



Distribution and Ecology of the Alpine Newt *Ichthyosaura alpestris* (Laurenti, 1768) (Amphibia: Salamandridae) in Bulgaria

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Abstract: The Alpine newt *Ichthyosaura alpestris* is a glacial relict in Bulgaria, occurring only in several mountainous regions. Results of a long-term herpetological survey in Bulgaria are presented, including from sampling specifically targeting the aquatic phase of *I. alpestris*. The known ranges of its vertical and geographical distribution are now expanded – it occurs autochthonously in seven mountains (in 42 10×10 km UTM squares, 22 of which previously unreported). We detected it for the first time in two mountains: Pirin and Karvav Kamak. However, several potentially suitable mountains remain under-sampled. A Maxent model with strong predictive power (test AUC = 0.988) based on 10 uncorrelated variables revealed that about 5% of the territory of the country are potentially suitable as habitats for this species. Elevation is the best predictor in the model, followed by slope, while precipitation seasonality and the potential evapotranspiration signify the importance of the presence of (seasonal) water bodies. *Ichthyosaura alpestris* seemingly prefers to breed in natural and semi-natural shallow water bodies with muddy bottom, grassy banks, limited reed/bulrush, without fish, near or in forested habitats. The registered relative abundances (0.002–1.183 ind./trap-hour), the estimated instantaneous population densities (0.2–7.1 ind./m²) and the sex ratios (from 0.25♂ : 1♀ to 7.29 : 1) vary widely among and within mountains.

Key words: Caudata, Balkan Peninsula, mapping, modelling, habitat, abundance, sex ratio, Maxent

To Andro and Niki, true friends

Introduction

The Alpine newt *Ichthyosaura alpestris* (Laurenti, 1768) is widely distributed in Central and Western Europe, ranging from introduced populations in Great Britain, reaching southern Denmark to the

north, western Ukraine and eastern Romania to the east, and northern Spain, southern Italy and southern Greece to the south (see SILLERO et al. 2014). However, after SILLERO et al. (2014), no updates were published on the distribution of *I. alpestris* in Europe, while for other newts the latest data have

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been compiled (see WIELSTRA et al. 2014, 2018 for the genera *Triturus* and *Lissotriton*). The vertical distribution of *I. alpestris* also ranges widely, from ca. 0 to ca. 2500 m a.s.l. (ROČEK et al. 2003, THIESMEIER & SCHULTE 2010). This broad occurrence of the species has inspired multiple studies on its habitat selection and population parameters but these were carried out mostly in Central Europe (reviewed in THIESMEIER & SCHULTE 2010). Publications for the Balkan Peninsula are few (e.g. KLETEČKI 1995, DENOËL 2004, ČIROVIĆ et al. 2008).

In Bulgaria, *I. alpestris* is considered as a glacial relict, found in mountains (Stara Planina, Sredna Gora, Osogovo, Rhodopes and Rila) between 900 and 2500 m (STOJANOV et al. 2011, BESHKOV 2015). It was introduced to Vitosha (NAUMOV & LANGOUROV 2008). The first national mapping by BURESCH & ZONKOW (1941) was further updated with thematic maps by BEŠKOV & BERON (1964), BESHKOV (1985, 2015; but the data in the latter cover only up to 2011), and STOJANOV et al. (2011). The only study specifically targeting *I. alpestris* was a diploma thesis on its distribution and habitats (VESELINOV 1993), which remained unpublished.

The Alpine newt is included in the Red Data Book of the Republic of Bulgaria (BESHKOV 2015) and the Annex III of the Biological Diversity Act.

The aim of the present work is to analyse the available data (new and published) for the distribution, habitats, abundance and sex ratio of *I. alpestris* in Bulgaria.

Materials and Methods

Study area

The territory of Bulgaria covers ca. 111,000 km² (comprised of 970 whole and 287 partial squares of the 10×10 km Military Grid Reference System). The relief is highly diverse, ranging from extensive plains and lowlands to subalpine and alpine mountains. Five hypsometric belts were identified (SIMEONOV & TOTZEV 1997): lowlands (0–200 m a.s.l., 31.4% of the territory), hills (200–600 m a.s.l., 41%), low mountains (600–1000 m a.s.l., 15.3%), mountains of average height (1000–1600 m a.s.l., 9.8%) and high mountains (above 1600 m a.s.l., 2.5%). According to NIKOLOV & JORDANOVA (2002), the number of the distinct mountains is 37, ranging in maximum height from 710 m (Strandzha) to 2925 m (Rila); maps with their locations are given by HUBENOV (1997), NIKOLOV & JORDANOVA (2002) and STOJANOV et al. (2011).

Field studies and sampling

Field studies were carried out in 2000–2017; these were with unequal intensity and at various locations. However, the predominant amount of data (> 84%) was collected after 2007. Overall, the search effort was uneven and generally increased in time and scope. Sampling was likely biased, with favoured herpetological regions and habitats in proximity to roads visited more intensely. However, fieldwork was carried out by multiple researchers (often independently) through an extended period and was performed to serve different goals. Therefore, we regard the data collection overall as semi-haphazard. As we aimed at finding all native newt species (to be discussed in future publications) and not exclusively *I. alpestris*, the study area encompassed the whole country. This approach provides a generally more objective assessment of the perceived absences of the species at the national level compared to studies limited to locations where the species is expected to occur (e.g. high mountains).

Newts were sampled during their reproductive period (April – June) in temporary and permanent, standing and flowing water bodies (e.g. wetlands, lakes, puddles, slow streams, canals, water troughs). Presence was confirmed by captures using unbaited funnel traps (L50/H25/W25 cm, 0.5 cm mesh size) or a dip net, and, in limited cases, by visual observations from the bank and/or hand capture. Due to their highly secretive terrestrial phase, findings of individuals on land were extremely rare and limited to chance. The geographic coordinates of observations were recorded using a hand-held GPS (accuracy ±10 m) or, in rare cases, georeferenced using high-resolution aerial/satellite images. We considered as localities with “potential absence” (as true absence was not proven) water bodies where we carried out targeted sampling (funnel trapping and dip-netting), and found newts of the genera *Triturus* Rafinesque, 1815 and *Lissotriton* Bell, 1839 but no *I. alpestris*.

Distribution mapping

We compiled all available published presence data as well as unpublished observations (personal and contributed by colleagues). The mapping was done on a Universal Transverse Mercator (UTM) grid with a resolution of 10×10 km in the projection coordinate system “WGS 84 UTM 35N”. We used the codes of the Military Grid Reference System (MGRS; spatially identical with UTM) for the 10-km quadrates for map visualization and listing the localities. We reviewed herpetological publications related to Bulgaria (ca. 800 publications from 1892–

2018) for newt presence records. We considered as a separate presence record every original description of a location where an individual was found and which was different from descriptions in later publications. Hereafter, we use “locality” to denote a specific water body with a proven presence of *I. alpestris*; only as an exception, it signifies two or three water bodies in close proximity, or, when citing published data, the name of a geographical area that cannot be attributed to a particular water body. In most sources, settlements or geographical objects were given as reference points for the locations; we assigned these as best as possible to the UTM 10×10 km grid. Few presence records had already been reported as cells in a UTM/MGRS grid or with precise coordinates.

Habitats characteristics

We characterised the aquatic habitats where we encountered *I. alpestris* according to the following categories: (1) Origin – natural, semi-natural (e.g. as an unintentional result of human activity: puddles on dirt roads, inundated pits, rock quarries), or artificial (intentionally man-made, e.g. for watering livestock, actively maintained or abandoned); (2) Type (according to the popular hydronyms) – lake, swamp, stream, etc. (see DIMITROVA et al. 2007); (3) Form (in the topographic sense) – polygonal or linear; (4) Current speed – none, slow or fast; (5) Surface area (m²) – < 10, 10–100, 100–10000, > 10000; (6) Depth (maximum, m) – < 0.5, 0.5–1, 1–1.5, > 1.5; (7) Bottom substrate – stony (incl. cement), sandy, muddy; (8) Proportion of underwater vegetation (compared to the surface area) – no vegetation, < 1/3, 1/3–2/3, > 2/3; (9) Amount of reed/bulrush (compared to the surface area) – none, < 1/3, 1/3–2/3, > 2/3; (10) Dominant vegetation type on the bank – no vegetation, grasses, shrubs, trees; (11) Presence of fish (visually observed or trapped) – yes, no. Since we have only single individuals found in the immediate vicinity of water bodies (assuming a terrestrial phase), the likely terrestrial habitats were based on the level 3 data of the Corine Land Cover 2012 (CLC) in a 400-m buffer around water bodies with detected individuals. A similar approach for quantifying terrestrial habitats for newts was utilised by other authors (e.g. JOLY et al. 2001, THIESMEIER & SCHULTE 2010).

Relative abundance and density of populations

For the water bodies sampled with funnel traps, we estimated relative abundance (Ab), using the formula $Ab = N/(Tr \times H)$, where: N = count of captured adult newts; Tr = number of traps set; H = sum of

the hours of traps being set (with accuracy of ½ hour; see TZANKOV et al. 2014). The obtained values relate only to the adult individuals (present in the water during the mating season) and therefore underestimate the true size of the local populations. For five water bodies, we estimated instantaneous reproductive population size (and respective densities) with a 95% confidence limit using a Lincoln-Petersen method with capture-mark-recapture (cohort marking by toe clipping) (SUTHERLAND 2000).

