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On oviparous populations of *Zootoca vivipara* (JACQUIN, 1787) in south-eastern Central Europe and their phylogenetic relationship to neighbouring viviparous and South-west European oviparous populations

(Squamata: Sauria: Lacertidae)

Über eierlegende Populationen von *Zootoca vivipara* (JACQUIN, 1787) im südöstlichen Mitteleuropa und deren phylogenetische Beziehungen zu benachbarten lebendgebärenden und südwesteuropäischen eierlegenden Populationen

(Squamata: Sauria: Lacertidae)

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KURZFASSUNG

In Slowenien und in Kärnten wurden eierlegende Populationen der Bergeidechse, *Zootoca vivipara* (JACQUIN, 1887) entdeckt. Neben vorläufigen Vergleichen der Eihüllen und der Embryonalstadien beim Absetzen der Eier stellen wir eine phylogenetische Analyse dieser Art vor, die auf mitochondrialen DNA-Sequenzen von Stichproben aus Niederösterreich, Kärnten, Slowenien, Italien (Friaul) und Spanien beruht. Die Ergebnisse legen eine evolutive Divergenz innerhalb dieser Art nahe, die der zwischen manchen allopatrischen (Semi-)Spezies entspricht und uns veranlaßt, die südostmitteleuropäische eierlegende Linie als eigene Unterart, *Zootoca vivipara carniolica* ssp. nov. aufzufassen.

ABSTRACT

We discovered egg-laying populations of the Viviparous Lizard, *Zootoca vivipara* (JACQUIN, 1887) in Slovenia and Carinthia. Besides preliminary comparisons of egg membrane characteristics and embryonic stages at the time of egg deposition, we present a phylogenetic analysis of this species based on mitochondrial DNA sequences of several samples from Lower Austria, Carinthia, Slovenia, Italy (Friuli) and Spain. The results suggest an evolutionary divergence within this species comparable to that found in allopatric (semi)species. This leads us to recognise the south-eastern Central European oviparous lineage as a distinct subspecies *Zootoca vivipara carniolica* ssp. nov.

KEY WORDS

Reptilia, Sauria: Lacertidae; *Zootoca vivipara, Z. v. carniolica* ssp. nov.; egg membranes, reproductive modes; mtDNA sequences, phylogenetic analysis; Austria, Slovenia, Italy, Spain

INTRODUCTION

Concerning its reproduction, the Viviparous Lizard *Zootoca vivipara* (JACQUIN, 1887), is bimodal in being represented by both ovoviviparous and allopatric oviparous populations. Until now, oviparous populations have been known to exist only in northern Spain and south-western France, from the Cantabrian mountains to the Pyrenees and Aquitania (LANTZ 1927; BRAÑA & BEA 1987; BÖHME 1997; HEULIN 1988). On the other hand, ovoviviparous (hereinafter simply called viviparous) populations have a vast distribution from Central France and the British Isles to the North Cape in Scandinavia, eastwards as far as eastern Siberia, Sakhalin Island and Hokkaido Island, Japan. Apparently, there is no parapatric abutment of the Central French viviparous populations and their oviparous southern French conspecifics (HEULIN & GUILLAUME 1989). Bulgarian populations, despite their location on a similar southern limit of the species' distribution range, are nonetheless viviparous (GUILLAUME et al. 1997). Recently, HEULIN et al. (1999) presented first data of mitochondrial DNA se-

Table 1: Localities (and their abbreviations used in the text and in figure 2) and specimens of *Zootoca vivipara* used for DNA sequencing. Collection acronyms: NHMW - Naturhistorisches Museum Wien; NHMW-TC - Naturhistorisches Museum Wien - Tissue Collection.

Tab. 1: Fundorte (und deren im Text und in Abb. 2 verwendete Kurzformen) und DNA-sequenziertes *Zootoca vivipara* Material. Sammlungsakronyme: NHMW - Naturhistorisches Museum Wien; NHMW-TC - Naturhistorisches Museum Wien - Gewebesammlung.

Locality / Fundort	NHMW number NHMW Nummer	NHMW-TC number NHMW-TC Nummer	Abbreviation Kurzform
Austria, Carinthia, Carnian Alps, Doberbach Valley	35146	VK-1	C1
Austria, Carinthia, Carnian Alps, Straniger Alm	35854:3	VK-2	C2
Austria, Carinthia, Turracher Höhe	33573	TU-1	C3
Austria, Carinthia, Koralm	35823	VH-2	C4
Spain, Cantabrian Mts., Puerto de los Tornos	36075:2	VC-4	E1
Spain, Eastern Pyrenees, Aran Valley	35822, 35854:4	ZP-1, ZP-2	E2
Italy, Friuli, Julian Alps, Musi	- - -	ZF-1	F1
Austria, Lower Austria, Waldviertel, Heidenreichstein	35850:1	ZV-1	N1
Austria, Lower Austria, Schneeberg (terra typica)	36072:8	ZL-1	N2
Slovenia, Snežnik region, Cerkniško Jezero	- - -	ZE-1	S1

quences of samples of viviparous populations from a wide area in Europe and of oviparous populations of south-western Europe. According to the results these two groups are clearly differentiated.

