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LAND SNAIL *SPHYRADIUM DOLIOLUM* (STYLOMMATOPHORA, ORCULIDAE) IN UKRAINE AND MOLDOVA: DISTRIBUTION, HABITATS, VARIABILITY AND ORIGIN

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Land Snail *Sphyradium doliolum* (Stylommatophora, Orculidae) in Ukraine and Moldova: Distribution, Habitats, Variability and Origin. Balashov, I., Levenets, T., Markova, A., Kramarenko, A. & Kramarenko, S. — All existing data and most of the available materials on *Sphyradium doliolum* from Ukraine and Moldova are reviewed. The species is reported from 38 localities (some including several sites) on uplands east of the Carpathians, the Dnipro Upland and in the Crimean Mountains. It mostly inhabits the broadleaved forests, but in one locality was found in a forestless rocky meadow steppe at an altitude of *ca.* 1400 m in the Crimea. Statistical analysis of the morphometric shell characters shows a significant geographical variability between the populations of *S. doliolum* from the different parts of Ukraine and Moldova. The most prominent morphometric differences were found between the populations from the Crimean Mountains and from all other East European populations combined. An origin of *S. doliolum* in Eastern Europe is discussed. The results of the morphometric analyses support the hypothesis of the two different origins during the two separate periods for the populations of *S. doliolum* in the Crimean Mountains and in the East European Plain. These two groups of populations could be representing the two different lineages that perhaps could be of the two different subspecies, but their status requires additional studies involving molecular genetic methods and material from a wider area.
Key words: Mollusca, Gastropoda, Pupilloidea, terrestrial mollusks, Eastern Europe.

Introduction

Land snail *Sphyradium doliolum* (Bruguière, 1792) is a medium-sized pulmonate mollusk (shell up to 6 mm) distributed from Western and Southern Europe to southwestern and Central Asia. Its range includes eastern Spain, France, Italy, Belgium, Netherlands, Luxembourg, southern Germany, Switzerland, Austria, Slovakia, Czechia, Hungary, southern Poland, Ukraine, Moldova, all Balkan countries, Anatolia, Georgia, Armenia, Azerbaijan, northern Iran, Uzbekistan, southern Kazakhstan and Tadzhikistan (Kerney & Cameron, 1979; Schileyko, 1984; Hausdorf, 1996; Wiktor, 2004; Welter-Schultes, 2012; Schileyko & Rymzhanov, 2013). This

species is mainly found in the broadleaved forests, usually compound of beech, hornbeam or oak, in the leaf litter or among the rocks. Tend to prefer humid, shaded places and heavily calcified soil. It often inhabits the distinct landforms, such as ravines or hillsides, often on the limestone outcrops or in the karst areas. Usually, *S. doliolum* does not live in the significantly transformed habitats, although in Czechia it was reported to live on the calcium-rich walls of the old castle ruins (Horsák et al., 2013). At the higher altitudes in the mountains *S. doliolum* sometimes lives in the more or less open habitats: the sparse forests or even the forestless rocky outcrops. Some specimens of *S. doliolum* have been found at an altitude up to 1600 m in the Swiss Alps and up to 1900 m in Bulgaria (Kerney & Cameron, 1979; Schileyko, 1984; Hausdorf, 1996; Welter-Schultes, 2012; Horsák et al., 2013).

Sphyradium doliolum is not a globally threatened species (Neubert et al., 2019), although it is regionally threatened or near threatened in many European countries (Wiktor & Riedel, 2002; Beran et al., 2005; Rüetschi et al., 2012; Welter-Schultes, 2012; Balashov, 2016 a, etc). In Ukraine, this species is officially protected since 2021 by the Red Book of Ukraine (officially listed as a protected species by the Decree of the Ministry of Ecology of Ukraine from 19 January 2021, in effect from 12 March 2021).

In Moldova, *S. doliolum* was only reported from the several sites of Codru Nature Reserve (Baidashnikov, 1993). In Ukraine, it is a rare species that occurs in some western and central regions, as well as in the Crimean Mountains (Balashov, 2016 a, b). In Western Ukraine, it is known to occur since the studies of Bąkowski (1880, 1881, 1882, 1884; Bąkowski & Łomnicki, 1891) who reported it from one locality in Ciscarpathia (eastern foothills of Carpathians) and from the six locations in the western part of Podolian Upland. Most of these old materials are preserved in the collection of State Natural History Museum in Lviv and were reviewed recently (Gural-Sverlova & Gural, 2020). From the Podolian Upland, this species was reported also from several locations by Baidashnikov with coauthors (Baidashnikov, 1987, 1996, 2002; Balashov & Baidashnikov, 2012; Balashov et al., 2013). For the Crimean Mountains, *S. doliolum* was reported only from the four exact locations (Lindholm, 1926; Puzanov, 1927; Pogrebnyak & Sedysheva, 2015), although in many works related to the Crimea *S. doliolum* was listed or described without precise data on the distribution. Three isolated areas with populations of *S. doliolum*, far from its main range, were also found in Central Ukraine on the Dnipro Upland (Korniushin, 1988; Balashov & Baidashnikov, 2010).

Although overall distribution and habitat preferences of *S. doliolum* in Eastern Europe are known, it was never reviewed in one source. The numerous details on the habitats and distribution were remaining unpublished. Variability of *S. doliolum* has not been ever studied in Eastern Europe, despite existence of the distinct regional morphs.

