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Tracing the viviparid evolution: soft part morphology and opercular characters (Gastropoda: Architaenioglossa: Viviparidae)

With 12 SEM Photographs and 87 Figures

ANDRZEJ FALNIOWSKI[1)], KRYSTYNA MAZAN[1)],
MAGDALENA SZAROWSKA[1)] & ANDRZEJ KOZIK[2)]

Abstract. The paper describes the soft part morphology and opercula of five European viviparid species: *Viviparus acerosus* BOURGUIGNAT, 1862; *V. ater* (CRISTOFORI et JAN, 1832); *V. contectus* (MILLET, 1813); *V. hellenicus* (CLESSIN, 1879); and *V. viviparus* (LINNAEUS, 1758). Descriptions, drawings and SEM photographs, as well as measurements are presented. The reproductive organs are given special attention. It is stressed that among the viviparids wide infraspecific variation is coupled with a few characters interspecifically variable. The interspecific differences are slight. In 20 characters (14 of them biometrical) the interspecific differences are statistically significant. These characters have been applied to reconstruct phylogeny, using MACCLADE and PAUP. Since the characters are rather weak, and the reconstruction not much consistent, another MPR is constructed, based on all the 38 characters of the operculum, soft part morphology, radula and embryonic shell, found ealier by the authors.

Kurzfassung. **Auf den Spuren der Evolution der Viviparidae: Merkmale der Morphologie des Weichkörpers und des Operculums (Gastropoda: Architaenioglossa: Viviparidae).** – Die Arbeit beschreibt die Morphologie des Weichkörpers und die Opercula von fünf europäischen Arten der Viviparidae: *Viviparus acerosus* BOURGUIGNAT, 1862; *V. ater* (CRISTOFORI et JAN, 1832); *V. contectus* (MILLET, 1813); *V. hellenicus* (CLESSIN, 1879); und *V. viviparus* (LINNAEUS, 1758). Beschreibungen, Zeichnungen und REM-Photos ebenso wie Maße werden vorgelegt. Die Fortpflanzungsorgane werden speziell betrachtet. Es wird darauf hingewiesen, daß bei den Viviparidae eine breite infraspezifische Variabilität kombiniert ist mit wenigen Merkmalen, welche interspezifisch variieren. Die interspezifischen Unterschiede sind gering. Bei 20 Merkmalen (davon 14 biometrischen) sind die interspezifischen Unterschiede statistisch signifikant. Diese Merkmale wurden benutzt, um mittels der Kladistikprogramme MACCLADE und PAUP die Phylogenie zu rekonstruieren. Da die Merkmale ziemlich schwach ausgeprägt und nach diesen Programmen keine einheitlichen Aussagen möglich sind, wurde die phylogenetische Rekonstruktion nach MPR (most parsimonious reconstruction) ermittelt, welche auf 38 Merkmalen des Operculums, der Morphologie des Weichkörpers, der Radula und der embryonalen Schale (die von den Autoren schon früher ermittelt wurden) basiert.

Introduction

The relationships of the Architaenioglossa, to which the family Viviparidae belongs, are not clear (HASZPRUNAR, 1988; BERTHOLD, 1989). The same concerns the interrelationships of the old and archaic family. There are a few characters that discriminate species, the morpholo-

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Addresses of the authors:
1) Department of Malacology, Institute of Zoology, Jagiellonian University, ul. R. Ingardena 6, 30-060 Kraków (Poland)
2) Department of Biochemistry, Institute of Molecular Biology, Jagiellonian University, ul. Mickiewicza 3, 31-120 Kraków (Poland)

gical distinctness of which is hardly recognizable in many cases (FALNIOWSKI, 1989a, 1990a). Recently an undoubtedly hybrid specimen (*Viviparus contectus* x *V. viviparus*) has been found (FALNIOWSKI, KOZIK & SZAROWSKA, 1993). It seems that evolution within the Viviparidae has slightly affected the morphological characters, thus the speciation events have brought about only a minor morphological change. To test the above hypothesis and explain the relationships among chosen European species, we have combined morphological and electrophoretic (allozymic) studies.

The following viviparid species have been considered: *Viviparus acerosus* BOURGUIGNAT, 1862; *V. ater* (CRISTOFORI et JAN, 1832); *V. contectus* (MILLET, 1813); *V. hellenicus* (CLESSIN, 1879); and *V. viviparus* (LINNAEUS, 1758). The present paper describes the results of the studies on the soft part morphology and anatomy. *V. hellenicus* is given special attention as the least known species from among the viviparids concerned. The objective of the study is neither the comparative nor functional anatomy of the Viviparidae – there are a number of papers dealing with these subjects, summarized by VAIL (1977). We are rather seeking soft part (and opercular) characters that are interspecifically variable.

Material and methods

Numerous specimens of *Viviparus contectus* (MILLET, 1813) and *V. viviparus* (LINNAEUS, 1758) were collected from various localities in Poland. Most of them are the same as in FALNIOWSKI (1989a). *V. acerosus* BOURGUIGNAT, 1862 were collected from drainage canals (Zupny kanal) by the Danube River north of Calovo (SW Slovakia); *V. ater* (CRISTOFORI et JAN, 1832) were collected at Sirmione, the South coast of the Garda Lake (North Italy); *V. hellenicus* (CLESSIN, 1879) were picked from the Trichonis Lake near Agrinio (West Greece).

The gastropods were fixed in 4% formalin, and then kept in 70% ethanol. They were dissected under a stereoscopic microscope. The heads and selected anatomical parts were freeze-point dried, glued to a holder, coated with gold and examined with a Jeol JSM-35 scanning electron microscope. The other anatomical characters were examined under a stereoscopic microscope. Measurements were taken using a calibrated ocular micrometer, figures were drawn with a drawing apparatus.

Descriptive statistics (Table 1 and 2) was performed on a MacIntosh IIvx microcomputer using the SYSTAT 5.2.1 package (WILKINSON, HILL & VANG, 1992). Since the samples were scarce, and the distribution of the variables, especially the countable characters (SOKAL & ROHLF, 1987), was apparently not normal, both parametric univariate techniques and multivariate techniques had to be abandoned. On the other hand, there was no cause to contradict either the continuity or one distribution shape of every studied populations (species). Thus we applied Kruskal-Wallis nonparametric tests (with SYSTAT), analogous with the independent groups t-test and one-way ANOVA for each character. The characters, the states of which had shown statistically significant ($p = 0.01$) interspecific differences, were then recoded into multistate characters (Table 3). Since the distributions were as a rule not normal, usually not means but medians were recoded, because of the robustness of the latter (with a breakdown point as high as 0.5: JAJUGA, 1993). Similarly the qualitative characters were coded (Table 3). Table 3 was used as an input for the Macintosh MACCLADE (MADDISON & MADDISON, 1992). Its Nexus-format file was processed by PAUP (SWOFFORD, 1991) with the exhaustive search option to find the shortest, most parsimonious (SWOFFORD & OLSEN, 1990; WEIR, 1990) tree. The latter was then processed by MACCLADE to analyze character evolution and print cladograms.

Results

Opercula and soft part morphology

FALNIOWSKI (1989a) has illustrated the opercula of *V. contectus* and *V. viviparus*. The opercula of the other three species are similar to the illustrated ones. All the interspecific differences are marked in opercular biometry (Table 1 and 2), namely in the proportions of operculum length to shell height, and of operculum nucleus distance (measured to the operculum edge at the left upper „corner“) to operculum length (Table 3, characters 1 and 2, respectively).

Tab. 1: Descriptive statistics of the operculum and anatomy – females.

