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Shell biometry characters in species discrimination and classification within the genus *Viviparus* (Gastropoda: Architaenioglossa: Viviparidae)

With 36 Figures and 4 Tables

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Abstract. In the paper the authors describe and illustrate the shells of five viviparid species: *Viviparus contectus* (MILLET, 1813) from the Lake Sarąg (Masurian Lake district, northern Poland) and drainage canals near Kielce (southern Poland); *V. viviparus* (LINNAEUS, 1758) from the Radunia River (Pomeranian Lake district, northern Poland); *V. acerosus* BOURGUIGNAT, 1862 from drainage canals (Zupny kanal) by the Danube River near Calovo (SW Slovakia); *V. ater* (CRISTOFORI et JAN, 1832) from Sirmione, the Lake Garda (North Italy); and *V. hellenicus* (CLESSIN, 1879) from the Lake Trichonis near Agrinio (West Greece). Interspecific differences marked in 14 shell biometry characters are analyzed, for males and females separately and for the sexes combined, by means of descriptive statistics, ANOVA, ANCOVA, PCA, nonlinear MDS, and discriminant analysis. To illustrate phenetic grouping, the UPGMA cluster analysis was performed, also not ultrametric, „phylogenetic" grouping by means of neighbor-joining was computed. The results are compared with the MPR based on soft part morphology, radula, embryonic shells and opercular characters, published earlier by the authors. The results strongly indicate the overlapping variability ranges of the species, the within species (*V. contectus*) interpopulation differences not less pronounced than the interspecies ones, and, in general, shell biometry characters too weak to allow even for species discrimination. The shell biometry was also found completely useless for any phylogenetic inference. The results are noteworthy since all the systematics within the Viviparidae is still based on the shell alone.

Kurzfassung. **Biometrische Merkmale der Schale bei der artlichen Differenzierung und Klassifikation innerhalb der Gattung *Viviparus* (Gastropoda: Architaenioglossa: Viviparidae).** - In dieser Arbeit beschreiben und illustrieren die Verfasser die Schalen von fünf Arten der Viviparidae: *Viviparus contectus* (MILLET, 1813) aus dem Sarąg-See (Masurisches Seengebiet, Nord-Polen) und aus Entwässerungskanälen bei Kielce (Süd-Polen); *V. viviparus* (LINNAEUS, 1758) aus dem Fluß Radunia (Pommersches Seengebiet, Nord-Polen); *V. acerosus* BOURGUIGNAT, 1862 aus Entwässerungskanälen (Kanal Zupny) an der Donau bei Calovo (Südwest-Slowakei); *V. ater* (CRISTOFORI et JAN, 1832) von Sirmione, Garda-See (Nord-Italien); und *V. hellenicus* (CLESSIN, 1879) aus dem Trichonis-See bei Agrinio (West-Griechenland). Interspezifische Unterschiede auf der Grundlage von 14 biometrischen Merkmalen der Schale werden (für männliche und weibliche Tiere getrennt als auch für beide Geschlechter kombiniert) mit Hilfe von Computerprogrammen der deskriptiven und multivariaten Statistik und zur Rekonstruktion der Phylogenese analysiert, darauf basierend werden phänetische Gruppierungen ermittelt und die phylogenetische Stellung der Arten rekonstruiert. Die Ergebnisse werden mit früheren Untersuchungen verglichen, bei denen die phylogenetische Verwandtschaft auf der Basis morphologischer Merkmale des Weichkörpers, der Radula, der embryonalen Schale und des Operculums

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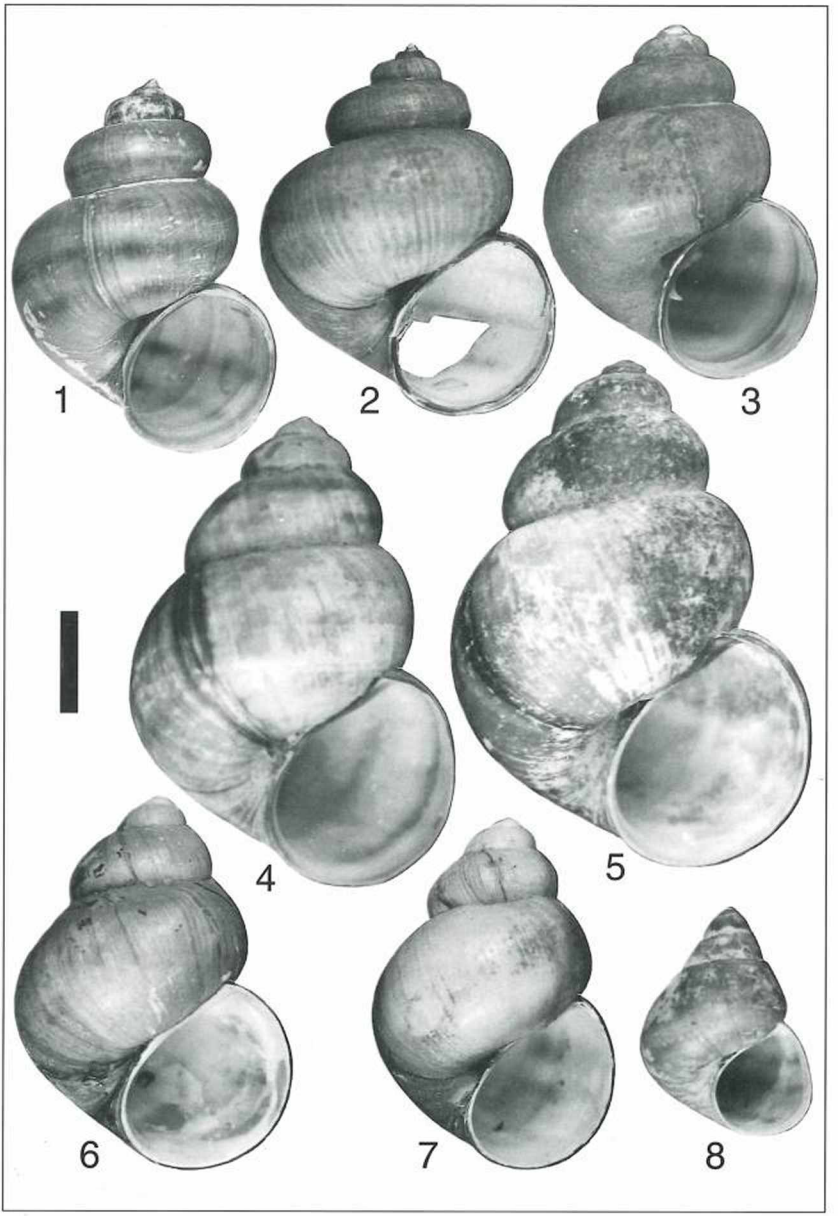
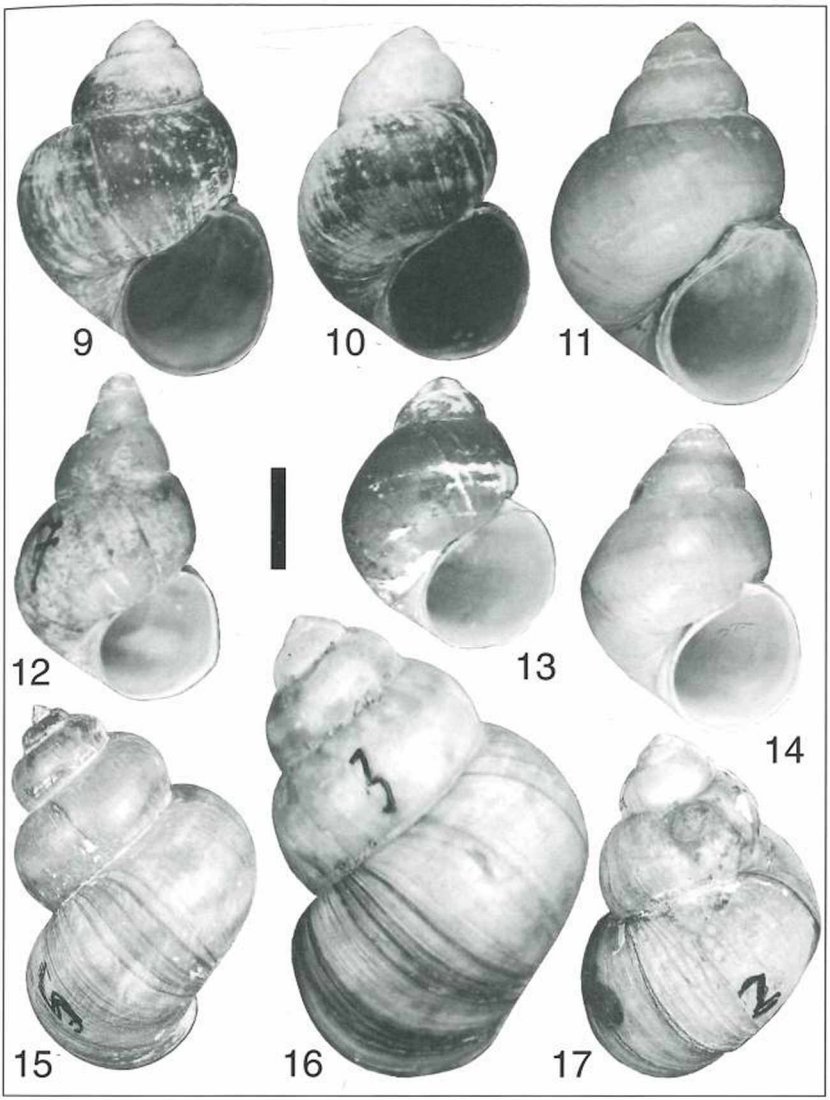


