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FUNCTIONAL MORPHOLOGY AND MORPHOLOGICAL VARIABILITY OF THE OPERCULUM OF *RAPANA VENOSA* (GASTROPODA, MURICIDAE)

 I. P. Bondarev

A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS, Sevastopol, Russian Federation
E-mail: *igor.p.bondarev@gmail.com*

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The gastropod *Rapana venosa* has spread from the Western Pacific to the Black Sea, Mediterranean Sea, and coastal areas on both sides of the Atlantic Ocean largely due to its ecological and morphological plasticity. Numerous works have been devoted to the study of the variability of the rapa whelk shell. The functional morphology and morphological variability of the *R. venosa* operculum have been insufficiently studied, and the description of this exosomatic organ is given only schematically. Based on the analysis of 190 *R. venosa* specimens sampled in two areas of the Black Sea, detailed description is given, and trends in the morphological variability of the operculum are shown depending on the specimen age and size. The characteristics determining the normal and aberrant development of the operculum are evaluated. It is shown for the first time that *R. venosa* has regenerative capabilities, up to the restoration of the lost operculum, and morphogenetic adaptive potential. A manifestation of this potential is the formation of a hypertrophied large operculum, with the shape that is not characteristic of any other Muricidae species and gastropods in general. Apparently, the abnormal size and shape of the operculum are a defensive response to pressure from predators, especially crabs. The previously unknown ability to regenerate the operculum broadens the understanding of the physiological capabilities of the rapa whelk. The phenomenon of operculum formation with a unique shape for gastropods is another manifestation of morphological plasticity, which made *R. venosa* one of the most successful invasive species in the modern marine environment.

Keywords: variability, operculum, morphology, regeneration, *Rapana venosa*

The Western Pacific predatory gastropod *Rapana venosa* (Valenciennes, 1846), being transferred by ships, entered the Black Sea in the early 1940s and successfully adapted there [Bondarev, 2010, 2014; Chukhchin, 1961, 1984; Drapkin, 1953]. Then, this species spread its expansion to the Mediterranean Sea and the Atlantic Ocean waters off the coast of Europe and North and South America [Alien Species Alert, 2004; Bondarev, 2010; Xue et al., 2018].

The success of colonization of a wide range by the rapa whelk was due to the features of its biology: high fertility, development of eggs in durable capsules, the possibility of a long stay in the planktonic phase, and tolerance to abiotic environmental factors [Alien Species Alert, 2004; Chukhchin, 1984; Drapkin, 1953]. Successful adaptation to new habitat conditions and to their changes is largely determined both by ecological and morphological plasticity of *R. venosa* [Bondarev, 2010, 2013, 2015, 2016; Chukhchin, 1961; Kosyan, 2013]. The functional morphology and morphological variability of the species shell are most studied [Bondarev, 2010, 2013, 2016; Chukhchin, 1961, 1970; Kosyan, 2013]. Much less attention is paid to the analysis of the operculum. The monograph devoted to the functional morphology of the rapa whelk [Chukhchin, 1970] contains just a mention of the presence of a horny operculum, and the figure shows its position on a mollusc foot. "The Guide to Fauna of the Black Sea and the Sea of Azov" [Golikov et al., 1972] provides a brief description of the operculum for the genus *Rapana* Schumacher, 1817; however, the operculum is not described in the diagnosis of the species *R. venosa*. The features of the rapa whelk operculum morphology are discussed in several publications in terms of the possibility of determining the age of individuals by growth lines ("rings") [Choi, Ryu, 2009; Chukhchin, 1961; Kosyan, Antipushkina, 2011]. A detailed analysis in this field was carried out for the banded dye-murex *Hexaplex trunculus* (Linnaeus, 1758) [Vasconcelos et al., 2012]. The variability and functionality of the operculum under different ecological conditions were investigated in the Atlantic dogwinkle *Nucella lapillus* (Linnaeus, 1758). Based on the studies, it was concluded that *N. lapillus* operculum plays a more significant role in defense against predators, especially crabs, than in defense against desiccation on the littoral [Keppens et al., 2008].

R. venosa operculum also has an important protective function, but its functional morphology and variability have not been well investigated and have not been described. The aim of this study is to fill the gap based on the analysis of material that allows to trace age-related changes, sex-driven differences, and anomalies in the operculum development under the effect of external factors.

MATERIAL AND METHODS

The material was sampled in summer and autumn, 28.06.2020–28.11.2020, using freediving equipment at depths of 1.0–6.0 m in the Donuzlav Bay (the Northwestern Crimea) and in the bays of Sevastopol (the Southwestern Crimea) of the Black Sea (Fig. 1). In the Donuzlav Bay, 49 specimens were sampled (14 females and 35 males), and in Sevastopol bays, 145 specimens (10 juveniles, 48 females, and 87 males). *R. venosa* sample for statistical analysis of the operculum characteristics consists of 190 specimens (10 juveniles, 62 females, and 118 males), with a shell height from 10.4 to 135.0 mm and age from 5–6 months to 15 years.

Fig. 1. Schematic map of sampling areas: 1, the Donuzlav Bay; 2, Sevastopol bays

The main morphological parameters of *R. venosa* shell and operculum are shown in Fig. 2A and 3A: Hs, shell height; Ds, width or maximum diameter of the last whorl of the shell; Ha, aperture height; Da, aperture width; Ho, operculum height; and Do, operculum width. The sex of individuals (F, female; M, male; and J, juveniles) was determined by the presence/absence of a penis in males/females, respectively, and the color of the gonads (Fig. 2B); the age was determined by spawning marks [Bondarev, 2010, 2015; Chukhchin, 1961, 1970; Kosyan, Antipushkina, 2011].

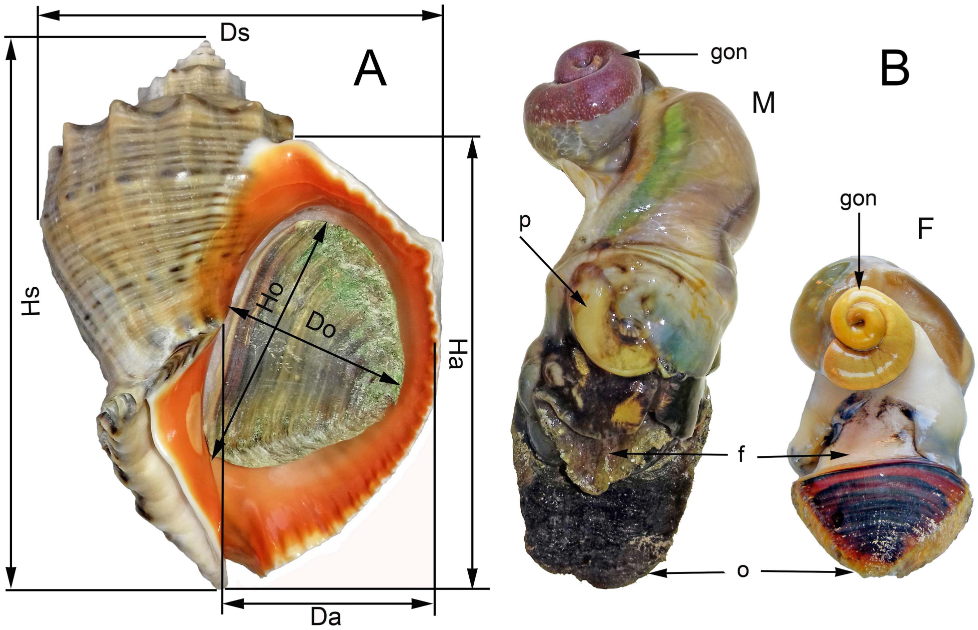

Fig. 2. A, main morphological parameters of *Rapana venosa* shell and operculum (in parentheses, there are the dimensions of the pictured specimen, male, 3 years old): Hs, shell height (108.8 mm); Ds, width or maximum diameter of the last whorl (77 mm); Ha, aperture height (91 mm); Da, aperture width (39 mm); Ho, operculum height (50.3 mm); Do, operculum width (33.8 mm). B, *R. venosa* soft body with operculum (o): M, male, 12 years old, ventral view (p, penis; f, foot; gon, gonad); F, female, 3 years old, dorsal view

