Comparative karyological analysis of five species of viviparus (gastropoda: prosobranchia)

Baršiene, J ; Ribi, G ; Baršyte, D

DOI: https://doi.org/10.1093/mollus/66.2.259

J. Moll. Stud. (2000), **66**, 259–271

COMPARATIVE KARYOLOGICAL ANALYSIS OF FIVE SPECIES OF *VIVIPARUS* (GASTROPODA: PROSOBRANCHIA)

[1]J. BARŠIENE, [2]G. RIBI, [1,3]and D. BARŠYTE
[1]*Institute of Ecology, Akademijos 2, 2600 Vilnius, Lithuania,* [2]*Zoologisches Museum der Universitat Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland,* [3]*School of Biological Sciences, Stopford Building, Oxford Road, Manchester University, M13 9PT Manchester, U.K.*
(Received 29 March 1999; accepted 19 October 1999)

ABSTRACT

Karyological analysis was performed on *Viviparus ater* (Cristofori & Jan, 1832), *V. acerosus* Bourguignat, 1862, *V. mamillatus* (Kuster), *V. viviparus* (Linnaeus, 1758) and *V. contectus* (Millet, 1813), collected from different freshwater bodies of Switzerland, Hungary, Albania, Italy and Lithuania. The karyotypes of *V. acerosus* and *V. mamillatus* are described for the first time. The diploid number of chromosomes in *V. contectus* equals 14, whereas in diploid sets of other studied species, 18 chromosomes are present. The karyotype formula is in *V. contectus* (5m + 2sm, NF = 28, in V. *ater*, 7m + 1sm-m + 1 sm, in *V. acerosus* and *V. viviparus*, 8m + 1sm, NF = 36, in *V. mamillatus*, 6m + 1m-sm + 1sm-m + 1sm, NF = 36. In females of V. *ater*, *V. mamillatus* and *V. acerosus*, the heteromorphism of chromosome pair no. 8 was observed, with a sex-determining mechanism—ZW female /ZZ male. Although, Z and W chromosomes are metacentric, significant differences ($P < 0.05$, or $P < 0.001$) in their size were determined. The interspecific significant differences ($P < 0.05$) in karyotypes of *V. ater*, *V. mamillatus*, *V. acerosus* and *V. viviparus* were detected by using one-way ANOVA and Bonferroni's Multiple Comparison tests. Only chromosomes of the pair no. 5 were of similar shape in all of these species. The smallest interspecific difference was between *V. viviparus* and *V. acerosus*. The intraspecific karyological differences in relative chromosome length and centromeric index of *V. contectus* from lakes Garda (Italy), Olauka and Asveja (Lithuania) were observed in the chromosome pair no. 5.

INTRODUCTION

Taxonomic questions concerning the genus *Viviparus* in Europe arise for two reasons. First, there is morphological variation within and between populations, which is often difficult to interpret, and secondly the same species names have often been used for different taxa by different authors (Franz, 1932). A recent identification guide mentions five *Viviparus* species in Europe (Fechter & Falkner, 1990). *V. viviparus* (Linne), which used to be called *V. fasciatus* (O.F. Müller); *V. ater* (Christofori & Jan), including former *V. pyramidalis* (O.G. Müller); *V. acerosus* (Bourguignat), including *V. hungaricus* (Hazay); *V. mamillatus* (Kuster); and *V. contectus* (Millet), which was called *V. viviparus* by authors for a long time (Fechter & Falkner, 1990).

Viviparus contectus can easily be distinguished from all other species by a wide shell with shouldered whorls separated by a deep suture, and a pointed apex. The remaining four species all have less prominent whorls and usually a blunt apex. The variability within species is so high that a shell of unknown origin can rarely be assigned to the species. Thus, the species identification of the four species is mainly based on their geographical provenance. *V. viviparus* lives in northern and eastern Europe. *V. ater* is restricted to northern Italy and to some Swiss lakes, where it was introduced (Roth, 1906). *V. acerosus* lives in the Danube River, *V. mamillatus* on the Balkan Peninsula. In contrast to these rather limited areas, *V. contectus* is distributed throughout Europe, overlapping the distributions of the remaining four species. *V. contectus* lives sympatrically with *V. viviparus* and *V. ater* at many places, and it has been recorded to hybridize with both these species (Falniowski, Kozik, Szarowska, Fialkowski & Mazan, 1993: Katoh & Ribi, 1996). This is astonishing insofar as *V. contectus* has 7 chromosomes in the haploid set, whereas *V. viviparus* and *V. ater* both have 9 chromosomes (Franz, 1932; Rainer, 1963; Baršiene, 1991, 1994; Baršiene, Baršyte & Virbickas, 1996; Baršiene & Ribi, in press).