The goal of this study was to review all existing data and material on *S. doliolum* from Ukraine and Moldova, to reveal its exact distribution and habitat preferences and to study geographical variability of this species and its possible implications.

Material and methods

All used materials, as well as all published data from Ukraine with no preserved materials, are listed in table 1. We have examined and confirmed identifications only for those materials that are kept in the Collection of terrestrial mollusks of I. I. Schmalhausen Institute of Zoology of National Academy of Sciences of Ukraine (Kyiv, hereinafter SIZK). Most of these materials were collected by A. Baidashnikov (all SIZK materials from 1985–1994, see table 1), other by A. Korniushin, I. Balashov, Z. Shvydka and T. Levenets. Materials of the Collection of State Natural History Museum of National Academy of Sciences of Ukraine (Lviv, hereinafter SNHM) were reviewed by Gural-Sverlova & Gural (2020) and of the Collection of National Museum of Natural History of National Academy of Sciences of Ukraine (Kyiv, hereinafter NMNH) by Pogrebnyak & Sedysheva (2015). One sample that includes unknown number of the *S. doliolum* specimens from the Crimea was sent by A. Baidashnikov to the collections of Zoological Museum of Moscow University (hereinafter ZMMU) as indicated in his notes with a list of species and description of locality (see table 1), but we have not checked if this material still exists in this institution.

Material was collected, handled and identified using common methods of work with terrestrial mollusks (Kerney & Cameron, 1979; Schileyko, 1984; Balashov, 2016 b).

The preparation of the geodata and creation of the map was carried out using QGIS 3.22.13 software platform. Free spatial data from DIVA-GIS (www.diva-gis.org/gdata) was used for the borders of countries, rivers, seas and relief (elevation).

We have indicated the coordinates of areas where this protected species occurs because the collecting of specimens is not a significant threat to its populations (Balashov, 2016 a). Moreover, most of these coordinates are not exact and referring to the forest massifs where species is present on some isolated sites, not to the sites themselves.

Table 1. All records of *Sphyradium doliolum* in Ukraine and Moldova

No	Localities (areas)	Coordinates (ca.)	Altitude, m (ca.)	Years	Habitats	Sites	Specimens	Material
			Moldova					
1	Codru Nature Reserve*	47°5'15" N 28°25'29" E	250–300	1990 1991	hof of	10	56	SIZK
			Chernivtsi Region					
2	Kuzmyna forestry	48° 8'57" N 25°59'48" E	350	1985	hbf	1	1	SIZK
3	n. Kulykivka village	48° 4'16" N 26°10'9" E	350	1985	hbf	1	3	SIZK
4	Rukhotyn forestry	48°30'40" N 26°10'41" E	250	1985	bf	1	6	SIZK
5	n. Revne village	48°19'17" N 25°48'54" E	250	1985	hbf	2	3	SIZK
			Ivano-Frankivsk Region					
6	n. Tekuche village	48°22'51" N 24°49'20" E	500	1881	blf?	1	3	SNHM
7	n. Vovchynets village	48°57'5" N 24°44'45" E	250	1879	blf?	1	11	SNHM
			Lviv Region					
8	n. Hubychi village	49°37'13" N 22°45'36" E	300	2016	bf	1	3	SIZK
			Ternopil Region					
9	Medobory Nature Reserve	49°16'25" N 26°9'25" E	300–400	~1880 1990	blf	2	14	SNHM, SIZK
10	n. Zalishchyky town	48°39'33" N 25°44'3" E	200	1990	hf	1	9	SIZK
11	n. Horodok village	48°37'41" N 25°51'20" E	150	~1880	blf?	1	6	SNHM
12	n. Skala-Podilska village	48°51'9" N 26°11'55" E	250	~1880	blf?	1	1+	Bąkowski, 1880
13	n. Ivane-Zolote village	48°43'31" N 25°38'7" E	250	~1880	blf?	1	4	SNHM
14	n. Markova village	49°9'24" N 25°3'9" E	350	~1880	blf?	1	5	SNHM
15	n. Kudryntsi village	48°37'25" N 26°16'41" E	250	~1880	blf?	1	6	SNHM
			Khmelnytskyi Region					
16	Panovetska Dacha reserve*	48°36'55" N 26°36'13" E	200	1990	hof	2	17	SIZK
17	Sovynyi Yar reserve	48°39'50" N 26°52'35" E	150	1991	hmf	2	3	SIZK
18	Knyazhpilskyi reserve	48°41'19" N 26°47'30" E	150	1991	hof	1	6	SIZK
19	Karmaliuk's Mountain reserve	48°46'20" N 26°39'11" E	300–350	1991	hbf hmf	2	6	SIZK
			Vinnytsia Region					
20	Brytavka forestry	48°9'21" N 29°10'37" E	250	1991	hof	2	14	SIZK
21	Zhuravlivkyi Forest reserve*	48°34'24" N 28°43'27" E	300	1990	hof	2	39	SIZK
22	Tsybulivka forestry	48°20'54" N 29°5'5" E	300	1991	blf	1	1	SIZK
23	Kotiuzhany forestry*	48°44'49" N 27°42'3" E	200	1991	hof	1	56	SIZK
			Cherkasy Region					
24	Kaniv Nature Reserve*	49°43'18" N 31°28'48" E	150–200	1980 1982 1983 2005 2006 2019	hmf	3	283	SIZK, SNHM, NMNH

