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MORPHOMETRIC ANALYSIS OF SOME POPULATIONS OF LYMNOCARDIID SPECIES (MOLLUSCA: BIVALVIA) FROM RAZELM LAKE COMPLEX (ROMANIA)

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Abstract. In this paper we report the morphometric analysis of some populations of Lymnocardiid species from the lakes Razelm and Goloviţa. We used three measurements ratios to perform a discriminant analysis and a multivariate analysis of variance (MANOVA), in order to compare the species *Hypanis colorata* vs. *Hypanis angusticostata* in the two lakes, *H. colorata* in Razelm vs. Goloviţa, and *H. angusticostata* in Razelm vs. Goloviţa. From this analysis we concluded that the multivariate means of the morphological variables used in this study were highly significantly different (p=3.2e-05) between the two species. Concerning the geographical variability, in both species, the analysis showed no significant difference between the populations in the two investigated lakes. We also determined from a fitting curve analysis, that the growth pattern of both species shows length-height isometry and width-length and width-height negative allometry.

Résumé. Les auteurs présentent l'analyse morphométrique de quelques populations de Lymnocardiidae des lacs Razelm et Goloviţa. Dans cette étude on a mesuré la longueur, l'hauteur et l'épaisseur des valves, les rapports de ces valeurs étant utilisés afin de réaliser l'analyse discriminante et l'analyse multivariée de la variance pour comparer ces populations. Les résultats ont montré l'existence d'une différence statistique significative (p=3.2e-05) entre les moyennes multivariées des populations de *Hypanis colorata*, en comparaison avec celles de l'espèce *Hypanis angusticostata*. Dans le cas de la comparaison des populations conspécifiques des deux espèces provenant des deux lacs étudiés, on n'a constaté aucune variabilité des paramètres morphologiques analysés. On a déterminé aussi le modèle de croissance des deux espèces comme étant isométrique en ce qui regarde la relation longueur- hauteur, respectivement négatif allométrique en ce qui concerne les relations épaisseur-longueur et épaisseur-hauteur.

Key words: *Hypanis colorata, Hypanis angusticostata*, *Monodacna*, isometry, allometry, growth pattern, geographic variability.

INTRODUCTION

The bivalves subfamily Lymnocardiinae (Bivalvia: Cardiidae) includes several fossil genera and two extant genera, *Hypanis* and *Didacna*. In the former genus there are two described subgenera, *Monodacna* and *Adacna*. The subgenus *Monodacna* comprises about 20 living species and/or subspecies distributed in the Black, Azov and Caspian Seas as well as in the connected brackish lakes (Nabozhenko, 2005).

In the Romanian fauna there are five species described in the genus *Hypanis*: *H. fragilis* (Milaschewitsch, 1908), *H. plicata* (Eichwald, 1829), *H. colorata* (Eichwald, 1829), *H. angusticostata* (Borcea, 1926) and *H. pontica* (Eichwald, 1829). The variability occurred within this subfamily is considered an adaptation to the brackish water and/or their living substrate, changes appearing to different direction, either concerning the shell thickness, the ribs or the dentition structure, in the siphon or the pallial sinus elongation (Grossu, 1962). The morphological criteria used in the species identification rise confusions and uncertainties (Borcea, 1926).

In Romania, the subfamily Lymnocardiinae was studied by Grossu (1962, 1973), Tudorancea (1972), Negrea & Negrea (1975) and Sarkany-Kiss (1995). Also, at the international level, the studies concerning the Lymnocardiinae species are scarced. Taxonomic studies based exclusively on morphological features were performed mainly in Ukraine (Munasypova-Motyash, 2006; Yurishinets & Korniushin, 2001; Yurishinets et al., 2002).

In this study we measured the morphological variability of the two species of the genus *Hypanis* in the Razelm-Sinoe Lake Complex, *Hypanis colorata* and *Hypanis angusticostata*.

MATERIAL AND METHODS

Samples were collected from the Romania Lagoons of the Black Sea, the Razelm Golovița Lake Complex. Figure 1 depicts the distribution of the collecting points for the Lymnocardiinae species presented in table 1. Specimens used in this study were collected with a Băcescu dredge.