Ecological niche model (ENM)

We modelled a presence-only ecological niche of *I. alpestris* in Bulgaria by combining unpublished field observations with precise coordinates collected after 2000 into a georeferenced database. We manipulated, visualised and analysed GIS data with ArcGIS v. 10.3 (ESRI, Redlands, CA, USA), generated the ENM with Maxent 3.4.1 (PHILLIPS et al. 2017) and ran statistical tests with Statistica 10.0 (StatSoft, Inc., Tulsa, OK, USA). We used a set of climatic and topographic variables available as digital layers to obtain the ENM. The climatic variables included the 19 parameters of the freely available WorldClim v. 2 (original resolution ≈ 1-km² cell; average for 1970–2000; FICK & HIJMANS 2017). We added the Global Aridity Index and the Global Potential Evapotranspiration (CGIAR-CSI Global-Aridity and Global-PET Geospatial Database; TRABUCCO & ZOMER 2009). We summed monthly values from WorldClim v. 2 to obtain the yearly solar radiation. We chose to resample the original rasters to 40-m cells, using “cubic convolution”, for the following reasons: at lower resolutions (that is, larger pixel size), crucial aquatic habitats cannot be included adequately in the model; furthermore, we considered a relatively small territory, with the primary goal to use the results for future conservation work at the local scale. The topographic variables were elevation (meters above sea level), and aspect (°) and slope (°) that we derived from a 40-m resolution Digital Elevation Model (DEM) using Spatial Analyst.

In addition, we tested models including habitat layers (surface waters) that resulted in a prediction with limited variance. Thus, we suspect that the species has very specific microhabitat requirements for which our habitat layers were not of sufficient quality, and we ultimately chose not to use them. Furthermore, important aquatic habitats such as temporary ruts on forest dirt roads are not available as digital layers.

We used systematic sampling to correct sampling bias as it consistently outperforms other methods (FOURCADE et al. 2014), especially because we do not know the specific type of sampling bias in

our data. Thus, we matched presence records to the 40-m pixels and randomly sampled one occurrence per grid cell.

To obtain the final ENM, we minimised collinearity using the “Absolute value of correlation coefficients ($|r|$)” method (DORMANN et al. 2013). We generated 100,000 random points and associated to them the corresponding values from all the variables. We calculated the Spearman Rank Order Correlations, setting $|r| = 0.7$ as a threshold. We created a preliminary ENM with the following settings: all variables included, 10 repetitions with „Bootstrap“, 25% of the presence records randomly chosen for verification in each repetition, 100,000 background points, logistic output, with a regularisation multiplier of 1. We then kept or removed variables as follows: we kept the variable with the highest percentage contribution to the ENM and removed all correlating variables; we kept the remaining variable with the second highest percentage contribution and removed all remaining correlating variables. This continued until all variables were considered. Thus, 10 continuous variables remained (Table 1, Appendix 3). We then ran the model with the same settings as before, but for 100 repetitions. The resultant “mean values” ENM was thresholded into unsuitable/suitable space using the “Maximum test sensitivity plus specificity logistic threshold” calculated by Maxent as maxSSS is one of the best thresh-

old selection method for presence/absence data (LIU et al. 2005, 2016). We classified the suitable space into Low, Medium and High suitability using Jenks Natural Breaks in ArcGIS.

Results

Mapping and ENM

Ichthyosaura alpestris was located in 121 (18.53%) of all the water bodies where newts were found ($n = 653$; Fig. 1). In the lowlands and hills (< 600 m a.s.l.), *I. alpestris* was not found. In the low mountains (600–1000 m a.s.l.), it was identified in 13.98% of the water bodies, in the mountains of average height (1000–1600 m a.s.l.) – in 47.01%, and in the high mountains (> 1600 m a.s.l.) – in 86.89%.

Original data for the distribution of the species in Bulgaria were found in 22 publications, listing roughly 50 localities (Appendix 1). We confirmed 10 of these localities and identified 112 previously unpublished (new) ones (Appendix 2). In total, the number of complete and incomplete UTM squares (10×10 km) with previously reported and new localities is 42 (3.34%; excluding one square in Vitosha Mts. where the species was introduced). We report it for the first time for 22 squares and confirm it in 11 (Fig. 2).

The resultant ENM fitted well to our expert knowledge of the ecology and distribution of this

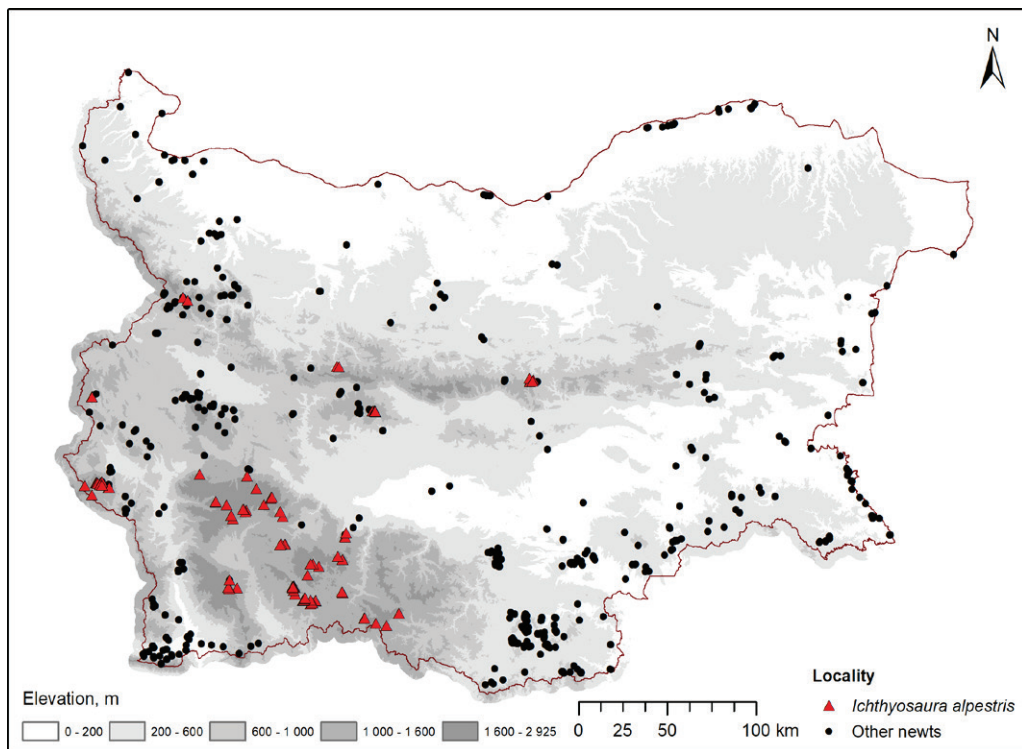


Fig. 1. Distribution of water bodies in which newts were deliberately sampled and detected in Bulgaria, during field sampling in 2000–2017.

species. Statistically, the model had very high predictive power: the average test AUC for the replicate runs was 0.982 and the standard deviation was 0.002. However, the high AUC value might be partially elevated as a result of the somewhat narrow range of the species in Bulgaria. Overall, *I. al-*

pestris could be widely distributed in mountainous areas (Fig. 3). The Low suitability area represented 2.6% (290.2 km²), the Medium – 1.7% (183.2 km²) and the High – 0.8% (85.2 km²), mostly clustered around high elevations.

Clearly, the most important factor predict-

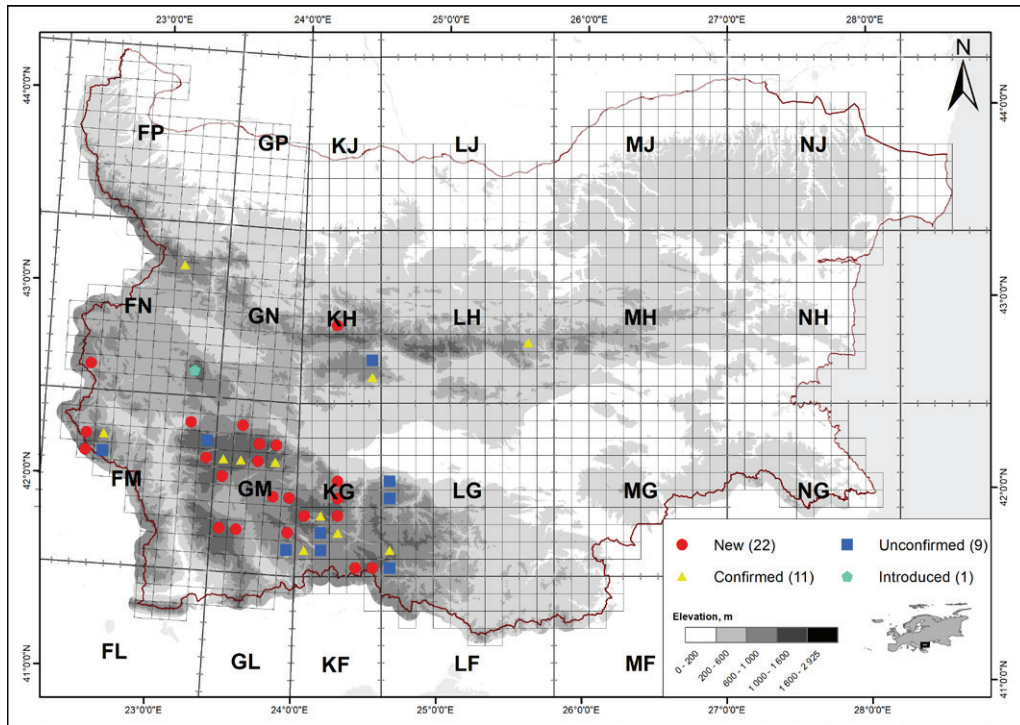


Fig. 2. Distribution of *Ichthyosaura alpestris* in Bulgaria, based on a 10 km MGRS grid.