In the present paper, we report cytogenetical data for *Viviparus contectus*, *V. viviparus*, *V. ater*, *V. acerosus* and *V. mamillatus*. The main aim of this study was to examine peculiarities of interspecific karyological differentiation.

MATERIALS AND METHODS

Karyological studies were carried out on somatic cells of embryos and juveniles, and gonadel cells of males. Snails were collected in the lakes Murten, Lauerz, Zurich, Constance (Switzerland), Scutari (Albania), Garda (Italy), Olauka and Asveja (Lithuania), in the rivers Neris and Nemunas (Lithuania) and in Viragoskuti Halasto canal (Hungary) (Table 1). Blocking somatic (including embryonic) and gonadal cell divisions at metaphase was achieved by injection of a 0.1–0.2% aqueous solution of colchicine into the adult snails 4–10 hours before they were dissected. Juvenile stages were placed directly in 0.01–0.02% solution of colchicine. The different stages of sample fixation, chromosome preparation and identification were the same as those previously described by Baršiene (1978) and Baršiene & Grabda-Kazubska (1988). Chromosomes were stained with a 4% solution of Giemsa, prepared in phosphate buffer, pH = 7.0. Metaphase plates, suitable for karyological analysis, were photographed and photomicrographs were used for construction of karyotypes. Karyometric characteristics have been determined by measuring chromosomes in 15 karyotypes from each species and different populations of *V. ater*. The following karyometric parameters were used: the absolute length of chromosomes in micrometers, their relative length and centromeric index. The chromosomes were classified by the system of Levan, Fredga & Sandberg (1964). Where the standard deviation of the centromeric index was at the borderline between two types of chromosomes, the nomenclature for both chromosome types is given.

Statistical significance of inter- and intraspecific differences in karyotypes was determined by using ANOVA single factor and Bonferroni's Multiple Comparison tests.

RESULTS

Chromosome counts in 6241 cells of 455 specimens were made. The diploid number of chromosomes of *V. ater*, *V. acerosus*, *V. mamillatus* and *V. viviparus* was 2n = 18 (Fig. 1). Fourteen chromosomes in diploid sets were observed in the majority of cells of *V. contectus* inhabiting lakes Garda, Asveja and Olauka (Fig. 2). Seven bivalents were determined at the prophase I in the male meiosis of *V. contectus*. In the meiosis of males of *V. ater,* V. acerosus. *V. mamillatus* and *V. viviparus* there were nine bivalents (Fig. 3). Modal sets were observed in 82.2–89.8% of the studied cells. Nuclei with less than 2n = 18, or 2n = 14 chromosomes were found in 4.9–10.9%, whereas polyploid sets were found in 0.6–11.4% of the cells. Hyperdiploid cells were rarely observed (Table 2).

Table 1. Material for karyological studies of *Viviparus*

Species	Locality	No of snails studied
Viviparus ater	Lakes Murten, Zürich, Lauerz, Constance (Switzerland)	290
V. acerosus	River Viragoskuti Halasto (Hungary)	13
V. mamillatus	Lake Scutari (Albania)	20
V. viviparus	Rivers Vilnia, Neris, Nemunas (Lithuania)	54
V. contectus	Lake Garda (Italy)	21
	Lakes Asveja and Olauka (Lithuania)	28

Table 2. Chromosome number variability of *Viviparus*

Species	Hypodiploid cells, %	Modal cells, %	Hyperdiploid cells, %	Polyploid cells, %	No. of cells studied
V. ater	10.9	83.6	0.5	5.0	4049
V. acerosus	9.6	89.8	–	0.6	345
V. mamillatus	10.5	87.9	0.1	1.5	825
V. viviparus	4.9	82.2	1.5	11.4	611
V. contectus					
(Italy)	8.4	88.8	–	2.8	36
(Lithuania)	10.9	86.6	0.2	`2.3	385

Karyotype structure of V. contectus

Karyological studies of *V. contectus* from Lake Garda were performed only in embryonic cells. However, mitotic activity was low and we were able to study chromosomes in 36 mitotic metaphase and to make chromosome measurements in 5 karyotypes. Pairs no. 1 and 2 consists of large, pairs no. 6 and 7 of small metacentric chromosomes. There are three pairs (no. 3–5) of medium-sized biarmed chromosomes (Table 3).

