range in Greece (Nilson and Andrén, 1988) and southern Albania (Korsós, Barina & Pifkó, 2008; Mizsei and Üveges 2012). Greek meadow vipers inhabit high elevation subalpine-alpine meadows, which normally occur above the tree line, beginning at altitudes of 1600 m a.s.l. and extending upwards of 2200 m unless meadows are interrupted by rocky slopes and peaks (Nilson and Andrén, 1988; Mizsei & Üveges, 2012).

Prior to our investigation, *V. u. graeca* was known from only eight mountains in Greece (Dimitropoulos, 1985; Chondropoulos, 1989; Nilson and Andrén 1988, 2001) and had one record in southern Albania (Korsós, Barina and Pifkó, 2008). Former studies mentioned *V. u. macrops* as the only subspecies occurring in Albania (Kopstein and Wettstein, 1920; Bruno, 1989; Haxhiu 1998) until Korsós, Barina & Pifkó (2008) reported the occurrence of *V. u. graeca* from one site in Nemerçkë Mountain in southern Albania on the basis of a single photo. This observation raised the possibility that *V. u. graeca* also occurs on other mountains of southern Albania.

Given the scarcity of information about the distribution of *V. u. graeca* and uncertainty surrounding the identity of *Vipera ursinii* populations in southern Albania, our study had three aims: (i) to model the potential distribution of *V. u. graeca* across the Balkan Peninsula using species distribution modelling (SDM); (ii) to empirically test the model results by field surveys in the potential habitats identified by the SDM exercise with the aim of detecting meadow viper populations, and (iii) to confirm the identity of meadow vipers in southern Albania using morphological and molecular data.

Material and Methods

Species distribution modelling

We used MaxEnt (Phillips, Anderson and Schapire, 2006) to predict the potential distribution of *Vipera ursinii graeca*. MaxEnt is a species distribution modelling approach that performs consistently comparable with the highest performing methods (Elith et al., 2006; Merow, Smith and Silander, 2013) even when only a few data points are available for modelling (Hernandez et al., 2006; Wisz et al., 2008).

Presence data of *V. u. graeca* were collected from scientific literature (Dimitropoulos, 1985; Chondropoulos, 1989; Nilson and Andrén, 1988; 2001) and personal communications (see Acknowledgements). At the time of creating our model (2010) only one presence data was known from Albania (Korsós, Barina and Pifkó, 2008), which we considered uncertain due to the lack of detailed morphological measurements, the reported extraordinary size of the specimen and contradictions with previous findings (Kopstein and Wettstein, 1920; Bruno, 1989; Haxhiu, 1998), which reported only *V. u. macrops* form Albania. Therefore, we omitted this occurrence point from the model. In total, we used 20 non-overlapping occurrence points from eight mountains in the model, including all published locations of the subspecies from Greece (fig. 1): Koziakas (n = 1), Lakmos (Peristeri) (n = 8), Metsovon (n = 3), Oiti (n = 1), Tsouka Karali (n = 1), Tzoumerka (Athamanika) (n = 3), Tymfristos (n = 1), Vardoussia (Akamanika) (n = 2). Because geographic coordinates were not available, we georeferenced these presence data using satellite imagery (Google Earth, Google Inc.) by choosing a random point within habitats based on descriptions and elevations above sea level as given in the literature. This method has been used before to accurately establish coordinates for sensitive, e.g. endangered, taxa for which accurate occurrence data are not typically given in the literature (Boakes et al., 2010). All data were entered and stored in a GIS using Quantum GIS 2.6 (http://www.qgis.org/), which was also used to prepare data for analysis and to visualize results.