		V.acerosus	V.ater	V.contectus	V.hellenicus	V.viviparus	hybrid (V.c.xV.v.)
	n	10	10	8	10	10	3
shell height	min./max.	22.20-44.89	26.50-33.50	17.50-35.00	21.40-33.10	18.30-22.90	30.90-38.50
	mean/med.	38.96/40.14	28.84/28.20	26.31/27.30	26.46/26.60	20.38/20.60	34.20/33.20
	sd/C.V.	6.27/0.16	2.31/0.08	7.82/0.30	3.56/0.13	1.40/0.07	3.90/0.11
operculum length	min./max.	15.90-22.00	13.10-16.87	8.65-18.20	9.45-12.99	9.82-12.20	16.20-19.60
	mean/med.	19.57/19.88	14.54/14.35	13.18/13.92	11.77/12.04	10.93/11.05	17.80/17.60
	sd/C.V.	1.64/0.08	1.05/0.07	3.96/0.30	1.04/0.09	0.76/0.07	1.71/0.10
operculum breadth	min./max.	12.90-16.50	10.10-12.83	6.94-14.60	7.52-10.34	7.58-9.70	12.30-15.30
	mean/med.	15.22/15.43	11.07/10.90	10.57/10.87	9.37/9.52	8.44/8.46	13.97/14.30
	sd/C.V.	1.10/0.07	0.79/0.07	3.21/0.30	0.86/0.09	0.63/0.08	1.53/0.11
operculum nucleus distance	min./max.	4.19-5.76	3.28-4.31	2.20-4.58	2.75-4.32	2.49-3.41	4.06-4.98
	mean/med.	5.16/5.20	3.72/3.70	3.36/3.58	3.68/3.78	2.77/2.76	4.50/4.45
	sd/C.V.	0.49/0.10	0.29/0.08	1.01/0.30	0.48/0.13	0.28/0.10	0.46/0.10
ctenidium length	min./max.	19.00-32.75	16.24-22.24	14.18-31.44	11.90-16.37	13.35-16.64	21.88-26.85
	mean/med.	28.87/29.58	19.20/19.33	21.39/20.42	14.80/15.21	14.88/14.94	25.11/26.59
	sd/C.V.	3.81/0.13	1.87/0.10	6.94/0.32	1.57/0.11	1.05/0.07	2.80/0.11
ctenidium breadth	min./max.	3.93-6.29	2.10/3.41	2.60-6.55	2.24-3.54	2.62-3.80	3.80-4.45
	mean/med.	5.30/5.40	2.70/2.67	4.01/3.61	2.77/2.74	3.09/2.98	4.15/4.19
	sd/C.V.	0.71/0.13	0.42/0.15	1.46/0.36	0.37/0.13	0.37/0.12	0.33/0.08
lamellae number	min./max.	229.00-272.00	197.00-253.00	102.00-215.00	151.00-206.00	148.00-177.00	183.00-224.00
	mean/med.	255.40/256.50	217.40/213.50	156.25/161.50	187.10/188.00	164.00/166.00	198.67/189.00
	sd/C.V.	15.03/0.06	17.32/0.08	48.06/0.31	14.50/0.08	11.19/0.07	22.14/0.11
osphrad. length	min./max.	4.85-6.29	3.67-5.25	2.35-4.85	2.86-4.58	2.62-3.41	4.45-5.37
	mean/med.	5.66/5.72	4.53/4.70	3.56/3.73	3.54/3.52	2.99/3.05	4.89/4.85
	sd/C.V.	0.43/0.08	0.53/0.12	1.05/0.30	0.50/0.14	0.27/0.09	0.46/0.09
osphrad. breadth	min./max.	0.25-0.33	0.26-0.42	0.19-0.52	0.24-0.39	0.24-0.39	0.52-0.55
	mean/med.	0.27/0.27	0.37/0.39	0.32/0.31	0.32/0.33	0.30/0.30	0.53/0.52
	sd/C.V.	0.02/0.08	0.05/0.13	0.11/0.36	0.06/0.17	0.04/0.15	0.02/0.03
vagina length	min./max.	3.01-9.30	4.06-7.60	4.45-6.94	4.06-6.42	2.49-3.93	5.11-5.89
	mean/med.	7.51/8.19	6.22/6.36	6.01/6.29	5.14/4.98	3.05/2.88	5.59/5.76
	sd/C.V.	1.89/0.25	0.97/0.16	0.92/0.15	0.72/0.14	0.45/0.15	0.42/0.07
vagina breadth	min./max.	1.31-3.67	1.70-3.14	1.44-2.49	1.44-2.36	0.92-1.44	1.31-1.83
	mean/med.	2.79/2.95	2.17/2.10	2.05/2.10	1.94/2.03	1.13/1.12	1.57/1.57
	sd/C.V.	0.61/0.22	0.45/0.21	0.32/0.16	0.33/0.17	0.16/0.15	0.26/0.17
rs length	min./max.	3.00-5.90	2.62-4.06	3.14-4.85	2.49-3.93	1.83-2.62	4.06-5.50
	mean/med.	5.25/5.42	3.67/3.74	4.11/4.39	3.04/2.88	2.36/2.49	4.89/5.11
	sd/C.V.	0.83/0.16	0.42/0.11	0.67/0.16	0.50/0.16	0.26/0.11	0.74/0.15
rs breadth	min./max.	0.39-0.91	0.52-0.92	0.39-0.77	0.39-0.59	0.39-0.52	0.46-0.52
	mean/med.	0.73/0.83	0.78/0.79	0.58/0.60	0.51/0.52	0.45/0.43	0.50/0.52
	sd/C.V.	0.19/0.26	0.13/0.16	0.12/0.21	0.05/0.10	0.06/0.14	0.03/0.07
embryo number	min./max.	6.00-18.00	3.00-9.00	18.00-34.00	1.00-6.00	10.00-25.00	9.00-17.00
	mean/med.	12.78/15.00	6.20/6.50	26.60/25.00	3.33/4.00	17.30/16.50	13.00/13.00
	sd/C.V.	5.09/0.40	1.99/0.32	6.47/0.24	1.73/0.52	4.47/0.26	5.67/0.44

The gross external morphology and anatomy of the female *V. hellenicus* is presented in Fig. 1, and the other studied species resemble *V. hellenicus*. In all the species the head and foot are intensively pigmented black, often with an admixture of yellow or orange. The head (Figs. 1 and 2) has a broad and massive snout and relatively short and massive tentacles with eyes situated on small knobs at the bases of the tentacles. In males the right, markedly broader tentacle functions as a penis containing the terminal section of the vas deferens (Figs. 2, 7–8, 18, 29–48 and 49–55). The foot is relatively short and broad. The head and foot proportions are highly variable and do not show interspecific variation among the studied

Tab. 2: Descriptive statistics of the operculum and anatomy – males.

		V.acerosus	V.ater	V.contectus	V.hellenicus	V.viviparus
	n	10	10	10	10	10
shell height	min./max.	34.17-42.88	21.60-31.60	16.00-33.22	20.20-25.50	18.10-21.70
	mean/med.	37.49/37.52	27.03/27.10	25.92/26.90	23.31/23.85	19.48/19.30
	sd/C.V.	2.53/0.07	2.54/0.09	5.85/0.23	1.89/0.08	1.14/0.06
operculum length	min./max.	16.85-21.00	10.80-15.00	8.68-17.81	10.52-12.77	9.87-11.40
	mean/med.	18.38/18.23	13.76/14.02	14.12/14.30	11.70/11.77	10.63/10.57
	sd/C.V.	1.22/0.07	1.20/0.09	2.99/0.21	0.74/0.06	0.54/0.05
operculum breadth	min./max.	13.20-15.70	8.40-12.20	6.83-13.98	8.62-10.38	7.80-9.00
	mean/med.	14.02/13.80	10.57/10.78	11.11/11.40	9.58/9.74	8.40/8.35
	sd/C.V.	0.75/0.05	1.01/0.10	2.47/0.22	0.64/0.07	0.47/0.05
operculum nucleus distance	min./max.	4.45-5.76	2.65-3.60	2.47-5.20	2.75-3.93	2.60-3.27
	mean/med.	5.04/4.95	3.37/3.46	4.02/4.19	3.47/3.58	2.80/2.70
	sd/C.V.	0.42/0.08	0.28/0.08	0.87/0.22	0.37/0.11	0.25/0.09
ctenidium length	min./max.	20.70-39.30	8.42-19.91	12.71-27.77	12.09-14.68	13.51-15.59
	mean/med.	28.45/28.02	15.85/17.63	20.59/20.74	13.45/13.56	14.55/14.51
	sd/C.V.	4.60/0.16	4.18/0.26	5.45/0.26	0.94/0.07	0.79/0.05
ctenidium breadth	min./max.	4.59-6.03	1.85/2.75	2.40-5.24	2.23-2.85	2.49-3.93
	mean/med.	5.19/5.07	2.45/2.49	3.92/3.83	2.51/2.51	3.33/3.44
	sd/C.V.	0.48/0.09	0.25/0.10	0.97/0.25	0.23/0.09	0.40/0.12
lamellae number	min./max.	232.00-291.00	165.00-221.00	104.00-212.00	154.00-196.00	161.00-183.00
	mean/med.	255.70/255.50	205.70/211.00	171.70/183.50	178.40/179.50	173.40/174.50
	sd/C.V.	17.08/0.07	16.71/0.08	36.69/0.21	13.21/0.07	8.24/0.05
osphrad. length	min./max.	3.41-6.29	3.44-5.50	2.53-5.46	2.41-3.51	2.49-3.54
	mean/med.	5.19/5.21	4.43/4.52	4.29/4.46	3.10/3.25	2.93/2.88
	sd/C.V.	0.82/0.16	0.61/0.14	0.95/0.22	0.43/0.14	0.29/0.10
osphrad. breadth	min./max.	0.13-0.32	0.26-0.39	0.26-0.52	0.33-0.41	0.26-0.39
	mean/med.	0.22/0.22	0.31/0.31	0.44/0.52	0.36/0.37	0.33/0.33
	sd/C.V.	0.05/0.22	0.04/0.12	0.11/0.24	0.03/0.08	0.05/0.15
distal lobe of testis length	min./max.	11.00-14.80	6.29-11.79	6.16-11.66	4.85-7.34	5.37-8.38
	mean/med.	13.21/13.30	7.46/7.14	9.51/10.09	5.95/6.03	6.81/6.75
	sd/C.V.	1.03/0.08	1.58/0.21	2.18/0.23	0.72/0.12	0.76/0.11
distal lobe of testis breadth	min./max.	6.55-8.65	3.41-6.81	3.80-7.28	3.01-4.98	3.80-6.16
	mean/med.	7.38/7.21	4.74/4.72	5.78/6.09	3.43/3.21	4.65/4.45
	sd/C.V.	0.80/0.11	0.89/0.19	1.41/0.24	0.62/0.18	0.74/0.16
prostate length	min./max.	28.95-36.42	14.15-19.91	14.67-22.53	12.05-17.29	11.00-15.06
	mean/med.	32.69/33.28	16.09/15.00	18.50/19.12	14.97/15.59	13.10/13.10
	sd/C.V.	2.49/0.08	2.31/0.14	3.25/0.18	1.87/0.12	1.27/0.10
prostate breadth	min./max.	5.50-6.55	2.62-3.67	1.96-4.94	2.23-4.45	2.36-3.14
	mean/med.	5.86/5.77	3.24/3.35	3.41/3.41	3.48/3.41	2.83/2.82
	sd/C.V.	0.37/0.06	0.35/0.11	0.92/0.27	0.74/0.21	0.28/0.10

species. In all the species there are numerous cilia on the ventral side of the male right tentacle (Fig. 9). On the other hand, the hyperciliation of the dorsal side of the male right tentacle has been observed only in *V. hellenicus* (Fig. 6).