Figs. 1-8: Shells of *Viviparus*: 1-3 - *V. contectus*, 4-5 - *V. acerosus*, 6-7 - *V. viviparus*, 8 - *V. hellenicus*. Bar equals 10 mm.

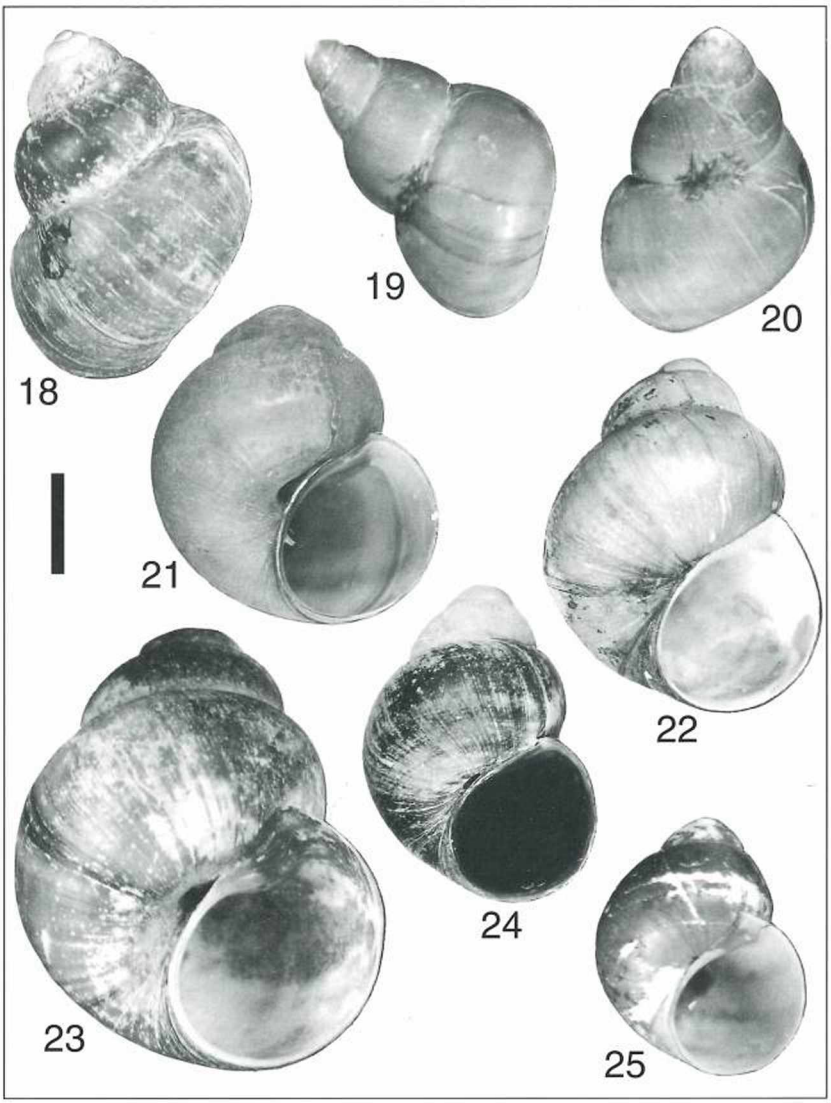
rekonstruiert wurde. Die Ergebnisse zeigen klar, daß sich die Schwankungsbreiten der Variabilität überlagern, daß die intraspezifischen Unterschiede zwischen den Populationen einer Art (*V. contectus*) nicht weniger stark ausgeprägt sind als die interspezifischen und daß die biometrischen Merkmale der Schale im allgemeinen zu schwach ausgebildet sind, um die Trennung der Arten zu ermöglichen. Die Biometrie der Schale ist für phylogenetische Aussagen ganz ungeeignet. Die Resultate sind insofern bemerkenswert, als die Systematik innerhalb der Viviparidae noch auf Schalenmerkmalen basiert.



Figs. 9-17: Shells of *Viviparus*: 9-11 - *V. ater*, 12-14 - *V. hellenicus*, 15 - *V. contectus*, 16 - *V. acerosus*, 17 - *V. viviparus*. Bar equals 10 mm.