We used the MaxEnt 3.3.3e (Phillips, Anderson and Schapire, 2006) software for modelling the potential distribution based on basic Bioclim variables (Busby, 1991). Climate data were obtained from the WorldClim database, version 1.4 (http://www.worldclim.org, Hijmans et al., 2005), at a resolution of 30 arc seconds. We selected climate variables based on the ecology of alpine reptiles (Monasterio et al., 2009) and on correlation tests between potential predictors. Although MaxEnt is more robust in controlling for correlations between variables than stepwise regression (Elith et al., 2011), strongly correlated variables ($r > 0.75$) were excluded to avoid multicollinearity in the models (Stohlgren et al., 2010; Elith et al., 2011). For example, altitude appeared to be an important predictor, because the known habitats of *V. u. graeca* are restricted to elevations above the tree line, we excluded it from the model due to its strong correlation with BIO1. We selected four variables for modelling: (i) annual mean temperature (BIO1), (ii) temperature seasonality (BIO4), (iii) annual mean precipitation (BIO12) and (iv) precipitation seasonality (BIO15). Temperature is a limiting factor for the annual activity period of ectothermic animals. Precipitation was considered important due to its strong influence on the vegetation type and structure.

Since all collected points of presence were located in Greece, we first used the area of that country as a background for model training to counterbalance our sampling bias from Albania. In the second step, the model was projected to the Balkan Peninsula (Croatia, Bosnia and Herzegovina, Montenegro, Serbia, Former Yugoslav Republic of Macedonia and Albania and the western two-thirds of Bulgaria; fig. 1). The discrimination ability of the model is tested by the Area Under Curve (AUC) metric, which is a measure of model performance in correctly distinguishing between sites associated to presence and background. The value of AUC varies between 0.0 and 1.0, where 1.0 is considered perfect prediction and 0.5 or less is considered no better than random (Fielding and Bell, 1997; Franklin, 2009). We calculated the importance of predictor variables by jackknife tests using MaxEnt. MaxEnt was run using the default settings except the fade by clamping function, which was applied for projection. Logistic output was used to visualise the climate suitability for the studied subspecies. We calculated the planimetric area of the predicted suitable habitats using Quantum GIS 2.6 (http://www.qgis.org/) (table 1).

Model testing by field surveys

Based on the model predictions (see Results section), we selected 10 separate mountains in southern Albania to test the subspecies distribution model and search for previously unrecorded populations (fig. 2). None of these mountains were known to harbour meadow viper populations prior to our study. Furthermore, we included the Nemerçkë Mountain in our field surveys in order to confirm the existence of *V. u. graeca*. We visited these potential habitats between 2010 and 2014 (table 1).

With consideration to the low detectability of vipers in the field, we also estimated detection probability to quantify how long one must search in order to obtain proof of presence of the subspecies and to eventually prove the model right or wrong for a given locality. We estimated the detection probability of vipers by tracking the movements of observers in potential habitats using GPS receivers (Garmin Ltd. Schaffhausen, Switzerland) as the searches were being made. We recorded track length, as well as the number of individual meadow vipers found on each track (Garmin BaseCamp 4.4.2). To test how track length predicts detection probability, we used a generalised linear mixed model (GLMM) with binomial error distribution, constructed using the lme4 package (Bates et al., 2014) in the R statistical environment (R Core team, 2013). Presence-absence data was used as the binary dependent variable, while the length of track was included as fixed explanatory variable. Observer and locality (mountain) were included in the GLMM as random factors to control for the non-independence of observations in these categories.

Subspecific identification

When we found a viper, the date, time, GPS coordinates of capture, sex, total body length and body mass were recorded in the field; furthermore, all specimens were photographed in order to collect more morphological data (scale counts) in the laboratory. For morphological identification, only dorsal scales were counted on site to minimize the time of handling and disturbance of the snakes. Tissue samples (a small piece from a ventral scale or tip of the tail) were also collected from each individual for genetic analyses and stored in 96% ethanol. After handling, animals were released where they were caught. In addition, shed skins of the target subspecies were also collected, which were insufficient for morphological measurements, but useful for genetic data collection. Morphological identification was determined based on the subspecific description (Nilson and Andrén, 1988). Meristic characters (preventrals, ventrals, subcaudals, apicals, supralabials, sublabials, circomoculars, loreals, crown scales) were counted using the photos in the program ImageJ 1.47v (Rasband, 2012).