25	n. Moshny village	49°28'56" N 31°47'36" E	150	1984?	blf	1	1+	Korniushin, 1988, SIZK?**
26	Kholodnyi Yar National Park*	49° 8'33" N 32°15'51" E	200	1994	hof	1	8	SIZK
				Crimea				
27	Mangup-Kale mountain	44°35'30" N 33°48'5" E	500	1913	blf?	1	1+	Lindholm, 1926
28	Bijuk-Sjuren area	44°39'9" N 33°49'13" E	300	1913	blf?	1	1+	Lindholm, 1926
29	around Pyliaky mountain*	44°25'47" N 33°58'15" E	600–900	~1927 1987	bhf	2	21+	Puzanov, 1927; SIZK
30	Chatyr-Dagh lower plateau	44°48'4" N 34°17'2" E	800–1100	1988 2006	bf of hof	4	12	SIZK
31	Chatyr-Dagh upper plateau*	44°44'60" N 34°17'60" E	1400	2011	rs	1	20	SIZK
32	Grand Canyon of Crimea*	44°31'28" N 34°0'51" E	650	1989	bf hmf	2	70	SIZK
33	Mala Chuchel mountain	44°38'13" N 34°14'25" E	900–1100	1987	bf bhf	2	3+	SIZK, ZMMU
34	Agarmysh mountain	45°2'10" N 35°1'59" E	550	1986	blf?	1	5	NMNH
35	around Tarpan-Bair ridge and Uzundzha River	44°26'53" N 33°53'52" E	400–900	1987 1988 2003 2008	hof blf	4	14	SIZK, NMNH
36	Basman mountain	44°34'7" N 34°12'6" E	1400	1988	bf	1	3	SIZK
37	Babugan plateau (south)	44°37'35" N 34°19'39" E	900	1988	bf	1	8	SIZK
38	Merdven-Kaya mountain*	44°25'25" N 33°50'58" E	600	1988	bhf	1	127	SIZK

*The series of shells from these areas were used in our statistical analysis.

**Species was reported by Korniushin (1988), but it has not been located yet in his collection (SIZK) among the species from the vicinities of Moshny village, although this part of collection is not sorted in detail by the species and it is still possible that material exists somewhere in SIZK.

Abbreviations: n. — near; habitats: bf — beech forest, bhf — beech-hornbeam forest, blf — broadleaved forest (not specified in the records), hbf — hornbeam-beech forest, hmf — hornbeam-maple forest, hof — hornbeam-oak forest, of — oak forest, rs — rocky meadow steppe; material — see abbreviations in "Material and Methods".

For the statistical analysis we have used the 11 series of shells with enough number of the adult specimens from the 10 areas (marked with an asterisk in table 1). We used the following codes for the populations: Cr1 – No. 31 (see numbers of localities in table 1 here and hereafter), Cr2 – No. 29, Cr3 – No. 38, Cr4 – No. 32, Mol1 and Mol2 – No. 1 (different sites within a single forest massif), Pod1 – No. 16, Pod2 – No. 21, Pod3 – No. 23, Dn1 – No. 24, Dn2 – No. 26. The eight shell's characters were measured: number of columellar teeth (1 or 2), number of whorls (NW), height of shell (HS), width of shell (WS), height of aperture (HA), width of aperture (WA), width of a visible part of the 5th whorl in adult shells (W5W), same for the 6th (W6W) and 7th (W7W) whorls. Characters were measured using an original micrometer in an ocular of MBS-1 microscope with scale bar 0.1 mm (measurements by T. Levenets).

Statistical analysis was performed using the STATISTICA 7.0 (StatSoft Inc.) software package. To detect significant differences a one-way analysis of variance was conducted for each character between populations. To divide populations into the homogeneous subsets a Turkey's multiple comparison test was conducted for each character. Moreover, a nested analysis of variance was conducted to detect significant differences between regions (Moldova, Podolian Upland, Dnipro Upland, Crimea) and between populations within the region. To compare populations by the number of the apertural teeth a Pearson's chi-squared test was used (Sokal & Rohlf, 1995).

Both inter- and intrapopulation variability was analysed using two multivariate statistical methods: principal component analysis and discriminant analysis to illustrate overlaps. Discriminant analysis was conducted both on the level of the individual specimens and on the level of the groups of specimens. We used the four criteria with different groupings of the specimens (11 populations; 4 regions; 3 regions with Podolia and Moldova in one group; Crimea and non-Crimea; see further details in the "Results") (Manly & Alberto, 2017).

Results

Present data (table 1) showing that *S. doliolum* is currently known to occur in the 38 localities (areas) in Ukraine and Moldova, including 12 in the Crimean Mountains, 1 on the Central Moldavian Plateau, 3 on the Dnipro Upland (Central Ukraine), 17 on the Podolian Upland, 3 on the Khotyn Upland (between Prut and Dniester rivers) and 2 in the distant parts of the Ciscarpathia (eastern foothills of the Carpathians) (fig. 1). Some of these localities include up to 10 sites where *S. doliolum* was found within the same forest massifs. In the 9 of these 38 localities the species was found more than 100 years ago only. We are reporting *S. doliolum* for the first time from Lviv region of Ukraine here, in Ciscarpathia (2016, collected by Z. Shvydka).