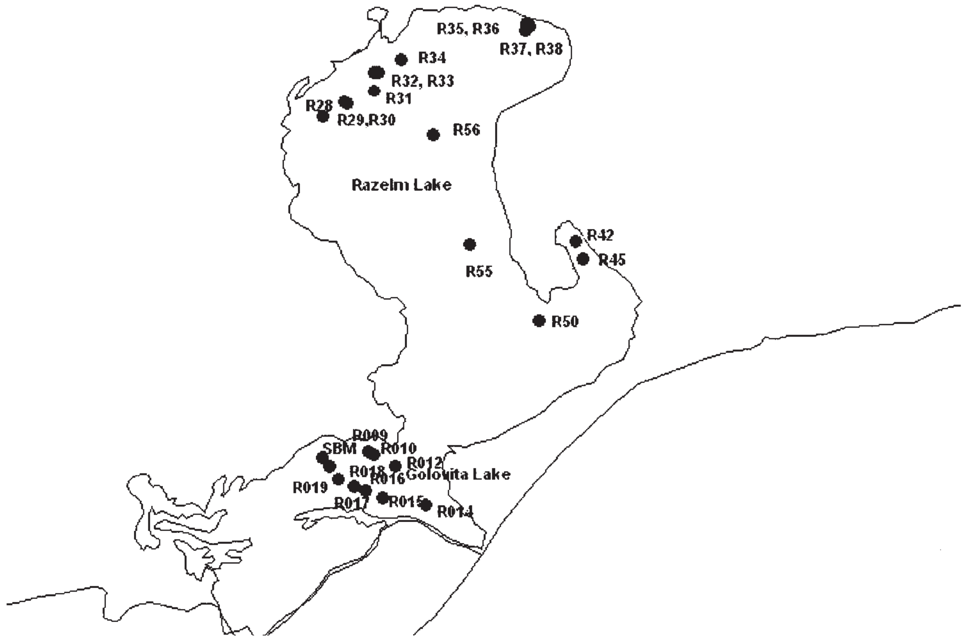

Fig. 1- Distribution map of the collecting points.

Morphological identification of the collected individuals was performed according to criteria such as the shape of the ribs (higher or more flattened), how dense these are, the dentition of the cardinal platform and the thickness of the valves, according to Munasypova-Motyash (2006), Logvinenko & Starobogatov (1968) and Zhadin (1952). The distinguishing of the species *H. colorata* and *H. angusticostata* was made according to the shape of the ribs, with narrow, sharp ribs in *H. angusticostata*, and flattened ribs in *H. colorata*.

The following species/population pairs were analysed: *H. colorata* vs. *H. angusticostata, H. colorata* from Golovița Lake vs. Razelm Lake, and *H. angusticostata* in Golovița Lake vs. Razelm Lake. The following three

Table 1

Collecting points of the analyzed Lymnocardiinae samples.

WAYPOINT	LATITUDE	LONGITUDE	NO OF. INDIV	LOCALITY OF COLLECTION	COLL. DATE
R028	44.95568	28.87551	5	Razelm Lake	July 2009
R029	44.96349	28.89404	2		
R030	44.96283	28.89654	1		
R031	44.98213	28.94826	6		
R032	44.98203	28.94833	2		
R033	44.98206	28.94652	2		
R034	44.98219	28.94505	1		
R036	44.98027	29.09231	2		
R037	44.97949	29.0933	5		
R038	44.97823	29.09411	1		
R039	44.97814	29.09394	5		
R040	44.97553	29.09102	1		
R042	44.85952	29.10623	6		
R045	44.84797	29.11141	1		
R050	44.84942	29.03362	2		
R055	44.97038	28.94425	15		
R056	44.97848	28.97514	7		
R09A	44.74603	28.91976	12	Golovița Lake	August 2009
R010	44.74413	28.92408	1		
R012	44.71823	28.95958	5		
R014	44.69475	28.98316	3		
R015	44.69985	28.94775	13		
R016	44.70453	28.93269	7		
R017	44.70744	28.92326	1		
R018	44.72065	28.90324	2		
R019	44.7264	28.89792	6		
Total			114		

morphometric variables were measured: shell length (SL), the maximum antero-posterior dimension of the shell, shell width (SW), the maximum left-right dimension with both valves appressed and shell height (SH), the maximum dorsal-ventral dimension of the shell measured perpendicular to the length. The shells were measured with a digital calliper, to the nearest 0.1 mm. The measured shell length (SL), shell width (SW) and shell height (SH) were used to compute the SW/SL, SH/SL and SW/SH ratios. The multivariate normality was checked by computing Mardia's multivariate skewness and kurtosis, with tests based on chi-squared (skewness) and normal distributions (kurtosis). A powerful omnibus (overall) test (Doornik and Hansen) was also performed. A MANOVA analysis was performed with the species/location designation as independent variables and the three mentioned ratios as dependent variables. A Discriminant Analysis (DA) was performed to test the efficiency of the selected ratios to predict different group locations. At the same time, we performed the Hotelling's t^2 test for equality of multivariate means between our groups.