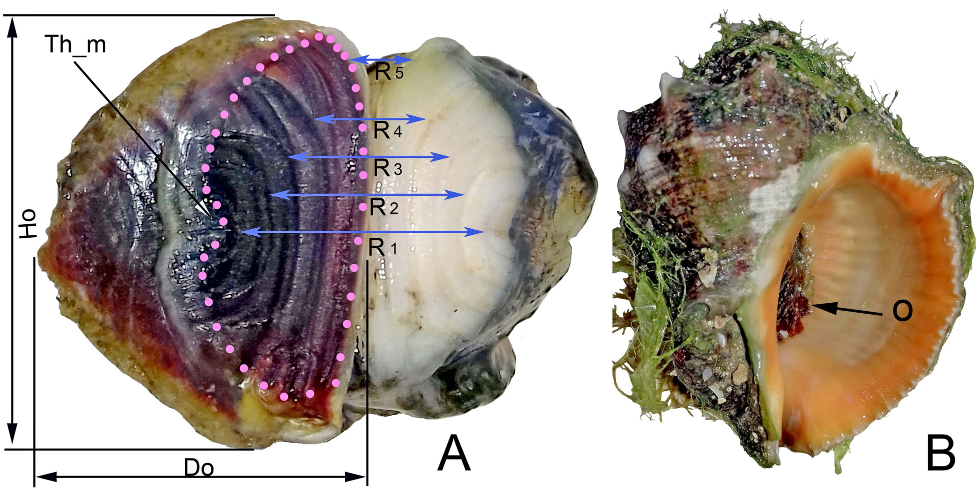

Fig. 3. A, the inner side of *Rapana venosa* operculum (Ho, 28.5 mm; Do, 23 mm) with 5 "rings" (shown by arrows R1–R5) and the imprint of its attachment site (light-colored surface) on the dorsal side of the foot. The arrow indicates the point of measurement of the operculum thickness (Th_m); the outline of light (pink) dots shows the area of the operculum attachment. B, *R. venosa* specimen (M; 4 years old; Hs, 68 mm; Ds, 48 mm) with the operculum (the parameters are given above, see A) retracted inside the aperture

The linear dimensions of shells and opercula were determined with a caliper with an accuracy of 0.1 mm. The operculum thickness (Tho) was measured with a caliper to the nearest 0.01 mm in the location shown by an arrow (Th_m) in Fig. 3A. To study Tho variability, we used 60 rapa whelk specimens (10 juveniles, 20 females, and 30 males) representing the entire size and age range of the general sample and reflecting the structure of the modern Black Sea metapopulation of *R. venosa*, where the proportion of males usually exceeds the proportion of females [Bondarev, 2010, 2014, 2016].

Morphometric characteristics of opercula regenerated after predator damage were not included in the data analysis.

Graphs were plotted and raw data were statistically processed applying standard algorithms of parametric and rank analysis with the use of SigmaPlot for Windows [2023] and MS Office Excel (v10).

RESULTS

R. venosa operculum consisting of a horn-like substance is located on the dorsal side of the mollusc massive foot (Figs 2B, 3A). The operculum is attached to the rapa whelk foot not by its entire inner surface, but only by its part (Fig. 3A). The operculum is the thickest outside the attachment site, where horn-like substance layering is maximum (shown by an arrow in Fig. 3A). The thickness naturally increases with mollusc shell size (Hs) and age (Fig. 4).

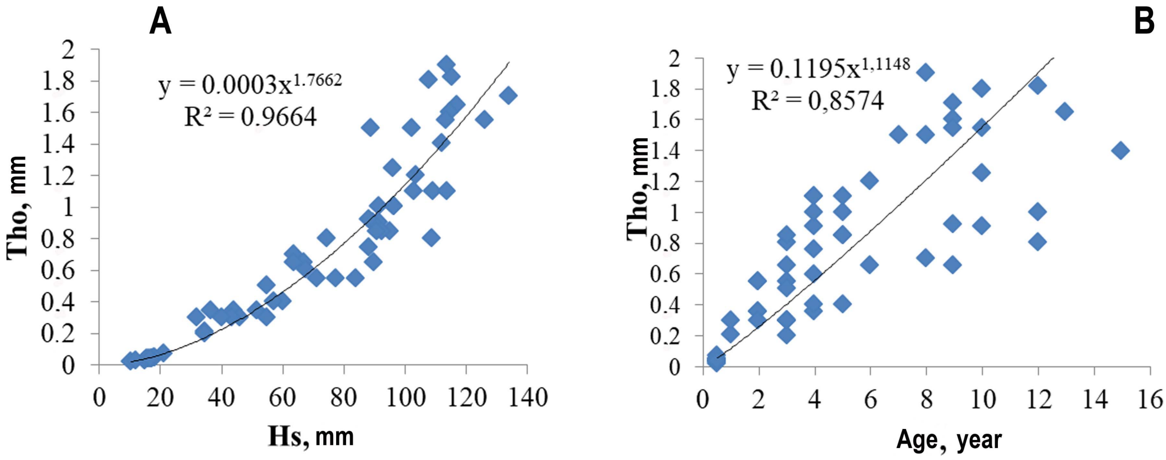

Fig. 4. Graphs of the dependence of *Rapana venosa* operculum thickness: A, on the shell height (Hs); B, on the age of the mollusc

The dependence of *R. venosa* operculum thickness on shell height is well approximated by a power function ($R^2 = 0.9664$) (Fig. 4A). The plot of the dependence on aperture height (no plot is shown) looks similar, but the coefficient of determination is slightly lower ($R^2 = 0.9627$). The operculum thickness correlates well with age ($R^2 = 0.8574$), but there are pronounced individual deviations (Fig. 4B).

In our sample, Tho values varied from 0.02 mm in a juvenile individual with a size (Hs) of 10.4 mm to 1.9 mm in an 8-year-old male with a shell height (Hs) of 114.0 mm. The operculum thickness of juvenile *R. venosa* about 5–6 months old with Hs ranging from 10.4 to 21.3 mm was 0.02–0.07 mm. The largest specimen in our sample (Hs of 135 mm), a 12-year-old male, had Tho of 1.62 mm. The mean thickness and the range of variation in the operculum thickness in females are lower than in males (Table 1).