In the studied regions, *S. doliolum* occurs at the altitudes from 150 m to 1400 m, but is nearly absent in the Ukrainian Carpathians, with two records on the eastern foothills (see fig. 1) at the altitude of up to 500 m. All records at higher altitudes are from the Crimean Mountains where *S. doliolum* occurs from the foothills up to almost the highest points, including the two highest plateaus (Babugan and Chatyr-Dagh).

Almost all records of *S. doliolum* in Ukraine and Moldova are from the natural broadleaved forests, mostly well-preserved in the protected areas. On the plains, it mostly lives in the various hornbeam-oak forests and its secondary deviates (e. g., hornbeam-maple forests where oak was replaced by maple as a result of cutting), while in the mountains it mostly inhabits the beech forests with various admixtures. Most of the *S. doliolum* records on the Podolian Upland are associated with the rocky outcrops. Some of the records referring to the large rocks with indication that species lives on them, not just near them, but there are no indications suggesting that those rocks are not within the forests. On the Dnipro Upland *S. doliolum* was never found near rocky outcrops, but is exclusively associated with the forest ravines. It is especially evident in the well-studied Kaniv Nature Reserve where species was mostly found along the bottom of a single medium-sized ravine without regular stream and a few times on the bottoms of two other large ravines with streams. The species was not found in the several other studied ravines within the same forest massif, as well as in the intermediate forests on the slopes and crests of the hills. It shows a very fragmented occurrence of *S. doliolum* within a single forest massif in Kaniv Nature Reserve with apparently isolated populations (or at least subpopulations) in the three different ravines. Some of the *S. doliolum* populations on the Podolian Upland are also associated with ravines, but some other are not. In Codru Nature Reserve (Moldova) the species was found from the bottoms of the ravines to the tops of the hills within a single forest massif.

The only case where we can confirm occurrence of *S. doliolum* in a completely open forestless habitat is on the upper plateau of the Chatyr-Dagh mountainous massif in the Crimean Mountains, at an altitude of *ca*. 1400 m (collected by I. Balashov in 2011). Mollusks were collected with a sample of a sod that was taken among the large rocks and grass vegetation next to a few low junipers (*Juniperus communis* L.). Vegetation around this place includes *Carex* sp., *Pulsatilla halleri* (All.) Willd., *Draba cuspidate* M. Bieb., *Alopecurus vaginatus* (Willd.) Pall. ex Kunth, *Festuca* sp., *Potentilla* sp., *Filipendula vulgaris* Moench, *Aconitum lasiostomum* Spreng., *Androsace villosa taurica* (Ovcz.) Fed., *Veronica* sp., *Allium* sp., *Cystopteris fragilis* (L.) Bernh., etc. This place is in a transition between the two types of habitats: the mountainous meadow steppes (grasslands) and the biotopes of rocky outcrops (Didukh, 2016). Other species of mollusks collected in the same sample of a sod together with *S. doliolum* were: endemics *Brephulopsis bidens* (Krynicki, 1833), *Thoanteus gibber* (Krynicki, 1833), *Mentissa gracilicosta* (Rossmässler, 1836), *Monacha fruticola* (Krynicki,

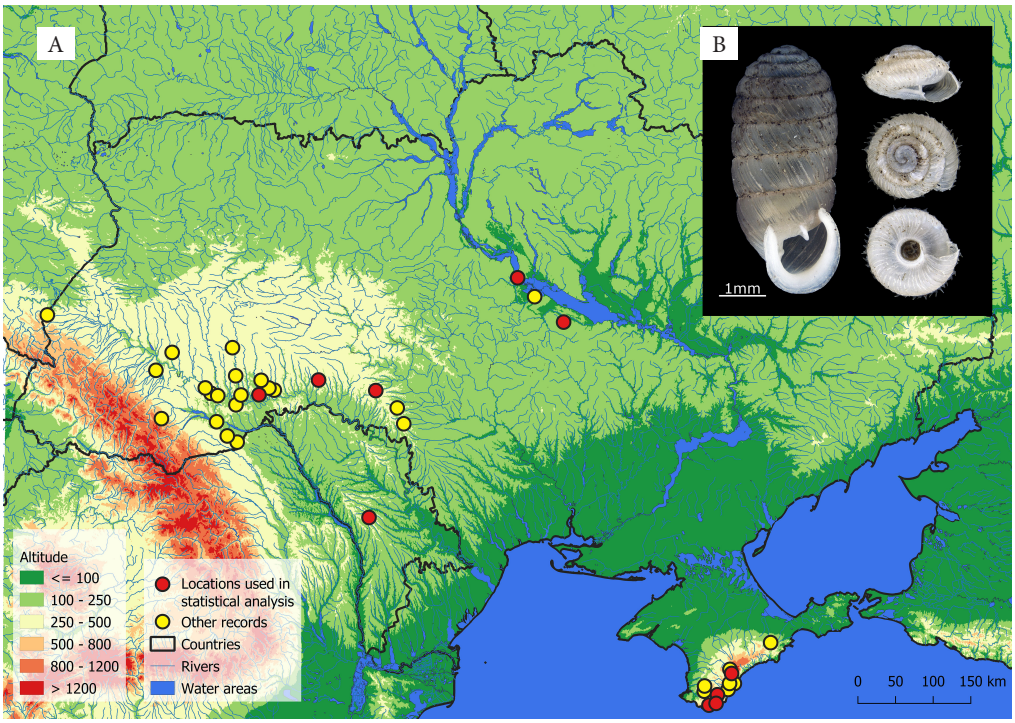

Fig. 1. *Sphyradium doliolum:* A — distribution in Ukraine and Moldova (map by A. Markova); B — shells (adult and juvenile) from the Kaniv Nature Reserve (photo by K. Martynova and I. Balashov).