Within the mantle cavity there is a food groove (Fig. 1) characteristic of the Viviparidae (Cook, 1949; Fretter & Graham, 1962; Purchon, 1977; Falniowski, 1989a), looking the same in all the studied species. Also the alimentary system, with a big, sac-shaped stomach (Fig. 1), shows no interspecifically variable characters.

The elongated ctenidium (Figs. 1 and 3–5) with about 200 fine and delicate lamellae (Table 1 and 2) looks similar in all the species. There are, however, interspecific differences in the number of the lamellae (Table 3: character 3), as well as in ctenidium length to shell height proportion (Table 3: character 4), ctenidium length to breadth proportion (Table 3: character

Tab. 3: Opercular and anatomical biometric and discrete characters and their states.

	V.acerosus	*V.ater*	*V.contectus*	*V.hellenicus*	*V.viviparus*
operculum/shell length prop. (1)	0	1	1/2	0	2
operculum nucleus distance/length (2)	1	0	1	2	0
ctenidium lamellae number (3)	3	2	0/1	1	0
ctenidium/shell length prop. (4)	2	1	3	0	2
ctenidium length/ /breadth prop. (5)	1	2	1	1	0
ctenidium lamellae n/ /shell height (6)	0	1	0	1	2
osphradium length/ shell height prop. (7)	1	0	0/2	2	1
osphradium length/ breadth prop. (8)	2	1	0	0	0
shell height/testis distal lobe length prop. (9)	0	1	0	1	0
testis distal lobe length/ /breadth proportion (10)	1	0	0/1	1	0
shell height/prostate length proportion (11)	0	1	1	1	1
shell height/length of receptaculum prop (12)	0/1	0/1	0	1	1
embryo number (13)	2	1	3	0	2
shell height/vagina length proportion (14)	0	0	0	0	1
cilia dorsally on right male cephalic tentacle (15)	0	0	0	1	0
pigmented zones at prostate section around villi (16)	0	0	0	1	1
villi within prostate (17)	0	2	1	2	2
receptaculum seminis (18)	0	1	2	1	1
embryos arrangement (19)	0	1	0	1	0
folds in female opening (20)	2	2	0	1	2

character states: (1): median: 0 - 0.499, 1 - 0.51, 2 - 0.54; (2): approximately: 0 - 0.25, 1 - 0.27, 2 - 0.31; (3): median approximates: 0 - 172, 1 - 185, 2 - 211, 3 - 257; (4): median approximates: 0 - 0.57, 1 - 0.65, 2 - 0.73 - 0.76, 3 - 0.80; (5): median approximates: 0 - 4.69, 1 - 5.29 - 5.48, 2 - 7.09; (6): median: 0 - 5.92 - 6.45 in females and 6.56 - 6.83 in males, 1 - 7.03 - 7.53 in females and 7.60 - 7.64 in males, 2 - 8.05 in females and 8.87 in females (sexual dimorphism - higher proportion in males); (7): median approximates: 0 - 5.97 - 6.11, 1 - 6.73 - 7.01, 2 - 7.43 - 7.49; (8): median: 0 - 10.00 - 11.22 in females and 8.66 - 9.78 in males, 1 - 12.47 in females and 14.23 in males, 2 - 20.80 in females and 23.10 in males; (9): median: 0 - 2.70 - 2.83, 1 - 3.86 - 3.91; (10): median: 0 - 1.47 - 1.57, 1 - 1.75 - 1.79; (11): median: 0 - 1.13, 1 - 1.46 - 1.70; (12) 0 - short, 1 - long; (13): median: 0 - 4.00, 1 - 6.50, 2 - 15.00 - 16.50, 3 - 25.00; (14): median: 0 - 4.25 - 5.06, 1 - 6.85; (15): 0 - absent, 1 - present; (16): 0 - absent, 1 - present; (17): 0 - "vestigial", 1 - straight and rather broad, 2 - wavy and delicate; (18): 0 - acerosus type, 1 - irregularly sac-shaped, 2 - contectus type; (19): 0 - in numerous rows irregular, 1 - in one regular row; (20): 0 - simple "u", 1 - "u" with folds and knob, 2 - radial folds

5) and ctenidium lamellae number to shell height proportion (Table 3: character 6). The osphradium (Figs. 1 and 3–5) spans along the base of the ctenidium lamellae and is rather long and narrow, elongate-cylindrical in shape. Its gross morphology is the same in all the species, but there are interspecific differences in the proportions of: osphradium length to shell height (Table 3: character 7) and osphradium length to breadth (Table 3: character 8). Despite the careful examination of the material, no more interspecific differences have been found except within the reproductive organs.

The male reproductive organs are similar to the ones described by VAIL (1977) for the North American *V. georgianus* (LEA, 1834). The testis is characteristically bilobate – its proximal (posterior) portion occupies the typical position along the apical part of the hepato-pancreas gland, but there is also the second, distal (anterior) portion („T" of VAIL, 1977), smaller than the proximal one and connected with the latter by a vas deferens. It is adjacent to the distal part of the hepato-pancreas. The proportions of: shell height to testis distal portion length (Table 3: character 9) and distal portion length to breadth (Table 3: character 10) are interspecifically variable.

Neither the vas deferens nor the seminal vesicle show interspecific variation. The most characteristic and striking structure within the male reproductive organs of the Viviparidae is their uncommonly large, cylindrical prostate gland (Figs. 14–23). It spans almost the whole

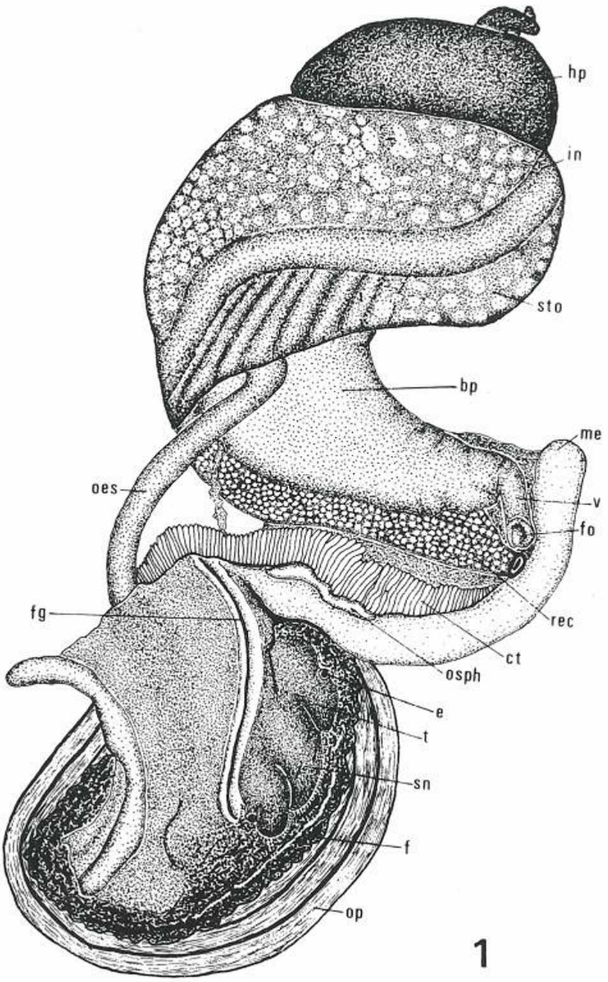
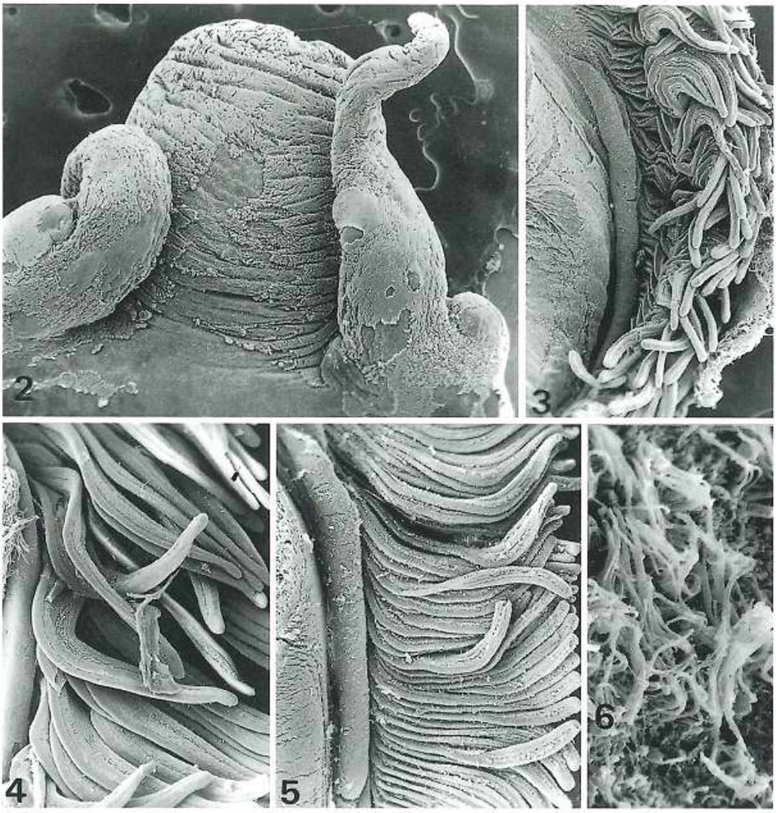