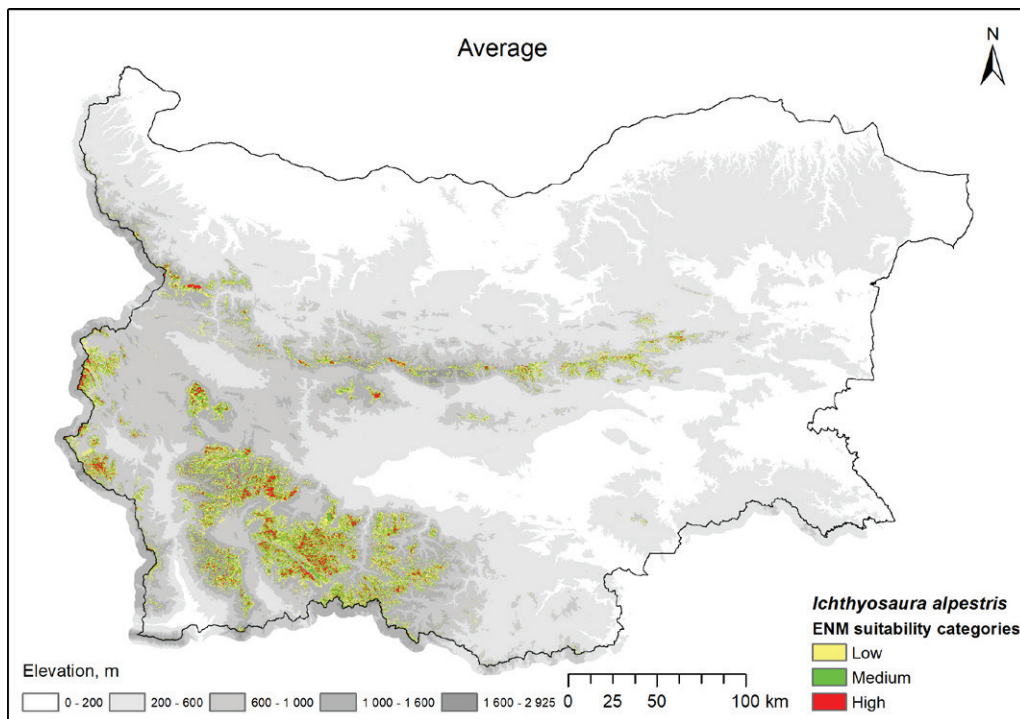


Fig. 3. Maxent average ENM for *Ichthyosaura alpestris* in Bulgaria. Suitability categories (thresholds): unsuitable (transparent), 0–0.0960; low suitability (yellow), 0.0961–0.2573; medium suitability (green), 0.2574–0.5069; high suitability (red), 0.5067–1.

ing the distribution of *I. alpestris* was the elevation (61.2% contribution; Table 1), which is expected for a glacial relict species. Slope (15.5%) reflected the need for relatively flat surfaces where water could be retained and was the variable that decreased the gain the most when omitted, suggesting it contained the most information not present otherwise. Since the species can reproduce even in tiny water bodies much smaller than the model’s cell size, such temporary waters can be situated on a range of slopes (e.g. steeper ones but with inundated ditches on dirt roads) and independently of the aspect (as favourable conditions can be found even on southern slopes in forested habitats). In Bulgaria, the species is known to inhabit mountainous regions above 800 m, where the precipitation maximums are usually in the end of winter/spring, coinciding with a warming of the temperatures, while during the summer the weather remains cooler and drier than in the lowlands. The model predicts similar results for the temperature and precipitation variables.

Clearly, elevation (as a proxy to broad climatic factors) and the potential evapotranspiration (as a proxy for presence of water required for mating) were very good predictors of the species’ potential distribution (Fig. 4). Based on the response curve, the predicted optimal elevation range for the species was around 900–2100 m a.s.l. (Fig. 5; see also Vertical distribution below). Slope, precipitation seasonality and the PET also signified the importance of the presence of (seasonal) water bodies for the reproduction of the species.

Vertical distribution

The vertical distribution covered a range of ~1700 m, with the lowest known locality being at 746 m above the Divchovoto Village in Stara Planina (this study), while the highest was at 2450 m near the Teodosievi Karauli Peak in Rila (VESELINOV 1993; height and location adjusted in TZANKOV et al. 2011). Considering the altitudinal belts, the most localities belonged to mountains of average height (51.28% of 156 localities, for which a hypsometric belt could be identified). Fewer localities were in high mountains (40.38%) and the least were in low mountains (8.33%). The distribution was close to symmetrical, with a clearly defined maximum between 1400 and 1700 m (Fig. 6).

Habitats

The water bodies where *I. alpestris* was found were highly diverse in terms of their type, size, origin, depth, etc. We summarised the frequency distribution of the characteristics of the water bodies for the

seven mountains with autochthonous populations (Table 2). Based on their origin, most water bodies were natural or semi-natural, with the artificial ones being much rarer. Sloughs were predominant among the natural habitats but lakes and swamps (combined together) were also important. Among the semi-natural water bodies, the pools predominated (these were always pools on dirt roads); the second type was not well-defined and unified water-filled, abandoned construction sites (quarries, construction pits, shafts). Artificial water bodies could be divided into two types: roadside ditches and micro-reservoirs (such as water sources for domestic animals or abandoned fishponds) but their frequency was the same. Puddles on dirt roads were the most frequent habitat type where we located *I. alpestris*. However, these results need to be analysed considering that

Table 1. Average percent contribution (PC) and permutation importance (PI) of uncorrelated variables in an Environmental Niche Model for *Ichthyosaura alpestris* in Bulgaria (T = Temperature, P = Precipitation, Q = Quarter, M = Month).

Variable	PC	PI
Elevation	61.2	18.1
Slope	15.5	10.5
Mean T, Wettest Q (BIO8)	8.1	51.5
Mean Diurnal Range (BIO2)	6.2	4.6
Aspect	3.4	2.0
Mean T, Driest Q (BIO9)	1.7	8.9
P Seasonality (BIO15)	1.6	2.0
P, Driest Q (BIO17)	1.2	1.0
Pot. Evapotranspiration	0.8	1.1
Min T, Coldest M (BIO6)	0.3	0.4

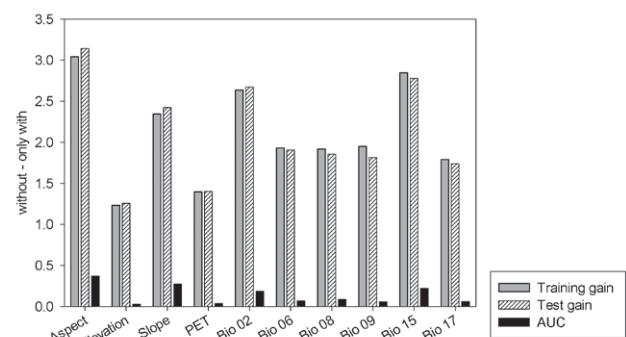


Fig. 4. Jackknife results for Maxent ENM for *Ichthyosaura alpestris* in Bulgaria. Shown are differences in average gain and Area Under the Curve (AUC) on test data between models built without and only with a given variable. Smaller differences both in gain and in AUC denote variables more highly related to the predicted distribution of the species.

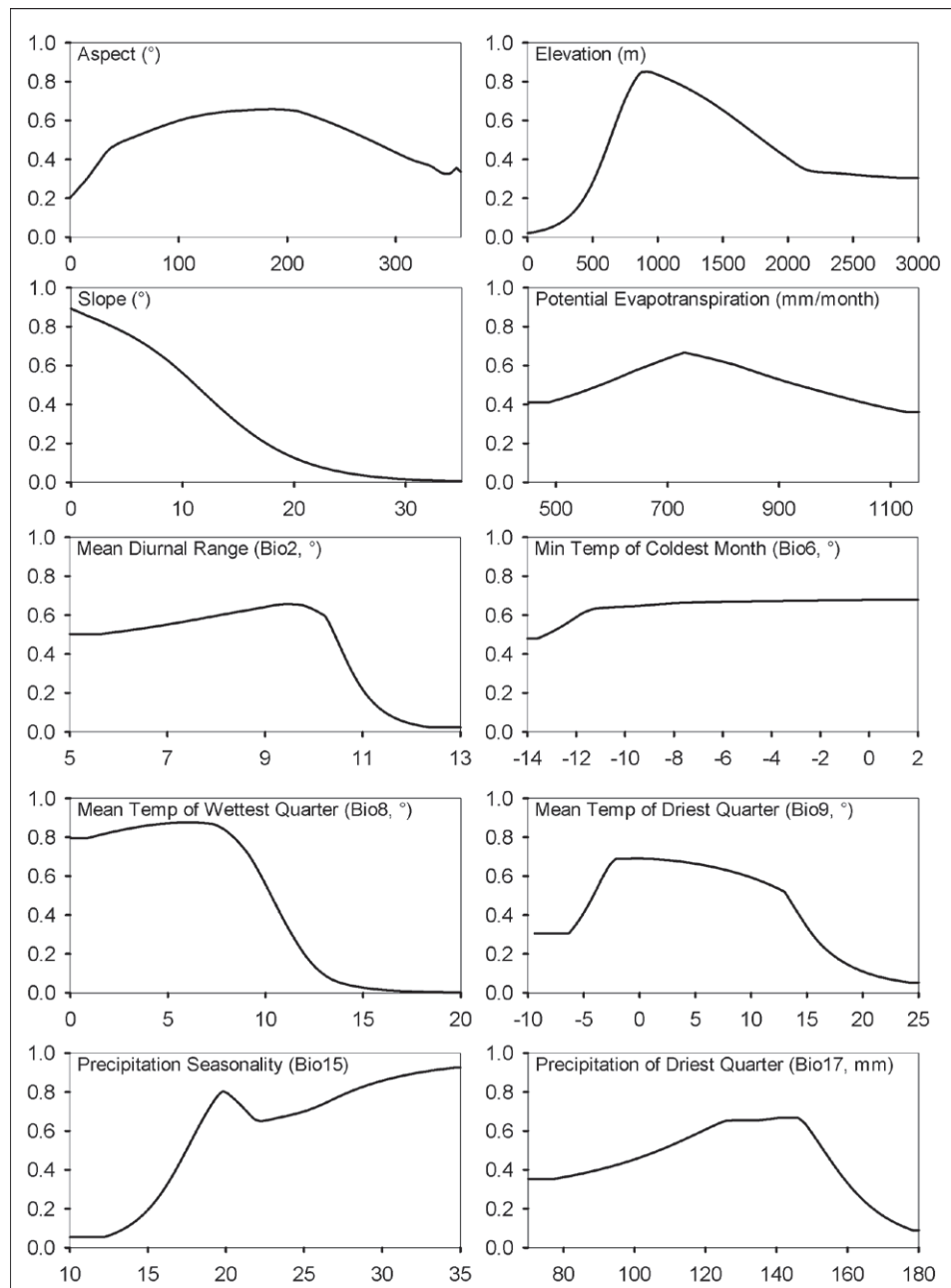


Fig. 5. Response curves of the variables contributing to the ENM for *Ichthyosaura alpestris* in Bulgaria. Log prediction is on the Y-axes.

newts are much easier to find in puddles compared to large lakes. The smallest number of observations of individuals (only two) was made in streams (including their runoffs). In this case, the subjective factor was probably not relevant since stream's width and depth were usually small (< 2 m and < 0.5 m, respectively) and the water was clear, thus finding newts was easier than in most of the other types of water bodies.