Introduction

The Viviparidae, a family of the Architaenioglossa, have a rather uniform shell morphology. The soft part morphology and anatomy have, in practice, not been studied so far at a species level, the diagnostic characters being scarce (FALNIOWSKI, 1989a, 1989b, 1990; FALNIOWSKI et al. 1996, 1996a, b, 1997). Hence, the phylogeny of the group is unclear, and controversies have arisen as to the species distinctness of numerous taxa belonging to the family (e.g. PRASHAD, 1928; PSARIANOS, 1953; EHRMANN, 1956; SCHÜTT, 1962; STAROBOGATOV, 1985; CHERNOGORENKO & STAROBOGATOV, 1987; FECHTER & FALKNER, 1990). The nominal taxa,



Figs. 18-25: Shells of *Viviparus:* 18 - *V. ater,* 19-20 - *V. hellenicus,* 21 - *V. contectus,* 22 - *V. viviparus,* 23 - *V. acerosus,* 24 - *V. ater,* 25 - *V. hellenicus.* Bar equals 10 mm.

especially those of the Balkan viviparids, are numerous (PRASHAD, 1928); PSARIANOS (1953) lists not less than 30 forms described from Greece alone. BUTOT & WELTER-SCHULTES (1994) give references for all the Greek taxa. The picture is yet more complicated due to interspecific hybridization which also occurs within the family (FALNIOWSKI, KOZIK & SZAROWSKA, 1993).

In our studies dealing with viviparid phylogeny (FALNIOWSKI et al., 1996, 1996a, b, 1997) based on the radulae, embryonic shells, opercula and soft part morphology and anatomy of the five species that are the subject of this study, we found slight morphological interspecific differences in all these character sets (FALNIOWSKI et al., 1996 a, b, 1997), coupled with quite large molecular (allozymic) differences among four of the species (FALNIOWSKI et al.,

Table 1: Results of ANOVA - significance of interpopulation differences.

	FEMALES and MALES			FEMALES			MALES		
Character	F	df	p	F	df	p	F	df	p
a	31.27	5 , 130	0.00	19.10	5 , 64	0.00	11.84	5 , 60	0.00
b	47.17	5 , 130	0.00	26.44	5 , 64	0.00	19.50	5 , 60	0.00
c	13.44	5 , 130	0.00	6.86	5 , 64	0.00	6.93	5 , 60	0.00
d	39.97	5 , 130	0.00	21.24	5 , 64	0.00	18.47	5 , 60	0.00
e	32.59	5 , 130	0.00	19.94	5 , 64	0.00	12.32	5 , 60	0.00
f	35.56	5 , 130	0.00	20.17	5 , 64	0.00	14.88	5 , 60	0.00
g	16.21	5 , 130	0.00	7.32	5 , 64	0.00	9.75	5 , 60	0.00
h	19.43	5 , 130	0.00	9.42	5 , 64	0.00	10.18	5 , 60	0.00
i	10.02	5 , 130	0.00	5.63	5 , 64	0.00	4.46	5 , 60	0.00
j	9.84	5 , 130	0.00	5.78	5 , 64	0.00	3.94	5 , 60	0.00
k	6.67	5 , 130	0.00	4.70	5 , 64	0.00	2.28	5 , 60	0.06
l	7.69	5 , 130	0.00	5.87	5 , 64	0.00	2.39	5 , 60	0.05
m	15.87	5 , 130	0.00	6.22	5 , 64	0.00	10.03	5 , 60	0.00
N	16.70	5 , 130	0.00	10.84	5 , 64	0.00	6.33	5 , 60	0.00

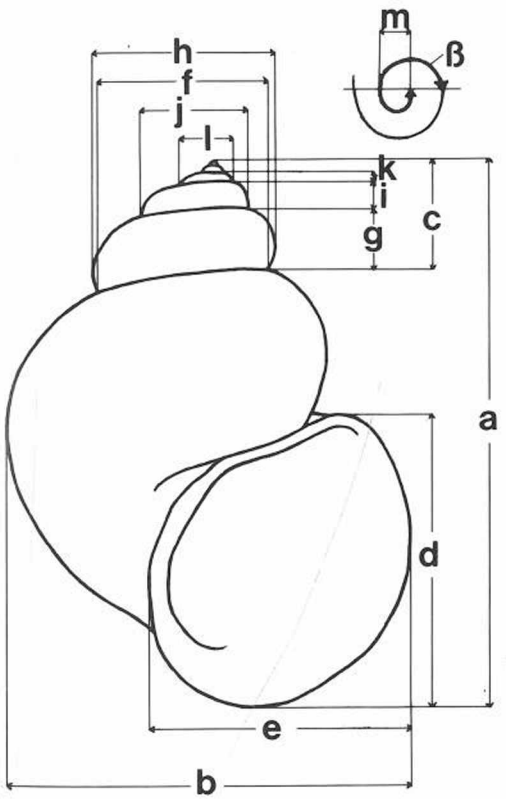


Fig. 26: Scheme of shell measurements: a - shell height, b - shell width, c - spire height, d - mouth height, e - mouth width, f - shell width at the suture between the body whorl and penultimate whorl, g - penultimate whorl height, h - penultimate whorl width, i - ante penultimate whorl height, j - ante penultimate whorl width, k - fourth from last whorl height, l - fourth from last whorl width, m - embryonic shell diameter, ß - first whorl (360°), to show how whorl number (N) was counted.

Table 2: Principal Component Analysis - eigenvalues and percent of variability explained. Sum of eigenvalues = 14.000; Average root: 10.000; Proportions of variance expected using broken-stick model.

	FEMALES				MALES			
PC no	Eigenvalue	Percent of variability explained	Cumulative percent	Expected percent	Eigenvalue	Percent of variability explained	Cumulative percent	Expected percent
1	6.18955	44.2111	44.2111	23.2254	9.33687	66.6919	66.6919	23.2254
2	3.43531	24.5379	68.7490	16.0826	2.48610	17.7578	84.4498	16.0826
3	1.17553	8.3966	77.1456	12.5112	0.65468	4.6763	89.1260	12.5112
4	0.93754	6.6967	83.8423	10.1302	0.53723	3.8373	92.9634	10.1302
5	0.74073	5.2910	89.1332	8.3445	0.28661	2.0472	95.0106	8.3445
6	0.41922	2.9944	92.1277	6.9159	0.21530	1.5379	96.5485	6.9159
7	0.32970	2.3550	94.4827	5.7254	0.20246	1.4461	97.9946	5.7254
8	0.26560	1.8971	96.3798	4.7050	0.12108	0.8649	98.8595	4.7050
9	0.23508	1.6792	98.0589	3.8122	0.06388	0.4562	99.3157	3.8122
10	0.13867	0.9905	99.0495	3.0185	0.03934	0.2810	99.5967	3.0185