We also performed a curve fitting analysis in order to identify the growing pattern of the studied species (allometric growth or isometric growth). For this purpose we analyzed shell length vs. shell height (SL-SH), shell width vs. shell length (SW-SL) and shell width vs. shell height (SW-SH). The growth patterns were considered to be isometric if the slope (a) of the regression line was 1, negative allometric if $a<1$, and positive allometric if $a>1$. All analyzes were performed with the software package PAST (Hammer et al., 2001).

RESULTS

We collected and measured several hundred bivalve specimens belonging to the Lymnocardiinae subfamily. Among the specimens with a length larger than 20 mm (to ensure a correct identification), we identified 79 specimens of *Hypanis colorata* and 35 *Hypanis angusticostata* specimens. The multivariate normality of the samples was checked by Mardia's kurtosis test (p_{normal}=0.89) and with the Doornik and Hansen omnibus test (p_{normal}=0.97). The assumption of homogeneity of variance covariance matrices was tested with Box's M test (p_{equal}=0.07).

H. colorata vs. H. angusticostata

The discriminant analysis shows quite unsatisfactory separation of the two groups (68.42% of the samples were correctly reclassified with the computed discrimination function), while the Hotelling's t^2 test indicates a highly significant difference between them (p_{same}=3.2e-05). The same result was revealed by the MANOVA analysis who rejected the hypothesis of equality of the means for the two species, both in Wilks's lambda test (p_{same}=8.8e-09) and the Pillai trace test (p_{same}=3.8e-09).

H. colorata from Golovița Lake vs. H. colorata from Razelm Lake

We analyzed 46 *H. colorata* specimens from Golovița Lake and 33 specimens from Razelm Lake. Both the discriminant analysis (54.43% of the samples were correctly reclassified) and the Hotelling's t^2 test (p_{same}=0.72) indicates there is no significant difference between the two analyzed populations of *H. colorata*. The same result was revealed by the MANOVA analysis which could not reject the hypothesis of equality of the means for the two species populations, both in Wilks's lambda test (p_{same}=0.21) and the Pillai trace test (p_{same}=0.20).

H. angusticostata from Golovița Lake vs. H. angusticostata from Razelm Lake

We analyzed 26 specimens of *H. angusticostata* from Golovița Lake and 9 specimens from Razelm Lake. As in the other species, both the discriminant analysis (68.57% of the samples were correctly reclassified) and the Hotelling's t^2 test (p_{same}=0.53) indicates that there is no significant difference between the two analyzed populations of *H. angusticostata*. The same result was revealed by the MANOVA analysis who could not reject the hypothesis of equality of the means for the two species populations, both in Wilks's lambda test (p_{same}=0.06) and the Pillai trace test (p_{same}=0.06) (Tab. 2).

Table 2

Discriminant analysis and MANOVA (Multivariate Analysis of Variance).

Discriminant Analysis		
Compared populations	Percent correctly classified	Hotelling's t^2 p (same)
H. colorata vs. *H. angusticostata*	68.42	3.2E-05
H. colorata Goloviţa vs. Razelm	54.43	0.72
H. angusticostata Goloviţa vs. Razelm	68.57	0.53
MANOVA		
Compared populations	Wilk's lambda p (same)	Pillai trace p (same)
H. colorata vs. *H. angusticostata*	8.8E-09	3.8E-09
H. colorata Goloviţa vs. Razelm	0.21	0.20
H. angusticostata Goloviţa vs. Razelm	0.06	0.06

Curve fitting and growth pattern

We used linear regression for fitting the data points to an exponential curve for the two species. This was done simply by fitting a straight line to the logarithms of the values of all three pairs constructed with the measured variables (shell length vs. shell height, shell length vs. shell width and shell width vs. shell length). When analysing shell length vs. shell height (SL-SH), in *H. angusticostata*, the slope (a) was found to be 1.01 (p = 0.94). The same analysis performed in *H. colorata*, revealed a slope (a) of 0.99 (p = 0.83). Both these situation correspond to an isometric growth pattern of the two species. When analysing the shell width vs. shell length (SW-SL) and the shell width vs. the shell height (SW-SH), in both species the growing pattern proved to be positive allometric (Tab. 3).