Fig. 1: Gross anatomy of *Viviparus hellenicus*: bp – brood pouch, ct – ctenidium, e – eye, f – foot, fg – food groove, fo – female opening, hp – hepato-pancreas, in – intestine, me – mantle edge, oes – oesophagus, op – operculum, osph – osphradium, rec – rectum, sn – snout, sto – stomach, t – cephalic tentacle, v – vagina.

length of the mantle cavity and terminates directly with a penis (FRETTER & GRAHAM, 1962; VAIL, 1977). Inside the prostate (Figs. 10–11 and 24–28) numerous villi of variable shape and size protrude toward a small, central lumen. The external appearance of the prostate (Figs. 14–23) is highly variable within a species, and uniform interspecifically. There are, however, clear differences among the species in the size of the organ, but they are correlated with differences in the general size of the animal and its shell. Thus we computed shell height/prostate length ratio (Table 3: character 11), to avoid the direct influence of general size differences. *V. acerosus* was found to have a relatively longer prostate than those of the other species.

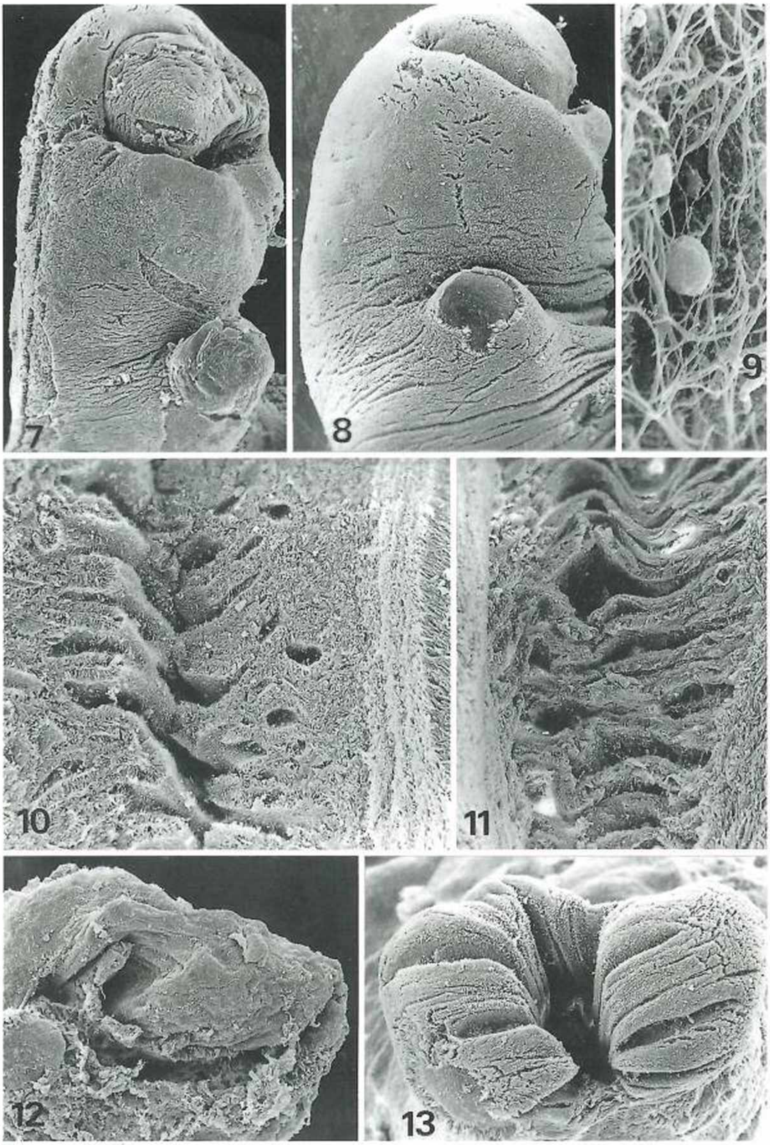
The villi inside the prostate (Figs. 10–11 and 24–28) are often intensively pigmented orange (not in *V. contectus*, but the pigmentation is highly variable in the other species – the



Figs. 2–6: Soft parts of *Viviparus*: 2 – *V. acerosus,* head of male (12x); 3–5 – osphradium and ctenidium (15x): 3 – *V. ater*, 4 – *V. viviparus*, 5 – *V. hellenicus*; 6 – *V. hellenicus*, cilia on dorsal side of male right tentacle (2 000x).

pigment may occur or not). Inside the prostate there also may occur black or dark grey pigment, forming differently pigmented zones (Figs. 27–28), usually (but not always) in *V. hellenicus* (Fig. 27) and *V. viviparus* (Fig. 28). The villi are widely variable as well within a species as within a given prostate, but some interspecific, rather constant differences are observable. In *V. acerosus* (Figs. 10 and 24) the villi are almost rudimentary: short, broad and slightly marked. In the other species the villi are longer and better marked (Figs. 11 and 25–28). In *V. contectus* they are rather broad and straight (Fig. 26), and in *V. ater* (Fig. 11 and 25), *V. hellenicus* (Fig. 27) and *V. viviparus* (Fig. 28) the villi are much narrower and often undulated („wavy“).

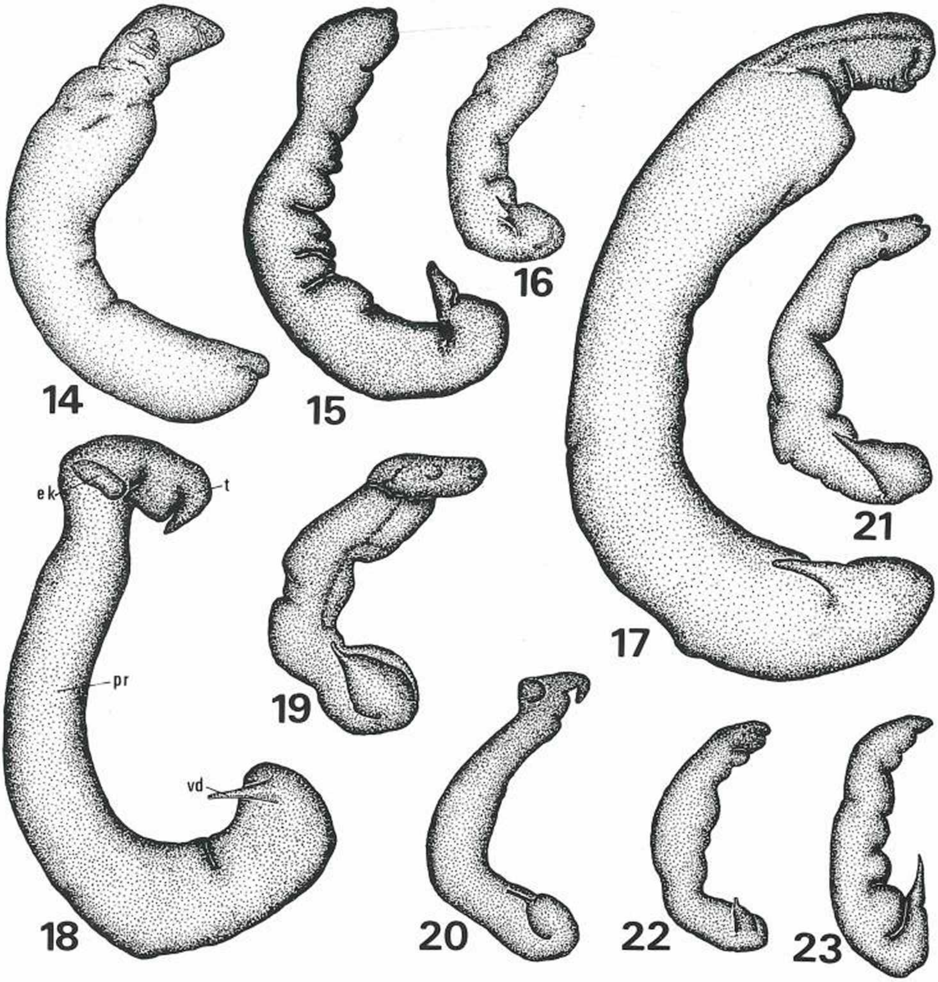
The penis runs inside the right cephalic tentacle (Figs. 7–8 and 29–55). There is a distinct eye knob at the base of the tentacle, the more or less elongated tip terminating with the male opening, and below the tip the accessory opening, situated and looking exactly like „the opening of the penis sheath“ of FRETTER & GRAHAM (1962). The external appearance of the tentacle presents wide variation, especially of physiological and artifact origin (Figs. 7–8 and 29–45). There are apparent interspecific differences in its size, but they are due to differences in the general size of an animal. Thus, all its variability presented (Figs. 29–45) renders the right cephalic tentacle not useful a character at the species level in the studied group. In a few specimens of *V. ater* (Figs. 46–48) there were found characteristic, pigmentless,



Figs. 7–13: Copulatory organs of *Viviparus:* 7–8 – right cephalic tentacle of male including penis inside (30x): 7 – *V. ater*, 8 – *V. viviparus*; 9 – *V. viviparus*, cilia on ventral side of male right tentacle (2 000x); 10–11 – longitudinal section of prostate (65x): 10 – *V. acerosus*, 11 – *V. ater*; 12–13 – *female gonoporus* (50x): 12 – *V. contectus*, 13 – *V. ater*.

whitish, bulbous papillae irregularly scattered on their ventral surface and the edge on a side of the snout. The papillae were probably of teratic character.