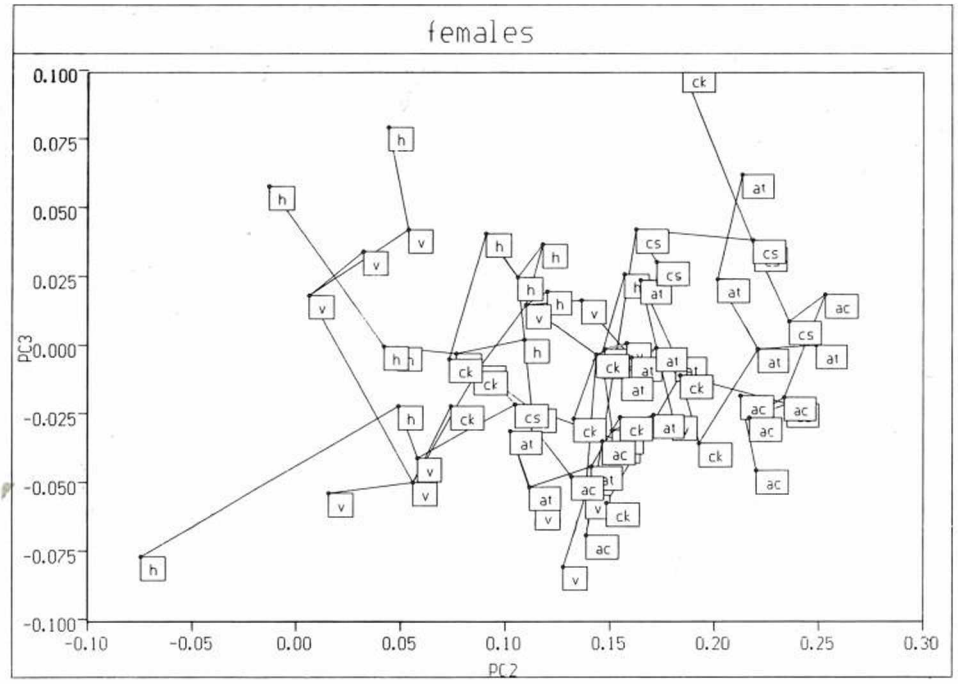


Fig. 27: Principal component analysis for all female specimens (ac - *Viviparus acerosus*, at - *V. ater*, ck - *V. contectus* Kieleckie, cs - *V. contectus* Saręg, h - *V. hellenicus*, v - *V. viviparus*), PC2 vs PC3.

Fig. 28: Principal component analysis for all male specimens (symbols as in Fig. 27), PC2 vs PC3.

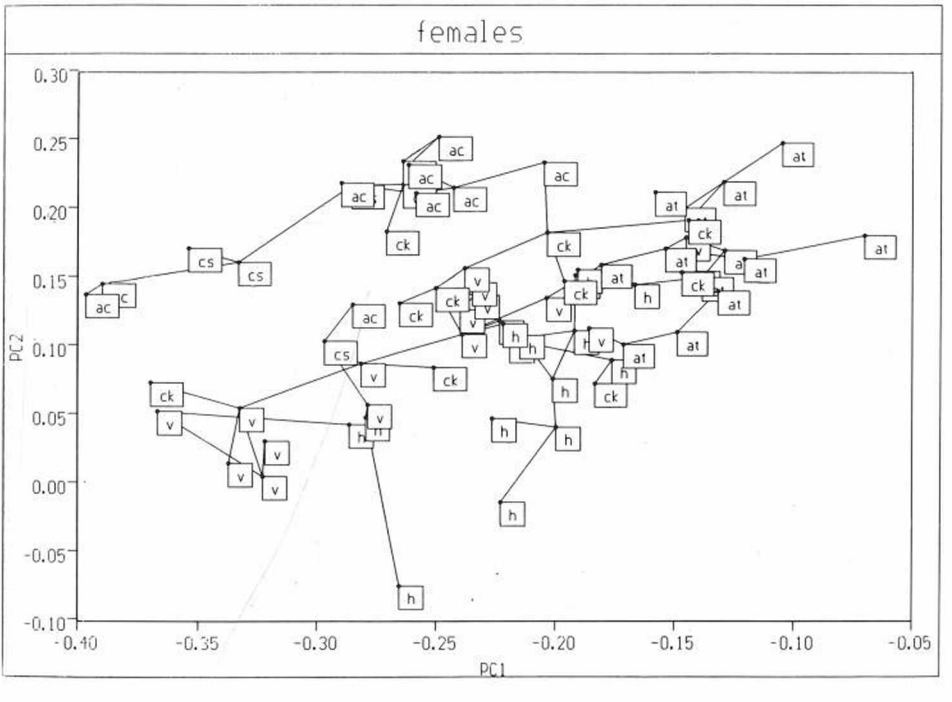


Fig. 29: Principal component analysis for all female specimens (symbols as in Fig. 27), PC1 vs PC2.

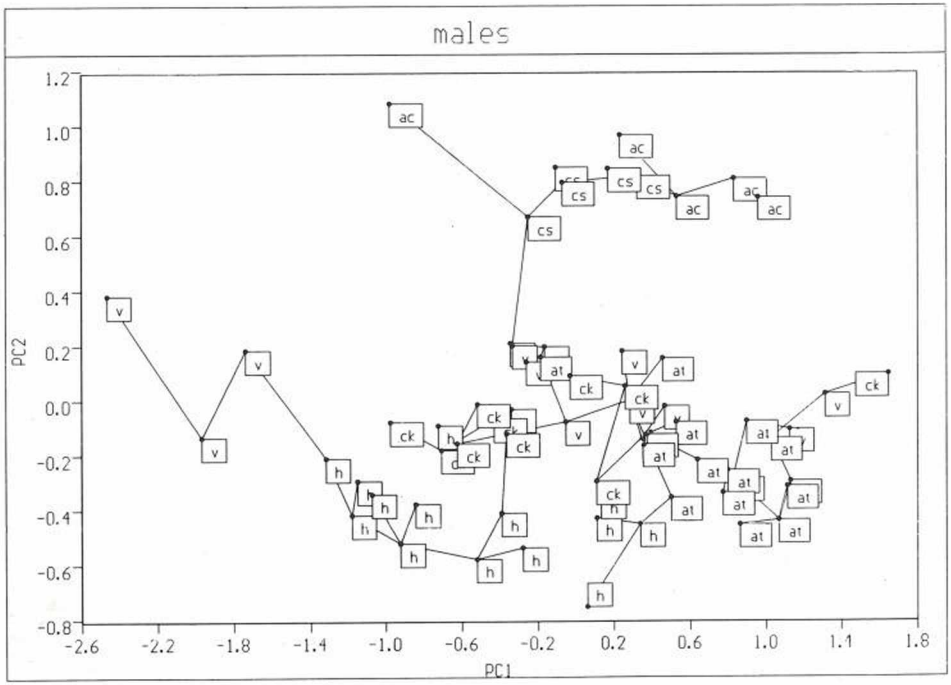


Fig. 30: Principal component analysis for all male specimens (symbols as in Fig. 27), PC1 vs PC2.

1996). The inferred phylogenetic relationships did not much differ between the two character sets (FALNIOWSKI et al., 1996).

In the literature, the descriptions of the taxa as well as opinions concerning their status and interspecies relationships are entirely based on shell characters. Thus, we have analysed the species distinctness and relations within the genus *Viviparus*, as marked in the shell characters, and compared the results with other character sets to test their usefulness in both species discrimination and phylogeny reconstruction. It must be stressed that we did not mean to describe the whole variability ranges of the considered species, but rather to test the differences one can find in a set of randomly chosen populations of various viviparid species.