Because some of the mountains predicted by the SDM as potential occurrences of the subspecies are relatively close (c. 80 km) to the southernmost known occurrences of *V. u. macrops*, we also used genetic data to confirm subspecies identity. We sequenced the mitochondrial DNA marker NADH dehydrogenase subunit 4 (*ND4*) of 20 specimens collected from Albania (table 2) since it had been successfully used to discriminate *V. ursinii* subspecies (Ferchaud et al. 2012). Three additional ND4 sequences of *V. u. graeca* specimens, published by Ferchaud et al. (2012), were used from GenBank. We used the DNeasy Blood & Tissue Kit (Qiagen) and the NucleoSpin Tissue kit (Macherey-Nagel) for extracting genomic DNA. Polymerase chain reaction (PCR) conditions followed the protocol of Ferchaud et al. (2012). PCR products were cleaned with High Pure PCR Product Purification Kit (Roche) or on NucleoFast 96 PCR plates (Macherey-Nagel) using vacuum filtering. We used BigDye v1.1 for cycle sequencing reactions. DNA sequencing was performed on an ABI 3130 (xl) capillary sequencer (Life

Technologies). Sequences were assembled and aligned using CodonCode Aligner v5 (CodonCode Corp.), and chromatograms were checked manually in order to clean the sequences. Sequences were then deposited to European Nucleotide Archive (ENA, http://www.ebi.ac.uk/ena) (table 2). A median-joining haplotype network was calculated with the software Network v4.6 (Bandelt et al., 1999).

Results

Potential distribution

The species distribution model predicted suitable habitats for *V. u. graeca* in treeless alpine meadows and mountaintops in the Pindos mountain range in both Greece and Albania (fig. 2) with high support values (AUC = 0.997). The distribution predicted by the model fits well with the known occurrences of the subspecies but also identified additional potential habitats in Greece. More importantly for this study, the model predicted potential occurrences for 11 mountains in Albania, ten of which meadow vipers were not known from (fig. 2).

The analysis of variable contribution showed that annual mean temperature (BIO1) had the highest importance (78%), whereas the precipitation variables (BIO12 and BIO15) contributed less, while seasonality of temperature (BIO4) did not contribute to the model at all (table 3). The permutation test showed an even higher importance of annual mean temperature (98%) to the model than the percentage of contribution by the same variable (table 3). The jackknife-regularized training provided additional support for this result, as BIO1 alone gave the most useful information for predicting the potential distribution of *V. u. graeca*, and the performance of the model was the lowest without BIO1.

Model testing by field surveys and discovery of new populations

During ten field expeditions in four years to the 11 mountains predicted by the model, we found one or more live individuals or shed skins of the Greek meadow viper on eight mountains (table 1, fig. 2). Six populations (Dhëmbel, Llofiz, Griba, Shendelli, Tomorr and Trebeshinë Mountains) are described here for the first time. We also verified the occurrence of the subspecies in Nemerçkë Mountain and found additional occurrences on Lunxhërisë Mountain (Mizsei and Üveges, 2012). Meadow vipers were not found on three of the 11 mountains visited (Cika, Bureto and Kulmak Mountains, table 1, fig. 2).

In total, we caught 78 vipers and collected 33 shed skins from eight different locations. During the field surveys, the observers covered a total of 457.2 km by walking. The probability of detecting at least one individual (or shed skin) increased with track length (GLMM, $n = 146$, $Z = 3.62$, $P = 0.0003$). Detection probability reached 50% after 4.9 km distance covered by a single observer on one track. In localities where no meadow vipers were found, track length was only 3.3 ± 0.45 km (mean ± SE, $n = 7$ tracks) due to logistical or weather constraints, and so the detection probability predicted for this distance was only 42.0%.

All of the visited Greek meadow viper habitats were characterized by a mosaic of open or closed grass and shrub communities formed on limestone. The annual mean temperature, averaged from BioClim data for all localities where the viper was observed, was 5.9 ± 0.5 °C (mean ± SE), and meadows were partially covered by snow until mid-summer. The south-facing slopes were usually more open and rocky than north-facing slopes. The open grasslands were dominated by different *Festuca*, *Poa* and *Sesleria* species, and characteristic shrubs present were *Juniperus sabina*, *Daphne oleoides* and *Astragalus creticus*. Most of the observed vipers were found close to shrubs or piles of stone in these south-facing habitat patches. In northern or north-eastern exposed slopes, open grasslands were replaced by closed grassland, where *Agrostis* species were dominant. All habitats were found to be used as sheep and goat pastures.