A more detailed understanding of *I. alpestris* preference for the characteristics of the water bodies could be obtained by analysing specific factors. Thus, we summarised the frequency distribution of

the water bodies with detected *I. alpestris* to the categories of the reported environmental factors for the seven mountains with autochthonous populations (Table 3). According to the shape (in topographical sense), polygonal water bodies predominated over the linear ones. According to the speed of the water flow, the number of the water bodies without current was close to that of the slow-flowing waters. In fast-flowing waters, newts were not found. These two factors were not directly related, i.e. in some surface waters there was a permanent inflow and outflow, which created a slow, weak current (noticeable only in small water bodies) and, in linear water bodies,

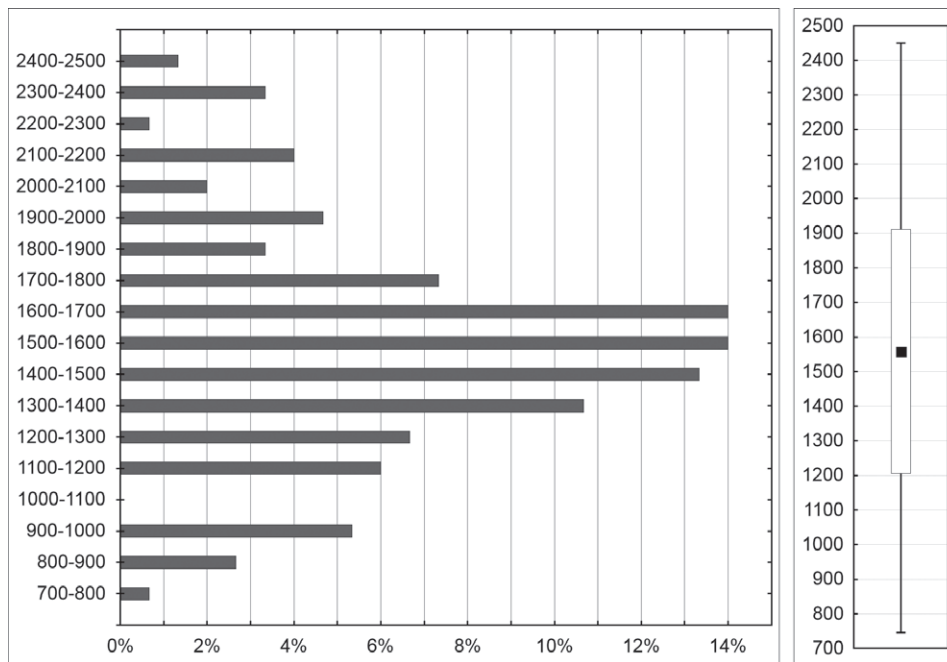


Fig. 6. Vertical distribution of the localities of *Ichthyosaura alpestris* in Bulgaria. On the left: based on 100-m altitudinal belts, as a percentage of all localities which could be attributed to a 100-m belt, n = 150); on the right: the arithmetic mean (\pm Standard deviation) and the range, based only on localities with precisely recorded altitude (n = 136).

Table 2. The frequency (%) of individual categories of origin and type of water bodies with established presence of *Ichthyosaura alpestris* in Bulgaria (generalized for 99 water bodies). Fr. (origin) is the frequency of the type in the relevant category of origin, and Fr. (total) is the frequency of the type relative to the total number of water bodies.

Origin (% from the total)	Type	Fr. (origin)	Fr. (total)
Natural (43.43)	Slough	51.16	22.22
	Lake	23.26	10.10
	Swamp	18.60	8.08
	Stream	4.65	2.02
	Stream runoff	2.33	1.01
Semi-natural (44.44)	Puddle	81.82	36.36
	Abandoned construction sites	18.18	8.08
Artificial (12.12)	Ditch	50.00	6.06
	Micro reservoir	50.00	6.06

there might or might not be current (respectively in streams or silted roadside ditches).

Although the area of the water bodies varied widely (from several square meters to about 23,000 m²), there was a progressive decrease in the number of water bodies with the increase in the area, with more than 80% of the water bodies being small (< 100 m²). A similar trend was observed with regard to the depth: most waters were shallow (< 0.5 m), suggesting that many of them probably dried annually, which led to absence of fish (cf. GIACOMA et al. 1988).

With regard to the nature of the bottom, water bodies with a muddy substrate predominated

(81%). This could be interpreted as meaning that the presence of mud favoured the typical newt escape strategy: burial in the bottom substrate. On the other hand, since most water bodies had no current (or had a slow one) and were located in areas with well-developed vegetation cover (thus, relatively deep soil), their bottoms could be expected to be muddy. Therefore, here we could not stipulate whether the data reflected a particular preference of the species to the bottom substrate or (more likely) depended on other factors.

In terms of the categories of underwater vegetation, no predominant type was detected but most water bodies lacked vegetation, while few were

Table 3. Frequency (%) of the individual categories of reported factors for the water bodies with established presence of *Ichthyosaura alpestris* in Bulgaria (N is the number of water bodies for which the relevant factor was assessed).

Variable	N	Category	Freq.
Form (topographic type)	104	Polygonal	92.31
		Linear	7.69
Current speed	104	None	54.81
		Slow	45.19
		Fast	-
Surface area (m ²)	96	< 10	51.04
		10–100	29.17
		100–10000	17.71
		> 10000	2.08
Depth (m)	103	< 0.5	72.82
		0.5–1	12.62
		1–1.5	9.71
		> 1.5	4.85
Bottom substrate	81	Muddy	81.48
		Sandy	12.35
		Stony	6.17
Underwater vegetation (area of the water body)	100	No vegetation	40.00
		< 1/3	25.00
		1/3–2/3	33.00
		> 2/3	2.00
Reed/Bulrush (area at the water surface)	100	None	90.00
		< 1/3	9.00
		1/3–2/3	1.00
		> 2/3	-
Dominant vegetation type on the bank	80	No vegetation	2.50
		Grasses	75.00
		Shrubs	3.75
		Trees	18.75
Presence of fish	100	No	92.00
		Yes	8.00

those completely overgrown. In most water bodies (90%), we found no Reed (*Phragmites australis*) or Bulrush (*Typha* sp.). This could hardly be interpreted as a species-specific requirement for the environment because plant stems and especially leaves that had fallen into the water could be used by newts for attaching eggs. More likely, the absence of reed/bulrush was due to periodic drying of the water bodies and/or to unsuitable conditions at high altitudes. Regarding the vegetation cover of the shores, most water bodies had banks dominated by grasses. Given the small area of most water bodies, the frequent absence of trees and shrubs along their shores

Table 4. Types of land cover (area expressed in percentage) within a radius of 400 m around *Ichthyosaura alpestris* localities in Bulgaria (generalized for 123 localities).

Corine Land Cover 2012 Level 3	Area
1.2.2. Road and rail networks and associated land	0.43
1.3.1. Mineral extraction sites	0.10
1.4.2. Sport and leisure facilities	1.43
2.3.1. Pastures	0.38
2.4.2. Complex cultivation patterns	0.10
2.4.3. Land occupied by agriculture, with significant areas of natural vegetation	7.87
3.1.1. Broad-leaved forest	12.52
3.1.2. Coniferous forest	34.40
3.1.3. Mixed forest	15.10
3.2.1. Natural grasslands	9.96
3.2.2. Moors and heathland	4.80
3.2.4. Transitional woodland-shrub	7.80
3.3.2. Bare rocks	1.29
3.3.3. Sparsely vegetated areas	1.61
5.1.2. Water bodies	2.21

likely provided more light by the sun, higher water temperature and better nutritional base, probably resulting in faster development of larvae compared to highly shaded water basins.

The presence of fish was recorded only in a few water bodies (8%). We identified three fish species of the family Cyprinidae occurring in two water bodies, i.e. Prussian carp *Carassius gibelio* (Bloch, 1782), Common rudd *Scardinius erythrophthalmus* (Linnaeus, 1758), and Gudgeon (*Romanogobio* sp.).

The terrestrial habitats of *I. alpestris* in Bulgaria at this stage cannot be analysed directly as field data are scarce (on land, we have found only a few individuals in the immediate vicinity of water bodies). Therefore, we apply here the approach widely used in such cases, based on the assumption that the newts depart no more than a few hundred meters from the breeding water bodies and describing the land cover around them represents the potential terrestrial habitats (JOLY et al. 2001, THIESMEIER & SCHULTE 2010).