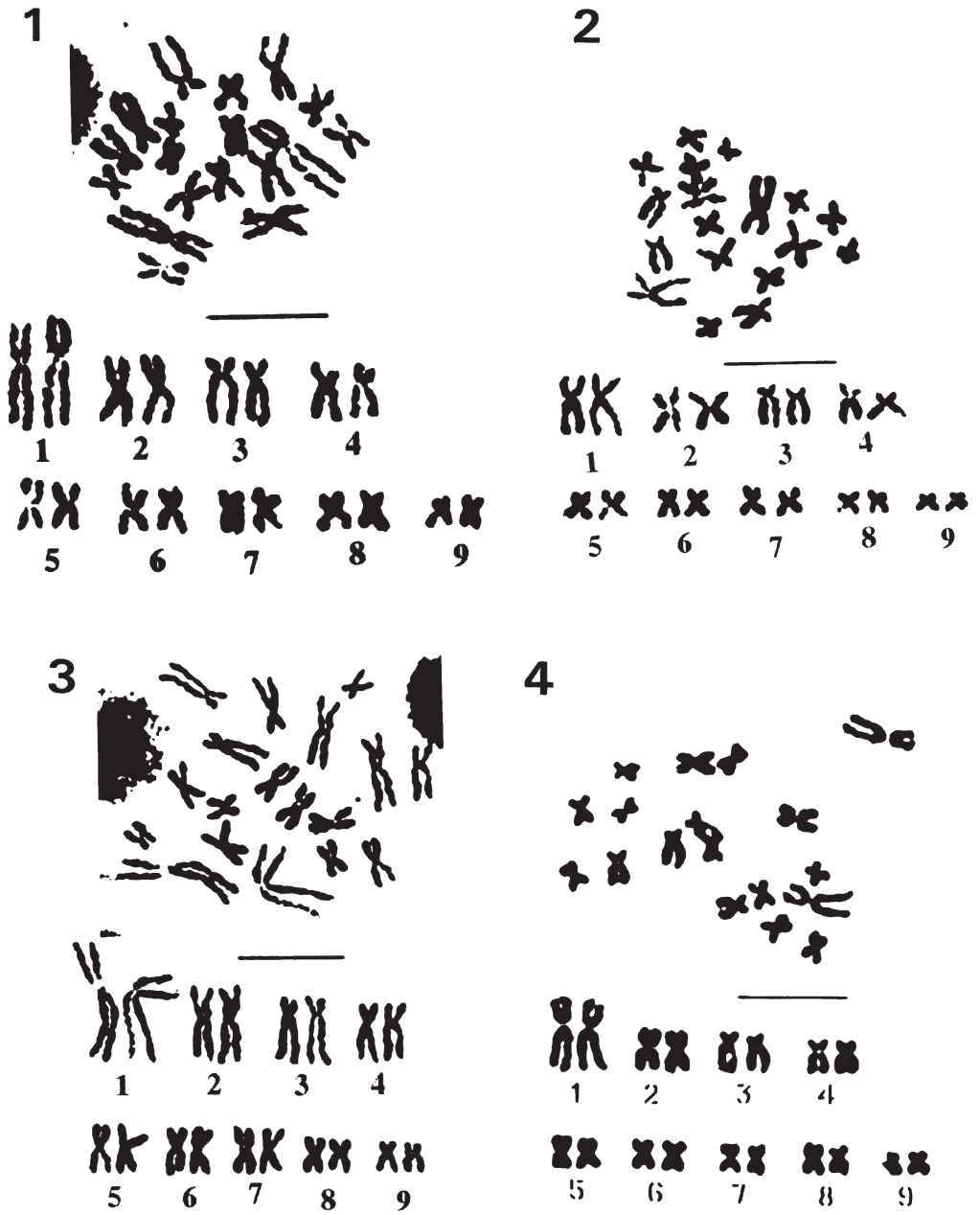

Figure 1. Metaphase chromosomes and karyotypes of: 1—*V. ater* from Lake Murten, 2—*V. acerosus*, 3—*V. mamillatus*, 4—*V. viviparus* from the river Nemunas. Scale is 10 μm.

Figure 2. Mitotic metaphases and karyotypes of *V. contectus*: above—from Lake Garda (Italy), below—from Lake Olauka (Lithuania). Scale is 10 μm.

Table 3. Measurements of chromosomes of *V. contectus* from Lake Garda (M; SD)

Pair no.	Chromosome length (μm)		Relative length (%)		Centromeric index		Chromosome type
1	7.00	1.83	20.70	0.24	38.75	0.91	m
2	6.55	1.90	19.25	0.63	38.85	0.14	m
3	4.70	1.41	13.75	0.49	45.30	0.84	m
4	4.65	1.34	13.70	0.42	31.95	0.31	sm
5	4.10	0.84	12.20	0.56	26.10	1.27	sm
6	3.60	0.70	10.75	0.63	49.70	0.42	m
7	3.25	0.63	9.62	0.49	49.65	0.49	m

A more detailed analysis of chromosome sets was carried out (in 385 cells) on males of *V. contectus* inhabiting Olauka and Asveja lakes in Lithuania. The karyotype structure was similar to that in *V. contectus* from Garda Lake. The centromeric indices in chromosome pairs no. 4 and 5 were at the borderline between submetacentric and metacentric chromosome types and the nomenclature for both centromere positions is shown (Table 4). Highly significant interpopulation differences in the relative length and centromeric index of chromosomes from pair no. 5 ($P < 0.0001$) were determined.