Table 1. Indicators of *Rapana venosa* operculum thickness (Tho) by sex and age groups (*N*, number of individuals; F, females; M, males; J, juveniles; Hs, shell height, mm; *M*, mean values; σ, standard deviation)

Sex	*N*	Age, years (min–max)	Hs, mm (min–max)	Tho			
				min	max	*M*	σ
J	10	0.5	10.4–21.3	0.02	0.07	0.04	0.014
F	20	1–15	34.6–126.0	0.2	1.55	0.76	0.46
M	30	1–12	36.8–135.0	0.3	1.90	0.97	0.50
J + F + M	60	0.5–15	10.4–135.0	0.02	1.90	0.74	0.55

Toward the periphery, the operculum decreases in terms of its thickness to a thin flexible film, which is minimum in the zone of growth on the inner edge, thinner than 0.01 mm. The flexibility and smooth surface of the peripheral zone ensure a tight adhesion of the operculum edges to the inner surface of the shell aperture. Moreover, such a structure of the operculum allows the mollusc to retract it deep inside the shell (Fig. 3B) and more effectively block the access of predators to the soft body.

The outer surface of the operculum is streaked with growth lines of varying relief [Figs 2A, B (F), 5A, D, 6A].

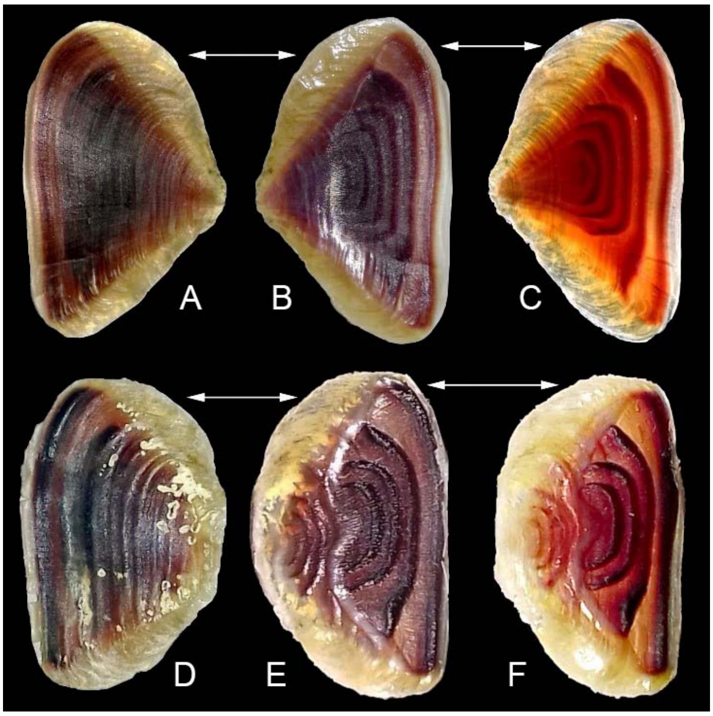

Fig. 5. A–F, opercula of two different-aged individuals of *Rapana venosa*. A–C, 2+ years; Hs, 64.2 mm; Ho, 27 mm; Do, 16.5 mm; number of "rings" (RN), 6. D–F, 4 years; Hs, 36.8 mm; Ho, 15.2 mm; Do, 9.3 mm; RN, 4. A, D, outer surface; B, C, E, F, inner surface (B, E, in reflected light; C, F, in transmitted light)

On the inner side of the operculum, the attachment site has a relief of concentric horseshoe-shaped ridges mirrored on the corresponding section of the dorsal side of the mollusc foot (Fig. 3A). The number of these ridges, commonly referred to as "rings" in the literature [Choi, Ryu, 2009; Chukhchin, 1961; Kosyan, Antipushkina, 2011], increases with age as the mollusc grows, as shown in Table 2.

Table 2. The number of "rings" (RN) on the inner side of *Rapana venosa* operculum for individuals of different age (years) and size (Hs, mm) (*N*, the number of individuals in the sample)

RN	2	3	4	5	6	7	8	9	10	11	12
Age	0.5	0.5	2–4	1–8	2–9	3–12	4–9	10–12	8–13	9–15	8–12
Hs	10.4–16.4	16.8–21.3	36.8–60.8	35.2–96.5	44–114	64–109.5	88.2–103	74.5–91.5	63.3–117.3	112.2–126	91.5–135
N	5	5	3	85	47	23	3	3	9	3	4

As follows from Table 2, the operculum of juvenile specimens has 2–3 "rings." Most (82%) of examined *R. venosa* have 5 to 7 "rings" on the operculum; out of them, 55% have 5 "rings." The age of those individuals varies from 1 to 12 years, and the size (Hs) ranges from 35.2 to 114 mm. The highest number of "rings" (8–12) is typical for large old individuals (Table 2). In molluscs up to and including 5 years, the "rings" on the inner side of the operculum usually differ quite clearly (Figs 3A, 5B, C, E, F). The "rings" are distinctly visible in transmitted light due to thickening relative to the base surface of the operculum (Fig. 5C, F). On the operculum of older individuals, the lines of "rings" are more often "intertwined" (Fig. 6B); sometimes, those are indistinguishable, and less often those are clearly distinguishable (Fig. 6C).

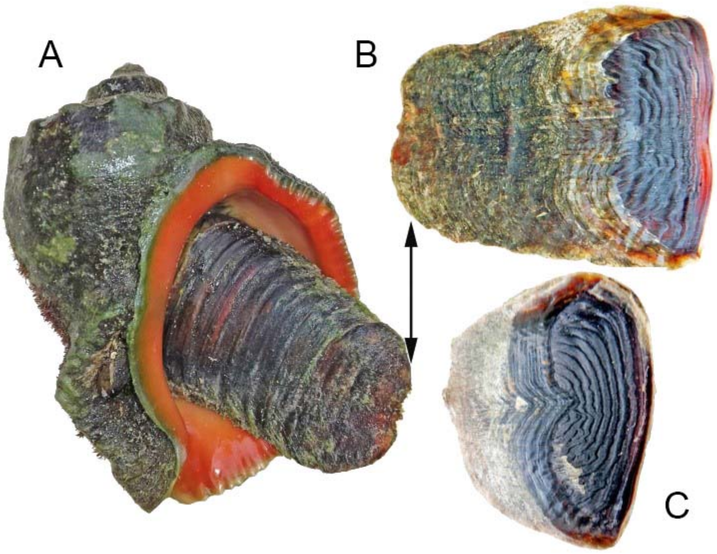

Fig. 6. A, *Rapana venosa*; male; 12 years old; Hs, 91.5 mm; with an abnormally wide operculum. B, its operculum (Ho, 45 mm; Do, 50.2 mm; RN, 12), inner view. C, operculum (Ho 62.5 mm; Do, 55.5 mm; RN, 10) of 12-year-old male *R. venosa* (Hs, 115.7 mm), inner view

Initially, the operculum of juvenile *R. venosa* has the shape of a triangle with rounded corners; this shape is typical for individuals up to 2–3 years old (Figs 2A, 5A–C). The long (inner) side of the triangle, oriented along the shell columella, is the growth zone of the operculum, and its nucleus is located on the opposite apex of the outer edge. With increasing age of the mollusc, the apexes of the corners become more and more rounded (Fig. 5D–F), and the operculum shape changes from subtriangular to irregularly oval, corresponding to the aperture shape. Such a change occurs due to abrasion of the nucleus area and a gradual decrease in the growth rate of the rapa whelk and its operculum as the mollusc becomes older.