On June 1, 1998, a gravid female *Z. vivipara* was found in Slovenia (Mt. Snežnik: approx. 8 km SE Mašun, 1,250 m a.s.l.) which laid two shelled eggs after two weeks; it failed, however, to expel seven more eggs (which remained in its oviducts) and died the following day (BÖHME et al. 1999). In 1999, oviparous females were discovered in Carinthia (Austria): three females, captured on June 6, 1999 in the Carnian Alps below the Straniger Alm (46° 36'N / 13°08'E, approx. 1,350 m a.s.l.), laid 10, 5, and 7 eggs on June 14, 16 and 20, 1999, respectively.

In this paper we present preliminary comparisons of the egg-shell surface gross morphology of these oviparous females. Based on mitochondrial DNA sequences, we furthermore analyse the phylogenetic relationships of the specimens as compared with Carinthian and Lower Austrian samples of viviparous specimens, as well as oviparous specimens from Spain.

MATERIALS AND METHODS

DNA Investigations

Zootoca vivipara specimens from four localities in Carinthia, two localities in Lower Austria, one locality each in Slovenia and Friuli (Italy), as well as from two localities in Spain were examined (table 1). DNA from tissue samples (liver, heart or tail tips) was extracted and purified using a phenol-chloroform standard protocol (SAMBROOK et al. 1989). For sequence comparisons we amplified sections of the mitochondrial genes coding for 12SrRNA und 16SrRNA producing a 12S fragment of about 460 bp and a 16S fragment of about 900 bp length (primer see table 2 und fig. 1). PCR products separated on agarose gel were purified using QIAquick® spin columns (Quiagen®) and reamplified with the same primers. Cycle-sequencing of PCR products was performed using biotin-labelled dideoxynucleotides and the sequencing primers listed in table 2 (see also fig. 1). Alignment of sequences (460 bp of 12S fragment and 410 bp of 16S fragment, 870 bp all together) including the corresponding sequences of *Lacerta trilineata dobrogica* (BEYERLEIN & MAYER 1999) [GenBank numbers AF149935 and AJ238177] was done with Clustal X® software (THOMSON et al. 1997) and corrected by eye. Neighbour Joining (NJ; p-distances) and Maximum Parsimony (MP) dendrograms were calculated using PAUP® version 4.0b3a (SWOF-

Table 2: Primers used for PCR and sequencing. References: (1) - KNIGHT & MINDELL (1993); (2) - TITUS & FROST (1996); (3) - KOCHER et al. (1989), modified; (4) - REEDER (1995), modified; (5) - KNIGHT & MINDELL (1993), modified.

Tab. 2: Die für PCR und Sequenzierung verwendeten Primer. Referenzen: (1) - KNIGHT & MINDELL (1993); (2) - TITUS & FROST (1996); (3) - KOCHER et al. (1989), modifiziert; (4) - REEDER (1995), modifiziert; (5) - KNIGHT & MINDELL (1993), modifiziert.

Primer	Sequence amplified / Amplifizierte Sequenz	P - PCR S - Sequencing / Sequenzierung	Reference / Literatur
L-1091	(5'-aaactgggattagatccccactat-3')	P	(1)
H-1298	(5'-gctacaccttgacctgacgt-3')	S	(2)
L-1318	(5'-acgtcaggtcaaggtgtagc-3')	S	inverse H-1298
H-1478	(5'-agggatgacgggcggtgtgt-3')	S	(3)
H-1557	(5'-gtacacttaccttgttacgactt-3')	P	(1)
L-2190	(5'-gtgggcctaaaagcagccac-3')	P	(4)
L-2510	(5'-cgcctgtttaccaaaaacat-3')	S	(5)
H-3056	(5'-ccggtctgaactcagatcacg-3')	P	(4)

FORD 1997) program package for all 870 bp together. All sequences are deposited at the GenBank (< http://www.ncbi.nlm.nih.gov/ >, numbers AF247043 - AF247053 and AF247368 - AF347378).

Egg-shells

Both eggs of the Mt. Snežnik female, eggs of the first and of the third clutch of the specimens from the Carnian Alps (locality C2 in table 1 and fig. 2) as well as eggs of a female from the eastern Pyrenees (locality E2) were fixed in 70% ethanol on the day of laying. Egg-shells were dehydrated in a graded ethanol concentration series (80-85-90-95-100%), critical point dried (CO_2) and gold-sputtered before being examined by means of scanning electron microscopy (JEOL® JSM 6301F). Developmental stages of the embryos were assigned according to DUFAURE & HUBERT (1961).

RESULTS

DNA

The MP analysis (exhaustive search, gaps treated as fifth character state) resulted in six equivalent most parsimonious trees with a length of 189 steps each the number of parsimony-informative characters being 40. The strict consensus tree and the NJ-Bootstrap tree are represented in figure 2. Using both kinds of calculation method, the same two main clusters were found: one including the samples C1, C2, S1, and F1 from south-eastern Central Europe (within distances 0.1% - 0.9%), the second comprising the samples C3, C4, E1, E2, N1, and N2. This second cluster contains both samples of viviparous as well as South-west European oviparous strains (within distances: 0.1% - 2.0%). The sequence differences between the clusters range from 2.5% to 3.7%.