Material and methods

The material was collected in 1985 - 1992. *Viviparus contectus* (MILLET, 1813) was taken from two localities: the Lake Sarąg (Masurian Lake district, northern Poland) and drainage canals near Kielce (southern Poland); *V. viviparus* (LINNAEUS, 1758) from the Radunia River (Pomeranian Lake district, Poland). Specimens of *V. acerosus* BOURGUIGNAT, 1862 were collected from drainage canals (Zupny kanal) by the Danube River north of Calovo (SW Slovakia); specimens of *V. ater* (CRISTOFORI et JAN, 1832) were collected at Sirmione, the south coast of the Garda Lake (North Italy); individuals of *V. hellenicus* (CLESSIN, 1879) were taken from the Trichonis Lake near Agrinio (West Greece).

The specimens were fixed in 4% formaldehyde solution, and then kept in 70% ethanol. The shells were photographed (Figs. 1-25). Measurements were taken under a stereo microscope, using a calibrated ocular micrometer.

A total of 14 continuous characters were analysed (Fig. 26). Descriptive statistics (SOKAL & ROHLF, 1995), was computed on a MACINTOSH IIvx microcomputer using the SYSTAT 5.2.1 package (WILKINSON et al., 1992) and STATISTICA (STATSOFT, 1991). Each of the

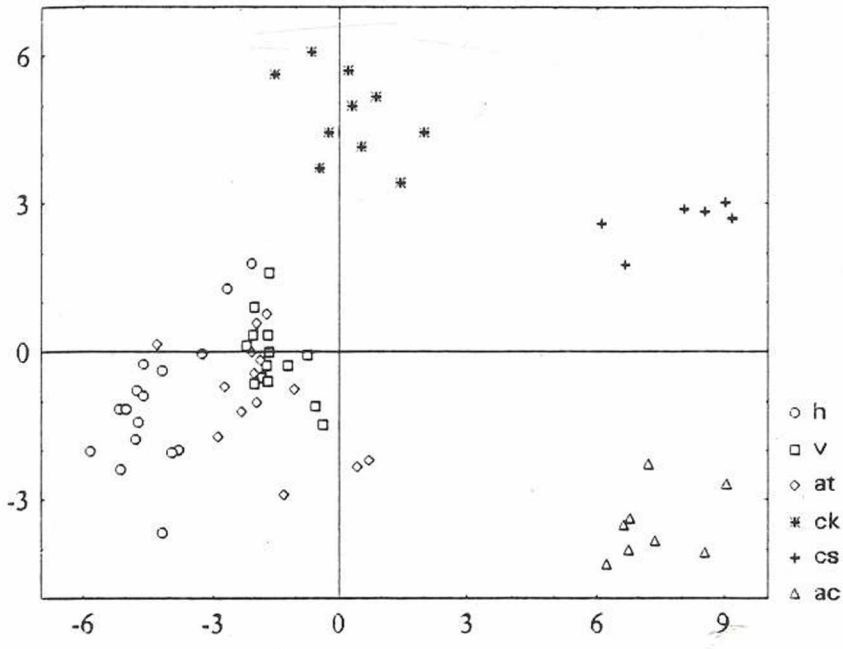


Fig. 31: Discriminant analysis for females (symbols as in Fig. 27).

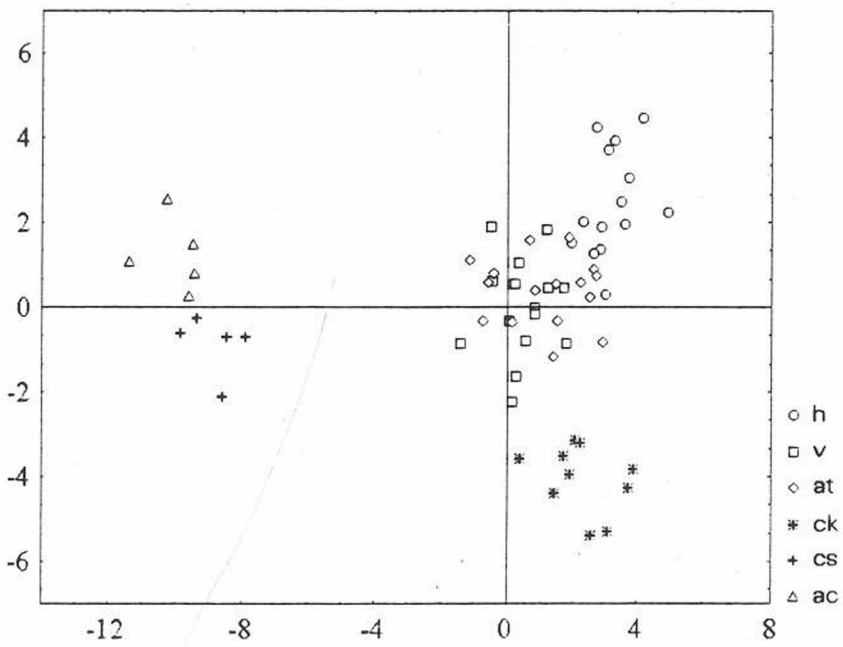


Fig. 32: Discriminant analysis for males (symbols as in Fig. 27).

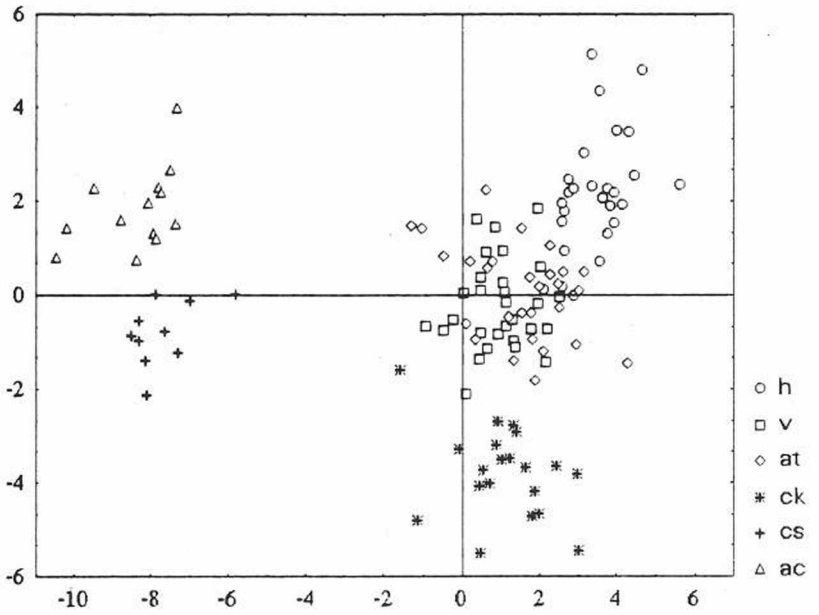


Fig. 33: Discriminant analysis for the sexes combined (symbols as in Fig. 27).

above-mentioned characters was tested by means of ANOVA (Table 1) for significance of differences among the studied populations. We also used this analysis to look for significant differences between males and females. To reduce the possible influence of shell size on character means, we repeated the analyses employing ANCOVA with shell height (a) and shell width (b) as covariates.