1833) and rather widespread *Cochlicopa lubricella* (Porro, 1838), *Cochlicopa lubrica* (Müller, 1774), *Vertigo pygmaea* (Draparnaud, 1801), *Truncatellina cylindrica* (Férussac, 1807), *Truncatellina costulata* (Nilsson, 1823), *Vallonia costata* (Müller, 1774), *Pyramidula pusilla* (Vallot, 1801), *Vitrina pellucida* (Müller, 1774), *Euconulus fulvus* (Müller, 1774). Such species composition of mollusks is rather typical for the meadow steppes and rocky outcrops on the higher plateaus across the Crimean Mountains, with the exceptions of *S. doliolum* and *T. costulata* (Balashov, 2016 b). The latter species is also rather a forest inhabitant, especially on the plains of Eastern Europe (Balashov, 2016 b).

Evaluated average values of the measured morphometric shell characters in the studied populations of *S. doliolum* in Ukraine and Moldova are presented in table 2. These values are showing a high level of an interpopulation variability regarding all measured characters (one-way analysis of variance: in all cases $P < 0.001$). Specimens of *S. doliolum* from the Crimean populations have the highest average number of the whorls and, as a result, the highest shell comparing to the specimens from the other measured series of shells. The only exception from this is a small series of shells from the Kholodny Yar National Nature Park on the Dnipro Upland (Dn2, No. 26 in table 1). Specimens of *S. doliolum* from this population appearing to be of the comparable size with that from the Crimean Mountains. In this population also the highest values of the shell's width were recorded. It is notable that a nearby population of *S. doliolum* in Kaniv Nature Reserve (Dn1, No. 24 in table 1) in contrast have the lowest values of the shell's height and width (see table 2).

We have determined that specimens of *S. doliolum* from the four different regions (Crimea, Podolian Upland, Moldova and Dnipro Upland) are significantly different in the size of shell and its other measurements. However, the significant differences were also found between the populations within the analysed regions (table 3). Therefore, we are dealing with an example of a large-scale geographic variability also complemented with a

Table 2. Estimated average values of the morphometric shell characters in the studied populations of *S. doliolum* in Ukraine and Moldova

Population codes	*n*	Shell characters							
		NW[1]	HS, mm	WS, mm	HA, mm	WA, mm	W5W, mm	W6W, mm	W7W, mm
Cr1 (31)	9	8.11^{ac}	5.44^{ad}	2.57^{ab}	1.79^{abc}	1.71^{abc}	2.39^{abc}	2.52^{a}	2.48^{fg}
Cr2 (29)	18	8.06^{a}	5.40^{a}	2.50^{ad}	1.87^{a}	1.79^{ac}	2.28^{de}	2.38^{cd}	2.35^{abc}
Cr3 (38)	111	8.02^{a}	5.40^{a}	2.51^{a}	1.85^{a}	1.79^{a}	2.30^{de}	2.40^{ce}	2.40^{c}
Cr4 (32)	53	8.02^{a}	5.47^{a}	2.45^{cd}	1.83^{ac}	1.78^{a}	2.27^{e}	2.34^{d}	2.32^{ab}
Mol1 (1)	17	7.59^{bde}	4.95^{bc}	2.48^{acd}	1.74^{bcd}	1.68^{b}	2.35^{abcd}	2.44^{be}	2.35^{abc}
Mol2 (1)	6	7.17^{d}	4.87^{bc}	2.52^{abcd}	1.77^{abcd}	1.76^{abc}	2.43^{bc}	2.49^{ab}	2.36^{abcde}
Pod1 (16)	15	7.80^{abce}	5.20^{abd}	2.56^{ab}	1.81^{abc}	1.75^{abc}	2.38^{abc}	2.50^{a}	2.47^{efg}
Pod2 (21)	18	7.50^{de}	5.01^{bc}	2.53^{ab}	1.79^{abc}	1.73^{abc}	2.41^{c}	2.48^{ab}	2.38^{bcd}
Pod3 (23)	44	7.86^{abc}	5.11^{bd}	2.53^{ab}	1.76^{b}	1.71^{bc}	2.35^{ab}	$2.^{47ab}$	2.44^{def}
Dn1 (24)	44	7.66^{bcde}	4.78^{c}	2.42^{c}	1.65^{d}	1.59^{d}	2.31^{ad}	2.38^{c}	2.32^{a}
Dn2 (26)	8	8.13^{abc}	5.48^{ad}	2.61^{b}	1.88^{abc}	1.77^{abc}	2.39^{abc}	2.52^{a}	2.53^{g}
SEM[2]	–	0.152	0.097	0.006	0.012	0.008	0.005	0.002	0.005
F(10; 332)	–	9.19	20.75	10.69	12.78	17.91	13.94	43.54	20.39
P	–	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

[1]Superscripts indicate test results of the ANOVA followed by Turkey's multiple comparison tests; [2]Pooled standard error of means.

high level of a low-scale variability within regions. The latter could be a result of the micro-habitat differences or some random factors (Kramarenko, 2016).