The operculum height and width increase as *R. venosa* shell grows. The operculum height (Ho) correlates better with the aperture height (Ha) (Fig. 7A) than with the shell height, where the coefficient of determination is slightly lower ($R^2 = 0.9764$) (no plot is shown). The operculum width (Do) is more closely ($R^2 = 0.9497$) related to the width of the last whorl of the shell (Ds) (Fig. 7B) than to the aperture width (Da) ($R^2 = 0.9199$) (no plot is shown).

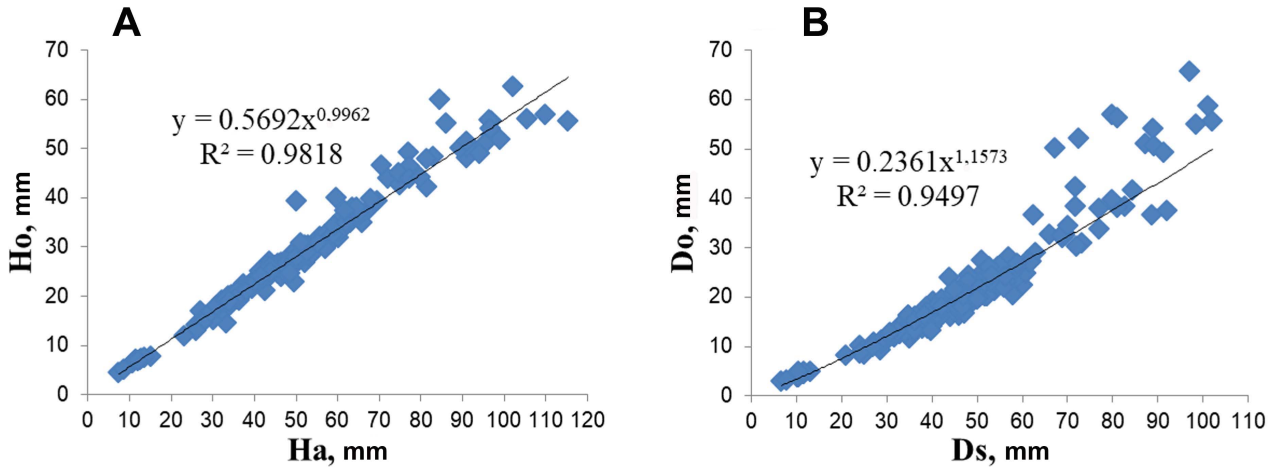

Fig. 7. Graphs of correspondence between the size of the operculum and the size of *Rapana venosa* shell: A, between the operculum height (Ho) and the aperture height (Ha); B, between the operculum width (Do) and the diameter of the last whorl (Ds)

At a high level of correspondence between the parameters of the operculum and the size of *R. venosa* shell, there are individual and repeated deviations. The highest Ho value (62.5 mm) was noted in a specimen with Hs of 115.5 mm, Ha of 102.0 mm, Ds of 102.0 mm, Da of 57.8 mm, and Do of 55.5 mm. The largest mollusc in our sample (with Hs of 135.0 mm, Ha of 115.2 mm, and Ds of 101.0 mm) has Ho of 56.8 mm, but the highest Do, 58.6 mm. These and several other individuals exhibited the phenomenon of a hypertrophied wide operculum (Figs 6A, B, 8A–C, 10D). This anomaly was registered only in male *R. venosa* and is typical for older individuals from the Donuzlav Bay, while in other areas, it is rare and much less pronounced (Fig. 8C).

The proportions of the operculum and the phenomenon of its abnormal width can be quantitatively assessed by the ratio of the parameters Do and Ho (Table 3).

Table 3. Indicators of the width-to-height ratio of *Rapana venosa* operculum (Do / Ho) of the areas of the Donuzlav Bay (DB) and Sevastopol bays (SB) and the entire sample (DB + SB) by sex groups (*N*, number of individuals; F, females; M, males; J, juveniles; *M*, mean values; *σ*, standard deviation)

Sex	Area											
	The Donuzlav Bay (DB)				Sevastopol bays (SB)				DB + SB			
	Min–max	*N*	*M*	*σ*	Min–max	*N*	*M*	*σ*	Min–max	*N*	*M*	*σ*
J	–	–	–	–	0.54–0.7	10	0.61	0.04	0.54–0.7	10	0.61	0.04
F	0.66–0.81	14	0.71	0.05	0.48–0.76	48	0.67	0.06	0.48–0.81	62	0.68	0.055
M	0.63–1.13	31	0.81	0.14	0.53–0.9	87	0.72	0.07	0.53–1.13	118	0.74	0.07
F + M	0.63–1.13	45	0.77	0.14	0.48–0.9	135	0.70	0.07	0.48–1.13	180	0.72	0.09
J + F + M	0.63–1.13	45	0.77	0.14	0.48–0.9	145	0.69	0.07	0.48–1.13	190	0.71	0.09

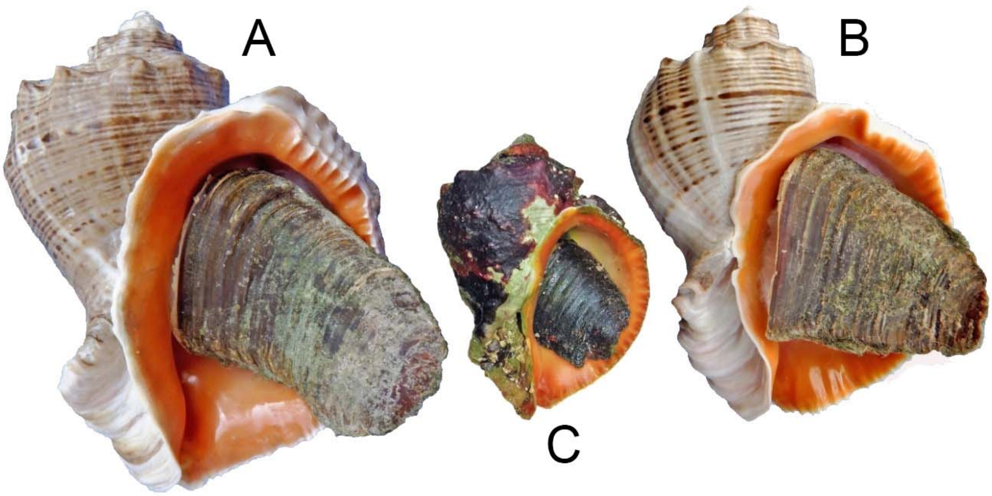

Fig. 8. Specimens of *Rapana venosa* males with an abnormally wide operculum. A, B, shells from the Donuzlav Bay; 10 years old. A: Hs, 107.8 mm; Ho, 48 mm; Do, 57 mm. B, Hs, 96.3 mm; Ho, 46 mm; Do, 52.2 mm. C, living specimen from the Solenaya Bay (Sevastopol); 8 years old; Hs, 64.5 mm; Ho, 26.5 mm; Do, 23.8 mm

The highest Do / Ho value, 1.19 (57.0 / 48.0 mm), was recorded in a 9-year-old male from the Donuzlav Bay with Hs of 107.8 mm, Ha of 91.0 mm, Ds of 80.1 mm, and Da of 42.0 mm (Fig. 8A). In Sevastopol bays, Do / Ho does not exceed 0.9 (23.8 / 26.5 mm) (Table 3), as in an 8-year-old male with Hs of 64.5 mm (Fig. 8B). In Sevastopol bays, the morphological parameters of *R. venosa* shells are characteristic of the Crimean coast; the operculum has characteristic shape and proportions as well. Do / Ho value of 0.7 for the rapa whelk in this area (Table 3) can be considered mean for the Crimea. *R. venosa* individuals from the Donuzlav Bay up to 5 years old have similar mean Do / Ho values as well. An example is Do / Ho of 0.67 (33.8 / 50.3 mm) of a fairly large (Hs of 108.8 mm) 3-year-old male *R. venosa* (Fig. 2A).