Egg-shells

Under SEM examination, the external surface of the membrane of the Mt. Snežnik female's eggs shows irregularly distributed mineral aggregates and also large areas without a mineral crust (fig. 3). In places without mineral crust, the underlying fibril layer is visible. The shape of the mineral aggregates resembles blossoms or leaves (fig. 3). Total thickness of the mineralized area of the egg-shell (fibril layer plus calcified crust) is 55 µm. The egg-shell of a female from Mt. Snežnik (="Monte Nevoso": Natural History Museum, Trieste [MCSNT 314]) does not show this inhomogenous surface structure and the eggs deposited by the Carinthian females also lack unmineralized areas and marked blossom- or leaf-like mineral aggregates; these egg-shells rather exhibit a uniformly mineralized external surface.

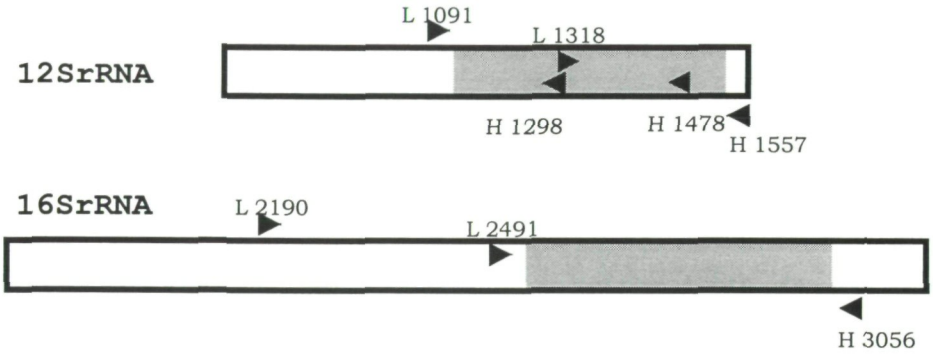


Fig. 1: Scheme of mt-rRNA genes with primers used for PCR and sequencing.
The sequenced regions are shaded (see also table 2).

Abb. 1: Schema der mt-rRNA Gene mit den für PCR und Sequenzierung verwendeten Primern.
Die sequenzierten Abschnitte sind schattiert dargestellt (siehe auch Tab. 2).

Embryos

One of the two eggs laid by the Mt. Snežnik female contained an embryo at developmental stage 30 (staging according to DUFAURE &HUBERT 1961). The embryo of the other egg was damaged during dissection, which prevented us from determining its developmental stage. Dissection of eggs laid by Straniger Alm females likewise revealed embryos of stage 30.