Karyotype structure of V. ater, V. acerosus, V. mamillatus *and* V. viviparus

The absolute lengths of chromosomes ranged from 2.08 to 8.99 μm (Table 5). Examination of the absolute length of the total haploid set showed that *V. acerosus* (30.60 μm) and *V. viviparus* (32.49 μm) had the smallest chromosomes. The total length of the haploid set of *V. ater* was 13–15 μm larger than that of *V. acerosus*. The variability in length was due to differences between embryonic cells and those of adult or juvenile specimens. Chromosome measurements in *V. acerosus* were made in the cells of juveniles (one year of age) whereas in *V. viviparus* gonadal cells of males were used. The same parameters of *V. ater* and *V. mamillatus* were obtained by measuring of chromosomes mainly in the cells of embryos. It is known that chromosomes in embryonic nuclei are less constructed (Nakamura, 1986).

In spermatogonial nuclei, all chromosome pairs of *V. ater*, *V. acerosus*, *V. mamillatus* and *V. viviparus* males were phenotypically homomorphic, while in females of *V. ater*, *V. acerosus* and *V. mamillatus* heteromorphism of size was observed in chromosome pair no. 8 (Fig. 4). Therefore, we suggest a ZZ–ZW sex-determin-

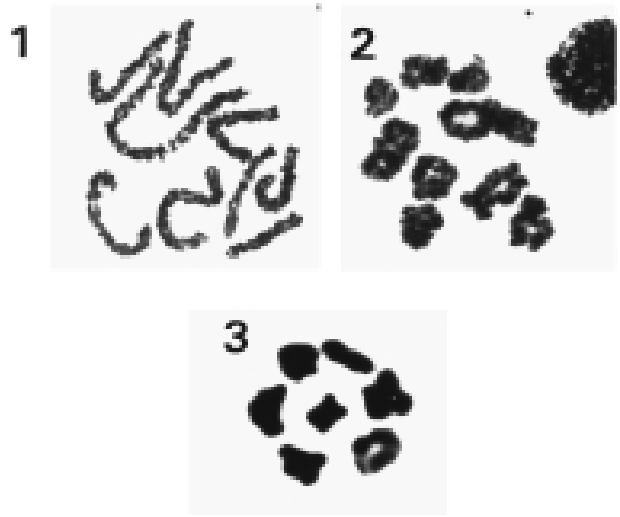

Figure 3. Meiosis in the cells of viviparid males: 1—pachytene of *V. ater*, n = 9; 2—diakinesis of *V. acerosus*, n = 9; 3—diakinesis of *V. contectus*, n = 7.

Table 4. Measurements of chromosomes of *V. contectus* from lakes Olauka and Asveja in Lithuania (M; SD)

Pair no.	Chromosome length (μm)		Relative length (%)		Centromeric index		Chromosome type
1	5.53	0.87	19.35	0.52	39.31	1.93	m
2	5.37	0.79	18.88	0.58	40.05	1.82	m
3	4.02	0.60	14.11	0.24	45.16	3.14	m
4	3.89	0.56	13.65	0.33	35.17	1.97	sm-m
5	3.78	0.46	13.31	0.47	36.15	1.88	sm-m
6	3.06	0.48	10.77	0.77	49.36	1.06	m
7	2.83	0.43	9.93	0.53	49.71	0.49	m