Subspecific identification

Specimens captured were identified as *V. u. graeca* based on the following morphological characteristics: number of ventrals (119-133), ventral base coloration white or pale brown, parietals frequently fragmented, generally fewer than 17 scale rows on posterior part of the body, 7 or 6 supralabial scales on one side of head, third supralabial scale always under the eye, posterior supralabials markedly smaller than anterior ones, no dark labial sutures, and nasal plate often partially divided (tables 4 and 5).

All obtained mtDNA *ND4* gene sequences (n = 23, 683 bp aligned length) were very similar, most were identical, including the reference *V. u. graeca* sequences from GenBank, which confirmed the morphological identification (fig. 3). The observed haplotype diversity was limited (fig. 3): sixteen (70%) of our *ND4* sequences shared the same haplotype with those *V. u. graeca* sequences published by Ferchaud et al. (2012), including the sequences which originated from the two shed skins collected in Griba Mountain (table 2). Four specimens belonged to two slightly different haplotypes, differing only in single nucleotide substitutions. The specimens from Shendelli Mountain had a single T→C substitution in position 222, while two of the four sequences from Lunxhërisë Mountain had a G→A substitution in position 384 (fig. 3).

Discussion

Our study verified the presence of the Greek meadow viper on eight mountains of southern Albania and expanded its known distribution by ca. 100 km to the north. The populations reported in this study double the number of populations previously known and the areas reported here comprise approximately one-third of the distribution area of the subspecies. We found evidence of the presence of Greek meadow vipers in seven previously unknown locations (including Lunxhërisë Mountain which has been mentioned in Mizsei and Üveges, 2012) and verified the former record of Korsós, Barina and Pifkó (2008) in Nemereçkë Mountain. The high proportion of the occurrences confirmed relative to the predicted ones (8 of 11 mountains or 73%) indicated that the prediction of our model was generally reliable for the northern part of the range of the subspecies.

Our distribution model for *V. u. graeca* based on macroclimatic factors (temperature, precipitation) performed well and highlighted the overwhelming importance of annual mean temperature. Annual mean temperature is known to have a direct effect on ectothermic organisms (Scali et al., 2011) and was recently demonstrated to play a key role in defining suitable habitat types for the closely related *V. u. ursinii*, while factors such as shelter or food availability have strong influence in determining the best patches within the habitat (Lyet et al., 2013). The availability of shelter and food, i.e., the vegetation and arthropod fauna of the suitable habitats, is in turn largely determined by precipitation (Marini et al., 2008), which may explain why annual precipitation and seasonality of the precipitation contributed more than 20% to our distribution model.

The match between model predictions and localities of observations suggested that the distribution model accurately predicted the macrohabitats such as mountain ranges, but did not differentiate at the microhabitat level such as among major plant associations. The microhabitats actually used by individuals varied among mountain ranges. For example, in the only habitat (Tomorr Mountain) where *Juniperus* species are present and common, vipers used this shrub for shade and hiding, similarly as *V. u. ursinii* uses junipers in France (Baron et al., 1966). On other mountain habitats where junipers are rare or missing, we usually found vipers close to shrubs (mostly *Astragalus* spp., *Daphne* spp.) which they used similarly as in the case of junipers. However, in localities where shrubs are absent (e.g. Trebeshinë Mountain), probably due to long-term grazing activity, the snakes were frequently observed in habitat patches characterized by *Festuca* spp. tussocks. These observations suggest that the biotic characteristics of the vegetation on a fine spatial scale within suitable habitats and its effects on the microhabitat choice and activity of snakes should be taken into account in the future ecological studies.