Studied populations of *S. doliolum* are not significantly different in the number of the columellar teeth in the shell aperture (Pearson's chi-squared test: $\chi^2 = 14.016$; $df = 10$; $P = 0.172$). The vast majority of specimens in all populations have two columellar teeth (86.7–100 %) (table 4).

Table 5 shows the factor loadings for the first three principal components of the variance–covariance matrix with morphometric shell characters in the studied populations of *S. doliolum* in Ukraine and Moldova. Overall, the first three principal components are covering more than 80 % of the total variance that makes them usable for the further analyses.

First Principal Component (PC1) covers 39.35 % of the total variance and shows the highest loadings for the height and width of the shell and of its aperture. This component can be described as the "general size of the shell".

Second Principal Component (PC2) covers 29.25 % of the total variance and shows, from the one side, the high loadings for the number of the shell's whorls (-0.576), as well as the width of the 5th and 6th whorls (+0.877 and +0.849 correspondingly) from the other side. This component divides the specimens with low number of whorls, but with wide 5th

Table 3. Results of the nested analysis of variance for the morphometric shell characters in the studied populations of *S. doliolum* in Ukraine and Moldova

Shell characters	Region		Population/Region	
	F (3; 332)	*P*	*F* (7; 332)	P
NW	21.74	< 0.001	3.81	< 0.001
HS	56.22	< 0.001	5.55	< 0.001
WS	12.53	< 0.001	9.90	< 0.001
HA	30.91	< 0.001	5.01	< 0.001
WA	46.73	< 0.001	5.56	< 0.001
W5W	27.86	< 0.001	7.97	< 0.001
W6W	77.04	< 0.001	29.19	< 0.001
W7W	18.81	< 0.001	21.07	< 0.001

Table 4. Absolute frequencies of the specimens with 1 or 2 columellar teeth from the studied populations of *S. doliolum* in Ukraine and Moldova

Population code	1 tooth	2 teeth	Total
Cr1 (31)	1	8	9
Cr2 (29)	0	18	18
Cr3 (38)	12	99	111
Cr4 (32)	3	50	53
Mol1 (1)	0	17	17
Mol2 (1)	0	6	6
Pod1 (16)	2	13	15
Pod2 (21)	0	18	18
Pod3 (23)	0	44	44
Dn1 (24)	2	42	44
Dn2 (26)	0	8	8
Total	20	323	343

and 6th whorls, from the specimens with high number of whorls, but with narrow 5th and 6th whorls. Considering that 5th and especially 6th whorls are the widest ones in *S. doliolum*, this component can be described as the "width of the shell's coiling".

Third Principal Component (PC3) covers 12.99 % of the total variance and related to the width of the 7th whorl and can be described as the "width of the 7th whorl" (see table 5).

Fig. 2 shows the centroid ordination of the shell series from the populations of *S. doliolum* in Ukraine and Moldova in the space of the first three principal components. In the space of PC1–PC2 the series from the Podolian Upland, Moldova and Crimea (except Cr1, No. 31 in table 1, the one from an open habitat on Chatyr-Dagh), composed the two clear isolated pools. Forming of a second pool may be explained by a close geographical position of the series from the Podolian Upland and from Moldova. Differences between the Crimean and non-Crimean specimens are evident mainly along the PC2 (see. fig. 2), i. e., regarding the width of the shell's coiling in *S. doliolum*.

The two analysed series of the shells from the Dnipro Upland are maximally distant from each other in the space of PC1 and PC2 (see. fig. 2, A). Differences between these two series are mainly regarding PC1, i. e., the general size of the shell. These differences were already pointed out above.

The series from an open habitat on Chatyr-Dagh (Cr1, No. 31 in table 1) is in the same trend of geographical variability with other series from the Crimea, despite being distant from them in the space of PC1 and PC2 (see dashed line in fig. 2, A).

All studied series of *S. doliolum* are forming a distinct V-shaped pattern of the geographical variability in the space of PC1 and PC2 (see fig. 2, B). All Crimean series are distributed along one axis (a "left wing"), while all other series are distributed along another axis (a "right wing"). There is an angle of 90° between these two geographical trends (see fig. 2, B), i. e., they are almost orthogonal to each other. This may be indicative of the different mechanisms in the formation of the upper part of the shell (first 5–6 whorls) and of its lower part (7th and following whorls with aperture). It results in a specific shape of the shell in *S. doliolum* with the widest part usually around the 5–6 whorls instead of the last whorl.