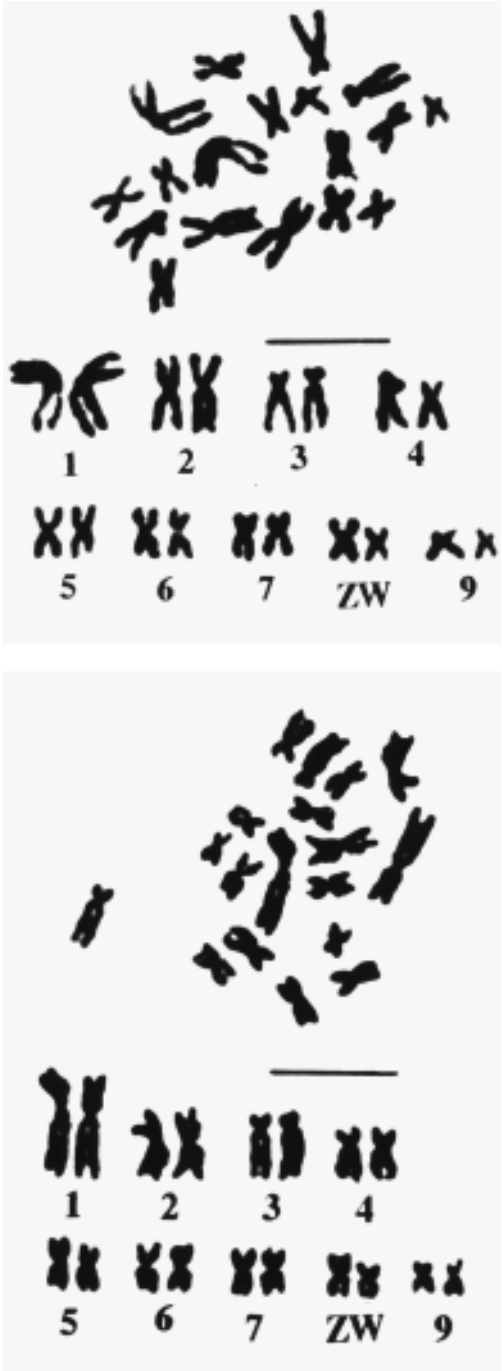

Figure 4. Mitotic metaphases and karyotypes of viviparid females: above—*V. mamillatus*, below—*V. ater* from Lake Lauerz. Scale is 10 μm.

ing mechanism for these species. The karyotypes of *V. viviparus* were analysed only in the gonadal cells of males and at present the sex-determining mechanism remains unknown.

Chromosomes of the first pair constitute 18.86–21.14% of the total length of the haploid complement. All other chromosomes were arranged in a row of units gradually decreasing in length (Table 6). There were significant differences between the relative length of the first chromosome pair of *V. mamillatus* and other species, as well as between these parameters of pair no. 3 of *V. viviparus* and *V. mamillatus*, *V. ater* (Lauerz population) (Table 6).

All chromosomes of the viviparid species studied were biarmed. Chromosome pair no. 3 was submetacentric in all species. Metacentric or submetacentric-metacentric chromosomes were determined in pair no. 4. All other pairs consist of metacentric chromosomes. The greatest interspecific differences between *V. mamillatus* and other species were expressed in the centromere location in pair no. 9 (Table 7).

Analysis of variance by using ANOVA one-way test showed that differences between relative lengths of the Viviparus species studied (for *V. ater* data of two populations were included) exist in all chromosome pairs excepting pair no. 5. Insignificant differences in centromeric indices were noticed in pairs no. 4 to no. 7 and in 8Z chromosome (Table 8). The significant differences between species were determined with Bonferroni's Multiple Comparison test. The smallest interspecific difference was between *V. viviparus* and *V. acerosus* (Table 9).

DISCUSSION

Chromosome sets within the family Viviparidae have been studied in representatives of three subfamilies. The haploid chromosome number ranges from n = 7 in *V. contectus* to n = 14 in the subfamily Lioplacinae (Patterson, 1969), whereas the haploid chromosome number in the subfamily Viviparinae ranged from n = 7 to n = 13 (Table 10).

Most of the earlier chromosome studies in viviparid snails were based on tissue sectioning, or squashing methods. As a consequence of this, there is some doubt regarding the accuracy of Franz's (1932) report on the chromosome number n = 10 in *V. viviparus*. In recent years, methods of karyological analysis have greatly improved, allowing more reliable hypotheses of phylogeny and evolution. We provide new data on karyotype structures of two re-examined

Table 5. Measurements of absolute chromosome length (M, μm; SD)

Pair no.	*V. ater* (L. Lauerz)		*V. ater* (L. Murten)		*V. acerosus*		*V. mamillatus*		*V. viviparus*	
1	8.99	2.00	8.32	1.21	5.82	1.03	7.65	1.79	6.29	0.94
2	6.44	1.47	6.07	0.65	4.29	0.51	5.23	1.11	4.30	0.60
3	5.78	1.13	5.83	0.77	3.96	0.61	4.57	1.07	4.13	0.68
4	4.87	1.02	4.93	0.65	3.39	0.41	3.92	0.91	3.65	0.68
5	4.62	0.89	4.33	0.53	3.06	0.44	3.56	0.79	3.25	0.54
6	4.45	0.93	4.25	0.52	2.97	0.35	3.36	0.75	3.19	0.50
7	4.23	0.91	3.98	0.52	2.77	0.32	3.17	0.76	2.93	0.54
8Z	3.77	0.61	3.43	0.47	2.60	0.35	2.87	0.67	2.46	0.48
8W	3.20	0.35	3.10	0.31	1.91	0.30	2.20	0.41	–	–
9	2.76	0.49	2.81	0.37	2.08	0.33	2.19	0.48	2.29	0.42

M—average means, SD—standard deviations.