The fact that we did not detect *V. u. graeca* on three of the 11 mountains does not necessarily mean that the subspecies is not present there. Usually, perfect detection of a secretive snake species is not possible during short visits (Kéry, 2002; MacKenzie et al., 2002). Our analysis of detection also suggests that our effort was insufficient to exclude viper occurrence (or confirm viper absence) at these locations, thus, further field surveys at these sites are needed in order to confirm the presence or absence of populations (see Lyet et al., 2013).

Our morphological and genetic identification of all southern Albanian populations of meadow vipers confirmed them being *V. u. graeca*, as proposed by

Korsós, Barina and Pifkó (2008). Although the morphological characteristics reported here agree well with the original description of *V. u. graeca* (Nilson and Andrén, 1988; 2001), we also found some inconsistency: none of the specimens displayed a nasal scale fused with nasorostralia (table 5), and we found a broader range of numbers of ventral, subcaudal and crown scales (table 4). Such differences in pholidotic characters can possibly be attributed to geographic variation or to greater sample sizes in the current study than in previous ones. Furthermore, two specimens out of 72 displayed brownish ventral colouration, whereas another six specimens showed grey ventral colouration caused by a densely spotted pattern, which was not typically reported for *V. u. graeca*. The latter specimens were all small juveniles (mean SVL ± SE: 137.3 ± 3.20 mm), thus this colouration probably represents an adolescent character state.

Mitochondrial sequences show that the sampled *V. u. graeca* populations have low *ND4* haplotype diversity (fig. 3). Only three different *ND4* haplotypes were found: N5 (Ferchaud et al., 2012) was detected in the entire distribution of *V. u. graeca* from the southernmost (Stavros Mountain, Greece) to the northernmost (Tomorr Mountain, Albania) population; LU (this study), was so far found only in Lunxhërisë Mountain, where the N5 haplotype was also present; and SH (this study) was found in the Shendelli Mountain population, the smallest known habitat for this taxa (table 1; 1.38 km^2). These results do not warrant strong conclusions with regard to conservation genetics, but may show that the number of *ND4* haplotypes can be lower in *V. u. graeca* than in other subspecies of *V. ursinii*, with the exception of the critically endangered *V. u. moldavica* (Ferchaud et al., 2012).

Despite our discovery of new populations, the known range of *V. u. graeca* is still narrowly confined to central and north-western Greece and southern Albania (high elevation meadows in the Pindos mountain range). Moreover, its potential distribution shows a highly fragmented pattern, with patch-like suitable locations separated from each other by tens of kilometres of unsuitable habitat. These potential populations are currently isolated by deep valleys with unsuitable forested habitats that most likely hinders gene flow between the high elevation meadows (>2000 m a.s.l.). This is especially relevant for a snake species that has limited movement capabilities, such as *V. ursinii* (Lyet et al., 2009). As *V. u. graeca* is constrained to specific habitats located on mountain tops, global warming could result in serious loss of its habitats, due to tree encroachment and upward movement of the tree line (Grace, Berninger and Nagy, 2002; Galbreath, Hafner and Zamudio, 2009). Extensive grazing could also be a significant threat as it can negatively influence the vegetation structure of habitats (Wilson, 1994; Beever and Brussard, 2004). Furthermore, the direct killing of snakes by local shepherds might also contribute to the decline of isolated and vulnerable populations.

With regard to these complex threats, the Greek meadow viper should be considered an important target for conservation research. Information vital for its conservation includes knowledge on the full distribution of the subspecies in Greece and Albania, along with knowledge on habitats (vegetation types, grazing pressure, soil erosion etc.) and microhabitats (vegetation structure, food/shelter availability etc.) and population characteristics and dynamics (population genetics, local threat factors, sex ratio and demographic structure, dispersion ability, etc.). Increased conservation attention is also warranted by its phylogenetic distinctiveness from other members of the *Acridophaga* subgenus (Ferchaud et al., 2012). Future international cooperation between experts is strongly recommended to conserve Europe's least known viperid snake.