Do / Ho for females has lower values than for males, and this is especially pronounced in the rapa whelk from the Donuzlav Bay (Table 3). With age, the "expansion" of *R. venosa* operculum increases, but in older specimens, individual characteristics are more pronounced (Fig. 9A, B). The relationship between Do / Ho and age is stronger for males (R^2 = 0.4325) (Fig. 9B) than for females (R^2 = 0.0365) (no plot is shown).

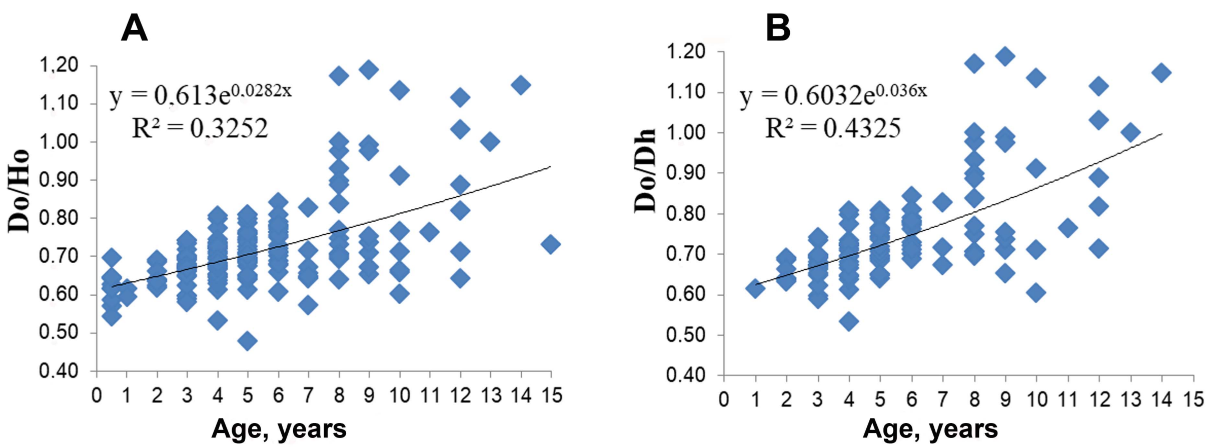

Fig. 9. Plots of Do / Ho dependence of *Rapana venosa* on the age of individuals: A, for the entire sample; B, for males

Based on the data in Table 3, the operculum of the mature rapa whelk with Do / Ho > 0.81 ($M + \sigma$) should be considered abnormally wide. Only 6-year-old males and older specimens have such Do / Ho values, and their proportion is 11.6% (22 ind.) of the total sample, 37.8% (17 ind.) of the sample from the Donuzlav Bay, and 3.5% (5 ind.) of the sample from Sevastopol bays. According to the results of the analysis of variance, Do / Ho distribution in the general sample by sex is not normal (Shapiro–Wilcoxon test, significance level of $P < 0.05$). Therefore, to compare two groups (all males *vs.* all females), Mann–Whitney rank test was applied. The differences between the samples in the median values of Do / Ho are statistically highly significant (at $P < 0.001$) and amount to 0.715 and 0.672 for males and females, respectively, *i. e.*, sex differences in Do / Ho are obvious.

According to the results of morphometric analysis of the operculum of females from two areas (the Donuzlav Bay and Sevastopol bays), the mean values of Do / Ho between groups do not differ statistically significantly (parametric test, $P > 0.05$). At the same time, the normal distribution of variants in the samples is observed (Shapiro–Wilcoxon test is passed, $P = 0.096$), and the variants are quite evenly and compactly grouped around the mean value (Fig. 10A–C). Do / Ho variation indicators, $M \pm \sigma$, are 0.71 ± 0.05 for the Donuzlav Bay and 0.67 ± 0.06 for Sevastopol bays (Table 3).

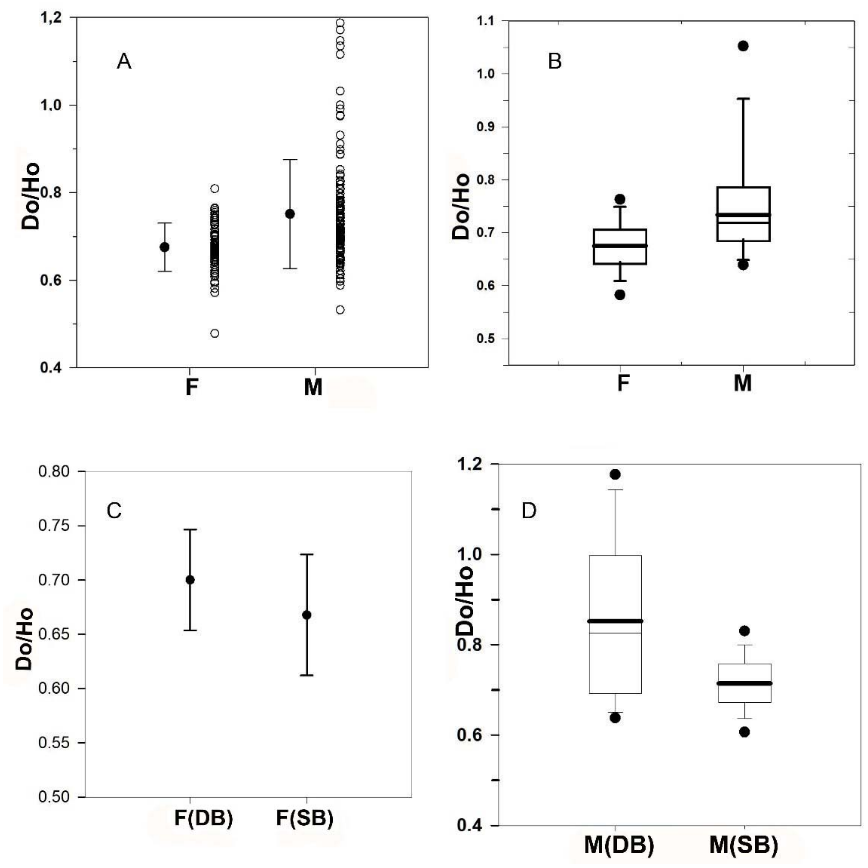

Fig. 10. Graphs of variation characteristics of Do / Ho for *Rapana venosa*: A, B, for females (F) and males (M) of the entire sample; C, for females (F); D, for males (M) of the Donuzlav Bay (DB) and Sevastopol bays (SB). A: vertical rows of "circles" correspond to a row of Do / Ho values. A, C: bold points show mean values (M); "whiskers" show ranges from mean values ($M \pm \sigma$). B, D: the lower and upper boundaries of the boxes correspond to 25% and 75% of the total number of measurements; "whiskers" are the intervals of the dispersion spread; bold points are the percentiles of the total number of measurements [5^{th} (bottom) and 95^{th} (top)]; the bold line inside the boxes is the mean value; the thin line is the median

When analyzing samples of males from two areas, it was established as follows: the median values differ significantly (rank test, $P < 0.001$) due to the special ratio of the operculum morphometric characteristics in individuals from the Donuzlav Bay. Do / Ho median for male *R. venosa* from this bay is 0.826, while for males from Sevastopol bays, the value is 0.710. This allows us to say that morphological deviations in the operculum proportions are inherent only in males. Those are more pronounced in the environmental conditions of the Donuzlav Bay: the upper limit of Do / Ho values (95th percentile) exceeds 1.1 (Table 3; Figs 9, 10D). The results of testing using the quantile method for extreme variants (possible statistical outliers) to belong to the sample showed that all the outliers in Do / Ho in some rapa whelk from the Donuzlav Bay statistically belong to the sample (for $P = 0.05$) and cannot be discarded in variational analysis as random anomalies.