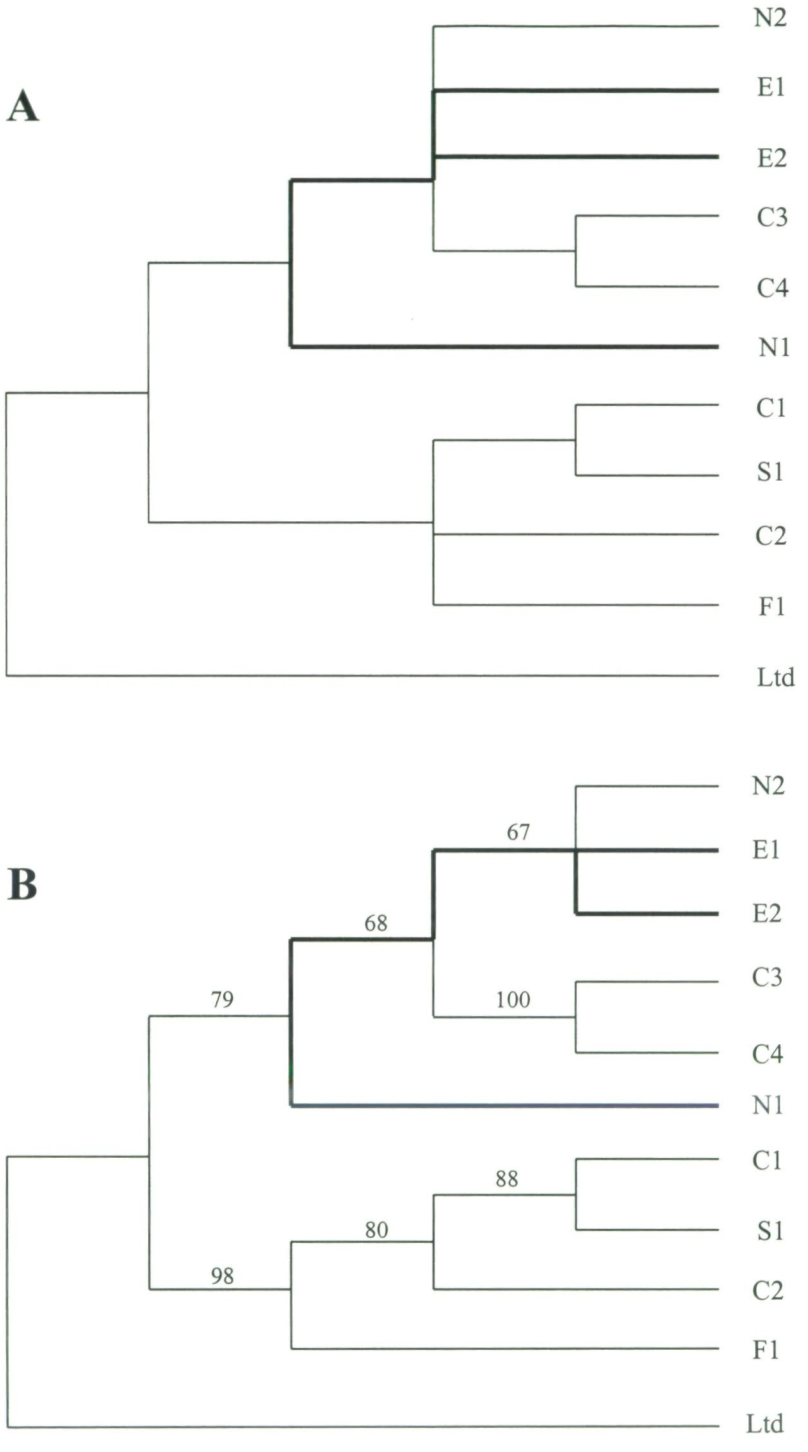
DISCUSSION

In *Zootoca vivipara* from south-eastern Central Europe, the egg-shell characteristics (calcification and thickness) and the stage of embryonic development at the time of oviposition are very similar to that of oviparous populations from Spain and south-western France. However, these characteristics differ strongly from those observed in the viviparous strains in which fully differentiated (stage 40) neonates surrounded only by a thin (9 µm) transparent uncalcified membrane are deposited (HEULIN 1990). By its incomplete calcification (blossom-shaped mineral aggregates), the egg-shell of the first described oviparous female from Mt. Snežnik resembled the egg-shell of laboratory-bred hybrids between oviparous and viviparous strains (HEULIN et al. 1992). A comparable incomplete calcification pattern was neither observed in the eggs of the second Mt. Snežnik female (Museum Trieste) nor in the clutches of any of the Carinthian females. Thus, we interpret the incomplete calcification observed in the above case as follows. Due to stress, the female discharged two eggs all too soon and died subsequently, so that additional eggs (the shells of which were partly studied by us, partly by B. HEULIN, see BÖHME et al. 1999) represented an immature stage of egg-shell mineralization. The "regular" morphological aspect of egg-shells of Slovenian and Carinthian *Z. vivipara* females seems to be

Figs. 2A and 2B (opposite Page) / Abb. 2A und 2B (gegenüberliegende Seite)

2A - MP (Maximum Parsimony) strict consensus tree; 2B - NJ (Neighbour Joining) 50% consensus bootstrap tree (1000 replicates, bootstrap values indicated on branches). *Lacerta trilineata dobrogica* (Ltd) was used as outgroup. Bold lines designate tree segments corresponding with the data given by HEULIN et al. 1999 (see discussion).

2A - MP (Maximum Parsimony) strict consensus tree; 2B - NJ (Neighbour Joining) consensus bootstrap tree (1000 Replikationen, Bootstrap-Werte sind an den Ästen angegeben).
Lacerta trilineata dobrogica (Ltd) wurde als Outgroup verwendet. Fette Linien bezeichnen Baumsegmente, die den von HEULIN et al. 1999 angegebenen Daten entsprechen (siehe Diskussion).



indistinguishable from that found in the Spanish and French oviparous populations. Likewise, the developmental stage of the embryos at the time of oviposition seems to be identical.

Comparison of the genetic data shows that the oviparous populations from south-western Europe are clearly more closely related to the viviparous strains than to the recently discovered oviparous lineage from south-eastern Central Europe. Therefore, the similarity of the two oviparous groups concerning the characteristics of their egg-shells has to be considered as a symplesiomorphic character state. We compared our sequences with data presented by HEULIN et al. (1999) who found three different haplotypes (OH1, OH2, OH3) of oviparous south-western European strains and four haplotypes (VH1, VH2, VH3, VH4) of viviparous strains. In an overlapping section of about 305 bp, VH1 is identical to our sequence N1, OH1 is identical to E2 and OH2 is identical to E1. The haplotypes VH2, VH3, and VH4 differ from VH1 by only one substitution, and are therefore only minor variants of a haplotype widely distributed from France to Bulgaria.

Our sample N1 stems from the southern margin of this area. However, a bit more southwards (in the eastern Alps), there occur haplotypes which differ considerably from N1 and from each other. In the 305 bp segment, samples N1, N2 (type locality), and the Carinthian samples (C3 + C4) differ from each other in 6 to 7 bp; their differences from south-western European samples range from 3 bp to 10 bp. With regard to our 870 bp sequences, the haplotypes of the viviparous populations from the eastern Alps (N2, C3, C4 - reproductive mode proven at least for the samples N2 [Schneeberg, Lower Austria, type locality of *Z. vivipara*] and C4 [Koralm, Carinthia]) are even more similar to the haplotypes of oviparous Southwest European populations (E1, E2) than to the group of haplotypes of the viviparous lineage studied by HEULIN et al. (1999) and represented in our data set by sample N1. The resulting phylogenetic hypothesis is shown in fig. 2.

