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MORPHOLOGICAL AND MOLECULAR STUDIES OF THE RAPA WHELK, *RAPANA VENOSA* (NEOGASTROPODA, MURICIDAE), FROM ODESA BAY

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Morphological and Molecular Studies of the Rapa Whelk, *Rapana venosa* (Neogastropoda, Muricidae), from Odesa Bay. Morhun, H., Son, M. O., Kovtun, O. O., Utevsky, S. — The gastropod *Rapana venosa* (Valenciennes, 1846) is a successful worldwide invader occurring in the Black Sea. The aim of this study is to overview specific population features of this mollusk from Odesa Bay through integrative systematic approach by means of morphological and molecular research. For this purpose, the mollusks were collected from the Black Sea and examined using morphological methods: traditional morphometry, which employs linear parameters of shells (height, width, whorl height, whorl width, height of the last whorl) and shell weight, and geometric morphometrics of the shell shape data. For a molecular genetic test, the COI gene region was used. Among all conchological variability, the two morphotypes were defined: the first has a "broad" shape — shells have a thick and durable last whorl and a low spire, and the second one — "extended" shape: shells are relatively slender with an elongated high-conical spire. According to the geometric morphometric data, *R. venosa* has statistically significant differences between defined morphotypes ($F = 4.12$, $p = 0.001$); however, the shapes in males and females are not significantly different ($F = 1.13$, $p = 0.318$). No genetic diversity, neither novel haplotypes were revealed by the molecular analysis: in Odesa Bay, the haplotype occurring also in other regions of invasion across the world is present.
Key words: COI, geometric morphometric analysis, invasive species, phylogenetics, rapa whelk, shell morphology.

Iintroduction

Rapana venosa (Valenciennes, 1846) is a well-known invasive mollusk occurring in the Black Sea for the last 70 years (Drapkin, 1953). It has a substantial influence on the environment as a highly effective predator (Mann, Harding, 2003; Bondarev, 2010; Pereladov, 2013) and significantly affects the local shellfish and benthic communities by displacing native bivalve species (Chukhchin, 1984; Rubinshtein, Hizniak, 1988; Marinov, 1990; Zolotarev, 1996), thus reducing the filtration potential of the region (Seyhan et al., 2003; Kurakin, Govorin 2008; Govorin, Kurakin, 2011).

The Black Sea is thought to be the first source of the initial introduction of the rapa whelk and then the mollusk has spread throughout the world by various vectors including ballast waters and/or intentional introductions (Chandler et al., 2008) to the Aegean (Koutsoubas, Voultsiadou-Koukoura, 1991) and Adriatic seas (Ghisotti, 1971, 1974; Mel, 1976; Cucaz, 1983; Rinaldi, 1985), France (ICES, 2004), USA (Harding, Mann, 1999), in the Rio de la Plata between Uruguay and Argentina (Scarabino et al., 1999; Pastorino et al., 2000; Giberto, Bruno, 2014), and the Netherlands (Nieweg et al., 2005). The effective invasion is explained by some specific reproductive and ontogenetic features of rapa whelk: extremely high fertility (15 million eggs laid by one female per season [Harding et al., 2002; Ware, 2002]), presence of plankton larva (veliger) (Harding, Mann, 2003), fast ontogenesis and maturation (Harding, Mann, 2003; Mann et al., 2006). All those features contribute to the potential for colonization and serve to a high invasive success of the rapa whelk.

Many researchers studying the populations of this mollusk in the Black Sea have noticed high ecological plasticity of the species, which may be due to specific biochemical peculiarities (Alakrinskaya, 1989) and great diversity of its conchological traits (Bondarev, 2010; Kos'yan, 2013; Slynko et al., 2020). In many papers, authors describe eco-morphs, metapopulations (Bondarev, 2010) and shell color forms (ICES, 2004, Savini et al., 2004; Micu et al., 2008; Bondarev, 2010), which outline the heterogeneity of morphological features; for some morphs, separate names were coined. For instance, an extremely prolonged morphotype is known in the literature as a "tower-shaped", and small-sized adults as "dwarf forms" (Bondarev, 2010). This phenotypic plasticity occurring among *R. venosa* is associated with some environmental conditions features of habitat (e. g. sediments type), food supply, potential prey (Shukshin, 1961; Bondarev, 2010; Kos'yan, 2013) — and their sex (Bondarev, 2010; Kovtun et al., 2014).

While high morphological variability of this species is observed, genetically very low nucleotide diversity is shown in populations from the regions of invasion (Chandler et al., 2008; Xue et al., 2018; Slynko et al., 2020). An examination from within the native range revealed high levels of genetic variation (110 haplotypes of COI and NADH gene regions), while specimens from all introduced populations — showed the complete lack of genetic diversity, and only a single haplotype was common to all introduced individuals, which occur also in Japan and Korea, especially from Jeju Island (Chandler et al., 2008). The low diversity is explained by an extreme genetic bottleneck occurred, while individuals from the native range were being introduced into the invasive range (Chandler et al., 2008).