Acknowledgements

We thank M. Schweiger and J. Spreyboeck for distribution data from Greece; P. Zaránd, G. Kulcsár and J. Jakó (Hungarian Land Rover Association) for logistic help, 24 volunteers of the Greek Meadow Viper Working Group for fieldwork; A.-L. Ferchaud, B. Halpern and J. Vörös for primers and sequences; O. Vincze, Z. Barina, Z. Korsós, G. Kardos and Y. Ioannidis for advice; G. Nilson for data on the population at the type locality and Paul Lambourne for proof-reading the manuscript. Financial support was provided by the CSEMETE Environmental and Nature Conservation Association; the Serpentes Foundation; the Department of Evolutionary Zoology, University of Debrecen; the Balassi Institute, Hungary (B2/1CS/11521/1, B2/1SZ/12851), and the Hungarian Meadow Viper LIFE+ programme (LIFE07 NAT/H/000322). Permits were provided by the Ministry of Environment of Albania (Research Permit Request No. 6584). We also thank the reviewers, S. Roussos and P. Tarroso for their useful comments and suggestions.

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Table 1. Localities (mountains) predicted by species distribution modelling and visited in this study with details and results of surveys to detect *V. u. graeca* in southern Albania.

Locality	Date month/year	Habitat area (km^2)	N. of active field days	N. of Observers	N. of Tracks	Track length (km)				N. of viper observations
						mean	SE	sum	range	Specimens (shed skin)
Bureto	6/2014	1.41	1	1	1	5.9	0.0	5.9	5.9	0 (0)
Cika	7/2014	4.31	1	4	4	3.1	0.2	12.6	2.8-3.5	0 (0)
Dhëmbel	7/2013, 6/2014	8.98	2	3	4	3.3	1.0	13.3	1.6-5.0	3 (1)
Griba	7/2013, 7/2014	27.32	3	6	6	1.0	0.4	6.2	0.1-2.2	0 (2)
Kulmak	6/2014	3.36	1	2	2	4.6	0.2	9.2	4.4-4.6	0 (0)
Llofiz	6/2014	1.46	1	5	5	4.3	0.6	21.7	2.6-5.6	1 (0)
Lunxhërisë	5/2011, 6/2014	21.51	3	9	10	3.4	1.1	34.3	0.2-10.3	4 (2)
Nemerçkë	5/2010, 5/2011	59.45	4	6	8	2.3	1.0	18.5	0.2-8.0	4 (1)
Shendelli	6/2014	1.38	1	8	8	2.7	0.1	21.5	2.0-3.0	1 (1)
Tomorr	6/2014, 9/2014	5.07	2	9	12	4.9	1.1	58.9	0.1-9.8	2 (0)
Trebeshinë	7/2011, 5/2013, 7/2013, 9/2013, 6/2014	7.58	14	23	93	2.7	0.2	249.8	0.1-11.8	56 (15)
Total		**141.83**	**33**	**76**	**153**	**38.2**	**6.1**	**451.9**		**71 (22)**

Table 2. *V. u. graeca* specimens sampled and sequenced from southern Albanian mountains and additional mtDNA *ND4* sequences retrieved from GenBank from Ferchaud et al. (2012) from Greek localities.

Voucher	Locality	Haplotype	Accession number	Reference
-	Stavros	N5	FR727018.1	Ferchaud et al. (2012)
-	Stavros	N5	FR727019.1	Ferchaud et al. (2012)
-	Stavros	N5	FR727020.1	Ferchaud et al. (2012)
DH14-1-02	Dhëmbel	N5	LN835177	This study
GR14-1-01EX	Griba	N5	LN835175	This study
LL14-1-01	Llofiz	N5	LN835176	This study
LU11-1-01	Lunxhërisë	LU	HG940669	This study
LU11-1-02	Lunxhërisë	N5	HG940670	This study
LU11-1-03	Lunxhërisë	N5	HG940671	This study
LU14-1-01	Lunxhërisë	LU	LN835172	This study
NE10-1-01	Nemerçkë	N5	HG940665	This study
NE10-1-02	Nemerçkë	N5	HG940666	This study
NE11-1-01	Nemerçkë	N5	HG940667	This study
NE11-1-02	Nemerçkë	N5	HG940668	This study
SH14-1-01	Shendelli	SH	LN835171	This study
SH14-1-02EX	Shendelli	SH	LN835170	This study
TO14-1-01	Tomorr	N5	LN835173	This study
TO14-2-01	Tomorr	N5	LN835174	This study
TR11-1-01	Trebeshinë	N5	HG940672	This study
TR11-1-02	Trebeshinë	N5	HG940673	This study
TR11-1-04	Trebeshinë	N5	HG940674	This study
TR11-1-05	Trebeshinë	N5	HG940675	This study
TR11-1-06	Trebeshinë	N5	HG940676	This study