The coefficient of variation (*CV*) of Do / Ho for the sample of males from the Donuzlav Bay was 21.2%, and for the sample from Sevastopol bays, 9.1%. For samples of females, *CV* values were even lower: 6.6% for the Donuzlav Bay and 8.4% for Sevastopol bays. The results obtained allow suggesting that, despite the significant variability in the initial data, we are dealing with a single sample of males from the Donuzlav Bay. For other groups (both females and males), the assertion that in each case this is a single sample is even more confirmed.

The shape of *R. venosa* operculum changes in accordance with changes in the growth rate of the mollusc. This relation can be seen especially clearly in spawning annual marks on the shell (Fig. 11). Those are noticeable on the shell surface cleaned of fouling by their orange-red color emphasizing the relief of an axial ridge, which corresponds to the stage of growth interruption and thickening of the outer edge of the aperture associated with spawning. A decrease in the distance between the marks on the shell and growth lines on the operculum corresponds to a drop in growth rates, and *vice versa*; this reflects age-related changes and (or) food availability. *R. venosa* individuals in Fig. 11 show an abnormally high increase in the shell and operculum growth in the year of sampling against the backdrop of a natural decrease in the rate of shell growth with age. The line of the outer edge from the nucleus to the inner side of the operculum is a conventional graph of the mollusc growth rate. An increase in the slope steepness of this line relative to an imaginary midline connecting the nucleus and the center of the inner edge corresponds to the stage of accelerating growth and increasing the operculum height (Fig. 11B–D).

Morphological changes in *R. venosa* operculum occur as a result of attacks by predators and damage to the protective outer layer of the mollusc (Figs 12, 13). In the Black Sea, such predators are crabs *Carcinus aestuarii* Nardo, 1847 and *Eriphia verrucosa* (Forskål, 1775). Damage remains on the shell as scars, even if it was inflicted at the early stages of the shell formation (Fig. 12A). Interestingly, the rapa whelk operculum, the foot, and the gland forming the operculum can be damaged to varying degree. As a result, a horny "pearl" can be formed on the mollusc foot next to the operculum – a round formation with a convex surface and an oval depression on the inside providing attachment to the foot (Fig. 12B). On the inner side of the operculum, an irregularly shaped "blister" can be formed (Figs 12C, 13B).

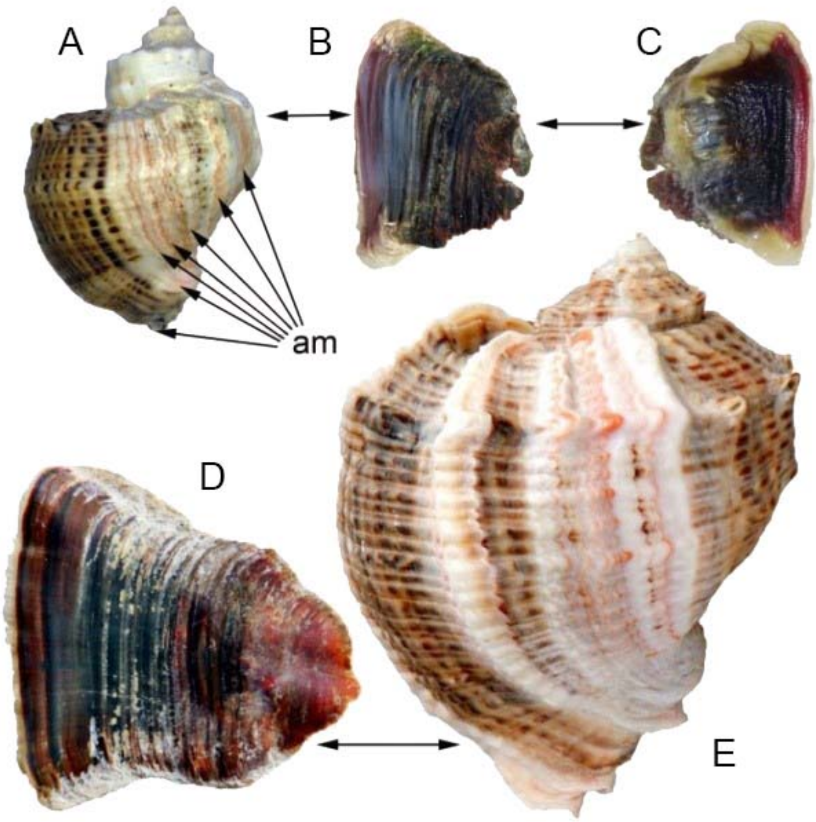

Fig. 11. Shells (A, E) of *Rapana venosa* (M, 9 years old) with annual spawning marks (am) and their opercula (B–D). A, the specimen from the Kazachya Bay (Sevastopol); Hs, 64 mm. B, C, its operculum (Ho, 26.5 mm; Do, 19.5 mm): B, outer side; C, inner side. D, outer side of the operculum (Ho, 55.5 mm; Do, 54.1 mm) of the specimen from the Donuzlav Bay, Hs, 115.5 mm, E

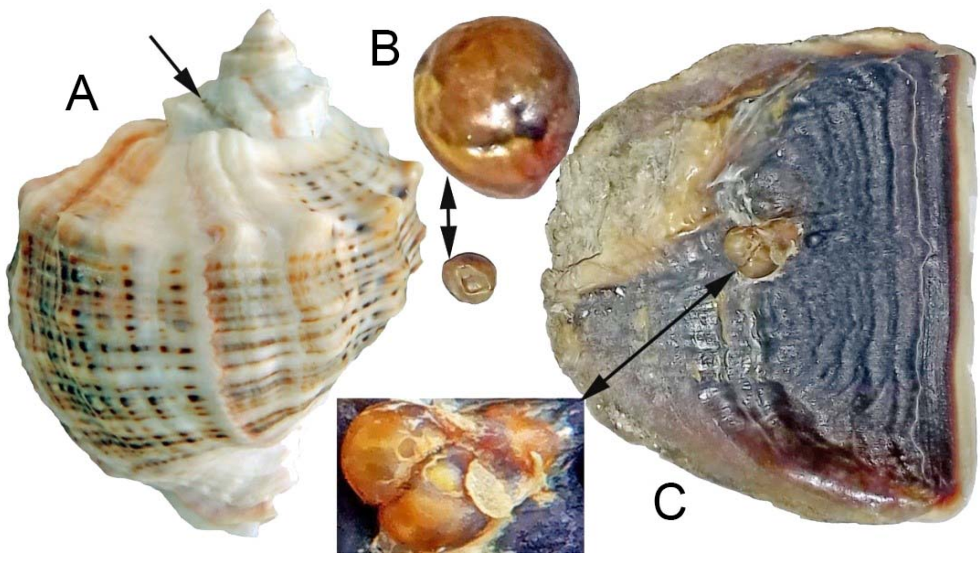

Fig. 12. A, specimen of *Rapana venosa* male; 8 years old; Hs, 88.2 mm; with damage at the apex (shown by the arrow). B, horny "pearl" of 4.2 × 3.3 × 2.9 mm [enlarged view from above; bottom view (below) on the same scale as the operculum]. C, operculum (inner side) of this individual (Ho, 43.1 mm; Do, 32.3 mm) with a "blister" of 5.2 × 3.8 mm

Shell damage that occurs in the first year can manifest itself throughout the life of the mollusc *via* the formation of ray "tracing" on the outer surface of the operculum (Fig. 13A). After rough damage to the shell (Fig. 13C) and almost complete detachment of the operculum from the attachment site on the foot, the rapa whelk can generate a new, duplicate operculum attached to the first one (Fig. 13D, E). If the operculum is severely damaged or completely detached, it can be regenerated in an aberrant form (Fig. 13F, G).