During a field survey of the rapa population in Odesa Bay, high conchological variability was noticed. The goal of the current research was to study samples of invasive rapa whelks *R. venosa* in order to evaluate both the genetic diversity and morphological variability by means of integrative approach.

Materials and methods

Sampling

Eighty adult (2–3 years old) rapa whelks were collected by scuba divers in the Black Sea near the Hydrobiological Station Odesa, I. I. Mechnikov National University (Ukraine), at a depth of 6–10 m in 2015. The habitat is characterized by mixed (shelly gravel and sand) sediments and high variability in water salinity (from 4 ‰ to 18 ‰ according to monitoring records of the Hydrobiological Station). A small amount of foot tissue from five individuals were taken and preserved in 95 % ethanol until further molecular processing.

Morphometric studies

The five linear distances of the shell — its height (H), width (W), aperture height (Ha), aperture width (Wa) and height of the last whorl (Ht) — were measured with a digital caliper as shown in fig. 1.1 and shells were weighed (Ms) with a hand scale. The ratios of height to width (H/W), height to width of the aperture (Ha/Wa) and height to height of the last whorl (H/Ht) were calculated to evaluate the elongation of each shell, and Ms/H was to evaluate the thickness of a shell. The ratios were used as thought to be "size-independent" and already corrected for allometry. To test the significant differences of linear measurements and ratios between morphotypes and sexes the Factorial ANOVA in Statistica v10 was performed.

For further geometric morphometric analysis, photos of each shell were captured and then saved in the JPG format. While taking photographs, we used a special hand-made equipment (see fig. 1.3) to keep all shells in the same position relative to the camera, thus avoiding the distortion error associated with the rotation. This equipment is aimed to fix the object by certain points. In our study each shell was fixed on three points: the top of the shell, the bottom edge and the extreme point of the aperture.

After photos were captured, all studied shells were visually assigned to the following morphotypes: the first has a thick and durable last whorl and a low spire, which makes the shell look "broad" (fig. 1.2, a), and the second one has a relatively slender shell with an elongated high-conical spire that looks like the "extended" morphotype (fig. 1.2, b).

Subsequently, the geometric morphometric analysis based on landmarks was performed. Fifteen landmarks of the shell were examined which are located as follows (fig. 1.1):

LM1 — extreme anterior point of siphonal canal;

LM2 — extreme anterior point of umbilicus;

LM3 — left side extreme point of body whorl;

LM4 — left side point on suture of 2nd whorl on spire;
LM5 — apex;
LM6 — right side point on suture of 2nd whorl on spire;
LM7 — posterior canal;
LM8 — extreme point of aperture;
LM9 — columellar fold on inner lip;
LM10 — curve on umbilicus;
LM11 — left spiral rib on body whorl;
LM12 — right spiral rib on body whorl;
LM13 — connection of aperture curve with body whorl;
LM14 — curve on posterior outer lip;
LM15 — curve on anterior outer lip.

Digitizing was done by using the TPSdig2 software (Rholf, 2013). The preliminary Procrustance ANOVA showed that the interaction between morphs and centroid size was not significant ($F = 0.5206$, $p = 0.883$), thus the residuals of a pooled within-group regression of shape on centroid size (accounting for 3.66 % of total variance, $p = 0.006$) were obtained to get a corrected for intra-specific allometry dataset. This dataset then was used in subsequent analyses.