The land cover near the water bodies with all new and confirmed records of *I. alpestris* included 15 categories (Table 4). The largest territory was lands covered by forests (62.02% combined for “Broad-leaved”, “Coniferous” and “Mixed” forests). The second type of territories were lands with transient vegetation (20.47% for “Transitional woodland-shrub”, “Moors and heathland” and “Land principally occupied by agriculture, with significant areas of natural vegetation”). The third type was open ar-

Table 5. Estimated instantaneous reproductive population sizes (N with 95% confidence interval, CI) and the respective densities (D as specimens per m²) of *Ichthyosaura alpestris* for five water bodies in the mountains of Stara Planina, Osogovo and Rhodopes (m² = surface area of the water body; Date = sampling period; Effort = number of trap sessions × number of funnel traps).

Locality	m2	Date	Effort	N	95% CI	D
Petrohan	70	08–10.05.2014	3 × 10	244	179–435	3.5
Krastets	377	28.04–30.06.2012	5 × 29	419	365–499	1.1
Novo Selo 1	800	24–26.04.2014	3 × 15	5662	4159–10072	7.1
Novo Selo 2	90	24–26.04.2014	3 × 10	579	497–706	6.4
Bistroto Lake	4172	26.05–11.07.2008	6 × 20	720	618–876	0.2

Table 6. Sex ratios (males : females) of *Ichthyosaura alpestris* in Bulgaria according to the size of the elementary samples (the samples with N ≤ 30 are not given separately, but the individuals are included in the total number, i.e. only in the last row).

Categories	Min ÷ Max	Median	Generalized
N > 30 (25 samples; 1966 specimens)	0.25:1 ÷ 7.29:1	1.79:1	2.04:1
N > 50 (18 samples; 1698 specimens)	0.25:1 ÷ 7.29:1	2.87:1	2.24:1
N > 80 (11 samples; 1284 specimens)	0.88:1 ÷ 5.13:1	2.95:1	2.65:1
N > 100 (4 samples; 675 specimens)	1.79:1 ÷ 3.18:1	2.51:1	2.50:1
N > 200 (1 sample; 213 specimens)	n/a	n/a	3.18:1
Total (148 samples; 2442 specimens)	n/a	n/a	1.89:1

areas (13.25% for “Pastures”, “Natural grasslands”, “Sparsely vegetated areas” and “Bare rocks”). The remaining five types of land cover comprised insignificant area (4.27% in total).

Relative abundance and density of populations

The relative abundance of *I. alpestris* in the Bulgarian mountains varied widely: in Stara Planina (a total of 15 traps set in 6 water bodies) – from 0.006 to 0.943 individuals per trap-hour; in Sredna Gora (a single sampling of 2 water bodies) – 0.500 and 1.086; in Osogovo (8 trappings in 2 water bodies) – from 0.437 to 1.183 (the highest relative number registered by this method); in Rila (one study of 4 water bodies) – from 0.004 to 0.012; in the Rhodopes (13 trappings in 7 water bodies) – from 0.002 to 0.579; in Pirin (a single study of 2 water bodies) – 0.006 and 0.013; in Vitosha (trapping twice in the water body where the species was introduced) – 0.039 and 0.046. The estimated instantaneous population density varied between 0.2 and 7.1 ind./m² (Table 5).

Sex ratio

We captured 1598 male and 844 female *I. alpestris* (summed up for all visits to all water bodies), indicating a male : female ratio of 1.89 : 1. By grouping individual samples (single samplings per water body) according to the number of caught newts and the subsequent exclusion of the lower categories

(with calculated sex ratio for each individual sample), we could obtain some idea of the impact of the sample size on the ratio obtained (Table 7). In individual samples with N > 30, the sex ratios ranged from 0.25 : 1 to 7.29 : 1, with the median at 1.79 : 1 (i.e. close to the ratio calculated for all individuals). In the single sample of N > 200, the ratio differed notably from that calculated for all individuals (3.18 : 1 vs. 1.89 : 1). The statistical tests performed (total number of newts compared to the sex ratio calculated for each of the mentioned categories, see Table 6) showed a significant negative correlation between the number of individuals captured and the sex ratio obtained (Spearman $r = -0.94$, $p < 0.05$; Kendall $\tau = -0.87$, $p < 0.05$).

Discussion

Mapping and ENM

During our field studies, *I. alpestris* was found only in areas with an elevation above 600 m, although the observations were conducted throughout the country (from the sea level to the highest mountains). Clearly, *I. alpestris* is a mountain-associated species in Bulgaria, as it has also been supported by previous descriptions (e.g. BURESCH & ZONKOW 1941, BESHKOV 1985, STOJANOV et al. 2011). The presented new data vastly increase knowledge of the distribution of this species in Bulgaria. Concerning the mapping on UTM 10 × 10 km, more than 52% of the

grid-cells are new. Regarding the known distribution at the continental level (50-km grid, see SILLERO et al. 2014), our data confirm the published presence data and add three new cells: GM2, KG3 and LH4. The ENM predicted a wider distribution of *I. alpestris* in Bulgaria (compared to the established by direct mapping) and confirmed that all of the potentially suitable areas are in mountains. Overall, 95% of the country is likely uninhabitable by this species and there are major environmental barriers isolating the known localities. The anthropogenic impacts, which are not discussed here, need to be considered further as they will exacerbate potential perils for small and (or) isolated populations.

Vertical distribution

Compared to the broader geographic range of *I. alpestris*, its vertical distribution in Bulgaria is very similar to that of Greece (700–2400 m, VALAKOS et al. 2008) but differs from that in Romania reported as from 150 to over 2000 m (COVACIU-MARKOV et al. 2009, COGĂLNICEANU et al. 2013). In the western Balkans, it may inhabit lower elevations 500–2300 m in Albania (SZABOLCS et al. 2017), and as low as 80 m in Croatia (KALEZIĆ et al. 1990). In the western parts of Southern Europe, it seems both low-altitude and high-altitude limits are lower than those in the Balkans: 35–2380 m in Italy (ANDREONE & TRIPEDI 2006) and 0–2200 m in Spain (RECUERO-GIL & MARTÍNEZ-SOLANO 2002).

Review of the distribution per mountain

Below we review the data for the presence (and potential absences) of *I. alpestris* in the mountains in Bulgaria and compare them to the ENM results.

Stara Planina (including the Predbalkan).

Until recently, *I. alpestris* was known only from the Petrohan Pass (Fig. 2: FN77). It was hypothesised that this was the sole locality for the mountain (BESHKOV 2015; however, the data here cover only until 2011). However, two other localities were recently reported, one of them close to Petrohan (POPGEORGIEV et al. 2014) and another far to the east, near the town of Tryavna (STOJANOV et al. 2011). We confirmed the known localities and identified seven new ones, mostly situated around the Tryavna region. Two locations above the Divchovoto Village (Fig. 2: KH74) are situated midway between Petrohan and Tryavna. All localities in Stara Planina are between 760 and 1400 m a.s.l., although large areas exist with higher elevation (the highest peak is 2376 m). In Western Stara Planina, the potential absence of the species was identified at 56 sampled water bodies (situated between 150 and 1680 m a.s.l.), and in Central and

Eastern Stara Planina – in 13 water bodies (130–880 m a.s.l.) and 23 (50–510 m a.s.l.) (Fig. 1). Although our data so far were concentrated in three general areas, the ENM suggested a wide potential distribution. Due to the low sampling effort so far there, we presume that the species will be found in multiple other localities, especially on the northern slopes, resulting in a more continuous distribution.

Sredna Gora. In this mountain, *I. alpestris* is known only from a small region in the central, highest part of the mountain: four closely situated localities near the peaks Bogdan and Malak Bogdan (CHICHKOFF 1914, VESELINOV 1993). In 2012 and 2014, we found the species in nine small water bodies in the same region (including in one reported by VESELINOV 1993). All known localities are situated at 1400–1500 m a.s.l., suggesting that the elevation of 1200 m given by CHICHKOFF (1914) likely is not correct. In the same part of the mountain (Sashtinska Sredna Gora), the potential absence of the species was identified at 10 sampled water bodies (440–1340 m a.s.l.). In the western part of the mountain (Ihtimanska Sredna Gora), we could not find the species in seven water bodies between 670 and 1070 m a.s.l., during multiple samplings in the past 25 years. However, we have not sampled in the eastern part (Sarnena Sredna Gora), which falls within the known altitudinal range for the species (the highest peak is 1235 m). The ENM covered predominantly the higher parts of the mountain, with more suitable areas in the western part of Sashtinska Sredna Gora.

Osogovo. The first record for this mountain is quite recent (1993), due to the limited research carried out there until then. Ten localities were described by VESELINOV (1993), NAUMOV (2005) and STOYNEVA & MICHEV (2007). We note that the elevation of 1200 m for the locality near Novo Selo in NAUMOV (2005) is in fact 920 m. In 2007–2016, we identified another nineteen localities and confirmed presence in three published ones. All known localities are situated between 920 m and 1760 m, west of the Novoselska River that approximately separates the higher western part of the mountain from the lower eastern one. We identified potential absences in seven water bodies: one in the western part (600 m) and six in the eastern part (630–880 m a.s.l.). The model predicted a wide distribution throughout the mountain, which partly matches the known distribution.