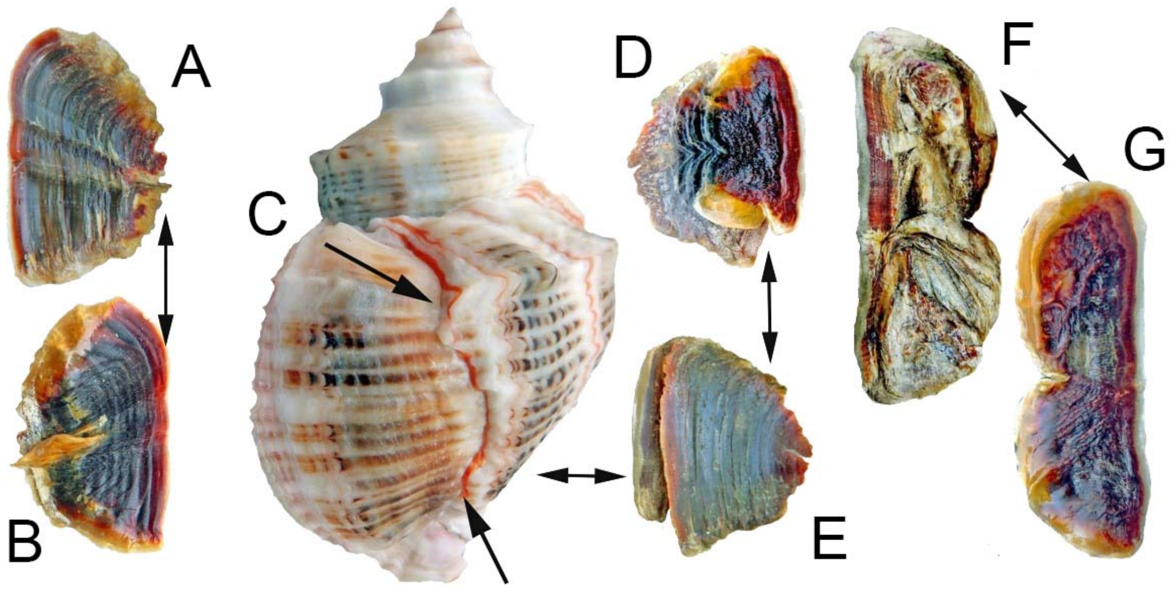

Fig. 13. A, B, operculum of *Rapana venosa* female; 12 years old; Hs, 74.5 mm; Ho, 34.2 mm; Do, 22 mm; with double-ray "tracing" of damage along the outer surface (A) and a "blister" on the inner side (B). C, *R. venosa* individual; M; 7 years old; Hs, 74.3 mm; with rough damage on the last whorl (shown by arrows). D, inner; E, outer side of its regenerated "duplicated" operculum (Ho, 28.6 mm; Do, 21.8 mm). F, outer; G, inner side of the regenerated operculum (Ho, 50 mm; Do, 18.5 mm) of *R. venosa*; male; 9 years old; Hs, 122 mm

DISCUSSION

In general terms, *R. venosa* operculum corresponds to the description for representatives of *Rapana* genus: large, horny, with a nucleus shifted to the outer edge and concentric growth lines [Golikov et al., 1972]. The deeper examination of the morphology of the rapa whelk operculum allows us to more fully assess the functionality and variability of this exosomatic organ.

Flexible and smooth edges of the operculum, which do not grow to the foot, act as a cuff and contribute to its tight adjacency to the inner surface of the aperture; also, those provide the ability to retract the soft body deep inside the shell (Fig. 3). This "option" is extremely important in defense against crabs capable of breaking off significant fragments of the shell, especially at the stage of active growth of the mollusc, when its thickness is insufficient to withstand the efforts of a predator [Bondarev, 2013]. The operculum allows *R. venosa* not only to protect itself from the intrusion of a predator, but also to maintain a special environment of the mantle fluid which ensures the normal functioning of the rapa whelk under adverse conditions [Bondarev, 2013].

The ribbed structure of the inner surface increases the adhesive area of the operculum and the foot and allows it to more effectively resist mechanical stress in various directions, preventing breaking off. Horseshoe-shaped ridges, the number of which rises with the mollusc growth, are not annual rings. V. Chukhchin [1961] was the first to draw attention to this fact; according to him, even juveniles with a shell height of 10–20 mm may have 5 "rings" on the operculum, as well as adult rapa whelk with Hs of 70–80 mm. However, determining *R. venosa* age, especially age of old individuals, by spawning marks is quite laborious (due to the need for cleaning the shell surface of fouling), and sometimes, it is simply impossible (if the shell surface is severely damaged by boring parasites). Moreover, in some habitats, *R. venosa* is characterized by weakly visible spawning marks. These circumstances force researchers to continue attempts to use growth lines on the operculum to determine the mollusc age [Choi, Ryu, 2009]. Based on the analysis of the content of stable oxygen isotopes in shell carbonates, it was established that the obtained data for determining individual age corresponded to the results of calculating the years of life of *R. venosa* specimens using spawning marks. As assumed, single coincidences of the number of "rings" on the operculum and the established age of the mollusc are accidental [Kosyan, Antipushkina, 2011]. Studying the growth of layers in cross sections under a microscope, counting the number of visible "rings" on the operculum, and assessment of their correspondence to annual marks of *H. trunculus* shell also led to the conclusion that it is impossible to use these morphological elements of the operculum to determine the age of the mollusc [Vasconcelos et al., 2012].

Our data show as follows: the number of "rings" on the inner side of *R. venosa* operculum increases with age (from 2 to 12), and the values may coincide, mainly for the age group of 5–7-year-old rapa whelk, since most individuals (82%) have 5–7 "rings" (Table 2). According to our material, 1-year-old molluscs (Table 2) and even younger ones [Chukhchin, 1961] can form the operculum with 5 "rings." The proportion of individuals with 5 "rings" on the operculum is 55% of the sample, but their age is from 1 to 8 years. Thus, obviously, the correspondence between the number of "rings" and the number of years is random, and determination of *R. venosa* age cannot be based on counting the "rings" of the operculum.