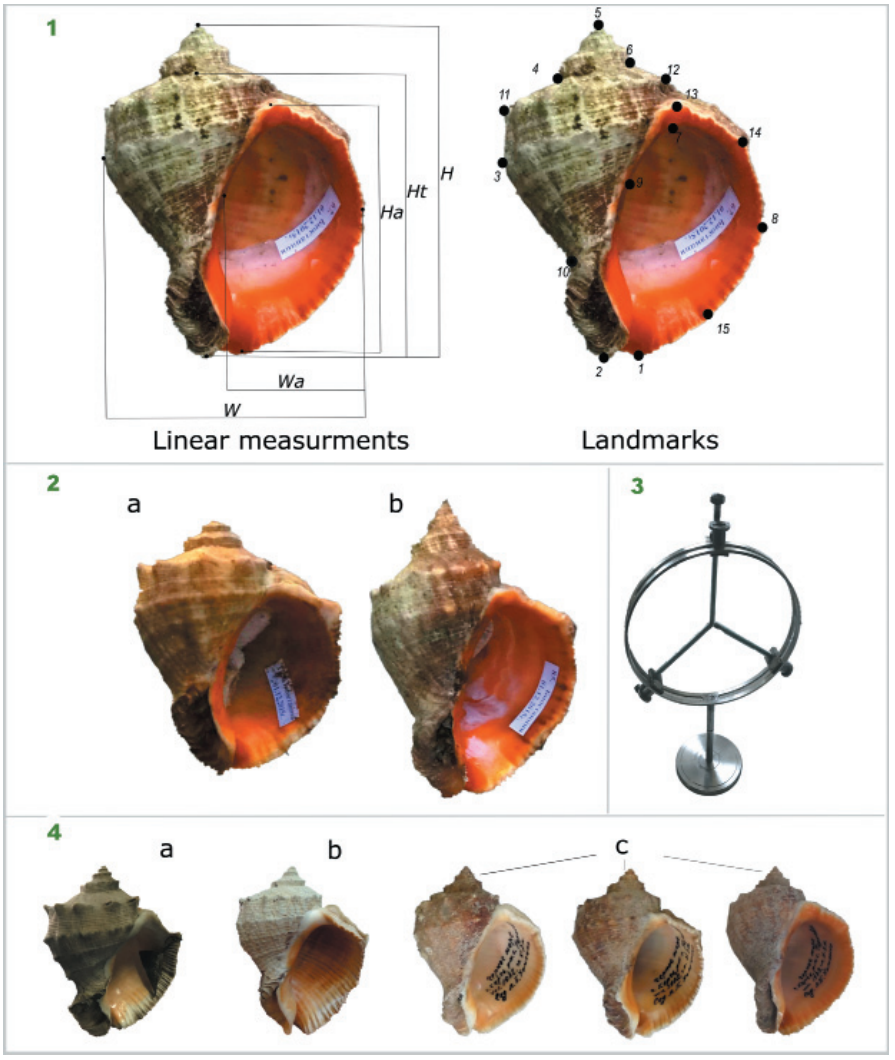

Fig. 1. *Rapana venosa* aggr.: 1 — linear measurements and landmarks (LM) used for GMM analysis (1–9 — fixed LM; 10–15 — semi LM); 2 — morphotypes; 3 — tool for fixation; 4 — museum material: a — Japan Sea, 1877, Natural History Museum of V. N. Karazin Kharkiv National University (H: 16.3 cm); b — Japan Sea, 1983, National Museum of Natural History at the National Academy of Sciences of Ukraine, Kyiv (16.1 cm); **c** — Black Sea, Kerch, 1972, National Museum of Natural History at the National Academy of Sciences of Ukraine, Kyiv (7.9, 7.8, 9.2 cm).

To test for statistical differences in shell shapes between morphotypes, as well as the effect of sex on the shape within each morphotype, we used the Procrustes ANOVA evaluated for significance with the F-test (Goodall, 1991). Significance testing was achieved through permutation using a residual randomisation permutation procedure involving 1,000 permutations (Collyer et al., 2015). Shell shape variability and intergroup difference were analyzed through the Principal Component Analysis in *Morpho J* software (Klingenberg, 2011).

The strength of covariation between different morphometric approaches — traditional morphometry (ratios of linear measurements) and geometric morphometric data (PC scores of shell shape changes) — was evaluated by the linear correlation coefficient (Pearson) in Paleontological Statistic program (PAST v4.03) (Hammer et al., 2001).

DNA extraction and amplification

Five specimens of rapa whelk from the Black sea near the Hydrobiological Station were transferred to the molecular laboratory of the Department of Zoology and Animal Ecology, V. N. Karazin Kharkiv National University (Kharkiv, Ukraine) for a molecular analysis. Small pieces of muscle tissue from the foot were used for DNA extraction. Genomic DNA was isolated using a DNA Blood and Tissue extraction kit (Qiagen).

The mitochondrial cytochrome c oxidase subunit one (COI) fragment was chosen as considered to be a standard animal DNA barcode gene region (Hebert et al., 2003). It was amplified using the standard primers (Folmer et al., 1994): LCO1490, 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198, 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'; the amplification was conducted under the following PCR protocol: 94 °C for 3 min; 5 cycles of 30 sec at 94 °C, 1:30 min at 45 °C, and 1 min at 72 °C; 35 cycles of 30 sec at 94 °C, 1:30 min at 51 °C, and 1 min at 72 °C; 5 min of denaturation step at 72 °C (Hou et al., 2007). PCR products (5 μl) were cleaned up by SIGMA columns according to the manufacturer's guidelines and then sequenced by Macrogen Inc. (the Netherlands) using the same primers as at the amplification stage. The resulting sequences were manually assembled to a uniform length by removing the remaining parts of the primer regions in FinchTV v. 1.5.0 (Geospiza Inc.) and then submitted to GenBank (Accession: OL504957–61). The length of COI sequences is 615 bp.

Phylogenetic analysis

To perform the phylogenetic analysis, previously published nucleotide sequences from NCBI database in addition to our sequences and the gene of *Plicopurpura patula* (Linnaeus, 1758), *Rapana bezoar* (Linnaeus, 1767) and *Rapana rapiformis* (Born, 1778) employed as an outgroup were used for inferring a phylogenetic tree. The COI sequences were aligned using MAFFT v7.452 (Katoh et al., 2017) with FFT-NS-i strategy selected by the "Auto" option, and examined at the amino acid level for the absence of stop codons using MEGA X (Kumar et al., 2018).

The evolutionary history was inferred by using maximum likelihood in IQ-TREE v1.5.5 (Nguyen et al., 2015), with branch support estimated using 1000 replicates of both the SH-like approximate likelihood-ratio test (SH-aLRT; Guindon et al., 2010) and the ultrafast bootstrapping algorithm (Minh et al., 2013). The ModelFinder option was used to identify the optimal partitioning scheme and substitution models (Kalyaanamoorthy et al., 2017). Best-fit models were determined according to the Bayesian information criterion (BIC); HKY+F+I model of the COI gene was chosen. The tree is drawn with branch lengths measured in the number of substitutions per site.