HEULIN et al. (1993) proposed a biogeographic scenario based on Pleistocene events, which may have accounted for the evolution of viviparity and the existence of residual southern oviparous populations in this species. The Quarternary glaciations would have split the original, plesiomorphically oviparous populations in two groups: a south-western group in the Pyrenean-Iberian refuge that remained oviparous and a south-eastern group (in a refuge somewhere between the Balkan Peninsula and the north of the Altai Mountains) that acquired viviparity. Viviparity, which is thought to be an adaptation to cold climatic conditions (see e.g., SHINE 1999) could have been strongly selected during glacial phases, and subsequently could have favored the recolonization of north-western and north-eastern parts of the extant range of *Z. vivipara* after the end of the last glaciation.

In the light of the discovery of an additional oviparous form in south-eastern Central Europe, the above concept must be extended in two aspects:

1. As the two oviparous lineages do not seem to be closely related, an additional glacial refugial area has to be postulated. Our molecular data indicate that viviparity evolved out of only one (the South-West European) of the two oviparous lineages.

2. The sequence divergence between the two clusters is of the same order of magnitude (average of 3%) as between sibling species pairs such as e.g. *Lacerta viridis / L. bilineata* or *L. trilineata / L. pamphylica* (MAYER & BEYERLEIN 2000). We therefore treat the two lineages within *Z. vivipara* as different taxa, at least on the subspecific level.

WERNER (1897) erected the name *Lacerta vivipara* var. *carniolica* for specimens from "Krain (Schneeberg)" (=Monte Nevoso, Mt. Snežnik) without providing a description nor designating any type material. He rather intended to name a colour morph which - according to him - can be observed almost only in females. According to Article 12 of The Code (ICZN 1999), we regard *carniolica* therefore as a *nomen nudum* as it is not "accompanied by a description or a definition of the taxon that it denotes (Article 12.1.)" nor by an indication in the sense of Article 12.2. Following the ICZN (1999), "a *nomen nudum* is not an available name, and therefore the same name may be made available later for the same or different concept; in such a case it would take authorship and date from that act of establishment", we decided to maintain WERNER's name for the description of

Zootoca vivipara carniolica ssp. nov.

Diagnosis: A subspecies of *Zootoca vivipara* which is distinguished from the nominotypic form by genetic properties (sequence differences between mitochondrial haplotypes) rather than by morphological peculiarities. As a main characteristic, it retains the oviparous reproductive mode, as it is the case in the populations of Spain and south-western France, which are, however, genetically only distantly related.

Holotype: ZFMK 68438, adult female, Slovenia: Mt. Snežnik: 8 km SE Mašun village, 1,250 m a.s.l., coll. U. & W. BISCHOFF, 1 June 1998 (figs. 4-6).

Head-body length 68.7 mm, head length 12.2 mm, tail length (partly regenerated) 71.6 mm; length of foreleg 16.8 mm, length of hindleg 25.1 mm, length of fourth toe 9.9 mm. Dorsal longitudinal scale rows at mid-body: 34, slightly keeled, smooth toward the flank region; ventrals in 27 transversal and 6 longitudinal rows; 18 marginals, 4/4 (left/right) supralabials, 5/5 supraciliars, no supraciliar granules; 2 longish anterior supratemporals on either side, followed each by a shield of half the length of the former; 24/17 temporals, masseteric shield very distinct, in touch with tympanic shield at the left side, separated from it at the right side; internasal and anterior parts of prefrontals subdivided into 9 scales of rather different size; 1/1 postnasal, 1/1 frenal, 1/1 preocular, 20 gulars, 9 collars, and 6 pairs of submaxillary scales, the most anterior three pairs in contact with each other, the 6th pair distinctly larger than the 5th; scales on tibia smooth and distinctly smaller than on dorsum; 12/12 femoral pores, 22/22 subdigital lamellae under 4th toe; caudal scales slightly keeled above, smooth ventrally; caudal whorls of equal length, 19 scales forming 6th whorl; 4 scales between the rows of femoral pores; 5 preanals; height to width of anal shield 76.3%.

Ground colour (in preservative) light reddish-brown, back lighter than the flanks. Dorsum separated from the darker, reddish-brown flanks by a relatively broad brownish-gray supratemporal line on either side. These lines continue anteriorly to the nostrils and separate the lighter pileus from the darker sides of the head. A dark brown middorsal line from the occiput to the 7th whorl of the tail regenerate, continuous in the neck region, becoming more interrupted caudally. From the occiput to the insertion of the forelegs, the light supratemporal lines are bordered by narrow, dark lines above and below, towards the head only below. Dorsum indistinctly reddish clouded. Flanks lighter towards venter, bordered by rows of ocellar spots above and below, the lower ones being much more distinct that the upper. The lower row of spots forms the continuation of the whitish sublabial line which is still continuous along the sides of the neck. Upper parts of hindlimbs as dark as flanks, with indistinct light spots. Regenerated tail plain, greyish; lower parts of head and throat whitish grey, nearly without patterning. Throat laterally with reddish-brown spots which are also present in the outer ventral scale rows. Few distinct dark spots on the chest. Subdigital lamellae darker than the rest of the ventral parts of the feet.