Table 3

Curve fitting analysis and growth patterns of the two species.

Species	SL-SH isometry	SW-SL allometry	SW-SH allometry
H. colorata	Slope (a):0.99 p(a=1): 0.83	Slope (a):0.79 p(a=1): 1.1E-05	Slope (a):0.78 p(a=1): 9.7E-06
H. angusticostata	Slope (a):1.01 p(a=1): 0.94	Slope (a):0.51 p(a=1): 7.1E-09	Slope (a):0.52 p(a=1): 5.4E-08

DISCUSSIONS

Although subtle, the morphological differences between *H. colorata* and *H. angusticostata* appear to be highly statistically significant when we performed a multivariate analysis of our samples. From a practical point of view, the only character distinguishing the two species is the shape of the shell ribs, with sharp, thin ribs in *H. angusticostata* and more flat ribs in *H. colorata*. This character could be explained as an adaptation to different living substrates of the two species. According to Munasypova-Motyash (2006), *H. colorata* lives on the surface of the substrate, while *H. angusticostata* borrow shallowly in the substrate. Savazzi and Sälgeback (2004) performed a comparative analysis of the shell shape of two cardiid bivalves species, *Cardium* and *Budmania*, and found that the sharp ribs offer strength to the shell under the pressure of the surrounding sediment, while also providing a larger shell area in contact with the sediment, which ensures a better anchoring of the animal in the substrate (in our case, *H. angusticostata*). On the other hand, the flat ribs ensure an even weight distribution preventing sinking for bivalve species living on the surface of substrate (in our case, *H. colorata*).

The growth model of the two species support also the same assertions. The negative SW-SL and SW-SH allometry is higher in *H. angusticostata* (a=0.51-0.52) than in *H. colorata* (a=0.78-0.79). These results indicate that for *H. colorata* the width increase over the height and length increase is superior to that of *H. angusticostata*. According to Eagar (1978), Gaspar et al. (2002) and Hinch & Bailey (1988), bivalve species shells become wider during growth in order to counter involuntary dislodgement by turbulence and currents. These environment effects are much likely to occur in a species living on the substrate (*H. colorata*) than in a species living borrowed in the substrate (*H. angusticostata*).

We also analysed two different populations, one in Golovița Lake, the other in Razelm Lake, for the two species of the genus *Hypanis*. In this case the analysis showed no differentiation between the two pairs of investigated populations. This finding could be explained by the fact that both lakes are part of the same system (the Razelm-Sinoe Lake Complex), and the salinity conditions are quite similar between Razelm and Golovița (0.4-0.6 g/l), according to Brețcan et al. (2009). In conclusion, we analysed some morphological variables in the two species of the subfamily Lymnocardiinae which are present in the Romanian fauna. The analysis showed the species *Hypanis angusticostata* and *Hypanis colorata* are different statistical populations. No differentiation could be observed for the populations of the two species of the genus *Hypanis* in the Lakes Razelm, respectively Golovița. Considering that a clear separation of both taxa on the basis of morphological characteristics is made difficult by a high degree of intra- and interpopulational variability, other characteristics, such as those provided by molecular analysis, should be taken into account for a more comprehensive interpretation of their taxonomic status.

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ANALIZE MORFOMETRICE REALIZATE ASUPRA UNOR POPULAȚII DE LYMNOCARDIINAE (MOLLUSCA: BIVALVIA) DIN COMPLEXUL LACUSTRU RAZELM-SINOE (ROMÂNIA)

REZUMAT

În lucrare autorii prezintă analiza morfometrică a populațiilor a două specii de Lymnocardiidae din lacurile Razelm şi Golovița. În studiu s-a măsurat lungimea, înălțimea şi grosimea valvelor, iar rapoartele acestor valori au fost utilizate pentru a se realiza analiza discriminantă şi analiza multivariată a varianței pentru compararea populațiilor analizate. Rezultatele au arătat existența unei diferențe semnificative statistic (p=3.2e-05) între mediile multivariate ale populațiilor de *Hypanis colorata*, în comparație cu populații ale speciei *Hypanis angusticostata*. În cazul comparării populațiilor conspecifice ale celor două specii, provenind din cele două lacuri studiate, nu s-a constatat nici o variabilitate a parametrilor morfologici analizați. De asemenea, a fost determinat modelul de creştere al celor două specii ca fiind izometric în ceea ce priveşte relația lungime-înălțime, respectiv alometric negativ, în ceea ce priveşte relațiile grosime-lungime şi grosime-înălțime.

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