Table 3: Results of Discriminant Analysis - importance of individual characters.

	FEMALES and MALES			FEMALES			MALES		
Variable	Wilks' Lambda	Partial Lambda	p-level	Wilks' Lambda	Partial Lambda	p-level	Wilks' Lambda	Partial Lambda	p-level
h	0.0035	0.4820	0.00	0.0013	0.4365	0.00	0.0017	0.4663	0.00
b	0.0032	0.5284	0.00	0.0013	0.4271	0.00	0.0015	0.5160	0.00
a	0.0029	0.5928	0.00	0.0009	0.6119	0.00	0.0016	0.4789	0.00
N	0.0024	0.7065	0.00	0.0014	0.3862	0.00	0.0010	0.8155	0.07
c	0.0024	0.7101	0.00	0.0009	0.6119	0.00	0.0010	0.8126	0.06
d	0.0023	0.7302	0.00	0.0010	0.5644	0.00	0.0012	0.6490	0.00
m	0.0020	0.8364	0.00	0.0007	0.8018	0.03	0.0010	0.7641	0.02
e	0.0020	0.8686	0.00	0.0007	0.8244	0.06	0.0011	0.7288	0.01
j	0.0020	0.8692	0.00	0.0007	0.7863	0.02	0.0009	0.8612	0.18
f	0.0019	0.8874	0.01	0.0006	0.8947	0.30	0.0012	0.6652	0.00
l	0.0018	0.9359	0.16	0.0006	0.8506	0.12	0.0010	0.8125	0.06
k	0.0018	0.9405	0.19	0.0007	0.7614	0.01	0.0009	0.8494	0.14

Table 4: Results of Discriminant Analysis - standardized coefficients for canonical variables (roots 1 and 2).

FEMALES and MALES			FEMALES			MALES		
Variable	Root 1	Root 2	Variable	Root 1	Root 2	Variable	Root 1	Root 2
h	1.4709	-1.2622	b	4.1773	4.3289	h	1.8956	0.9756
c	1.3937	-0.0482	a	1.3605	-3.1803	c	0.9376	-0.5118
e	0.7284	-0.1536	l	0.3823	0.4402	e	0.8788	0.5048
d	0.4746	0.8082	N	0.3456	1.7018	k	0.4627	0.3366
j	0.2872	0.5862	f	-0.0449	-1.1053	j	0.2965	-0.1834
m	0.2128	0.4210	k	-0.1026	-0.6970	l	0.1087	0.7655
l	0.0187	-0.2300	m	-0.3899	-0.3203	m	0.0311	-0.4906
k	-0.0270	-0.1534	j	-0.6729	-1.1123	N	-0.2184	0.4925
N	-0.1284	-0.6534	e	-1.0645	-0.3881	d	-0.3733	-0.5508
f	-0.3850	0.1346	h	-1.2411	0.7723	f	-0.9048	0.5617
a	-1.6952	3.8557	c	-1.3118	1.3193	b	-1.5220	3.0701
b	-2.2380	-3.3019	d	-1.6321	-1.9252	a	-1.5565	-4.9663
Eigenvalue	16.2567	3.7362		19.1675	6.4326		18.5641	4.3776
Cummulative Proportion	0.7020	0.8634		0.6290	0.8401		0.6758	0.8351

The multivariate techniques (JAJUGA, 1993) employed in our study included principal component analysis, multidimensional scaling, minimum spanning tree, and stepwise discriminant function analysis performed in order to find which of the above variables discriminated among the species, and how well they did. All the data were logarithmically transformed (ROHLF, 1994), and standardized. Although no apparent sexual dimorphism was found for the studied 14 morphometric characters, multivariate techniques were applied for the two sexes separately. The specimens were grouped in three sets: the first one consisted of females, the second of males, and - in some cases - the third of all the individuals together. All the analyses were performed twice or thrice, one time on each set.
To visualize the structure of the data with no *a priori* assumptions, principal component analysis was applied, with NTSYS (ROHLF, 1994). Euclidean distances between the specimens were computed, minimum-spanning trees (found with NTSYS) and ten eigenvectors with accompanying eigenvalues were extracted. Eigenvalues, per cent of the total variability explained by the subsequent eigenvalues, and the per cent variability explained by chance under the broken-stick model (ROHLF, 1994) are listed in Table 2. Then, the original data were projected into PC space, together with minimum-spanning trees to show local distorsions in the data. The resulting projection matrix was used as an initial configuration for the nonmetric multidimensional scaling, applied also with minimum-spanning tree (NTSYS: ROHLF, 1994).
The other technique, stepwise discriminant analysis, assuming *a priori* defined groups - populations - was applied by means of STATISTICA (STATSOFT, 1991). To illustrate phenetic relations among the populations, the UPGMA clustering technique based on mean Mahalanobis distances was calculated for males, females and the sexes combined.
Multivariate analysis, although useful where the internal and external relations of data are to be visualised, can hardly be applied directly to phylogeny reconstruction. Clustering, as widely used as it is, reflects overall similarity, but should rather not be applied to phylogeny reconstruction, because the data one deals with are usually not ultrametric (e.g. SWOFFORD & OLSEN, 1990; WEIR, 1990). The additive tree model behaves much better (EDWARDS &

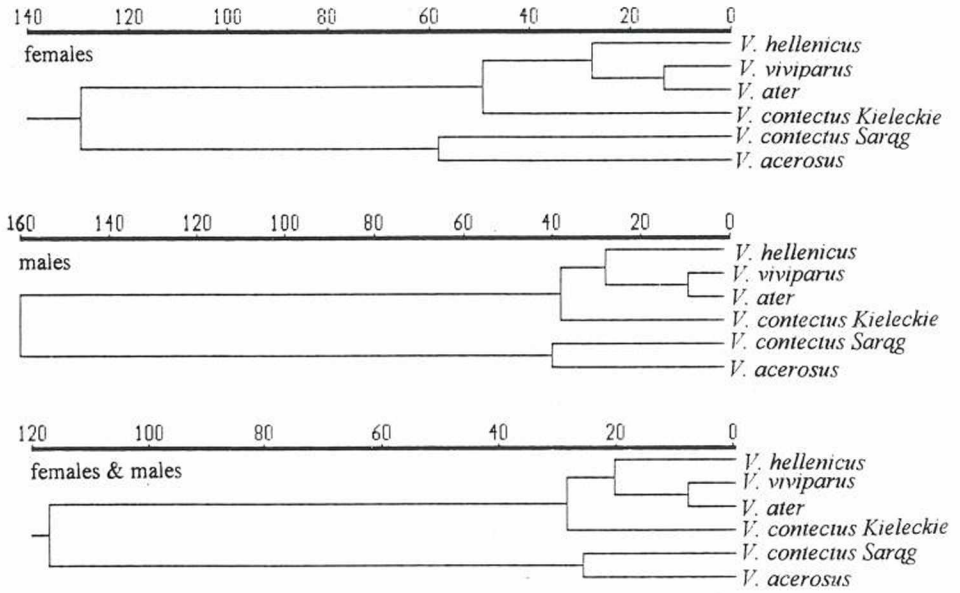


Fig. 34: UPGMA clustering ultrametric phenograms based on Mahalanobis' distances calculated on shell biometry characters, for males, females and the sexes combined.