Table 5. Factor loadings for the first three principal components of the variance–covariance matrix with morphometric shell characters in the studied populations of *S. doliolum* in Ukraine and Moldova (factor loadings above 0.5 are shown in bold)

Shell characters	Principal Component		
	PC1	PC2	PC3
NW	–0.583	**–0.576**	–0.473
HS	**–0.767**	–0.376	0.176
WS	**–0.659**	0.465	0.034
HA	**–0.738**	–0.236	0.386
WA	**–0.803**	–0.114	0.358
W5W	–0.086	**0.877**	0.329
W6W	–0.355	**0.849**	–0.236
W7W	–0.681	0.303	**–0.585**
% var.	39.35	29.25	12.99

Table 6 shows the results of the discriminant analysis of the morphometric shell characters for the different grouping criteria of the series from the studied populations of *S. doliolum* in Ukraine and Moldova. We have used the four criteria: (1) for the 11 populations, (2) for the four regions (Moldova, Podolian Upland, Dnipro Upland, and

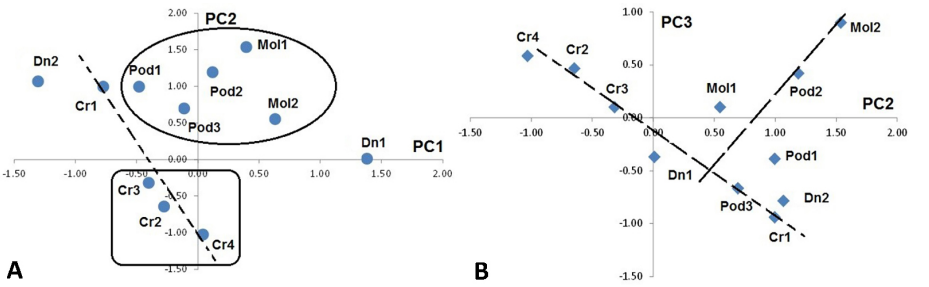

Fig. 2. Centroid ordination of the shells' series from the populations of *S. doliolum* in Ukraine and Moldova in the space of the first three principal components: A — for PC 1 and PC2; B — for PC2 and PC3.

Crimea), (3) for the three regions (Moldova+Podolian Upland, Dnipro Upland, Crimea) and (4) for the two regions (Crimea and non-Crimea). On each step of the series merging the quality of discriminant analysis is rises, i. e., the values of the Wilks's lambda test (Wilks's λ) are higher, as well as the classification accuracy of specimens to their groups.

Figure 3 shows the ordination of all measured *S. doliolum* specimens in the space of the first two canonical axes. The Crimean specimens (blue circles) are clearly separated from the specimens from Moldova and Podolian Upland (green diamonds), as well as from the Dnipro Upland (red squares), while the latter groups are significantly overlapping (see fig. 3).

Discussion

Distribution of *S. doliolum* in Eastern Europe, namely in Ukraine and Moldova, is limited mostly to the higher uplands east of Carpathians and to the isolated smaller areas on the Dnipro Upland and in the Crimean Mountains (see fig. 1). Mollusks of Ukraine are rather well-studied (see Balashov, 2016 a, b) and it is unlikely that *S. doliolum* was not found in the intermediate regions because of the insufficient studies. This species lives in the undisturbed

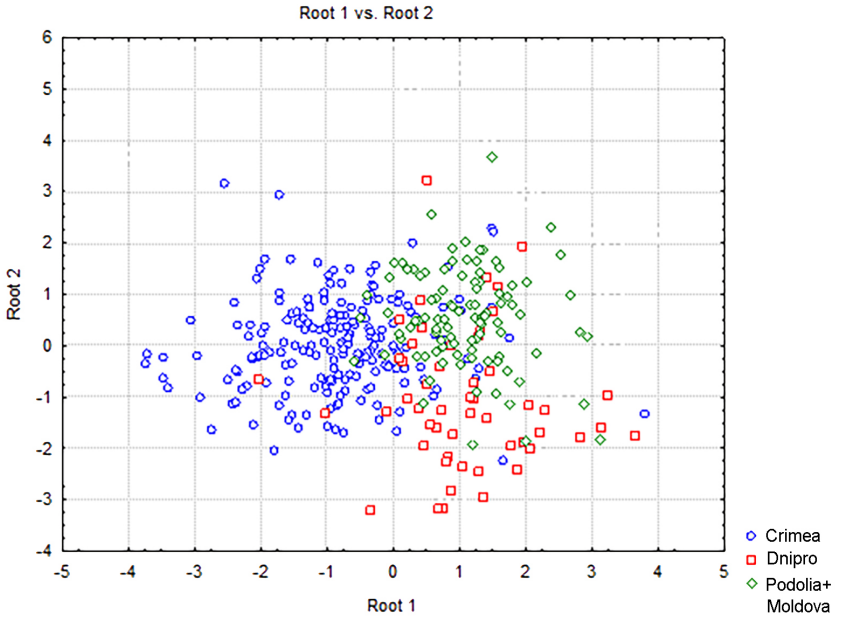

Fig. 3. Ordination of all measured *S. doliolum* specimens from Ukraine and Moldova in the space of the first two canonical axes.

natural forests and it is very unlikely that it could be transported into the new regions as a result of human activities. Therefore, the isolates of *S. doliolum* on the Dnipro Upland and in the Crimean Mountains are remains of the prehistoric natural migrations.