Rila. *Ichthyosaura alpestris* is known from nine precisely described localities (BURESCH & ZONKOW 1941, BESHKOV 1985, VESELINOV 1993, PESHEV et al. 2005, STOYNEVA & MICHEV 2007). BERON et al. (2000) mentioned the presence of the species in four park sections of the Rila National Park with-

out specifying localities; given the nature of the publication, we stipulate that the authors have summarised or confirmed previous data. The locality in the Yundola area (Fig. 2: GM36) is situated at the border between the Rila and Rhodopes Mountains. This has led some authors to attribute it to the Rhodopes (BESHKOV 1985, VESELINOV 1993, PETROV et al. 2006), to Rila (BESHKOV & STOYANOV 2000) or to both (STOYNEVA & MICHEV 2007). Based on the original published descriptions (as well as our observations in the same locality), *I. alpestris* individuals were found in the marshes occurring along the western bank of the Yundolska River (ca. 1–2 m wide), considered to separate the two mountains. Therefore, it is more accurate to attribute this locality to Rila, although we stipulate newts are likely found east of the stream as well. We identified twenty new localities and confirmed a published one, situated between 1350 and 2450 m a.s.l. Potential absence was recorded in four water bodies (480–1300 m a.s.l.). The southwestern part of the mountain (south of the Rilska and Iliyana Rivers, and west of Belishka River) as well as some of the lake clusters in the central part (including the region of Musala Peak) were not sampled. The model predicted a wide distribution throughout the mountain, which matches the known distribution.

Rhodopes. Eighteen localities have been published, mostly by BURESCH & ZONKOW (1941), VESELINOV (1993) and PETROV et al. (2006). We confirm two localities and identify fifty new ones, all situated between 840 and 1980 m a.s.l. The localities are found only in the higher western part of the mountain (Western Rhodopes), with the easternmost being at Zdravets Hut (BESHKOV 1985), the Studenets Resort (PETROV et al. 2006) and around the Smolyan Lakes (BURESCH & ZONKOW 1941, BATCHVAROV 1970, VESELINOV 1993, present study). In Western Rhodopes, the species was potentially absent from three water bodies (270–740 m a.s.l.), while in the Eastern Rhodopes – in all 168 sampled water bodies (160–660 m a.s.l.). The model predicted a wide distribution throughout the western portion of the mountain, which matches the known distribution. An extensive part in the eastern Western Rhodopes is possibly suitable but so far insufficiently sampled. The Rhodopes is the mountain with the highest number of known localities of *I. alpestris* in Bulgaria (both previously published and new). However, it is important to note that the Rhodopes is much larger than most other mountains, except for the much less studied Stara Planina.

Pirin. No confirmed published reports for *I. alpestris* exist for Pirin. VESELINOV (1993) mentioned

unconfirmed data for individuals found in the Vlahinski Lakes and the region of Pirin Hut but during his investigations of these (and other) regions he did not find any newts. However, in 2012 and 2014, we found *I. alpestris* in six water bodies (1390–2160 m) in the bottom parts of the Kremenski and Popovoezeren Cirques, in the valley of the Disilitsa River and in the region on the Shtrekova Skala Peak. It is worth noting that, as well as in Stara Planina and Rila, large territories of Pirin are at high elevations (the highest peak is 2914 m), also shown as high suitability based on the ENM. However, we have not carried systematic searches in Pirin (trapping and dip-netting) but only non-systemic, short-time visual observations at some of the multiple lakes and marshes there. In the lower parts of the mountain (650–770 m a.s.l.), we have not found the species in nine sampled water bodies.

Karvav Kamak. For this or the other multiple mountains in the Kraishte Region, *I. alpestris* has not been previously reported. In 2012, we have found three adults in two temporary pools at about 950 m near Bazovitsa Village. In the only other sampled water body (near the Dalga Luka Village at 840 m), the species has not been found. The ENM has predicted a wide distribution, which has to be verified by further sampling. The closest known localities are in Serbia in the region of the Vlasinsko Lake, 10–15 km west and north-west of Bazovitsa (see SOTIROPOULOS et al. 2007).

Vitosha. We are unaware of extant autochthonous *I. alpestris* in Vitosha. In the year 2000, it has been introduced in two water bodies. The long-term survival in one of them (at 1720 m) has been proven (NAUMOV & LANGOUROV 2008, TZANKOV et al. 2014; unpublished observations in 2015), while the second water body (at 1450 m) was not investigated after the introduction. The species is potentially absent, based on 22 sampled water bodies (670–1930 m a.s.l.). The ENM suggested that Vitosha is highly suitable (Fig. 3), supporting the deductive hypothesis of NAUMOV & LANGOUROV (2008) that native populations of *I. alpestris* either has inhabited the mountain in the recent past or still survive.

Other mountains. We have not located the species while sampling in 13 other mountains (Appendix 4). We have not specifically sampled for newts in the remaining mountains: e.g., Vlahina, Stargach, and most of the smaller mountains in the Kraishte Region. Based on the model's predictions and our expert opinion, mountains on which future sampling for the species needs to be concentrated include Ruy, Milevska, Zemenska, Vlahina, Maleshvska and Ograzhden (all of them situated at the

western country border). Although isolated patches of potentially suitable habitat are present around the Vishegrad Peak in Sakar, we believe it is unlikely that the species is present there.

Habitats

Our results indicate that in Bulgaria, *I. alpestris* inhabits quite various to their origin and type water bodies. Most frequently, the species has been found in the puddles on dirt roads, although this could be partly based on sample bias. Still, undoubtedly puddles play a very important role as suitable water bodies for reproduction, especially in regions heavily impacted by human activities that lead to loss and/or quality deterioration of the natural water bodies (e.g. deforestation, stocking with predatory fish). The fact that most puddles have a highly non-stable water regime (periodical or stochastic drying out based on the climatic conditions during a given year/season) likely does not influence significantly the newts' ability to use them. An interesting example in that respect was recorded in 1988 in Lower Saxony, Germany, by WINKLER & BRAUNS (1990): at the beginning of the summer, all 77 sampled puddles dried up completely leading to the mortality of the newt larvae; in the end of July, the same puddles re-filled with rainwater and adults re-entered for a second reproductive period; larvae successfully metamorphosed during the same year and next spring. The streams (according to our observations) seem to be the water habitat most avoided by Alpine newt. According to ROČEK et al. (2003), the observations of *I. alpestris* in streams are probably of individuals moving to breeding ponds. In this sense, streams should be considered mainly as bio-corridors, not as breeding sites. The other types of water bodies (excluding ditches) cannot always be properly distinguished from each other. For example, analysing literature on the aquatic habitats of *I. alpestris* in Germany, THIESMEIER & SCHULTE (2010) found that the predominant type was "Teich, Tümpel, Weiher" [pond, pool, pond] but explicitly stated that this type cannot be precisely defined and includes all kinds of natural or artificial water basins. Large variations in the area and depth of the waters inhabited by *I. alpestris* (established by our research) have been repeatedly found elsewhere, e.g. SZCZERBAK & SZCZERBAN (1980) reported areas between 1.5 and 70000 m² and DENOËL (2004) provided areas between 0.6 and 25000 m² and depths between 0.3 and 4.95 m.

Concerning the water vegetation, our observations indicate that the species occurs in fully overgrown water bodies as well as in ones without any vegetation. In contrast, BESHKOV (2015) has assert-

ed that waters inhabited by *I. alpestris* always have abundant underwater vegetation. The data from other parts of the range of *I. alpestris* on this issue are also ambiguous, e.g. for Albania, HAXHIU (1994a) has mentioned that the species prefers waters with scarce or no vegetation; in Montenegro, Ćirović et al. (2008) have found newts predominantly in water bodies with both macrophytes and algae but also only with algae, only with macrophytes or without any vegetation. It should be borne in mind that this factor is highly dependent on the time of the year, i.e. vegetation may not have yet developed in the early spring, whereas in summer it may be abundant. The role of vegetation in the reproduction of newts is mainly in the provision of space for egg deposition – the female attaches the individual eggs to underwater leaves of aquatic plants. Still, the presence of tufts of grass or fallen leaves and twigs is sufficient for this purpose (THIESMEIER & SCHULTE 2010).

The registered cases of coexistence of *I. alpestris* and fishes in Bulgaria are very rare, which well corresponds to the widely accepted views on this topic. Most publications treating the presence of fish suggest that *I. alpestris* avoids fish-inhabited waters, especially if there is no vegetation (see, e. g., THORN 1968, ROČEK et al. 2003, COVACIU-MARKOV et al. 2009). An exception is the data obtained by Ćirović et al. (2008) in Montenegro: in almost half of the aquifers with *I. alpestris* there are unspecified fish species (could be either predatory, omnivorous or plant eating). According to multiple authors, the presence of predatory fish has a strong negative impact on newt populations (e.g. BRANA et al. 1996, ORIZAOLA & BRANA 2006, HARTEL et al. 2007, BESHKOV 2015).

If we summarize the results for the aquatic habitat characteristics, we obtain the following picture: in Bulgaria, *I. alpestris* prefers natural and semi-natural, small, shallow, standing water bodies with muddy bottom, exposed (at least in part) to direct sunlight and devoid of fish species. This largely coincides with the summary of VESELINOV (1993) based on a survey of a total of 23 water bodies (5 of them surveyed also by us) in five mountains: "The alpine newt prefers small, shallow and highly vegetated water bodies located on marshy meadows and open places that are well sun-lit as well as roadside ditches". According to STOJANOV et al. (2011) (without the authors having conducted a specific study), the species inhabits different types of water bodies with well-developed underwater vegetation, including temporary puddles. BESHKOV (2015) (also without referencing specific data) mentions the following habitat characteristics: "stagnant or poorly flowing wetlands, including such that are insignifi-

cant in their area and depth but always with ample underwater vegetation, some also with overwater vegetation”. It is interesting to note that national summaries for other countries almost always mention a wide variety of water bodies inhabited by *I. alpestris*. For Greece, VALAKOS et al. (2008) wrote: “It occurs in both deep and shallow water bodies, permanent or temporary, such as pools, ponds, lakes, ditches, slow flowing streams, with clear, cool water”. For Montenegro, ĆIROVIĆ et al. (2008) have found that it inhabits mainly natural lakes. For Albania, HAXHIU (1994b) have mentioned it is “living in pure, cold water, mainly mountain lakes and more rarely streams or pits full of water”. For Germany, THIESMEIER & SCHULTE (2010) describe as the most suitable for the species the small and medium-sized forest water bodies with a shallow coastal area, which are not entirely overgrown with underwater vegetation. ROČEK et al. (2003) summarised literature data from different parts of Europe and suggested that breeding ponds range from very small puddles to large lakes.