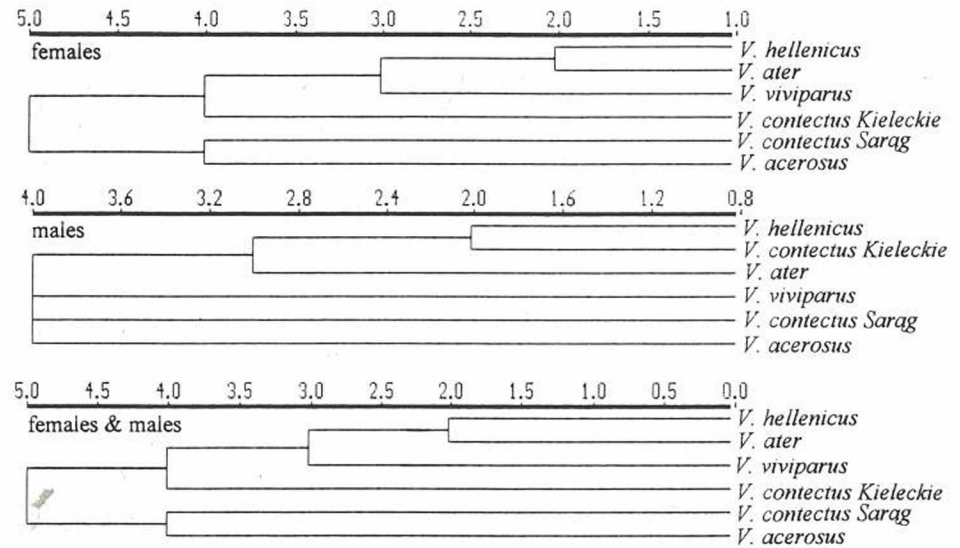


Fig. 35: Neighbor-joining additive trees based on Mahalanobis distances calculated on shell biometry characters, for males, females and the sexes combined.

CAVALLI-SFORZA, 1964; CAVALLI-SFORZA & EDWARDS, 1967; FITCH & MARGOLIASH, 1967; FELSENSTEIN, 1984). Neighbor-joining technique (SAITOU & NEI, 1987) behaves like FITCH-MARGOLIASH for additive data (ROHLF, 1994), thus we computed, with NTSYS, also neighbor-joining trees (SWOFFORD & OLSEN, 1990; ROHLF, 1994).

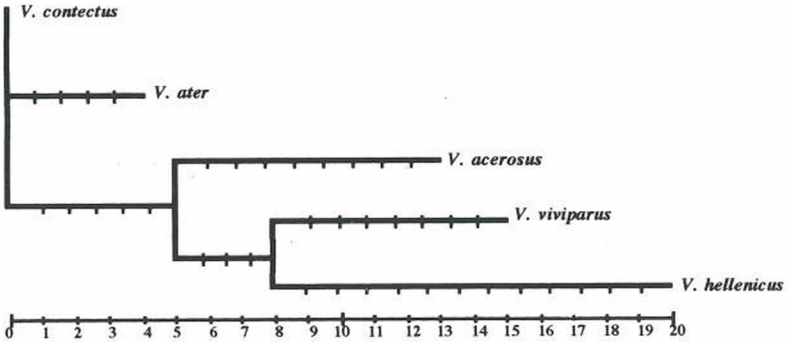


Fig. 36: Phyllogram based on FALNIOWSKI, MAZAN, SZAROWSKA & KOZIK (1997), showing most parsimonious phylogeny reconstruction based on radular, embryonic shell, opercular, and soft part external/internal characters. Only unambiguous changes considered. Each bar equals one change. Branch lengths proportional to amount of change.

Results of shell biometry were compared with the phylogeny reconstructed using radular, embryonic shell, opercular and soft part morphology and anatomy characters (FALNIOWSKI et al., 1996, 1996a, b, 1997). This was done with MACCLADE (MADDISON & MADDISON, 1992) and PAUP (SWOFFORD, 1991) using the exhaustive search option to find the shortest, most parsimonious (SWOFFORD & OLSEN, 1990; WEIR, 1990) tree.

Results

The shells of the studied viviparid species (Figs. 1-25) show rather minor interspecific differences, usually coupled with a wide intraspecific variability. *V. contectus* shell (Figs. 1-3, 15 and 21) has a deep suture, convex whorls (Figs. 1-3 and 15), and a conspicuous umbilicus (Fig. 21). The shell of *V. acerosus* (Figs. 4-5, 16 and 23) resembles the one of *V. contectus* in the form of the umbilicus (Fig. 23), but is much bigger and more oval in outline. The shell of *V. viviparus* (Figs. 6-7, 17 and 22) has a narrower spire, less convex whorls and a shallower suture, the umbilicus usually covered (Fig. 22). The shells of the other two species (Figs. 8-14, 18-20 and 24-25) bear a closer resemblance to the shells of *V. viviparus* than to those of *V. contectus* or *V. acerosus*. *V. hellenicus* (Figs. 8, 12-14, 19-20 and 25) is characterized by the smallest shell, but also by the widest range of shell variability, despite that all the examined shells of this species came from one locality. As it could be seen in the photographs, however, the shells of *V. hellenicus* resemble the shells of *V. viviparus* (Figs. 6-7, 17 and 22) not less than those of *V. ater* (Figs. 9-11, 18 and 24).

The results of ANOVA (Table 1) were almost the same as those of ANCOVA, so we will discuss only the former. The analyses showed no significant differences between sexes. This confirms the fact that there is no sexual dimorphism in *Viviparus* as far as the shell is concerned. The analyses did not show significant differences among the studied populations in the case of all the characters employed. Only the fourth from last whorl height (k) in males did not differ significantly among populations (Table 1). However, the *post-hoc* tests revealed that there were many cases in which individual populations did not differ significantly from each other. The best resolution was achieved in the case of mouth width (e: symbols as in Fig. 26), shell width at the suture between the body whorl and penultimate whorl (f), and shell width (b), the poorest one in the case of ante penultimate whorl height (i), ante penultimate whorl width (j), fourth from last whorl width (l), and fourth from last whorl height (k). Interestingly, the only significant difference shown by the following characters: spire height (c), penultimate whorl height (g), ante penultimate whorl height (i),

penultimate whorl width (h), and ante penultimate whorl width (j) was that between *V. ater* from the Lake Garda and the remainder of populations considered in this study. The characters did not differ significantly within the latter group.