Table 6. Measurements of relative chromosome length (M, %,; SD)

Pair no.	*V. ater* (L. Lauerz)		*V. ater* (L. Murten)		*V. acerosus*		*V. mamillatus*		*V. viviparus*	
1	19.66	1.01	18.96	0.97	18.86	1.51	21.14	1.01	19.37	1.01
2	14.18	1.12	13.86	0.67	14.00	0.54	14.39	0.92	13.21	0.72
3	12.78	0.58	13.36	0.65	12.85	0.57	12.60	0.57	12.71	0.57
4	10.73	0.42	11.33	0.46	11.05	0.47	10.75	0.43	11.22	0.41
5	10.13	0.45	9.88	0.42	9.95	0.40	9.79	0.46	10.02	0.31
6	9.73	0.40	9.68	0.33	9.66	0.39	9.27	0.46	9.81	0.30
7	9.21	0.47	9.08	0.47	9.03	0.46	8.69	0.36	9.03	0.61
8Z	8.21	0.62	7.89	0.42	8.49	0.30	7.89	0.61	7.55	0.61
8W	6.66	0.44	6.96	0.37	6.24	0.47	6.09	0.43	–	–
9	6.13	0.76	6.41	0.43	6.76	0.30	6.04	0.45	7.05	0.60

Table 7. Centromeric indices and classification of chromosomes (M; SD)

Pair no.	*V. ater* (L. Lauerz)		*V. ater* (L. Murten)		*V. acerosus*		*V. mamillatus*		*V. viviparus*	
1	40.03	1.74 (m)	39.92	2.10 (m)	40.45	1.87 (m)	38.53	2.42 (m)	37.88	1.42 (m)
2	44.81	2.16 (m)	48.69	1.79 (m)	48.55	1.52 (m)	47.20	1.86 (m)	47.39	2.15 (m)
3	27.72	2.58 (sm)	27.06	3.21 (sm)	29.69	1.61 (sm)	27.62	3.27 (sm)	30.90	3.03 (sm)
4	35.29	3.80 (sm-m)	34.61	3.24 (sm-m)	39.48	2.86 (m)	35.60	2.99 (sm-m)	39.28	2.86 (m)
5	49.26	0.90 (m)	49.59	0.51 (m)	49.88	0.28 (m)	49.08	1.94 (m)	49.89	0.41 (m)
6	49.78	0.40 (m)	49.50	1.16 (m)	49.59	0.46 (m)	48.63	2.27 (m)	49.83	0.29 (m)
7	49.86	0.24 (m)	49.43	1.48 (m)	49.48	0.66 (m)	49.04	1.61 (m)	49.57	0.50 (m)
8Z	49.07	0.46 (m)	49.03	1.07 (m)	49.50	0.34 (m)	49.27	1.06 (m)	49.80	0.45 (m)
8W	47.23	3.30 (m)	47.15	0.71 (m)	49.26	0.23 (m)	49.06	1.00 (m)	–	–
9	45.11	3.16 (m)	48.39	1.33 (m)	45.88	2.60 (m)	37.67	3.02 (m-sm)	47.75	1.51 (m)

M—metacentric, sm—submetacentric, st—subtelocentric chromosomes.

species—*V. viviparus* and *V. contectus*. The karyotypes of *V. acerosus* and *V. mamillatus* are described for the first time.

Ideograms of mean values of the relative lengths and centromeric indices of chromosomes showed karyological similarity between *V. ater*, *V. viviparus*, *V. acerosus* and *V. mamillatus*. The most remarkable interspecific differences were expressed in morphology of chromosomes from pair no. 1 and 9 (Figs 5, 6). However, the ANOVA analysis of statistical significance showed that interspecific differences exist in chromosomes of most pairs. Bonferroni's Multiple comparison test showed significant differences between *V. mamillatus* and other studied species. Only a slight karyological dif-

Table 8. The level of statistical significance (ANOVA) in the relative length and centromeric indices of *V. ater* (Lauerz), *V. ater* (Murten), *V. acerosus*, *V. viviparus* and *V. mamillatus*

Chromosome pair No	Relative length	Centromeric index
1	<0.0001	<0.0001
2	0.0028	0.0013
3	0.008	<0.0001
4	0.0005	<0.0001
5	n.s.	n.s.
6	0.0025	n.s.
7	<0.0001	n.s.
8Z	0.0001	n.s.
8W	<0.0001	0.001
9	<0.0001	<0.0001

n.s.—not significant

Table 9. Significant differences in chromosome relative length and centromeric indices (according Bonferroni's Multiple Comparison Test)

Species	*V. acerosus*/ chromosome pairs	*V. mamillatus*/ chromosome pairs	*V. viviparus*/ chromosome pairs
V. ater (Lauerz)	7,9; **2,4,8W**	1,6,7,8W; **8W,9**	2,4,7,8Z,9; **1,2,3,4,9**
V. ater (Murten)	8Z,8W; **1,4,8W,9**	1,3,4,6,8W; **8W,9**	3,9; **3,4**
V. acerosus		1,8Z,9; **4,9**	8Z; **1**
V. mamillatus			1,2,4,6,9; **3,4,9**