In addition, the number of base differences per site (p-distances) between sequences and their standard errors were calculated. All positions containing gaps and missing data were eliminated.

Haplotypes were determined using DnaSP version 6.12.03 (Rozas et al., 2017). There were a total of 615 base pairs in the final dataset.

Results

Morphometric studies

The morphometric measurements of population and the significant difference among morphotypes and sexes are shown in table 1.

The results of two-way ANOVA using linear measurements and the ratios revealed significant differences in values among the morphotypes for H, H/W, and Ht/H. The difference between the sexes was revealed for H, Ha, Wa and H/W, Ha/Wa ($p < 0.05$, table 1). No difference in linear measurements between the sexes within each morphotype was found ($p > 0.05$, table 1).

As a result of the geometric morphometric analysis, the significant difference in the shape of shells between the defined morphotypes was revealed ($F = 4.12$, $p = 0.001$) (table 2). Yet, no significant shape variation between the sexes was found ($F = 1.13$, $p = 0.318$), also no difference in shape between the sexes within each morphotype ($F = 0.22$, $p = 0.989$) was revelaed.

Table 1. Measurements of *R. venosa* from Odesa Bay

Morph, N	Sex, N		Linear Distances, mm					Ms, mg	Ratios			
			H	W	Ha	Wa	Ht		H/W	Ha/Wa	Ht/H	Ms/H
Extended	♀ 13	min	64.470	59.540	50.710	29.640	56.323	32.160	1.082	1.540	0.822	0.447
36		max	78.620	69.300	59.710	37.090	67.899	55.370	1.202	1.859	0.874	0.726
		average	72.663	63.691	56.578	33.082	62.298	43.148	1.142	1.714	0.857	0.592
	♂ 23	min	68.630	58.490	51.740	30.090	59.524	27.810	1.105	1.553	0.833	0.379
		max	86.640	76.430	68.470	42.190	75.027	83.240	1.261	1.745	0.896	0.982
		average	77.482	66.586	60.046	36.503	66.556	52.115	1.165	1.647	0.859	0.666
Broad	♀ 13	min	61.730	56.350	53.530	29.800	52.714	33.790	1.095	1.608	0.807	0.491
44		max	88.140	74.320	66.320	41.250	76.745	81.010	1.232	1.840	0.874	0.919
		average	77.125	65.164	58.811	34.196	65.073	48.094	1.183	1.725	0.844	0.617
	♂ 31	min	69.470	60.020	54.210	28.730	58.330	32.080	1.147	1.557	0.810	0.439
		max	89.910	77.030	75.800	40.940	78.164	82.260	1.316	1.974	0.878	0.930
		average	78.898	65.592	60.139	35.491	66.622	50.780	1.203	1.700	0.844	0.637
p (morph)			**0.024**	0.829	0.278	0.947	0.236	0.548	**0.000**	0.123	**0.001**	0.958
p (sex)			**0.012**	0.136	**0.027**	**0.003**	**0.017**	0.055	**0.018**	**0.025**	0.797	0.116
p (morph*sex)			0.236	0.267	0.318	0.164	0.259	0.297	0.850	0.301	0.906	0.370

Note. Coefficients with significant $p < 0.05$ are highlighted in bold.

The further principal component analysis of geometric data was performed. In total, 26 principal components were revealed, with PC1 accounting for 26.37 % of variation and PC2, 20.55 %. A cumulative proportion of these first components are 46.92 % (fig. 2). The remaining PC's each contributed around or less than 10 % of the total variation and are not discussed further.

PC1 is associated with sliding of the extreme point of the whorl of the shell (see fig. 2). All samples were scattered along the PC1 axis but did not show any inter-specific variation within each morphotype. The high and significant correlation between PC1 scores and ratios from traditional morphometric measurements was not revealed ($p > 0.05$, table 3, supplem.