In PCA (Table 2) the first eigenvectors were primarily not used. They mainly reflect size differences, and would not be appropriate here the more that the shells of *V. acerosus* and *V. contectus* from the Lake Sarąg were markedly bigger than the shells of the snails from the other populations. Thus the second and third eigenvectors, explaining also much of the variability and representing mainly shape differences, were used. In females (Fig. 27) practically all the populations overlapped - nearly all the populations included some outliers, whose projections in the PC space were situated far from the centroid of a group. In general, *V. acerosus* was mixed with *V. contectus* from the Lake Sarąg, and grouped marginally along the second axis, whereas on the other side of the axis there were situated *V. hellenicus* and *V. viviparus*. Along the centre there were scattered the specimens of *V. contectus* from Kieleckie, mixed with *V. ater* and some specimens of *V. viviparus* and *V. hellenicus*. Multidimensional scaling for females showed even a less clear picture, with all the popultions completely, irregularly mixed along the second and third axes.

PCA for males (Fig. 28) showed a much more separated group of *V. acerosus* with *V. contectus* from the Lake Sarąg. For the other populations, with the exception of some outliers, *V. contectus* from Kieleckie was separated along the third axis, *V. viviparus* was in the centre, and *V. ater* was mixed with *V. hellenicus*. Multidimensional scaling for males showed, like PCA, the distinctness of the group comprising *V. acerosus* and *V. contectus* from the Lake Sarąg, several outliers for each population, the wide range of *V. viviparus* along the second component axis, the rather good separation of the *V. hellenicus* and *V. ater* group, and the central position of *V. contectus* from Kieleckie. For both sexes, the populations were little distinct, and interpopulation differences within *V. contectus* were as clearcut as the ones between different species.

The above analysis based on the second and third PC, although following the procedure widely used in such studies, may have not reflected the true relationships among the objects, since the percent of variability explained by the third PC was lower than the one expected by chance (Table 2), thus the third PC may have not reflected any true relationships between the variables. Therefore, despite the „size-dimorphism-polymorphism" nature of the first PC, it was necessary to include it in analysis. For females (Fig. 29), the species are also mixed along the first axis. *V. hellenicus* specimens are distributed more closely to *V. viviparus* than to *V. ater*. Surprisingly, the first PC coordinates of individuals did not always reflect size differences (e.g. the big shells of *V. acerosus* are mixed with the small ones of *V. hellenicus* and *V. viviparus)*. Similar remarks concern first PC in males (Fig. 30).

The discriminant analysis gave the same results irrespective of the set of individuals used. Two distinct groups of populations were distinguished. One consisted of *V. acerosus* from drainage canals north of Calovo and *V. contectus* from the Lake Sarąg. The other group comprised all the remaining populations. Two of them, *V. viviparus* from the River Radunia and *V. ater* from the Lake Garda, practically could not be told apart (Figs. 31-33). The population of *V. contectus* from southern Poland was more distinct from the two just mentioned than was the population of *V. hellenicus* from the Lake Trichonis. Very few individuals from the latter were placed by the analysis among those belonging to either *V. viviparus* or *V. ater*.

From among the characters measured those describing the whole shell, its mouth and the penultimate whorl, weighted most in the discriminant analysis (Table 3). The older a whorl, the less significant was it in discriminating between species. Canonical variables also depended mainly on the overall shell size, interestingly the breadth of the shell and of the penultimate whorl weighted more than the height of the shell and the number of whorls (Table 4). Like in PCA and MDS, the two populations of *V. contectus* and *V. acerosus* were well discernible from each other, and from each of the other three populations studied;

V. acerosus was closer to *V. contectus* from the Lake Sarąg than the latter population to *V. contectus* population from Kieleckie; the other three populations constituted one continuous group, their centroids ordered: *V. ater* - *V. viviparus* - *V. hellenicus*.
The UPGMA clustering computed from Mahalanobis distances showed the same branch pattern for males, females, and both sexes together, the only difference being in branch lengths (Fig. 34). Also this technique enabled the cluster connecting *V. acerosus* with *V. contectus* from the Lake Sarąg to be detected. *V. ater* and *V. viviparus* were clustered together, *V. hellenicus* being the closest to this cluster, and *V. contectus* from Kieleckie less close. Neighbor joining (Fig. 35) inferred different trees for males than for females and both sexes together. For every neighbor-joining tree the same cluster as in UPGMA, joining *V. acerosus* with *V. contectus* from the Lake Sarąg was detected. The neighbor-joining for both females and the sexes combined differed from UPGMA in joining *V. ater* with *V. hellenicus*, not *V. viviparus*. In males, surprisingly, *V. hellenicus* was joined with *V. contectus* from Kieleckie, *V. ater* was closer, and *V. viviparus* less close to *V. hellenicus* and *V. contectus* from Kieleckie.
Finally, the most parsimonious tree, based on other data sets: of the radula, embryonic shell, operculum, and soft part external morphology and anatomy (FALNIOWSKI et al., 1996, 1996a, b, 1997) was constructed (Fig. 36). It was completely different, in both topology and branch length, from all the above, shell-based trees.

Discussion

In our earlier paper (FALNIOWSKI et al., 1997) concerning the embryonic shell, radula, operculum and soft part morphology, we found *V. hellenicus* more similar to *V. viviparus* than to *V. ater*. On the other hand, all the literature opinions on phylogenetic relationships among the viviparid taxa were based on shell characters alone. When the level of differences observed between given taxa is considered, not knowing the reconstructed phylogeny - the evolutionary history of a group - it may be pointless to use them to support decisions on the taxonomic status of the taxa (CRACRAFT, 1989). As long as a phylogeny inferred based on non-shell characters is concerned, *V. hellenicus* cannot be a subspecies of *V. ater*, as acknowledged by e.g. FECHTER & FALKNER (1990). Considering the overall similarity of the shell, as presented by UPGMA, *V. hellenicus* is the closest to the cluster *V. ater* + *V. viviparus*, and not grouped in a cluster with *V. ater*. Thus, the species distinctness of *V. hellenicus* has been confirmed by the shell characters also.
All the results present the same picture of high intraspecific variation coupled with slight interspecific differences, and within-species interpopulation differences of the same rank as the interspecific ones. Thus, the shell characters are useful in neither discriminating species nor inferring phylogeny in *Viviparus*. This is a rule in almost all groups of prosobranchs, if not of gastropods (FALNIOWSKI, 1989b, 1990). The conditions illustrated above are noteworthy, the more that all the taxonomy within *Viviparus* is entirely based on the shell. On the other hand, shell biometry, when described by only 14 randomly chosen characters, may not characterize the shells adequately. The shells have also several qualitative characters coupled with numerous characteristics based on a taxonomist's experience. Their descriptions are usually not much clear and univocally understood. That they may often reflect the real biologically sound taxa is confirmed by the fact that as little as the species of *Viviparus* differ in morphology between each other, they are quite distinct when molecular characters are concerned (FALNIOWSKI et al., 1996). However, all such characters are hard to be defined and tested in any more rigorous, not arbitrary way.
On the other hand, all the shell characters, as pointed out above, are weak. Moreover, the various data generated by the multivariate techniques applied, although useful in analysis, are not really existing, and need not be evolutionary sound enough to reconstruct the phylogeny upon. Thus, phylogeny reconstruction must be supported by other data sets, and

this was done based on our earlier studies (FALNIOWSKI et al., 1996, 1996a, b, 1997). The curious results of neighbor joining, completely different for males and females, well illustrate the more than restricted usefulness of the viviparid shells in any phylogenetic inference.

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