Bold—significant differences in centromeric indices

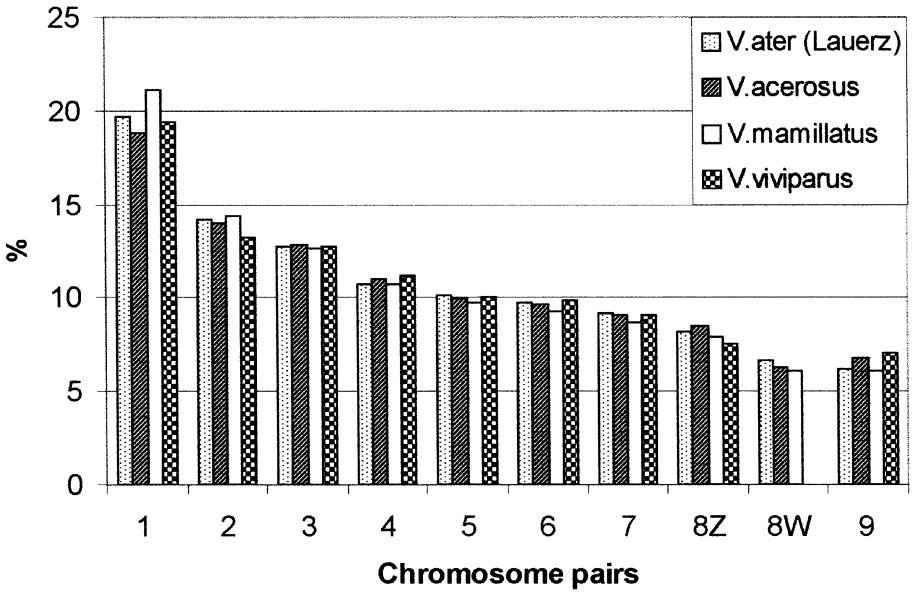

Figure 5. Ideograms of the relative length of chromosomes.

Table 10. Chromosome numbers and karyotype formulae in the subfamily Viviparinae

Species	Chromosome number n	2n	Karyotype formula	Country	Reference
Viviparus contectus	7	14		Germany Austria Lithuania	Franz, 1932 Rainer, 1963 Baršiene et al., 1996
	7	14	5 m + 2 sm	Italy	This study
	7	14	5 m + 2 cm-m	Lithuania	This study
V. ater	9	18		Germany Switzerl.	Franz, 1932 Rainer, 1963
	9	18	7m + 1sm-m + 1cm; (ZZ/ZW)	Switzerl.	Baršiene, Ribi, in press
V. viviparus	10			Germany	Franz, 1932
	9	18		Netherl.	Rainer, 1963
	9	18		Lithuania	Baršiene, 1991, 1994
	9	18	8 m + 1sm	Lithuania	This study
V. acerosus	9	18	8m + 1sm; (ZZ/ZW)	Hungary	This study
V. mamillatus	9	18	7m + 1m-sm + 1sm-m + 1sm; (ZZ/ZW)	Albania	This study
V. dissimilis		22	6 m + 5 sm	India	Ramomoorthi, 1958
V. bengalensis		22	19=0 m + 1 sm	India	Ramomoorthi, 1958
V. georgianus	12	24		USA	Pollister, Pollister, 1940
V. malleatus	9	18		USA	Pollister, Pollister, 1940, 1943; Inaba, Tanaka, 1953
V. intertestus	13			USA	Pollister, Pollister, 1940
V. contectoides	13			USA	Pollister, Pollister, 1940
V. subpurpureus	13	26	7 m + 3 sm + 2 st; (XX/XY)	USA	Stern, 1975
Tulotoma magnifica	12			USA	Pollister, Pollister, 1943
T. angulata	13		(XX/XY)	USA	Patterson, 1965

m — metacentric, sm—submetacentric, ZW—sex-determining chromosomes, Switzerl.—Switzerland, Netherl.—Netherlands.

ferentiation between *V. acerosus* and *V. viviparus* was detected. Close relationships between these two species were indicated by the analyses of morphological (non-shell) characters and molecular (allozyme) data (Falniowski *et al*., 1996). Taking into account that there are slight morphological, molecular and karyological differences between *V. viviparus* and *V. acerosus*, future studies to confirm *V. acerosus* as a distinct species are necessary. Moreover, *V. acerosus* and *V. ater* are often placed as a subspecies of *V. viviparus* (Fretter & Graham, 1978). Differential staining of chromosomes as well as methods of molecular cytogenetics to determine the variation at the gene level—fluorescence in situ hybridisation (FISH) and primed in situ DNA synthesis (PRINS) are necessary for the verification of *V. viviparus* and *V. acerosus* as distinct species. Additionally, hybridization experiments could be valuable.