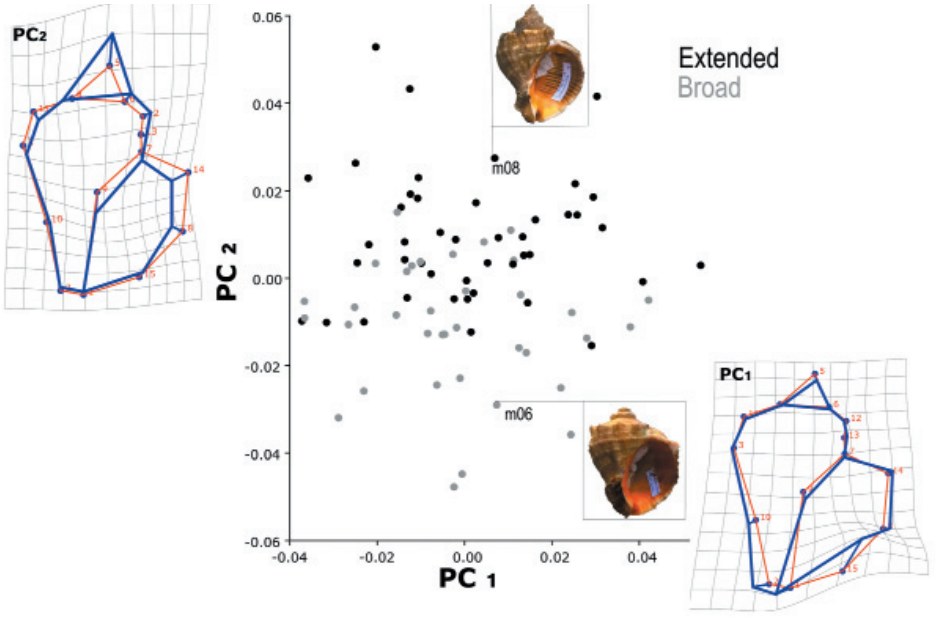

Fig. 2. Morphospace defined by the two first principal components (PC's) of shape variance using landmark data. Shape changes associated with scores of each PC axis are shown as warped surface on a transformation grid (see text for details).

Table 2. Procrustes ANOVA evaluating variation in shape between morphotypes and between sexes within each morphotype

	Df	SS	MS	Rsq	F	Z	Pr (> F)
CS	1	0.009725	0.0097254	0.04838	4.0784	3.1615	0.001
Morph	1	0.009909	0.0099092	0.04929	4.1555	3.2605	0.001
Sex	1	0.002692	0.0026916	0.01339	1.1288	0.5135	0.320
CS:Morph	1	0.001241	0.0012413	0.00617	0.5206	–1.2089	0.882
CS:Sex	1	0.001986	0.0019859	0.00988	0.8328	–0.1443	0.544
Morph:Sex	1	0.000770	0.0007702	0.00383	0.3230	–2.2098	0.989
CS:Morph:Sex	1	0.003027	0.0030274	0.01506	1.2696	0.7102	0.242
Residuals	72	0.171691	0.0023846	0.85401			
Total	79	0.201042					

Note. *P*-values based on 1,000 random residual permutations.

Table 3. Correlation coefficient of linear measurements and PC scores (under diagonal) and p-value after permutation test (above diagonal)

	H	W	Ha	Wa	Ht	Ms	H/W	Ha/Wa	Ht/H	PC1	PC2
H		*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.002*	*0.240*	*0.555*	*0.732*	*0.030*
W	0.865		*0.000*	*0.000*	*0.000*	*0.000*	*0.122*	*0.004*	*0.001*	*0.495*	*0.618*
Ha	0.881	0.837		*0.000*	*0.000*	*0.000*	*0.176*	*0.458*	*0.003*	*0.423*	*0.851*
Wa	0.781	0.852	0.846		*0.000*	*0.000*	*0.560*	*0.000*	*0.005*	*0.129*	*0.141*
h	0.959	0.913	0.921	0.822		*0.000*	*0.130*	*0.185*	*0.002*	*0.488*	*0.727*
Ms	0.822	0.870	0.857	0.802	0.889		*0.773*	*0.056*	*0.000*	*0.336*	*0.870*
H/W	**0.341**	–0.174	0.153	–0.066	0.171	–0.033		*0.002*	*0.000*	*0.528*	*0.000*
Ha/Wa	–0.133	**–0.322**	–0.084	–0.599	–0.150	-0.214	0.339		*0.422*	*0.090*	*0.009*
Ht/H	0.067	0.353	**0.329**	**0.310**	**0.343**	**0.395**	**–0.527**	–0.091		*0.168*	*0.000*
PC1	0.039	0.077	0.091	0.171	0.079	0.109	–0.072	–0.191	0.155		*1.000*
PC2	**0.242**	–0.057	–0.021	–0.166	0.040	–0.019	**0.579**	**0.290**	**–0.677**	0.000	

Note. Coefficients with significant $p < 0.05$ are highlighted in bold.

fig. 1). PC2 relates with elongation of shell shape: with negative PC2 scores shell appeared to be broad, while with positive PC2 scores shells are prolonged (fig. 2). All specimens showed inter-specific variation being scattered about the PC2 axis within each morphotype. There was a significant correlation between PC2 scores and the H/W and Ht/H ratios values revealed ($r = 0.58$, $p < 0.05$ and $r = –0.68$, $p < 0.05$ respectively, table 3, supplem. fig. 1).

Molecular studies

The phylogenetic analysis of our samples and GenBank data revealed the evolutionary history of *R. venosa*, which is illustrated using the resulting phylogenetic tree (fig. 3). It was found that all 5 specimens from Odesa Bay are identical and shared the same haplotype; this haplotype is identical to sequences previously published in GenBank, including samples from the native range — Mikawa Bay (Japan), Jeju-do (Korea) — and from the invasive range — the Black Sea, Adriatic Sea, Quiberon Bay in France, the Netherlands, and Chesapeake Bay in the USA (sequences beginning with the codes EU and MH in fig. 3) (Chandler et al., 2008). Particularly in the Black Sea, this haplotype currently known from Anatolia (sequences with code KP and KU), Crimea (sequences were not deposited in a Genbank by Slynko et al., 2020; personal communication), and the north-eastern Black Sea (sequences with the code EU) (Chandler et al., 2008).