Each of the above-mentioned environmental factors has a relatively high tolerance, i.e. nearly every possible condition (according to the defined categories) of the relevant factor is present. This, to some extent, confirmed the opinion of THIESMEIER & SCHULTE (2010) that no single natural factor directly determines the presence or absence of *I. alpestris* in a given water body. However, in this sense, there seems to be at least one exception: the species has not been found in Bulgaria (and as far as we know anywhere else) in fast-flowing mountain streams (“linear water body” and “high water velocity”). It is obvious that some individual conditions are interconnected (e.g. in temporary water bodies one does not expect fish) and probably depend on other, unexplored factors. Long-term studies based on a wider set of data are needed to clarify the complexity of the habitat factors.

Published data on the habitats of *I. alpestris* in Bulgaria during its terrestrial phase are virtually absent. BESHKOV (2015) mentions that “it spends the summer and the autumn on the ground under stumps, in soil cavities, etc.”, but this can only be interpreted as daily shelters (in both locations where we find the species on land, the individuals are under stones). We cannot find specific analyses of the terrestrial habitats of *I. alpestris* for the Balkan Peninsula, although there are some (probably purely deductive) generalisations, e. g. for Greece VALAKOS et al. (2008) wrote that breeding ponds are located “in forests, forest meadows, alpine grasslands and sometimes in rocky alpine plateaus”. On the other

hand, publications from Central Europe show that forested areas are always the predominant terrestrial habitat of *I. alpestris* (e.g. SZCZERBAK & SZCZERBAN 1980, DENOËL & FICETOLA 2008, COVACIU-MARKOV et al. 2009, THIESMEIER & SCHULTE 2010), matching our analysis for Bulgaria. It should be borne in mind that with regard to the terrestrial habitats, both here and in the reviewed publications, these are theoretical considerations and not true habitat preferences, i.e. the indicator was only the area of the individual land cover categories, without the presence of the species in them being taken into account.

Relative abundance and density of populations

It seems that the method used in our study for identifying the relative abundance has been used very rarely so far (at least in studies on the Balkans). In our opinion, it provides a higher degree of uniformity (respectively, objective results) in large-scale monitoring studies, compared, for example, with dip-netting or counting newts from the shore. Although more long-term and robust estimations are needed, likely *I. alpestris* can attain large local population sizes at certain localities (given also suitable terrestrial habitats around the water body). Available data on the density of *I. alpestris* populations in neighbouring countries are scarce. DENOËL & SCHA-BETSBERGER (2003) established (by mark-recapture) densities of 0.3 and 4.4 ind./m³ in two high-altitude mountain lakes in Greece, which, recalculated to the areas of the lakes (provided by DENOËL 2004), is respectively 0.5 and 3.7 ind./m². For Albania, HAXHIU (1994b) has mentioned a density of 13–15 ind./m².

It is worth mentioning that we have captured 47 adult newts with a single sweep of a round dip net (30 cm diameter) in less than 1 m² of water surface in a flooded ditch along the SW coast of the Dospat Reservoir in the Rhodopes (28.05.2008). Similar data for Bulgaria were only mentioned in BESHKOV (2015): “In some habitats (Yundola, 1995; Osogovska Mountain, 2001–2006), it is abundant – up to about 30 individuals have been observed on an area of 1 m² in the shallow places of the wetland inhabited”. Furthermore, based on our experience, other newt species (genera *Lissotriton* and *Triturus*) sometimes concentrate in particular parts of the water body while missing in others, possibly due to microhabitat differences. Therefore, we strongly urge any sampling (especially concerning density estimations) to be as exhaustive as possible and to cover as much of the water body as feasible.

The relative abundances and densities presented here have been obtained during short-term surveys (either single visits or multiple but during

one season). Therefore, they must be compared only with caution with published long-term research data (such as those conducted in Central and Western Europe). Medium- and long-term studies in all cases show large or very large variations in the population numbers, while studies conducted over only one or two years represent only snapshots in a highly dynamic system (THIESMEIER & SCHULTE 2010).

Sex ratio

Results of the present study suggest that as the sample size increases, the difference between the number of males and females decreases, therefore, it can be assumed that the real sex ratio is close to the theoretically expected (1 : 1); still, a much larger sample is needed to prove this. The literature data on sex ratios of *I. alpestris* vary considerably. For Bulgaria, such data have only been published by STOJANOV et al. (2011) reporting a ratio of 1.01 : 1 based on 257 individuals from Osogovo (not included in this study). VESELINOV (1993) has not specifically addressed this question but mentions that he has found 177 males and 156 females (from Rila, Rhodopes, Osogovo, Sredna Gora and Stara Planina), which equals to 1.13 : 1 when transformed according to the way of presentation here. GLANDT (1978) has recorded that males were more numerous than females and gave ratios of 6.14 : 1, 2.26 : 1, 1.86 : 1, and 0.75 : 1 (converted from the original percentages) based on a sample of 441 individuals from 4 water bodies in Germany. KLETEČKI (1995) has found a slight predominance of males, an average of 1.31 : 1 and 1.26 : 1 (converted) based on 1344 and 219 individuals, respectively, captured during three-year studies of two water bodies in Croatia. SZCZERBAK & SZCZERBAN (1980) have observed significantly more females than males, with a ratio of 0.39 : 1 (converted), based on 214 individuals from the Ukrainian Carpathians. FABER (1996) has also determined a prevalence of females and reported an average of 0.69 : 1 (converted) based on thousands of specimens captured during triennial studies of 50 water basins in Austria. In the same work, a review of 27 publications from Central and Western Europe provides highly varying ratios: in some cases the males predominate (1.1 : 1 to 6 : 1) and in others the females are more abundant (from 0.91 : 1 to 0.22 : 1).

Most likely, the variances found by different authors are due both to the (mostly small) sizes of the respective samples and to the natural variations in the proportion of the sexes in the water bodies throughout the breeding season. Here, we do not specifically investigate this issue but we should mention the following observation: during one rainy night (4/5.05.2012) near a water body in Osogovo

we have observed about 30 adult *I. alpestris* that are apparently involved in reproduction (the males with well-developed crests) but temporarily outside the pond; the movement of these animals is slow and varies in direction, which excludes the possibility of targeted migration. Such manifestations of terrestrial activity at the height of the breeding season can be explained by the demand for food caused by high newt density in the water itself and the resultant strong intra- or interspecific competition. In the case described above, the water body is the one in which the highest relative abundance of *I. alpestris* was established in Bulgaria; besides, this water body is one of the few known ones, where three newt species are present (see NAUMOV 2005). The same idea has been suggested by KLETEČKI (1995): “Sex ratio changes [...] indicating existence of migration in and out of a puddle during the aquatic life phase. The newts mating intensity changes with the change of sex ratio, and this is maybe one more way (if these ratios harmonise among species in [a]syntopic population) of competition decrease among species”. It is possible that such potentially short-term terrestrial forays occur frequently but remain unnoticed; for example, in our case, the observation was accidental, only because during the night we have been camping near the water body. On the other hand, such phenomena undoubtedly influence the results of sex studies and the relative abundance of newts. Sex ratio dynamics in breeding ponds have not been sufficiently explored, and existing claims on this issue are ambiguous. According to SZCZERBAK & SZCZERBAN (1980), the males leave the water first, followed by the females one or two weeks later. According to FABER (1996), at the beginning and at the end of the breeding season, the number of males in the water is higher than that of females, whereas during courtship and the eggs deposition it is the opposite. According to ANDREONE & TRIPEPI (2006), both sexes remain in the water throughout the breeding season.

In view of the above, it can be assumed that the sex ratio in the water bodies during the breeding season is quite dynamic and is determined by locally specific factors. We further stipulate that such dynamics might vary between different types of water bodies (e.g. temporary vs permanent, temperature, prey availability), influencing the duration of the mating season and the respective behaviour of the individuals.

Conclusions

The alpine newt has a wider distribution in Bulgaria than previously reported and occurs naturally in at least seven separate mountains. Rila, the high-

est mountain on the Balkans, contains the highest known locality on the peninsula, and one of the highest in general. Even though elevation is the best ENM predictor, the distribution of *I. alpestris* is not directly correlated to the absolute altitude of the separate mountains – e.g. it has not been identified on Vitosha and Slavyanka, which are higher than the Rhodopes where it is widespread. The localities in the region of Tryavna are the easternmost, which is significant in terms of delineating the range of the species on the continental level.

The absence of *I. alpestris* from the plains, lowlands and valleys (along with the low mountains in SE Bulgaria) are likely due to factors related to local conditions (low elevation, high mean temperatures during rainy months, etc.) that do not match the environmental requirements of the species. On the other hand, *I. alpestris* is missing from two mountainous regions that are visibly similar in characteristics to localities with known occurrences: 1) the southern parts of the Western Stara Planina, Ihtimanska Sredna Gora, Vitosha, Plana, Verila, Konyavska, Lyulin and the mountains in the Tran district; 2) the mountains south of Osogovo, including Slavyanka. Some of those mountains have not been surveyed but others were extensively sampled (Western Stara Planina, Vitosha, Maleshevska, Belasitsa). Currently, we cannot explain the potential absence of the species there.

According to the extensive sampling of various habitats, it seems that *I. alpestris* prefers (as breeding sites) shallow water bodies with muddy bottom, grassy banks, limited reed/bulrush and free of fish.

The results for the population parameters of the species (relative abundance, density, sex ratio) have to be treated with caution as the data were collected during different months, at different elevations, and in different types of water bodies. Still, other similar data for newts in Bulgaria are very limited, so these results should serve as the foundation for future more detailed studies, especially considering the ongoing global climate changes that will further limit suitable habitats.