Based on a comparison of the time of damage to the shell and regeneration of the operculum, we can say that *R. venosa* is capable of complete restoration of the operculum with 6 "rings" within a year (Fig. 13). As already noted, after rough damage to the shell by a predator (Fig. 13C) and almost complete detachment of the operculum from its attachment site on the foot, the mollusc is able to form a new, duplicate operculum attached to the old one (Fig. 13E, F). A 9-year-old male (Hs of 122 mm), with the shell bearing traces of a severe attack by a predator at 8 years, has the operculum that is not fully restored (Fig. 13D, E). However, taking into account the width (18.5 mm) and thickness (0.95 mm), probably, this is a newly formed operculum, since in males of this age and size from the Donuzlav Bay the operculum is 2–3 times wider and up to 2 times thicker.

The formation of the previously undescribed horny "pearl" and "blisters" on *R. venosa* operculum (Fig. 12B) seems to occur according to the same principle as the well-known mineral and organic influx in bivalves when glands or tissues are irritated and start secreting dense covering material layer by layer.

Already in the first months of shell formation, the proportions of the operculum (Do / Ho) correspond to the shape inherent in sexually mature individuals (Table 3). In the first 3 years, the growth rate of *R. venosa* is maximum, and this determines the subtriangular shape of the operculum (Figs 2A, 5A–C).

An age-related decrease in growth rates and abrasion of the nucleus area result in a change in shape to a more oval one (Fig. 5D–F). A rise in the relative operculum width with increasing age is more pronounced in males (Fig. 9B). The lowest Do / Ho values were recorded in females, 0.48 (in a 5-year-old rapa whelk with Hs of 62.1 mm from Sevastopol bays). The maximum Do / Ho values, 0.76 for females of the same area (Table 3), were also registered in a 5-year-old *R. venosa* with a shell height of 64.5 mm. Against the backdrop of individual growth characteristics, a general tendency for the relative width of the operculum (Do / Ho) to rise as the size of *R. venosa* shell increases can be traced for the entire sample (Fig. 9).

Of particular interest is the phenomenon of an abnormally wide operculum, found in *R. venosa* mainly from the Donuzlav Bay (Figs 6, 8, 11D). Such a shape of the operculum, with the width that can exceed the width of the shell aperture, has not previously been described for any Muricidae representatives or even for any Gastropoda species. The operculum thickness in abnormal individuals (1.0–1.9 mm) is 1.5–2.5 times greater than the mean value for the sample, 0.74 mm (Table 1). Since this anomaly in development was found in males alone, it is logical to assume that it is driven by the rapa whelk sex. Male *R. venosa* have a fairly large penis (Fig. 2B), which can be damaged by predators during copulation. Such an adaptation, as the operculum with a wide and free edge not attached to the body (Fig. 2B), can be an effective additional protection for the penis, even if the soft body is not retracted inside the shell which occurs during copulation. The Mediterranean green crab *C. aestuarii*, the most widespread rapa whelk predator in the Donuzlav Bay, is capable of causing significant damage to the soft body and even to rather thick-walled shell of *R. venosa* (Fig. 13C). Attacks of *C. aestuarii* on the rapa whelk were repeatedly observed by the author during the sampling of material. Apparently, increased pressure from crabs on this mollusc in the Donuzlav Bay is the reason for higher abundance of males with an abnormally wide operculum there (53%) than in Sevastopol bays (5.8%). It takes time for a wide operculum to be formed. Accordingly, individuals with abnormal Do / Ho are 6 years old and more, and the highest values are recorded in specimens of 9 years old and more. A significant proportion (53%) of individuals with an abnormally wide operculum among males from the Donuzlav Bay indicates that this phenomenon is not a random deviation from the norm, but the result of morphogenesis initiated by predators. This is confirmed by the analysis using the quantile method for extreme variants (possible statistical outliers) to belong to the sample. Specifically, it showed that all outliers in Do / Ho belong to the sample of *R. venosa* from the Donuzlav Bay and cannot be discarded as random anomalies (for $P = 0.05$). Despite the significant variability in the data in the sample of males from the Donuzlav Bay ($CV = 21.2\%$), we are dealing with a single sample. For other studied groups of *R. venosa*, *CV* of Do / Ho does not exceed 9.1%; therefore, the assertion that in each case the sample is single is even more confirmed.

According to molecular genetic studies, *R. venosa* in new habitats has an extremely low level of genetic variability compared to that for populations from its native range [Slynko et al., 2020; Xue et al., 2018]. However, with genetic monomorphism in the Black Sea, the rapa whelk demonstrates a wide polymorphism of its shell [Bondarev, 2010, 2015, 2016; Chukhchin, 1961; Slynko et al., 2020] and gonads [Bondarev, 2015]. The occurrence of a previously undescribed variant of the "expanded" operculum shows a new facet of the potential for morphological variability of *R. venosa*.

Conclusion. The operculum of *Rapana venosa* is an exosomatic organ changing its morphology with growth and age and demonstrating both regenerative capabilities and the morphogenetic potential of the species. A striking manifestation of this potential is the formation of a hypertrophied large operculum, with the proportions not being characteristic of any other Muricidae species and Gastropoda in general. The formation of an abnormally wide and thickened operculum in the rapa whelk can be explained by increased pressure from predators. The presence of an abnormal operculum exclusively in male *R. venosa* suggests that this feature is sex-driven and results from the adaptation, possibly promoting protection for the reproductive organ from predators.

The discovery of the ability to regenerate the operculum expands the understanding of the physiological capabilities of the rapa whelk. The phenomenon of the operculum formation with a unique shape for gastropods is another manifestation of morphological plasticity, which made *R. venosa* one of the most successful invasive species in the modern marine environment.

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ФУНКЦИОНАЛЬНАЯ МОРФОЛОГИЯ И МОРФОЛОГИЧЕСКАЯ ИЗМЕНЧИВОСТЬ ОПЕРКУЛУМА *RAPANA VENOSA* (GASTROPODA, MURICIDAE)

И. П. Бондарев

ФГБУН ФИЦ «Институт биологии южных морей имени А. О. Ковалевского РАН», Севастополь, Российская Федерация

E-mail: *igor.p.bondarev@gmail.com*

Брюхоногий моллюск *Rapana venosa* распространился из западной части Тихого океана в Чёрное и Средиземное моря и прибрежные районы по обе стороны Атлантического океана во многом благодаря своей экологической и морфологической пластичности. Исследованию вариабельности раковины рапаны посвящены многочисленные работы. Функциональная морфология и морфологическая изменчивость оперкулума *R. venosa* изучены недостаточно, описание этого экзосоматического органа приводится только схематично. На основе анализа выборки из 190 экз. *R. venosa*, собранных в двух районах Чёрного моря, дано детальное описание и показаны тренды морфологической изменчивости оперкулума в зависимости от возраста и размера особей. Оценены характеристики, определяющие нормальное и аберрантное развитие оперкулума. Впервые показано, что *R. venosa* имеет регенеративные возможности, вплоть до восстановления утраченной крышечки, и морфогенетический адаптационный потенциал оперкулума. Проявлением такого потенциала является формирование гипертрофированно крупной крышечки, форма которой не характерна ни для одного другого вида мурицид и гастропод в целом. Аномальный размер и форма крышечки, вероятно, являются защитной реакцией на давление хищников, прежде всего крабов. Ранее неизвестная способность регенерировать оперкулум расширяет представления о физиологических возможностях рапаны. Феномен формирования крышечки уникальной среди гастропод формы — ещё одно проявление морфологической пластичности, позволившей *R. venosa* занять место среди наиболее успешных видов-вселенцев в современной морской среде.

Ключевые слова: вариабельность, крышечка, морфология, регенерация, *Rapana venosa*