The number of base substitutions per site from averaging over all sequence pairs between groups and within groups are shown in table 4.

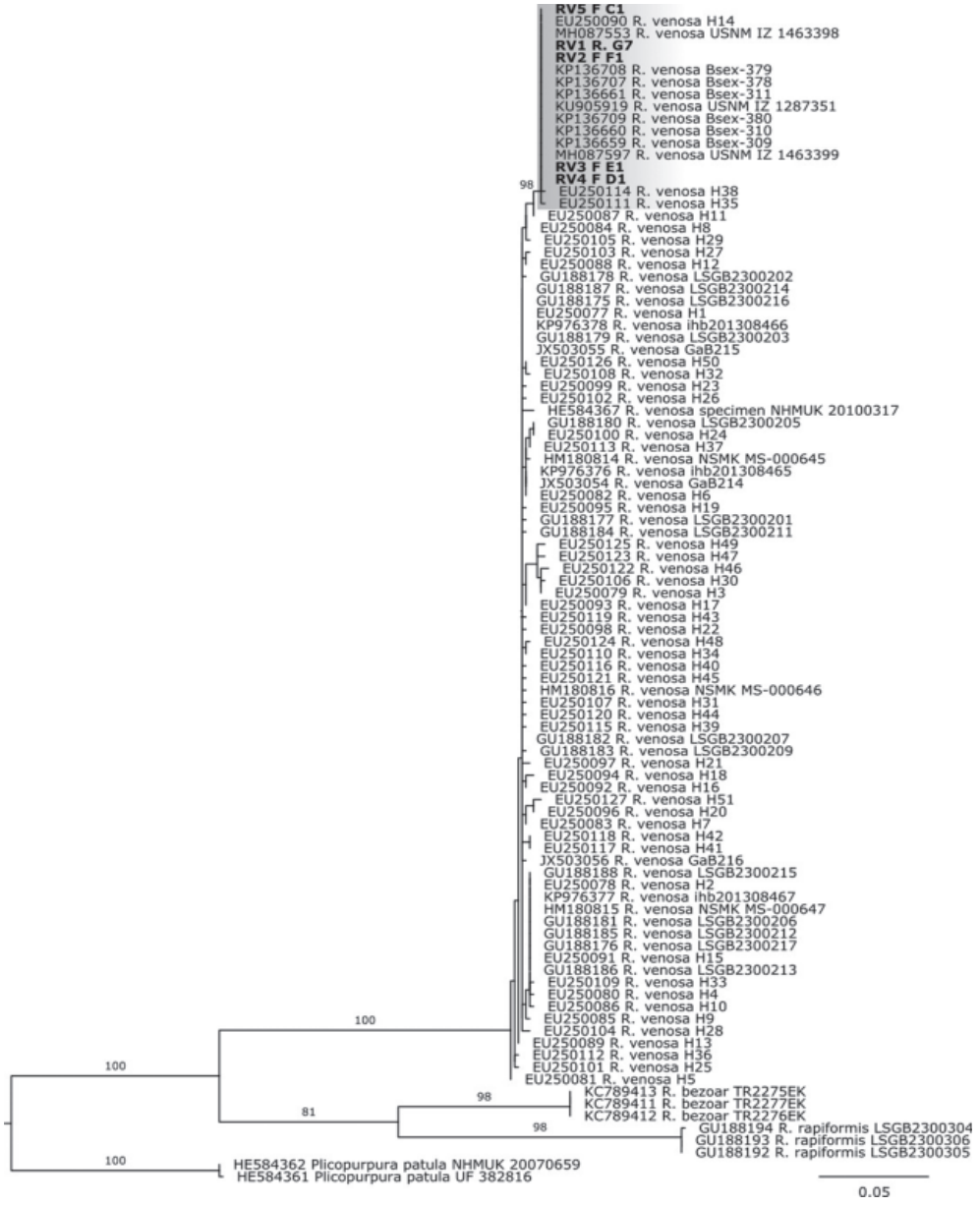

Fig. 3. Phylogenetic relationships between major groups of *Rapana* genus obtained by Maximum-Likelihood method and based on COI sequences (ultrafast bootstrap values are shown for clades; the tree is rooted at *Plicopurpura patula*). Sequences from the current research are highlighted in bold.

Table 4. Estimates of evolutionary divergence over sequence pairs of *R. venosa* dataset. The number of base differences per site (based on *p-distances*) between groups and within each group are shown

Group	*Between group*						Within group	
	1	2	3	4	5		p-dist	S. E.
1. *P. paluta*		0.150	0.134	0.110	0.105		0.001	*0.001*
2. R. bezoar	0.083		0.064	0.072	0.072		0.000	*0.000*
3. *R. rapiformes*	0.076	0.058		0.134	0.132		0.000	*0.001*
4. *R. venosa* Odesa	0.076	0.064	0.083		0.003		0.000	*0.000*
5. *R. venosa*	0.074	0.064	0.082	0.004			0.003	*0.002*

Note. Standard error estimates are shown in *italic*.