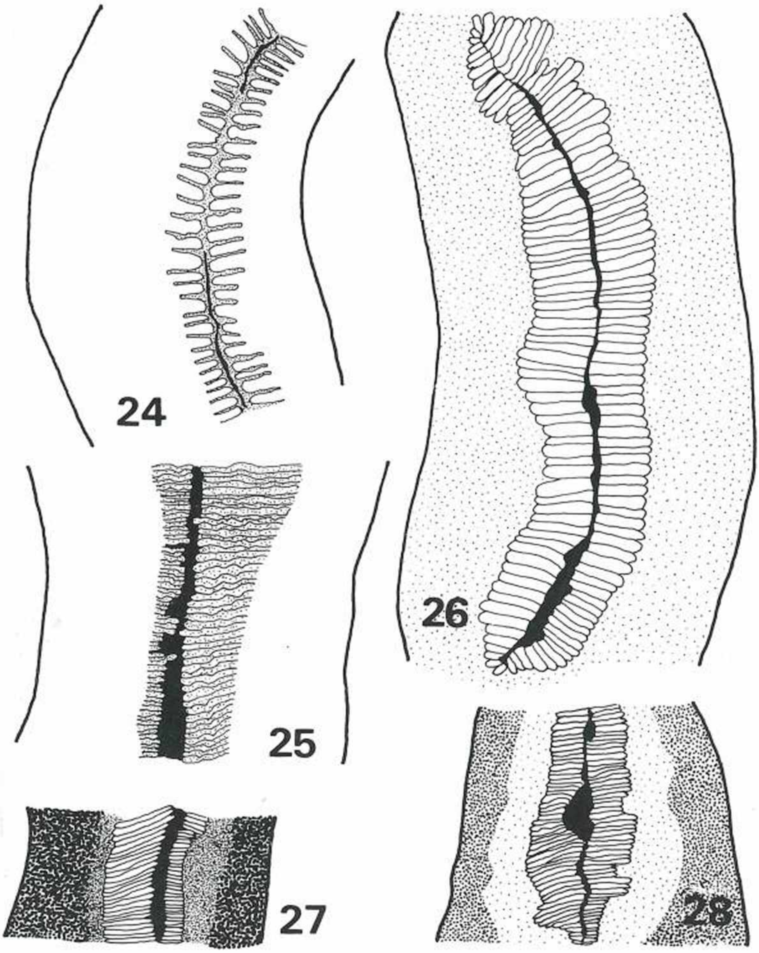
Figs. 49–55 illustrate cross-sections of the right cephalic tentacle. Figs. 51 and 53–55 show the vas deferens divided within the penis, forming two lumens: one of them terminated with a genital opening at the tip of the tentacle and the other terminated in the accessory opening mentioned above. The function of the accessory opening may be the same as that of



Figs. 14–23: Prostate and right cephalic tentacle including penis of studied *Viviparus* species (ek – eye knob, pr – prostate, t – tentacle tip with penis outlet, vd – vas deferens): 14–15 – *V. contectus*, 16 – *V. viviparus*, 17–18 – *V. acerosus*, 19–21 – *V. ater*, 22–23 – *V. hellenicus*. All drawings to same scale.

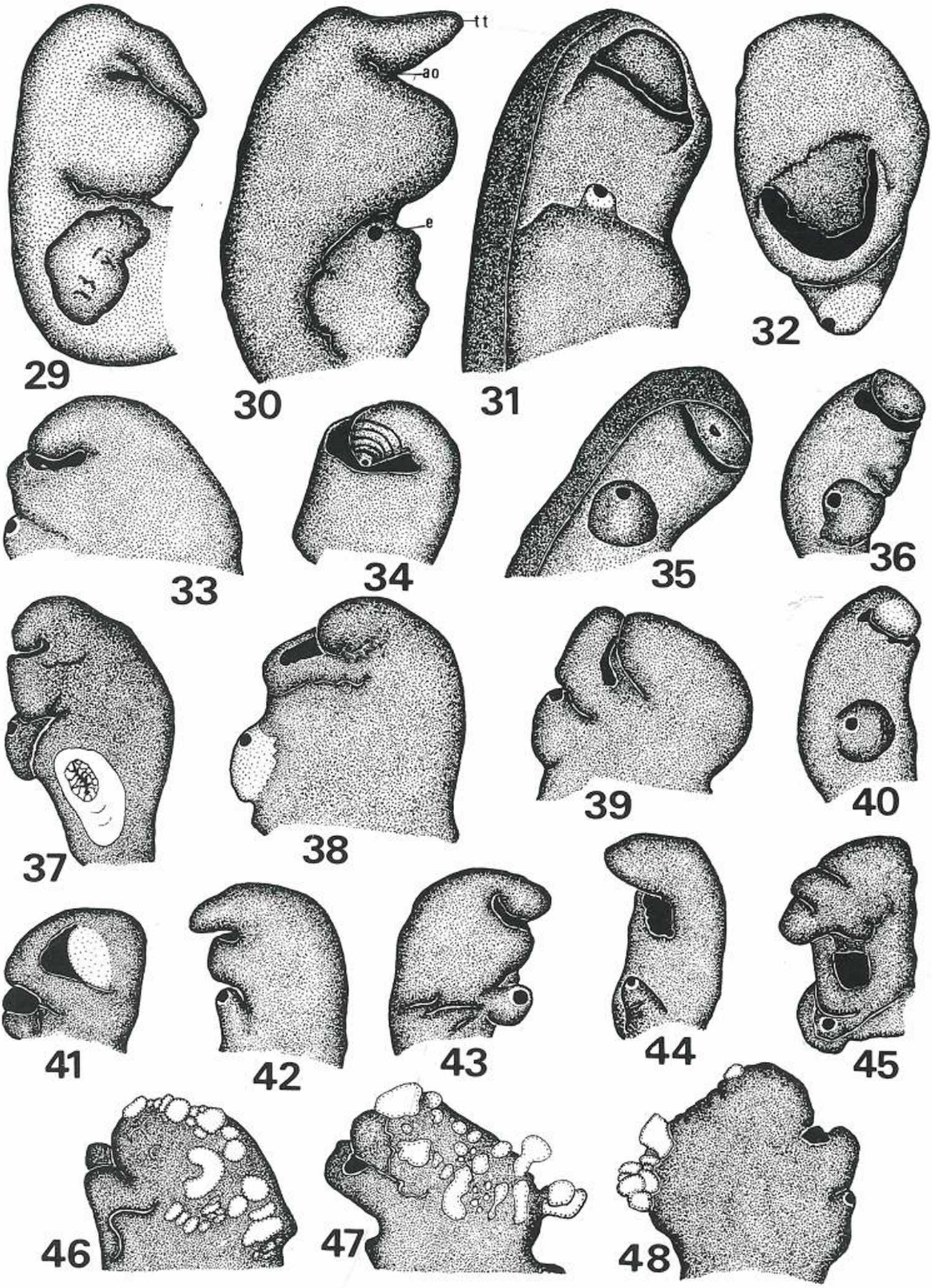
the accessory opening of the vas deferens into the mantle cavity, observed in numerous mesogastropods (FRETTER & GRAHAM, 1962). If the male is disturbed when copulating, the withdrawal of the penis and rapid contraction into the shell may only be possible with an escape of the seminal fluid from the vas deferens: the accessory opening of the vas deferens into the mantle cavity serves as an outlet for the sperm to relieve the pressure inside the vas deferens and prostate. The same function seems to have the accessory opening of the penis in *Viviparus*.