Paratypes: Naturhistorisches Museum Wien NHMW 11133:1-2, two adult females, Slovenia: Mt. Snežnik [original labelling: Krainer Schneeberg], coll. GINZBERGER, 1400-1600 m, 20 June 1908. - Museo Civico di Scienze Naturali Trieste MCSNT 314, adult female, Slovenia: Mt. Snežnik (original labelling: Monte Nevoso), coll. G. MÜLLER, June 1946.

The paratypes display considerable morphological variation. The counts of their dorsal longitudinal scale rows at mid-body range from 28 to 36, temporals from 12 to 26; only in NHMW 11133:1, the masseteric shield is present on both sides of the head while in the remaining paratypes it is differentiated on the left side only. Number of femoral pores varies from 11 to 13. The configuration of the anterior pileus scalation corresponds to that of the types A and G (sensu DELY & BÖHME 1984), whereas the holotype exhibits an intermediate position between the types C and E. In a similar manner, the colour pattern of the paratypes is variable.

Distribution: *Zootoca vivipara carniolica* ssp. nov. is known from Mt. Snežnik, Slovenia, and Straniger Alm, Carinthia, Austria. According to our DNA data (fig. 2), the specimens from Doberbach valley, Carnian Alps, Carinthia (NHMW

35854:3) and from Musi, Julian Alps, Friuli, Italy (NHMW 35854:2) also belong clearly to this subspecies. This distributional pattern makes it likely that the new taxon is distributed over larger parts of Slovenia, NE Italy, S Carinthia and possibly also NW Croatia. Possible zones of secondary hybrid contact or parapatric abutment with *Z. v. vivipara* have still to be identified, particularly in Carinthia where live-bearing populations (e.g. Koralm) occur north of the Drau valley.

Etymology: WERNER's name *carniolica* is a Latin feminine adjective meaning "belonging to the Carnian region" ("Krain" in German) i.e., the area where this new taxon seems to have its geographic and historical (refugial) center.

OUTLOOK ON FUTURE RESEARCH

Further work should concentrate on the determination of the distribution areas and borderlines of the various groups of haplotypes to identify their glacial refuges and postglacial recolonization routes. Special attention has to be paid to the possible existence of hybrid zones in the postulated contact areas versus parapatric abutment without or with only reduced genetic exchange. The latter situation would strongly favour the view of regarding *carniolica* even as a distinct species. It would also be important to intensively study the ecology of the newly discovered oviparous form, in comparison with that of their viviparous neighbours as well as that of their egg-laying South-west European relatives. The remarkable genetic distance between the latter and *carniolica* should make the new taxon also extremely important for Europe's conservationists!

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REFERENCES

BEYERLEIN, P. & MAYER, W. (1999): *Lacerta kulzeri* - its phylogenetic relationships as indicated by DNA sequences.- Natura Croatica, Zagreb; 8: 181-187.

BÖHME, W. (1997): *Lacerta vivipara* JACQUIN, 1787; pp. 358-359. In: GASC, J.-P. & CABELA, A. & CRNOBRNJA-ISAILOVIC, J. & DOLMEN, D. & GROSSENBACHER, K. & HAFFNER, P. & LESCURE, J. & MARTENS, H. & MARTÍNEZ RICA, J. P. & MAURIN, H. & OLIVERA, M. E. & SOFIANIDOU, T. S. & VEITH, M. & ZUIDERWIJK, A. (eds.): Atlas of amphibiens and reptiles in Europe, Societas Europaea Herpetologica & Muséum National d'Histoire Naturelle (JEGB/SPN), Paris.

BÖHME, W. & HEULIN, B. & BISCHOFF, W. (1999): First data on a second oviparous population of the viviparous lizard *Zootoca vivipara* JACQUIN, 1787 (Squamata:Lacertidae) from NW Slovenia.- Abstr. 10th Ord. Gen. Meet. SEH, Iraklion (Nat. Hist. Mus.Crete); pp. 34-35.

BRAÑA, F. & BEA, A. (1987): Bimodalité de reproduction chez *Lacerta vivipara*.- Bull. Soc. Herpetol. France, Paris; 44: 1-5.

DELY, O. G. & BÖHME, W. (1984): *Lacerta vivipara* JACQUIN 1787 - Waldeidechse; pp. 362- 393. In: BÖHME, W. (Ed.): Handbuch der Reptilien und Amphibien Europas, vol. 2/I, Wiesbaden (Aula).

DUFAURE, J. P. & HUBERT, J. (1961): Table de développement du lézard vivipare: *Lacerta* (*Zootoca*) *vivipara* JACQUIN.- Arch. Anat. micr. Morph. experim., Paris, New York, Barcelona, Milano; 50: 309-328.