Discussion

Studies of the morphological diversity of *R. venosa* from different parts of the northern Black Sea region have shown high capability of this mollusk to vary in its shell conchology under the influence of environmental conditions (Bondarev, 2010; Snigirov et al., 2013; Kos'yan, 2013). All systematic features of shells, including the general shape and color, the thickness of walls, the presence and development of axial and spiral ribs and grooves, spines and other sculptural surface elements of the shell, are variable. The most common and main driver for changes is assumed to be different trophic conditions in habitats (Bondarev, 2010; Kovtun et al., 2014). Depletion of food sources causes a slowdown in growth, a decrease in the size of individuals and also decrease in the size when maturity occurs (Chukhchin, 1961).

The results of our geometric morphometric analysis revealed significant differences in shell shape between the defined morphotypes in the spire elongation: shells vary from a tall (extended) to a squatted one (fig. 2).

Such a high variability of shell habitus might be explained based on previously published studies in which it is associated with habitat where the food objects occur: a narrow and long shell is more capable for moving on sandy seabed and hunting for mollusks burrowing into sand (Bondarev, 2010). Thus, high diversity of potential food objects in Odesa Bay, which *R. venosa* hunts for, is assumed to result in the high morphological heterogeneity of molluscs which we observed.

Although the shells vary in the thickness of their walls from thick-walled to thin-walled, the thickness is not a sign of either sexual dimorphism or morphotype (table 1, Ms/H, $p > 0.05$). It is believed that food abundance can affect on thickness: if the amount of nutrients is enough, the growth is more or less constant and the carbonate layer is lied on the inner surface of the shell evenly in a certain unit of time; however, if food is in short supply, the growth of the soft body (its weight and size) slows down significantly, but the shell itself continues to become thicker (Kos'yan, 2013).

The results of our geometric morphometric analysis also showed no differences in shape between the sexes within the population of Odesa Bay. Yet, according to linear morphological measurements (H, Wa, Ha) and some ratios (H/W and Ht/H), significant differences between sexes were revealed. Thus, the approaches we applied contributed to each other making the results more informative: although we detected significant differences between males and females in shell sizes (linear measurements), the sexes did not differ in their shell shape (geometric morphometrics).

Kovtun et al. (2014) also showed that males have larger shell sizes than females do. This fact is explained by the need for the latter to spend additional energy on reproduction of offspring. According to Bondarev (2010), the sex of individual can be determined based on both size and conchological traits of shell, especially shape: males have a higher and narrower shell than females. We also obtained significant differences in H/W and Ht/H ratios between sexes, but a more sensitive approach (geometric morphometrics) shows that this trend in shape morphology is absent for the Odesa Bay population.

Summarizing the results of morphological examination, the following features of population were revealed: no sexual dimorphism either in shell shape or in thickness was found, and the difference in size between males and females is caused by the need for the latter to reproduce. The diversity in elongation of individuals might be a result of a high variety of food objects in Odesa Bay.

The second essential part of our research was devoted to a molecular study of *R. venosa* from Odesa Bay. Our analysis revealed phylogenetic relationships and nucleotide diversity of the population. In particular, all specimens shared the same haplotype known from previous publications as the only one occurring in the regions of invasion around the world (Chandler et al., 2008). Our results are consistent with previous publications on the molecular diversity of *R. venosa* from the northern Black Sea region, especially from Crimea (Slynko et al., 2020).

The observed low genetic diversity can be interpreted as the evidence of an one-time invasion of the rapa whelk into the Black Sea and the further dispersal to other regions. Moreover, it can be assumed that the worldwide success of *R. venosa* invasion is resulted from this one specific haplotype, and the low genetic variation may be the consequence of successful adaptation to new environmental conditions affecting evolutionary rescue in the process if invasion aimed to establish the invader in a new region (Estoup et al., 2016).

The results obtained using the integrative approach (combining genetics and morphology) imply high morphological diversity and, at the same time, low genetic variability. This can be considered in a context of the "Genetic Paradox of Biological Invasions" concept (Estoup et al., 2016). Thus, a single-time introduction of a species (as was with rapa whelks in the 1940s) accompanied by the bottleneck effect usually leads to a depletion of the genetic variation and, accordingly, the reduced phenotypic diversity of an introduced population in general. This is because a small part of the population is introduced, and it does not carry all the genetic diversity of its species. However, in case of the rapa whelks from the Black Sea, we observe rather a high heterogeneity of morphological characteristics: high phenotypic plasticity, the emergence of new morphs in biotopes with different ecological characteristics, which suggests high adaptability in general; and all this is along with the complete absence of nucleotide diversity for both the COI gene (this study) and *nad2* (Chandler et al., 2008). Less conservative markers could be used in future as an attempt to reveal higher nucleotide diversity in the population.