Exact origin of *S. doliolum* is uncertain considering that *Sphyradium* Charpentier, 1837 is a monotypic genus with the widest range among all Orculidae and consists of no recognised subspecies. The family Orculidae in its current understanding includes six extant and one or two fossil genera with *ca*. 80 recognised species, distributed primarily in the mountain regions around the Mediterranean and Black seas (Harl et al., 2017). The fossil taxa of Orculidae are known starting from the Upper Paleogene of Europe, but overall fossil record of this family is rather sparse, *Sphyradium* is only recorded starting from the Pleistocene (Harzhauser & Neubauer, 2021). Recent phylogenetic reconstructions (Harl et al., 2017) are suggesting that the closest relative of *Sphyradium* is genus *Shileykula* Gittenberger, 1983 distributed from Anatolia (main range) to northern Iran and consisting of *ca*. 10 recognised species (Harl et al., 2020). Another two closely related genera (Harl et al., 2017) are *Orcula* Held, 1837, distributed in the mountains of Central Europe and Balkans (*ca*. 15 species), and *Orculella* Steenberg, 1925, distributed from Spain and North Africa to Iran (mostly in Balkans, Aegean Islands, Anatolia and Caucasus, ca. 40 species). Consequently, it appears to be more likely that *Sphyradium* has originated in Anatolia or Balkans, but this is not certain from the existing data.

Expansion of *S. doliolum* on the uplands east of Carpathians has probably happened during the Pleistocene as the local fauna consist no endemic molluskan taxa or other indications of the earlier origins. The species was recorded starting from the Late Pleistocene of the Podolian Upland (Kunitsa, 2007). Baidashnikov (1996) suggested that *S. doliolum* migrated to the Podolian Upland during some interglacial periods of Pleistocene from the Balkans through the regions southeast of Carpathians (e. g., Central Moldavian Plateau, where species is still present in the Codru Nature Reserve, see fig. 1). Other terrestrial mollusks representing the same interglacial migration from the Balkans were suggested to be *Laciniaria plicata* (Draparnaud, 1801) and *Deroceras turcicum* (Simroth, 1894) (Baidashnikov, 1996). Absence of *S. doliolum* in the Ukrainian Carpathians (see fig. 1) strongly supports this hypothesis. Although our present finding in Ciscarpathia near the border with Poland (see fig. 1) may suggest connections to the populations of *S. doliolum* in southern Poland north of Carpathians (Wiktor, 2004). Apparently, it is also possible that *S. doliolum* has migrated to the Podolian Upland from Central Europe north of Carpathians and from there later colonised Central Moldavian Plateau. Populations of *S. doliolum* on the Dnipro Upland are, apparently, representing a northeastern frontier of the same migration that colonised Podolian Upland.

Origin of *S. doliolum* in the Crimean Mountains is less certain. According to Baidashnikov (1990), several species of terrestrial mollusks have probably colonised the Crimean Mountains during the Late Pleistocene from the East European Plain. This is most evident regarding some forest-dwelling species that mostly inhabiting the beech forests in the Crimean Mountains, first of all *Macrogastra plicatula* (Draparnaud, 1801), *Macrogastra borealis* (Boettger, 1878) and *Vertigo substriata* (Jeffreys, 1833). These species absent in Caucasus, Anatolia and in most of the Balkans, therefore, there is no other way how they could appear in the Crimea, to the southeast from their main ranges, other than migrating through the East European Plain. There is no evidence of a continuous existence of the broadleaved forests in the Crimean Mountains before the Late Pleistocene (Didukh, 1992), as well as there are no endemic Crimean mollusks that inhabit exclusively the broadleaved forests (Balashov, 2016 b). Therefore, mollusks that are strongly associated with the beech forests of the Crimean Mountains are probably migrated to the Crimea rather recently from the East European Plain, maybe during the last interglacial (Eemian). Perhaps *S. doliolum*

was a part of this migration considering its association with the beech and other broad-leaved forests. In such a case this migration of *S. doliolum* was a continuation of migration that colonised uplands east of Carpathians (see above) and is happened in the Late Pleistocene. But considering that *S. doliolum* can live in the open rocky habitats of the Crimean Mountains it is also possible that this species represents an older part of the Crimean malacofauna associated with Caucasus and Anatolia (where this species is also present). In the Crimean Mountains there are numerous species mutual with Anatolia and /or Caucasus, but absent on the East European Plain (Balashov, 2016 b). Even more notable example is a rock-dwelling snail *Chondrina arcadica* (Reinhardt, 1881). In the Crimean Mountains this species is represented by *C. arcadica caucasica* Ehrmann, 1931, a subspecies mutual with Caucasus, while in Central Europe *C. arcadica clienta* (Westerlund, 1883) lives, whose range spans to the Podolian Upland on the east (Balashov, 2016 b). Both *S. doliolum* and *C. arcadica* are present in the nearby areas of the Podolian Upland (but not coinhabiting) and both living on the upper plateau of Chatyr-Dagh in the Crimean Mountains (Balashov et al., 2013; Balashov, 2016 b; our data). Perhaps these two species have similar history of migrations in Eastern Europe. In such a case the populations of *S. doliolum* on the uplands of the East European Plain and in the Crimean Mountains could have very different origins with colonization of these regions during different periods. It could mean that populations of *S. doliolum* in the Crimean Mountains are existing for a much longer time, during which they were likely isolated. This time could be enough for an endemic subspecies, or maybe even a species, to evolve.

Our morphometric analysis (fig. 2, 3; table 6) shows a separation between the populations of *S. doliolum* on the uplands of East European Plain and in the Crimean Mountains. This supports the hypothesis that these two groups of populations represent two separate lineages of different origin. The phylogeography of *S. doliolum* requires further study based on molecular genetic data and more material from a wider area.

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