GUILLAUME, C. P. & HEULIN, B. & BESHKOV, V. (1997): Biogeography of *Lacerta* (*Zootoca*) *vivipara*: reproductive mode and enzyme phenotypes in Bulgaria.- Ecography, Helsinki; 20: 240-246.

HEULIN, B. (1988): Données nouvelles sur les populations ovipares de *Lacerta vivipara*.- Comptes Rendus Acad. Sci. Paris; 306: 63-68.

HEULIN, B. (1990): Étude comparative de la membrane coquillère chez les souches ovipares et vivipares de *Lacerta vivipara*.- Canadian J. Zool., Ottawa; 68: 1015-1019.

HEULIN, B. & ARRAYAGO, M. J. & BEA, A. & BRAÑA, F. (1992): Caractéristiques de la coquille des oefs chez la souche hybride (ovipare x vivipare) du lézard *Lacerta vivipara*.- Canadian J. Zool., Ottawa; 70: 2242-2246.

HEULIN, B. & GUILLAUME, C. P. (1989): Extension géographique des populations ovipares de *Lacerta vivipara*.- Rev. Ecol. (Terre Vie), Paris; 44: 283-289.

HEULIN, B. & GUILLAUME, C. P. & ARRAYAGO, M. J. & BEA, A. (1993): Interprétation biogéographique de la bimodalité de reproduction du lézard

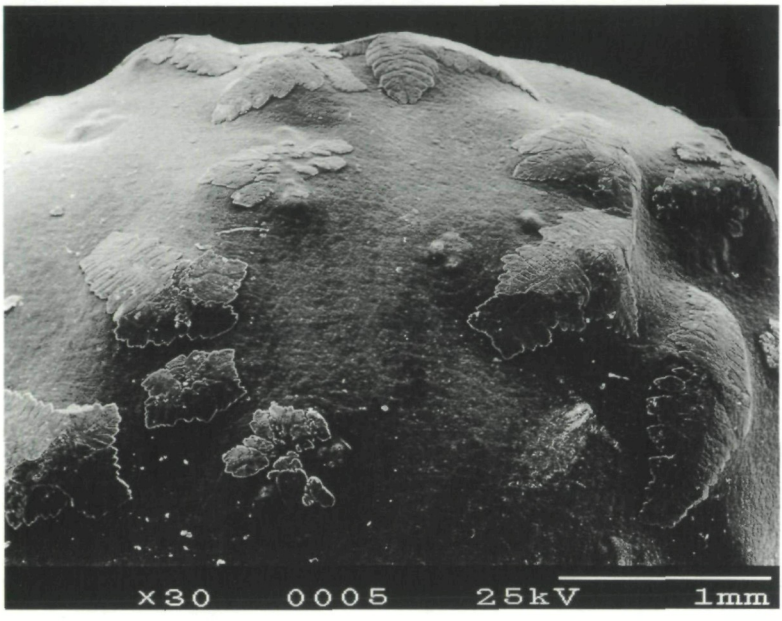

Fig. 3: SEM micrograph of the patchily mineralized egg-shell produced by the Holotype of *Zootoca vivipara carniolica* ssp. nov. (ZFMK 68438) (for details see text). Micrograph K. ULMEN-KÜRTEN, ZFMK.

Abb. 3: Rasterelektronische Aufnahme der nur teilweise mineralisierten Eischale wie sie vom Holotypus von *Zootoca vivipara carniolica* ssp. nov. (ZFMK 68438) produziert wurde (Einzelheiten siehe Text). Aufnahme K. ULMEN-KÜRTEN, ZFMK.

Fig. 4: Holotype of *Zootoca vivipara carniolica* ssp. nov., ZFMK 68438, from Mt. Snežnik, Slovenia. Dorsal aspect (in life). Photograph by W. BÖHME (Bonn).

Abb. 4: Holotypus von *Zootoca vivipara carniolica* ssp. nov., ZFMK 68438, vom Mt. Snežnik, Slowenien. Dorsalansicht des lebenden Exemplars. Foto W. BÖHME (Bonn).

Fig. 5: Holotype of *Zootoca vivipara carniolica* ssp. nov., ZFMK 68438, from Mt. Snežnik, Slovenia Ventral aspect (in life). Photograph by W. BÖHME (Bonn).

Abb. 5: Holotypus von *Zootoca vivipara carniolica* ssp. nov., ZFMK 68438, vom Mt. Snežnik, Slowenien. Ventralansicht des lebenden Exemplars. Foto W. BÖHME (Bonn).