To explore the phenomenon of phenotypic plasticity of the rapa whelks from the Odesa Bay in a comparative way, some available museum material from its native region (Japan) and from the Kerch Strait sampled in 1972 (about 25 years after invasion) were examined to compare conchological characters of those populations (fig. 1.4).

Firstly, a size difference is seen: the Odesa mollusks are smaller than the native ones — the average H is 70–80 mm, while shells from the Sea of Japan are 161 and 163 mm (fig. 1.4, a, b). Secondly, a difference in the sculpture and massiveness of the shells are observed: specimens from the native area (fig. 1.4, a, b) have more pronounced spines and thicker-walled shells than shells from Odesa Bay. The fact of reducing the shell size in the Black Sea rapa whelk population was previously recorded by other researchers (Ivanov, 1961; Bondarev, 2010). Yet, the studied shells from the Kerch sampled in 1972 have dimensions similar to the Odesa population — 79–92 mm. It can be assumed that the mollusks became finer rapidly after the invasion, and this reduction in size is related not only to the available amount of food. The populations of mussels and other bivalves in those years in the Kerch Strait were at a high level (both free-living populations and existing mussel farms) and there was a rather sufficient amount of food (Ivanov, 1987; Ivanov, Synegub, 2008). Thus, reduction in shell size can be a result of the influence of unknown environmental factors and / or the bottleneck effect occurred as a consequence of invasion.

The size of the rapa whelk can also be determined by a potential prey: if small *Chamelea gallina* (Linnaeus, 1758), *Anadara kagoshimensis* (Tokunaga, 1906) and *Mytilus* spp. are predominant in the diet, then the rapa whelk has a large size (Kos'yan, 2010, 2013). This is explained by the fact that the mollusk prefers the prey of particular size, thus compensating the energy for foraging (the opening of the shell valves of the prey) (Savini, Occhipinti-Ambrogi, 2006; Kos'yan, 2009, 2010, 2013).

Comparing the sizes of the rapa whelks in previous studies, the maximum size of the shell of *R. venosa* from the native range is 212.3 mm (Pisor, 2005); Bondarev (2010) found that shells from the Sea of Japan vary from 75 to 168.7 mm. In the non-native regions, the following shell lengths are observed: in the Chesapeake Bay (1998–1999) from 67 mm to 160 mm (ICES, 2004); in the estuary of the Rio de la Plata — 28–120 mm (Giberto et al., 2006); in the Mediterranean Sea near Venice — 78–139 mm (Cesari, Pellizzato, 1985); off the coast of Romania — from 50 to 95 mm (Micu et al., 2008; Sereanu et al., 2016); off the coast of Turkey — from 58 to 102 mm (Seyhan et al., 2003). The specimens from our

sampling had approximately the same size (table 1) with no extremes and are similar to those from the previous studies within invasive regions.

The tendency to decrease in size in comparison with the native area are seen in general; however, the aforementioned assumption about the influence of the bottleneck effect as one of the reasons should be further verified by carrying out appropriate molecular studies accompanied with morphological studies based on more extensive sampling (museum collections and fresh specimens).

Along with the genetic and statistical analyses, this work was aimed to test the applicability of such an advanced and sensitive method as geometric morphometrics on rapa whelks. Our research can be a starting point for a more detailed survey of morphological diversity among multiple morphotypes, eco-forms and forms of *R. venosa* inhabiting the Black Sea.

One of those morphs named "dwarf" is of particular interest as it has a size abnormality: a sexually mature mollusk is 4 times smaller in size than a regular adult one (Bondarev, 2010: fig. 5, A). These individuals are rare to find and rather unique in samples, but are the key to understand the ecology of the rapa whelk: the mature "dwarf" can maintain juvenile feeding strategy — drilling through the shell of its prey (Kingsley-Smith et al., 2003) — while large adults tend to open bivalve mollusks (Chukhchin, 1970; Savini et al., 2002, 2006; Bondarev, 2010). It is believed that, due to having this feeding strategy, rapa dwarfs can switch to another ecological niche, which may result in their genetic isolation (Bondarev, 2010). Similarly, by keeping this feeding strategy throughout a life it can impact on the shell shape in general; this hypothesis of the shape phenotypic plasticity of regular and dwarf forms will need specifically designed studies to be properly tested.

It is also reasonable to expand a study for detailed examination of *R. venosa* shape changes towards ecological abiotic factors (salinity, temperature, illumination, soil, depth); this could be done using *Partial least squares* analysis (Rohlf, Corti, 2000; Fruciano et al., 2011) or *Rarefaction* analysis of morphospace volumes (Foote, 1992; McClain et al., 2004). Similarly, to explore the contribution of heredity into the shell shape it is possible to perform through the *Mantel test* (Liu et al., 1996; Lynch, Walsh, 1998; Klingenberg, Leamy, 2001) but this requires a fairly large array of data.

In general, the methodology used in this paper (landmarks and photographing equipment) can be used for a future morphometric study of *R. venosa*. The applied geometric morphometric approach appeared to be a useful tool to visualize the morphological heterogeneity of the population of Odesa Bay in general, as well as to test and evaluate it statistically.

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