The female reproductive organs consist of a large ovary slightly distinguishable from the hepato-pancreas gland (Fig. 1), connected by an oviduct with the pallial section (Fig. 56), the most conspicuous structure of the latter being a vast brood pouch (Figs. 1 and 56). The oviduct reaches the proximal (posteriormost) end of the brood pouch and gets conspicuously broadened to form a thin-walled, spacious brood pouch terminating with a narrow,

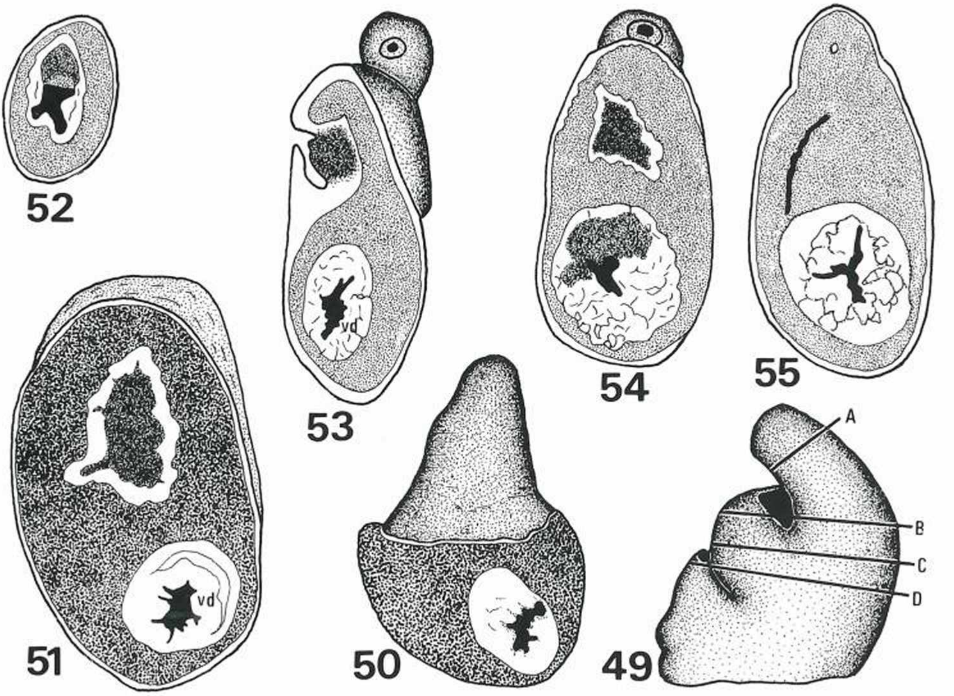


Figs. 24–28: Longitudinal section of *Viviparus* prostate: 24 – *V. acerosus*, 25 – *V. ater*, 26 – *V. contectus*, 27 – *V. hellenicus*, 28 – *V. viviparus*. All drawings to same scale.

thick-walled vagina. FRETTER & GRAHAM (1962) and VAIL (1977) describe a characteristic loop of the oviduct reaching the albuminoid gland and receptaculum seminis, both situated almost entirely ventral to the posterior end of the pallial oviduct (brood pouch). The drawings and descriptions of FALNIOWSKI (1989a, 1990) agree with the former descriptions in general but not in detail. The albuminoid gland is situated exactly as described, and spans along about $^{1}/_{3}$ of the brood pouch. The oviduct forms a sort of a loop. However, FALNIOWSKI (1989a, 1990) as well as the present study do not confirm the receptaculum seminis to have the form of a long, thick-walled ventral canal. The latter is rather short and thin-walled, and together with its duct it forms a U-shaped structure looking like the „oviduct“ shown by VAIL (1977). On the other hand, we have not studied the histology of those structures, thus we cannot resolve their homology. Anyway, as shown in Figs. 56–59, we use the term receptaculum seminis (with its duct) with regard to the thin-walled loop, and its dimensions and shape are employed as characters in the present study. The very flat sperm groove (Figs. 56–57) run-



Figs. 29–48: Right cephalic tentacle of *Viviparus* male, including distal part of penis (ao – accessory opening, e – eye, tt – tentacle tip with penis outlet); 33–34, 37–39, 41–42 and 44–47 – ventrally, 32 – apically, other – dorsally: 29–32 – *V. acerosus*, 33–34 – *V. contectus*, 35 – *V. viviparus*, 36–41 – *V. ater* (37 – visible cut surface with vas deferens cross section), 42–45 – *V. hellenicus*, 46–48 – abnormal penes of *V. ater*, with teratic (?) white papillae. All drawings to same scale.



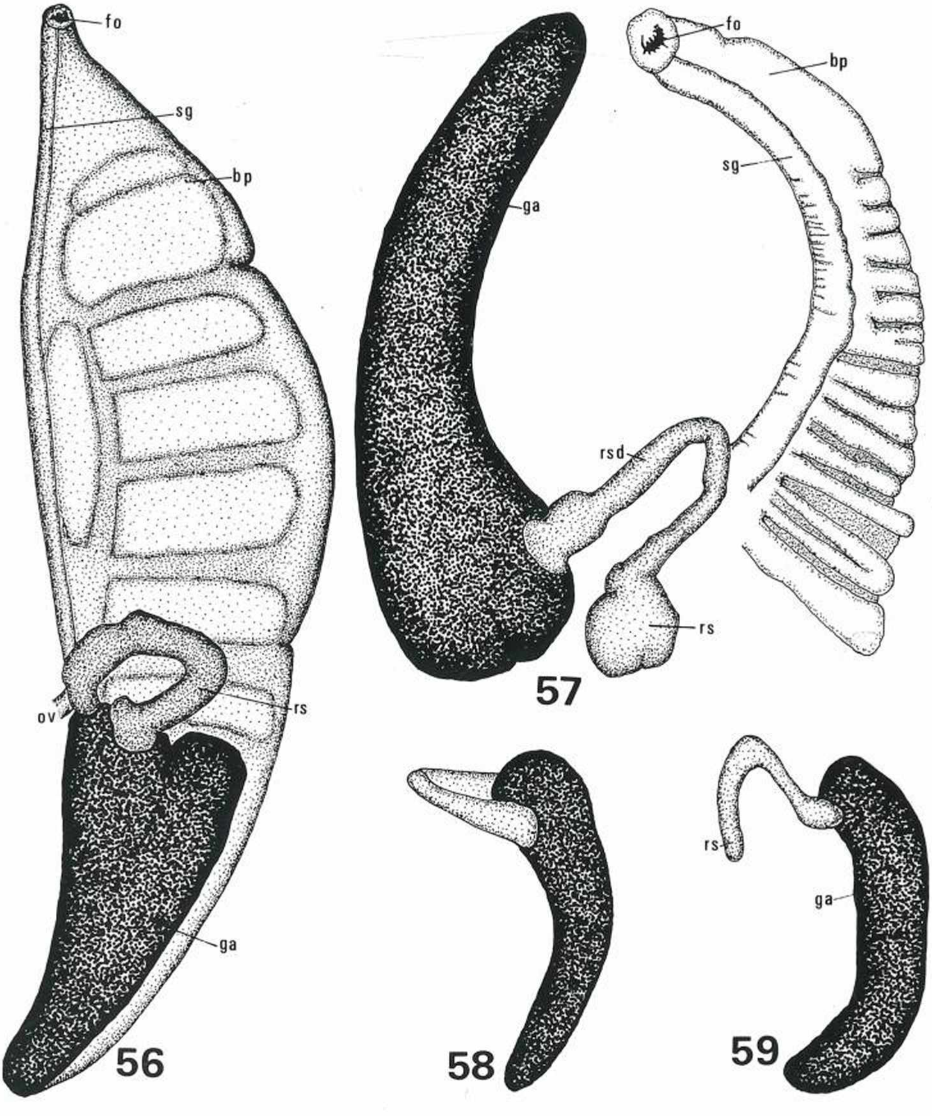
Figs. 49–55: Right cephalic tentacle of *Viviparus* male: 49 – habitus, ventrally, to show the position and direction of the following cross sections (A, B, C, D); 50–55 – cross sections of the tentacle, drawn to the same scale: 50–51 – *V. acerosus* (50 – at A, 51 – at C), 52–55 – *V. hellenicus* (52 – at A, 53 – at B, 54 – at C, 55 – at D) (vd – vas deferens).

ning ventrally, its poorly developed folds not separating the groove completely from the brood pouch, is similar in all the species and in *V. georgianus* described by VAIL (1977).

The shape of the receptaculum seminis with its duct (as defined above) is the only constant difference found between *V. contectus* and *V. viviparus* (FALNIOWSKI, 1989a, 1990). In the interspecific hybrid (FALNIOWSKI, KOZIK & SZAROWSKA, 1993) it was intermediate. We have found such intermediate receptacula in the population from the Hańcza Lake (Figs. 75–77), although the presumptive hybrids from the latter population have not been tested allozymatically. In the present study the receptaculum shape is widely variable infraspecifically, which is coupled with slight but rather constant interspecific differences (Figs. 60–74). The receptacula of *V. contectus* (Figs. 60–63) and *V. acerosus* (Figs. 67–68) differ in shape from each other and from the irregular sac-shaped receptacula of the other three species (Figs. 64–66 and 69–74). There also are interspecific differences in shell height to receptaculum length proportion (Table 3: character 12).

Within the brood pouch (Figs. 1 and 56), embryos may be scarce and packed in a single row in *V. ater* and *V. hellenicus*, or numerous and packed irregularly in a few parallel rows in *V. acerosus*, *V. contectus* and *V. viviparus*. There also are statistically significant interspecific differences in the median number of embryos within the pouch (Table 3: character 13).