Table 3. Climatic variables used in the species distribution model.

Variable		Contribution %	Permutation importance	Value at training points			
				Mean	±	SE	
BIO1	Annual Mean Temperature	78.4	98.1	5.39	±	0.22	°C
BIO15	Precipitation Seasonality	13.2	1.7	38.6	±	0.79	†
BIO12	Annual Precipitation	8.4	0.1	1016.2	±	8.36	mm
BIO4	Temperature Seasonality	0	0.1	6296.25	±	18.13	‡

† coefficient of variation, ‡ standard deviation×100

Table 4. Quantitative characteristics of *Vipera ursinii graeca* from seven Albanian localities (mountains) where live specimens were found and from the terra typica (Lakmos, Greece). Values are given as ranges and means ± SE. Values for Lakmos based on Nilson & Andrén (1988) and additional data cordially provided by G. Nilson (pers. comm.).

		Present study																			
Locality		Nemerçkë					Lunxhërisë					Trebeshinë					Dhëmbel				
Characteristic		n	range	Mean	±	SE	n	range	mean	±	SE	n	range	mean	±	SE	n	range	mean	±	SE
Preventrals		2	1				3	2				57	0-3	1.68	±	0.09	4	1-2	1.25	±	0.25
Ventrals	males	0		N/A			3	120-125	123.33	±	1.67	17	121-129	125.53	±	0.64	3	125-127	126.33	±	0.67
	females	2	127-130	128.5	±	1.5	0		N/A			35	119-133	128.34	±	0.56	1	132			
Subcaudals †	males	1	27				3	25-27	26.33	±	0.67	18	21-29	26.5	±	0.44	3	24-28	26.33	±	1.2
	females	3	13-21	18.33	±	2.67	0		N/A			38	17-26	20.11	±	0.38	1	22			
Dorsal scale row	neck	2	19				4	19-20	19.25	±	0.25	51	19-20	19.06	±	0.03	4	19			
	mid-body	2	19				4	18-19	18.75	±	0.25	51	19-20	19.06	±	0.03	4	19			
	posterior	2	15-18	16.5	±	1.5	4	16-17	16.5	±	0.29	51	13-18	15.39	±	0.11	4	16			
No. of apicals		4	1				4	1				60	0-1	0.98	±	0.02	4	1			
Supralabials ‡		4	12				4	12-13	12.25	±	0.25	60	12-15	13.4	±	0.12	4	12			
Sublabials ‡		4	16-19	17.5	±	0.65	4	16-18	17.25	±	0.48	60	15-19	16.88	±	0.16	4	17-18	17.75	±	0.25
Circomoculars ‡		4	16-19	17.5	±	0.65	4	16-19	17.5	±	0.65	60	14-19	16.92	±	0.13	4	18-19	18.5	±	0.29
Loreals ‡		4	3-7	5	±	0.82	4	2-5	3	±	0.71	60	3-8	4.52	±	0.13	4	4-7	5.25	±	0.63
Crown scales		4	10-14	12.5	±	0.87	4	12-14	13	±	0.41	59	10-20	14.58	±	0.3	4	9-14	11.5	±	1.04
Supralabials to level below eye ‡		4	6				4	6				60	6				4	6			

Legend: †: counted on left side of the tail, ‡: counted as right and left side together

Table 4. (Continued.)