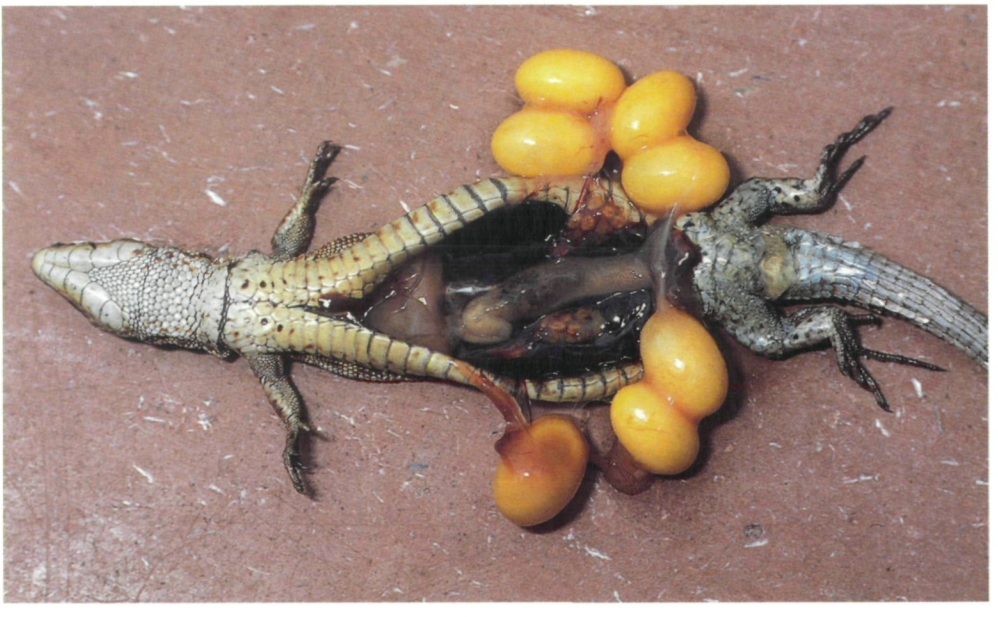

Fig. 6: Holotype of *Zootoca vivipara carniolica* ssp. nov., ZFMK 68438, from Mt. Snežnik, Slovenia. Situs viscerum. Photograph by W. BÖHME (Bonn).

Abb. 6: Holotypus von *Zootoca vivipara carniolica* ssp. nov., ZFMK 68438, vom Mt. Snežnik, Slowenien. Situs viscerum. Foto W. BÖHME (Bonn).

Lacerta vivipara: un modèle pour l'étude de l'évolution de la viviparité.- Biogeographica, Paris; 69: 1-11.

HEULIN, B. & SURGET-GROBA, Y. & GUILLER, A. & GUILLAUME, C. P.& DEUNFF, J. (1999): Comparisons of mitochondrial DNA (mtDNA) sequences (16S rRNA gene) between oviparous and viviparous strains of *Lacerta vivipara*: a preliminary study.- Molecular Ecology, Oxford; 8: 1627-1631.

ICZN (INTERNATIONAL COMMISSION ON ZOOLOGICAL NOMENCLATURE) (ed.) (1999): International code of zoological nomenclature. 4th edition. London (International trust for zoological nomenclature), 306 pp.

KNIGHT, A. & MINDELL, D. P. (1993): Substitution bias, weighting of DNA sequence evolution, and the phylogenetic position of Fea's viper.- Syst. Biol., London, New York, Philadelphia, Singapore; 42: 18-31.

KOCHER, T. D & THOMAS, W. K. &MEYER, A. & EDWARDS, S. V. & PÄÄBO, S. & VILLABLANCA, F. X. & WILSON, A. C. (1989): Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers.- Proc. Natl. Acad. Sci. USA, Baltimore, New York; 86: 6196-6200.

LANTZ, L. A. (1927): Quelques observations nouvelles sur l'herpétologie des Pyrénées centrales.- Bull. Hist. Nat. Applic., Toulouse; 8: 54-61.

MAYER, W. & BEYERLEIN, P. (in press 2000): Genetische Differenzierung des *Lacerta-viridis-bilineata*-Komplexes und von *Lacerta trilineata* in Griechenland: mitochondriale DNA-Sequenzen.- Mertensiella, Rheinbach; 12.

REEDER, T. W. (1995): Phylogenetic relationships among phrynosomatid lizards as inferred from mitochondrial ribosomal DNA sequences: substitutional bias and information content of transitions relative to transversions.- Molecul. Phylogenet. Evol., Orlando; 4: 203-222.

SAMBROOK, J. & FRITSCH, E. F. & MANIATIS, T. (1989): Molecular cloning. A laboratory manual. vol. 3, pp. E3, E4; 2nd edition. New York (Cold Spring Harbor Laboratory Press).

SHINE, R. (1999): Egg-laying reptiles in cold climates: determinants and consequences of nest temperatures in montane lizards.- J. evol. Biol., Basel; 12: 918-927.

SWOFFORD, D. (1997): PAUP: Phylogenetic analysis using parsimony, version 4.0.0 d55.- Smithsonian Institution, Washington DC.

THOMSON, J. D. & GIBSON, T. J. & PLEWNIAK, F. & JEANMOUGIN, F. & HIGGINS, D. G. (1997): The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools.- Nucl. Acid Res., Oxford; 25: 4876-4882.

TITUS, T. A. & FROST, D. R (1996): Molecular homology assessment and phylogeny in the lizard family Opluridae (Squamata: Iguania).- Molecul. Phylogenet. Evol., Orlando; 6: 49-62.

WERNER, F. (1897): Die Reptilien und Amphibien Österreich-Ungarns und der Occupationsländer. Wien (Pichler), 162 pp. + 3 plates.

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