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Appendix 1. Published localities for *Ichthyosaura alpestris* in Bulgaria (sorted alphabetically by the names of 10×10 km MGRS grid cells). To minimize the text size in most cases the original descriptions were shortened while allowing their identification within the original publications; confirmed localities are marked with an asterisk. Abbreviations: **FM36**: Tsarna Reka Reserve (VESELINOV 1993); **FM37**: Osogovo Hut*, 830 m from Osogovo Hut, 1.5 km from Osogovo Hut, Trite Buki Hut, 2.5 km from Trite Buki Hut, Plaviloto Place*, and Plavilski Vrah Peak (VESELINOV 1993), Novo Selo* (NAUMOV 2005, STOJANOV et al. 2011), IBW 9751 (STOYNEVA & MICHEV 2007); **FM97**: Suhoto Ezero Lake (BURESCH & ZONKOW 1941, PESHEV et al. 2005, STOYNEVA & MICHEV 2007 as “IBW 0384”, TZANKOV et al. 2011 as “N42 9 58.6 E23 25 12.1”), Sedemte Ezera Place (BESHKOV & STOYANOV 2000, BESHKOV 1985, STOYNEVA & MICHEV 2007 as “IBW 5746”); **FN77**: Petrohan* (summarized for three separate small wetlands, located close to each other) (BEŠKOV & BERON 1964, KABISCH 1966, VESELINOV 1993, STOYNEVA & MICHEV 2007 as “IBW 0662”), FN77C3* (POPGEORGIEV et al. 2014); **FN81**: SE of Aleko Hut* (NAUMOV & LANGUROV 2008, TZANKOV et al. 2014); **GM06**: below Teodosievi Karauli Peak (VESELINOV 1993, STOYNEVA & MICHEV 2007 as “IBW 9753”, TZANKOV et al. 2011 as “N42 7 2.5 E23 27 18.2”), Kirilova Polyana Area (PESHEV et al. 2005, TZANKOV et al. 2011 as “N42 8 29.5 E23 26 42.6”), IBW 0372 (STOYNEVA & MICHEV 2007); **GM16**: Yakorudsko Ribno Ezero Lake (BURESCH & ZONKOW 1941), between the lakes of Yakorudsko Ribno and Martvo (VESELINOV 1993), IBW 5747 (STOYNEVA & MICHEV 2007); **GM36**: Yundola Place* (BESHKOV 1985, VESELINOV 1993, BESHKOV & STOYANOV 2000, STOYNEVA & MICHEV 2007 as “IBW 0675”); Unmapped localities: the park area “R11”, “R13”, “R22”, “R31”, and “R32” in Rila National Park (BERON et al. 2000); **GM41**: Satovcha (SOTIROPOULOS et al. 2007); **KG51**: 6 km W of Dospat* (PETROV et al. 2006); **KG61**: Dospat Dam (PETROV et al. 2006); **KG62**: Dzhenevra Place (VESELINOV 1993); **KG63**: Forester’s lodge of Beglika (BURESCH & ZONKOW 1941), Golyam Beglik Dam (formerly Vassil Kolarov Dam) (BELCHEVA 1959, VESELINOV 1993), IBW 9715 (STOYNEVA & MICHEV 2007); **KG72**: Izvora Place and Suvarla Tepe Place (VESELINOV 1993), IBW 3157 (STOYNEVA & MICHEV 2007); **KH91**: 2.5 km from Chevira Hut* (VESELINOV 1993); **KH92**: Malak Bogdan Peak (KOVACHEV 1912, CHICHKOFF 1914), Bogdan Hut (VESELINOV 1993); **LG00**: Smolyan Lakes (BURESCH & ZONKOW 1941, STOYNEVA & MICHEV 2007 as “IBW 5750”), the lower Smolyan Lake, the middle Smolyan Lake, and the upper Smolyan Lake (BURESCH & ZONKOW 1941), Smolyan (ARANO & ARNTZEN 1987, RECUERO et al. 2014); **LG01**: Sinyoto (Bistroto) Ezero Lake* (BATCHEVROV 1970, STOJANOV et al. 2011 as “Blauer See”), the second lake above Smolyanski Ezera Hut (VESELINOV 1993); **LG04**: Studenets (PETROV et al. 2006); **LG05**: Zdravets Hut (BESHKOV 1985); **LH83**: an undefined point SSE from Tryavna* on the map given by STOJANOV et al. (2011); **Unmapped localities**: the park areas “R11”, “R13”, “R22”, and “R31” in Rila National Park (BERON et al. 2000).

Appendix 2. New localities for *Ichthyosaura alpestris* in Bulgaria (sorted alphabetically by the names of 10×10 km MGRS grid cells); the localities are described as the names of the closest settlements (or other geographic objects) and a direction relative to them. Abbreviations: **FM26**: W of the Sazhdenik village; **FM27**: N of the Ruen peak; **FM37**: 8 small wetlands S of the Choveko peak, 6 small wetlands in the area of the Osogovo hut, 3 small wetlands in the area of the Trite Buki hut; **FM88**: the Panichishte place; **FM96**: 3 lakes and 1 swamplet in the cirque of Manastirski Ezera; **FN21**: 2 small wetlands N of the Bazovitsa village; **GM02**: 2 lakelets in the Popovoezeren cirque; **GM05**: NE of the Semkovo resort; **GM06**: a lakelet in the cirque of Ribni Ezera; **GM12**: a small wetland SW of the Kremen village, 2 lakelets in the Kremenski cirque, a swamplet in the Prazo place; **GM16**: 4 small wetlands in the Nehtenitsa place; **GM18**: E of the Borovets resort; **GM26**: S of the Ortachal peak; **GM27**: 2 small wetlands NW of the Ibar peak and SE of the Belmeken peak; **GM34**: a small wetland SW of the Grashevo village, 2 small wetlands SE of the Krastava village; **GM36**: N of the Yundola place; **GM37**: 3 small wetlands along the NW shore of the Belmeken reservoir; **GM42**: a small wetland N of the Pletena village, 14 small wetlands W and SW of the Sarnitsa village; **GM44**: a small wetland SW of the Grashevo village; **KG51**: 3 small wetlands along the SW shore of the Dospat reservoir, 3 small wetlands N of the Osina village, 7 small wetlands NE of the Pletena village; **KG53**: a small wetland SW of the Golyam Beglik reservoir and 2 terrestrial locations NW of the same reservoir; **KG63**: a small wetland N of the Golyam Beglik reservoir; **KG72**: 2 small wetlands N of the Borino village; **KG73**: 2 small wetlands W of the Fotinovo village; **KG74**: SE of the Batak town; **KG75**: 3 small wetlands S of the Peshtera town; **KG80**: 2 lakes and 1 small wetland in the Chairski Ezera place; **KG90**: NW of the Dimovo village, the Gerzovitsa place; **KH74**: 2 small wetlands S and SW of the Divchovoto village; **KH91**: 8 small wetlands S and SE of the Bogdan peak; **LG01**: the Trevnoto Ezero lake; **LH83**: 5 small wetlands in the surroundings of the Krastets village.

Appendix 3. Descriptive statistics for variables obtained from presence records (new and confirmed localities; N = 123) of *Ichthyosaura alpestris* in Bulgaria (T = Temperature, in °C; P = Precipitation, in mm). The variables used for the final ENM are marked with asterix.

Variable	Median (25% ÷ 75%)			Min ÷ Max	
*Elevation (m)	1561	1375	1721	746	2352
*Aspect (°)	177.3	113.4	228.9	0	356.1
*Slope (°)	8.5	4.7	11	0	23.1
BIO1: Annual mean T	6.1	4.9	7	1.3	9.1
*BIO2: Mean diurnal range of T	9.3	8.5	9.8	7.7	11.2
BIO3: Isothermality	33.7	32.1	34.5	30.4	36.3
BIO4: T seasonality	684.3	679.8	696.7	658.5	748
BIO5: Max T of warmest month	18.7	17.5	20.3	13.4	23.8
*BIO6: Min T of coldest month	-8.7	-9.9	-7.9	-12	-7.1
BIO7: T annual range	27.6	26.7	28.6	25.2	31.8
*BIO8: Mean T of wettest quarter	9.3	8	10.4	4.1	15.7
*BIO9: Mean T of driest quarter	6.6	0.1	11.5	-3.5	14.5
BIO10: Mean T of warmest quarter	14.3	13.2	15.3	9.2	17.9
BIO11: Mean T of coldest quarter	-2.2	-3.6	-1.1	-7.1	0.3
BIO12: Annual P	680	665.5	724.6	597.7	761.9
BIO13: P of wettest month	78	76.1	84	69.9	98.1
BIO14: P of driest month	39.8	38	41	32.5	44.7
*BIO15: P seasonality	21	20.1	23.9	19.2	29.6
BIO16: P of wettest quarter	223.9	214.1	241.6	191.8	264
*BIO17: P of driest quarter	133.7	127	140.4	117.4	150.3
BIO18: P of warmest quarter	186.4	174.5	198.4	149.5	241.6
BIO19: P of coldest quarter	154.1	143.1	160.5	120.5	186.8
*Potential evapotranspiration (mm/month)	740	714	786	564	891
Aridity index (yearly)	9364	8480	10126	6143	14436
Solar radiation (WH/m ²)	159748	158364	160614	152208	162912

Appendix 4. Sampled mountains in Bulgaria with no *Ichthyosaura alpestris* detected (N = number of sampled water bodies).

Mountains	N	Elevation (m)
Strandzha	36	2–400
Belasitsa	19	190–1370
Sakar	17	90–510
Konyavska	11	600–870
Lyulin	8	770–1170
Ograzhden	8	330–850
Maleshevska	7	600–1200
Plana	2	1120, 1250
Slavyanka	2	780, 1650
Verila	1	1230
Milevska	1	950
Ruy	1	650
Zemenska	1	950