		Present study														Nilson and Andrén (1988)				
Locality		Tomorr					Llofiz		Shendelli		Albania (all localities)					Lakmos (*terra typica*)				
Characteristic		n	range	mean	±	SE	n	range	n	range	n	range	mean	±	SE	n	range	mean	±	SE
Preventrals		2	1-2	1.5	±	0.5	1	2	1	2	70	0-3	1.66	±	0.08	13	1-2	1.54	±	0.14
Ventrals	males	1	125				0	N/A	1	126	25	120-129	125.36	±	0.49	8	121-124	123	±	0.42
	females	1	130				1	127	0	N/A	40	119-133	128.45	±	0.5	5	123-127	125.4	±	0.81
Subcaudals †	males	1	25				0	N/A	0	N/A	26	21-29	26.42	±	0.33	8	20-27	24.13	±	0.87
	females	1	18				1	18	0	N/A	44	13-26	19.93	±	0.38	5	18-21	19.4	±	0.51
Dorsal scale row	neck	2	19				1	19	1	18	65	18-20	19.05	±	0.03	13	18-19	19	±	0.11
	mid-body	2	19				1	19	1	19	65	18-20	19.03	±	0.03	13	17-19	18.77	±	0.17
	posterior	2	15				1	16	1	17	65	13-18	15.55	±	0.11	13	13-17	15.15	±	0.25
No. of apicals		2	1				1	1	1	1	76	0-1	0.99	±	0.01	13	1-2	1.08	±	0.08
Supralabials ‡		2	12				1	12	1	12	76	12-15	13.12	±	0.11	13	12-14	12.62	±	0.24
Sublabials ‡		2	16-18	17	±	1	1	18	1	17	76	15-19	17	±	0.13	13	14-18	16.23	±	0.43
Circomoculars ‡		2	16-20	18	±	2	1	16	1	17	76	14-20	17.08	±	0.13	13	13-20	16.77	±	0.53
Loreals ‡		2	4				1	6	1	4	76	2-8	4.5	±	0.13	13	2-7	4.15	±	0.44
Crown scales		2	12-14	13	±	1	1	16	1	12	75	9-20	14.16	±	0.27	13	7-16	10.85	±	0.64
Supralabials to level below eye ‡		2	6				1	6	1	6	76	6				13	5-6	5.92	±	0.08

Legend: †: counted on left side of the tail, ‡: counted as right and left side together

Table 5. The prevalence of discriminating morphological characters in the examined *V. u. graeca* specimens.

Characteristic	n	Prevalence
Ventral colour whitish or pale brown	72	88.89 %
Ventral colour brownish	72	2.78 %
Ventral colour grey	72	8.33 %
Parietals divided	76	15.79 %
Parietals fragmented	76	68.42 %
Fewer than 17 scale rows on posterior part of the body	65	86.15 %
7 or 6 supralabial scales on either side ‡	152	97.37 %
Third supralabial under eye ‡	152	100 %
Size difference between anterior and posterior supralabials ‡	152	100 %
Dark labial sutures ‡	152	0 %
Nasalia divided ‡	143	11.88%
Nasalia partially divided ‡	143	60.14 %
Nasalia fragmented ‡	143	4.89 %
Nasalia united with nasorostralia ‡	152	0 %

Legend: ‡ calculated as right and left side occurrences together

Figure 1. Geographic area and presence points of *V. u. graeca* used in species distribution modelling. Grey shading indicates the area for model training, black indicates projection area. The distribution of *V. u. macrops* is also presented due to its proximity to the study taxon.

Figure 2. The prediction of MaxEnt model for *V. u. graeca* throughout the range and in southern Albania (inset). Field surveys were conducted on all mountains shown. Filled acronyms indicate mountains where the subspecies was found, open letters indicate those where no evidence of presence was found. Abbreviations: Bureto (BU), Cika (CI), Dhëmbel (DH), Griba (GR), Kulmak (KU), Llofiz (LL), Lunxhërisë (LU), Nemerçkë (NE), Shendelli (SH), Tomorr (TO), Trebeshinë (TR).

Figure 3. Median-joining haplotype network of *V. u. graeca* based on mitochondrial *ND4* sequences. Numbers 222 and 384 indicate the relative positions where the single step differences occur in sequence.*see table 2.