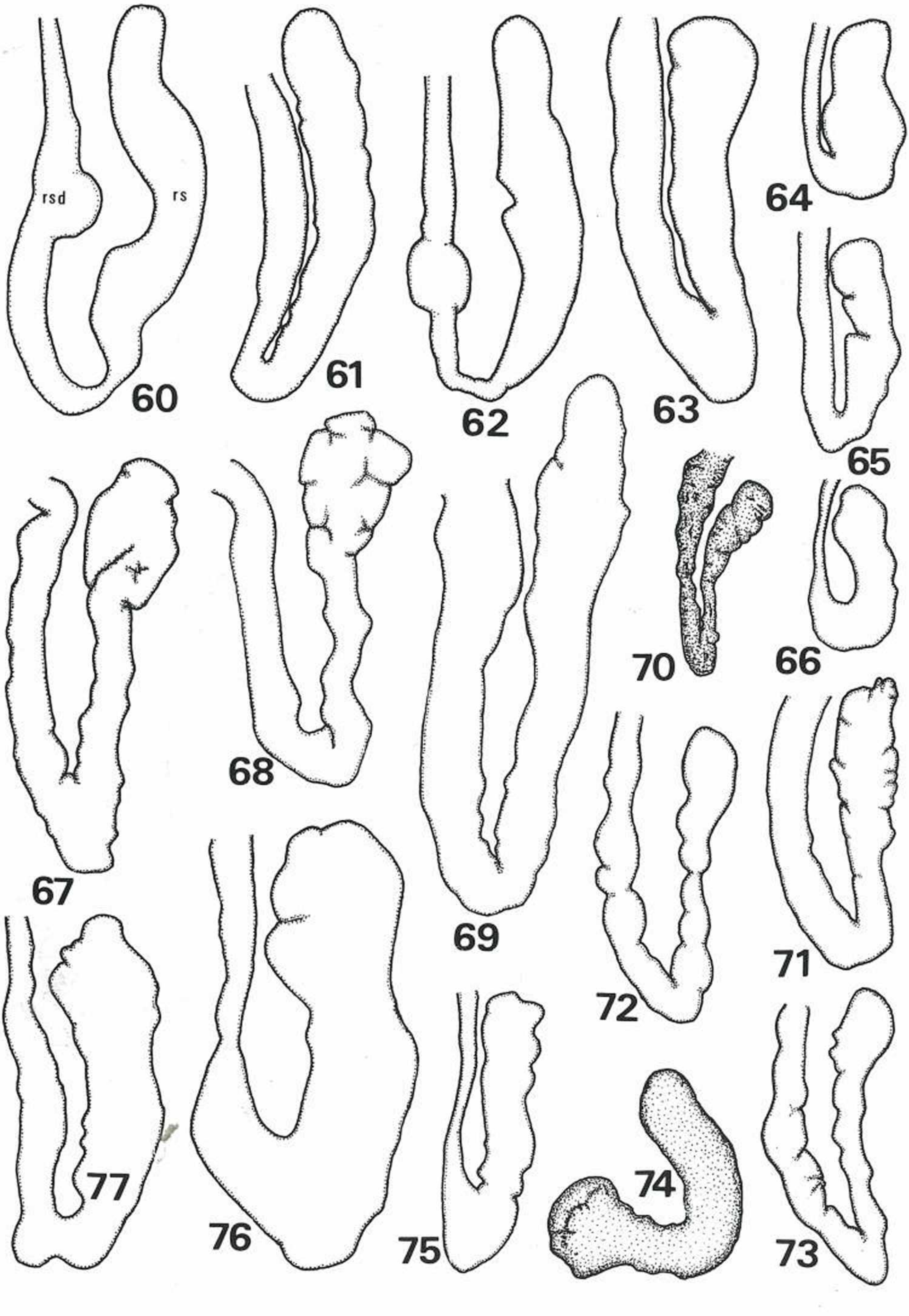
The vagina (Figs. 1 and 56–57) looks similar in all the studied species, but in *V. viviparus* it is relatively shorter than in the others (Table 3: character 14). It terminates with a female opening whose folds (Figs. 12–13 and 78–96), although variable, show species-characteristic patterns. The pattern found in the presumptive hybrid specimens (*V. contectus* x *V. vivi-*



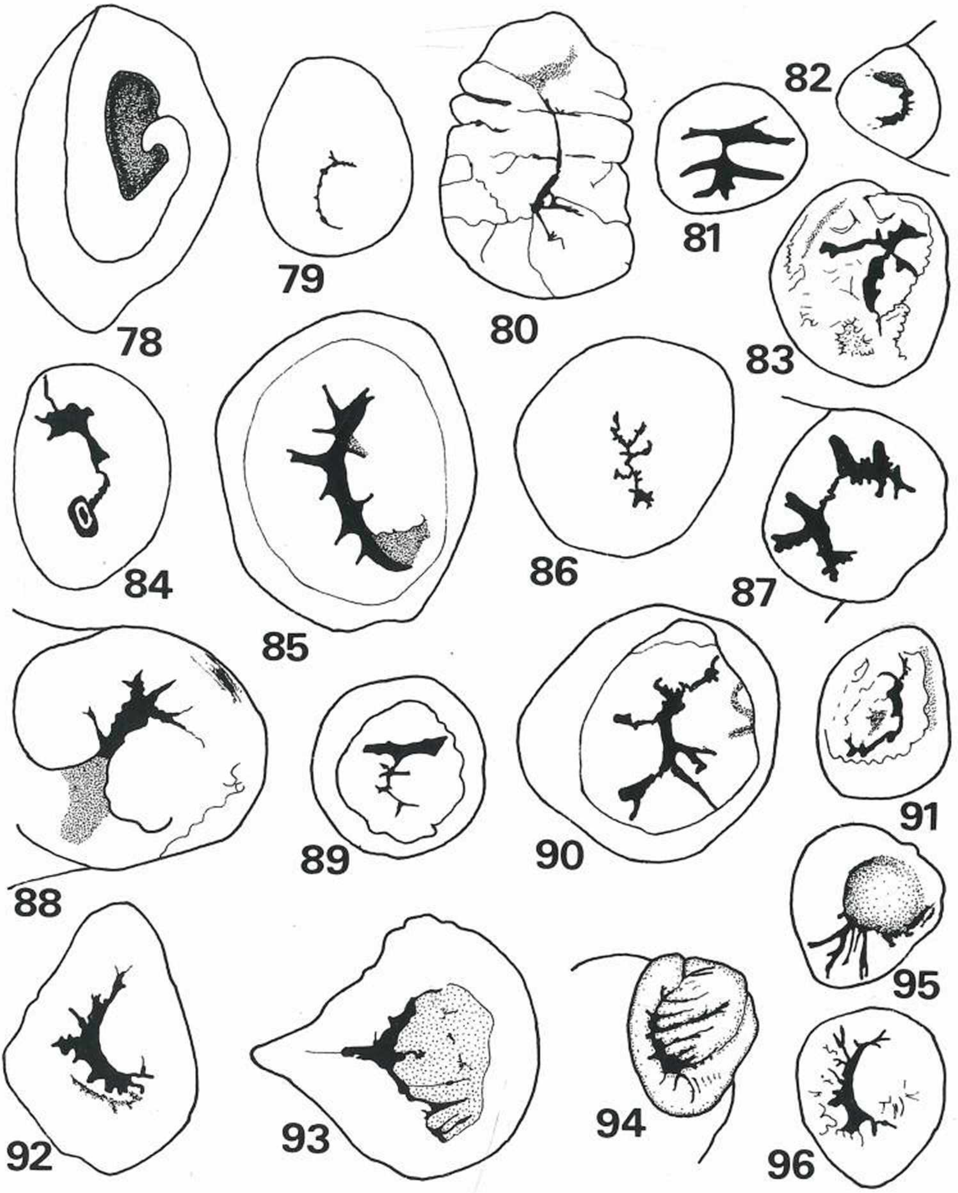
Figs. 56–59: Female reproductive organs of *Viviparus hellenicus*: 56 – whole pallial section; 57 – receptaculum seminis, albuminoid gland and distal part of empty brood pouch; 58–59 – receptaculum seminis and albuminoid gland (bp – brood pouch, fo – female opening, ga – albuminoid gland, ov – oviduct, rs – receptaculum seminis, rsd – duct of receptaculum seminis, sg – seminal groove).

parus) from the Hańcza Lake (Fig. 83) resembles the one characteristic of *V. acerosus* (Figs. 84–87); the same concerns the shell (FALNIOWSKI, KOZIK & SZAROWSKA, 1993).

In *V. contectus* (Figs. 12 and 78–79) the female opening is simple, u-shaped („u“ dichotomous at one tip), with no smaller folds. In *V. hellenicus* (Figs. 92–96) it is similar u-shaped, but with numerous folds and an elevated knob inside the „u“, which is absent in *V. contectus*. The opening in *V. acerosus* (Figs. 84–87), *V. ater* (Figs. 13 and 88–91) and *V. viviparus* (Figs. 80–82) differs between species, but in general it is one type: a radial pattern of folds resembling neither V. *contectus* nor *V. hellenicus*.



Figs. 60–77: Receptaculum seminis with duct in studied *Viviparus* species, ventrally (rs – receptaculum seminis, rsd – duct of receptaculum seminis): 60–63 – *V. contectus*, 64–66 – *V. viviparus*, 67–68 – *V. acerosus*, 69–71 – *V. ater*, 72–74 – *V. hellenicus*, 75–77 – hybrid specimens from the Hańcza Lake (*V. contectus* x *V. viviparus*). All drawings to the same scale.



Figs. 78–96: Terminal part of vagina showing folds in female opening in *Viviparus* species: 78–79 – *V. contectus*, 80–82 – *V. viviparus*, 83 – hybrid specimen from the Hańcza Lake (*V. contectus* x *V. viviparus*), 84–87 – *V. acerosus*, 88–91 – *V. ater*, 92–96 – *V. hellenicus*. All drawings to the same scale.

Phylogenetic implication of the opercular and soft part morphology characters

The characters that show interspecific variation are summarized in Table 3. This was used as an input for MACCLADE, and the shortest tree was found with PAUP (Fig. 97: only unambiguous changes shown in the tree). Its statistics is: tree length: 41, consistency index

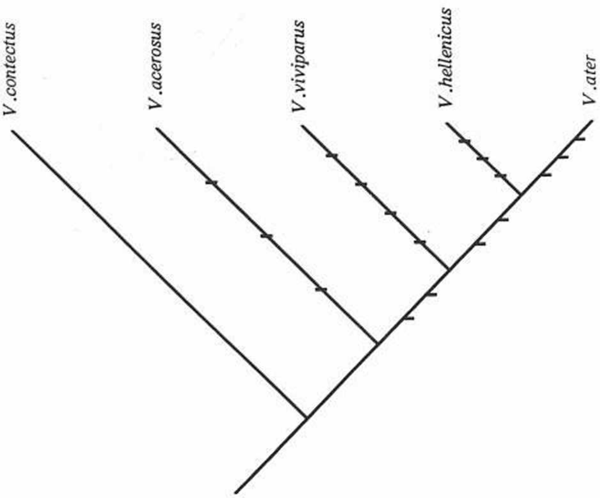


Fig. 97: MPR based on opercular and soft part morphology characters. Only the characters that change unambiguously on branch are shown by bars.

CI: 0.854, retention index RI: 0.400, rescaled consistency index RC: 0.341, minimum: 35, maximum: 45, 39 changes (Maddison & Maddison, 1992). However, the applied data set has resulted in an unresolved trichotomy, so it is impossible to define relationships between *V. contectus* and *V. acerosus* unequivocally. The data set has not enabled us to root the tree, either. Thus the latter has been done basing on radular character polarities concluded due to ontogenetic criteria (Falniowski, Mazan & Szarowska, 1996a).

From among the 20 characters, many are not evolutionary sound and/or allow for more than one reconstruction of the character evolution inferred from the tree shown in Fig. 97. Thus our conclusions as to the character evolution are limited. As a result of preliminary analysis of phylogeny and character evolution, characters: 3, 5, 6, 8, 9, 12, 14, 15, 16 and 19 are treated as ordered, and the others as unordered. In character 4 – ctenidium length to shell height proportion – there is an apparent evolutionary tendency to reduction of the relative length of the ctenidium (transformation series: 3 - 2 - (1, 0), thus the relatively longest ctenidium is the ancestral state. On the other hand, the ancestral state of ctenidium length to breadth proportion (character 5) is 1 (medium). The ancestral state of ctenidium lamellae number to shell height proportion (character 6) is 0 – the lowest value: a growing value is an advanced state reflecting probably the growing efficiency of ciliary feeding mechanism (Cook, 1949; Falniowski, Mazan & Szarowska, 1996a). In character 8 – osphradium length to breadth proportion – the ancestral state is 0, which means less slender; there are two autapomorphies, and an apparent sexual dimorphism (Table 3) in this character.

In character 9 the ancestral state is 0. That means that the decrease of the length of the distal lobe of the testis relatively to shell height is advanced. The number of embryos in the brood pouch (character 13) shows transformation series 3 - 2 - (1, 0), so the highest number is an ancestral state, and reduction is advanced. In character 16 – pigmented zones inside the prostate – the ancestral state is absent (0). Numerous irregular rows of embryos inside the brood pouch (character 19: state 0) is an ancestral and one regular row an advanced state. The simple „u“ seems morphologically basic for the other two states describing the female opening, this, however, is only one of the two equivocal reconstructions of the character evolution.

Although autapomorphies tell us nothing about relationships, their occurrence indicates

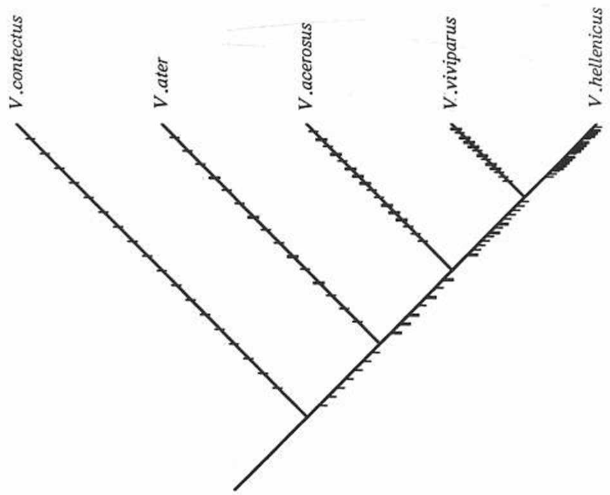


Fig. 98: MPR based on all opercular, soft part morphology, radular and embryonic shell characters. On each branch there are shown almost all possible character changes; the ones that change unambiguously are shown by thick bars.

quite well the amount of distinctness and anagenetic evolution within a clade. In the light of the phylogeny reconstruction presented in Fig. 97, there are no undoubtedly autapomorphic character states in *V. contectus*, three autapomorphies in *V. acerosus*, five in both *V. hellenicus* and *V. viviparus*, and six in *V. ater*. On the other hand, the total number of synapomorphies in the group/data set is relatively low, which makes the inferred phylogeny rather weak. Also the tree statistics indicates a relatively high amount of reversals and parallelisms. And, last but not least, the total number of 20 characters found in all the opercular and anatomical characteristics is strikingly low. In viviparids the above characters are found more useful as a basis for phylogeny reconstruction than the ones describing embryonic shells (FALNIOWSKI, MAZAN & SZAROWSKA, 1996b), but less than the characters of the radula (FALNIOWSKI, MAZAN & SZAROWSKA, 1996a).

Phylogeny inferred from operculum, soft parts, radula and embryonic shells

All the characters discussed above have been used together with the radular characters described by FALNIOWSKI, MAZAN & SZAROWSKA (1996a), and the embryonic shell characters given by FALNIOWSKI, MAZAN & SZAROWSKA (1996b). PAUP runs (exhaustive search option) have resulted in one MPR presented in Fig. 98. Its statistics is: length 77, CI: 0.831, RI: 0.381, RC: 0.317, minimum 64, maximum 85, 74–77 changes (MADDISON & MADDISON, 1992). Compared with the tree presented in Fig. 97, it presents even less consistent phylogeny. On the other hand, the cladogram is identical to the one constructed on the radular characters solely (FALNIOWSKI, MAZAN & SZAROWSKA, 1996a).

Fig. 99 shows the same MPR as a „phyllogram“ (MADDISON & MADDISON, 1992), with the branch lengths proportional to the amount of change along a branch. It reflects only unambiguous changes. *V. contectus* and *V. ater* are very close to each other and primitive (again, the tree rooted basing on the radular character polarities concluded due to ontogenetic criterion – FALNIOWSKI, MAZAN & SZAROWSKA, 1996a). *V. hellenicus* is the most advanced species, its clade shows the highest amount of change. *V. viviparus*, also advanced, is close to, but clearly different from *V. hellenicus*. *V. acerosus* is intermediate (between the primi-

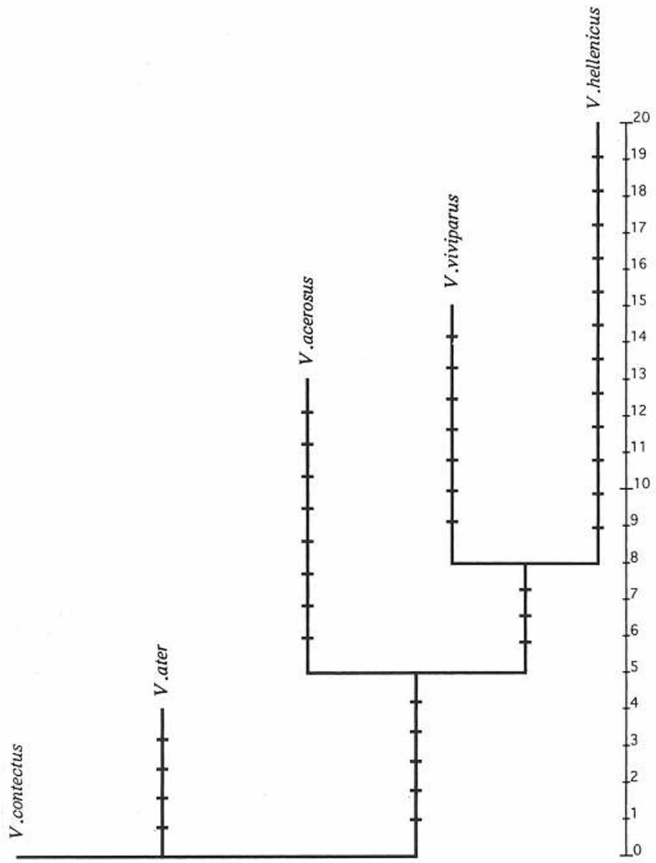


Fig. 99: The same MPR as in Fig. 98, but shown in the form of a „phyllogram" (Maddison & Maddison, 1992) with branches lengths made proportional to the amount of change along a branch, only unambiguous changes considered.

tive and advanced groups). The cladogram rejects the commonly acknowledged subspecies status of *V. hellenicus* (*V. ater hellenicus*, e.g. Schütt, 1962). In general, all the interspecific differences – unnumerous, in fact – are marked slight and, morphologically, the species seem very close to each other. This needs confirmation by a future study of another data set.

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Autor(en)/Author(s): Falniowski Andrezej, Mazan Krystyna, Szarowska Magdalena, Kozik Andrzej

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