In mollusc families, low chromosome number is often related to more evolved species (Butot & Kiauta, 1969; Hinegardner, 1974; Ahmed, 1976; Vitturi *et al*., 1982; Vitturi & Catalano,

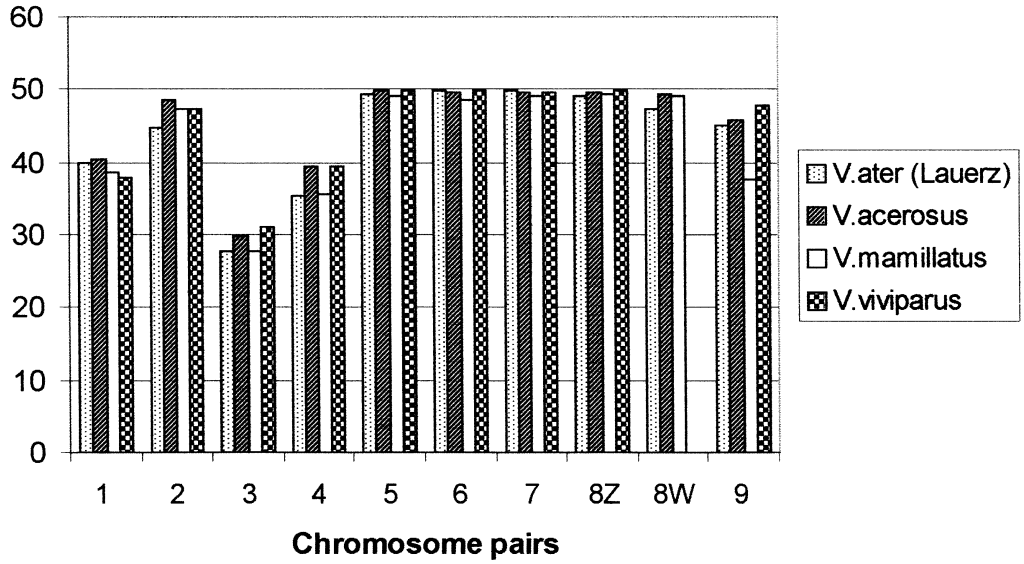

Figure 6. Ideograms of centromeric indices of chromosomes.

1984). Within the Viviparidae, a remarkable karyological differentiation took place, and chromosome numbers range from n = 7 to n = 14. The highest chromosome number, n = 14, was observed in derived groups such as the subfamily Lioplacinae and chromosome numbers of n = 11 (Bellamyinae) and n = 13) Viviparinae) are reported (Patterson, 1969). On the basis of karyological data it is clear that the trend of karyotype evolution in this family is chromosome number reduction from n = 14 to n = 7. Therefore, the karyotype of *V. contectus* may possess some of the most derived the features of viviparid karyotypes. On the other hand, ideograms of average values of the relative lengths and centromeric indices of chromosomes showed karyological conservatism in *V. contectus*, as only slight interpopulational differences in snails from Lake Garda and lakes in Lithuania were noticed (Figs 7, 8).

The comparative karyometrical analysis of *V. contectus* and other viviparids, led researchers to suggest that the main mechanism of evolutionary changes in this group of snails was Robertsonian fusion of chromosomes, originally proposed by Rainer (1963). According to this author, the chromosome pairs no. 4, 7, 8 and 9 of *V. ater* may be included in chromosomal rearrangements. However, it is more likely that chromosomes of pair no. 8 (sex-determining chromosomes) did not play an important role in the evolutionary changes.

The Neritidae provide the best evidence of the presence of sex chromosomes in gastropod molluscs. The detailed study of mitotic and meiotic chromosomes in various species showed the occurrence of the XX female/XO male type of sex-determining mechanism (Natarajan, 1969; Komatsu & Inaba, 1982; Nakamura, 1986). However, several different sex-determining types—XX/XY (Jacob, 1959; Burch, 1960; Patterson, 1965, 1967) and ZW/ZZ (Baršiene & Ribi, in press) have also been described. Among the Viviparidae, both the XX/XY type (*V. subpurpueus*, Stern, 1975; *Tulotoma angulata* (Patterson, 1965) and ZW/ZZ sex-determining type have been described. Females of *V. ater*, *V. acerosus* and *V. mamillatus* were heterogametic sex (ZW) whereas the males are the homogametic sex (ZZ). Although both W and Z chromosomes were metacentric, chromosome W is significantly smaller than Z (Baršiene & Ribi, in press).

The main mechanism of XY and WZ chromosome differentiation is the accumulation of highly repeated (satellite) DNA sequences on Y and W chromosomes (Schmid *et al.*, 1991). As a result, the highly derived Y and W chromosomes are completely heterochromatic, or contain distinctly more heterochromatin than their homologues, the X or Z chromosomes (Olmo *et al* 1987; Schmid et al., 1988, 1991). It should be stressed that in certain amphibian and fish families both ZW/ZZ and XX/XY sex-determining

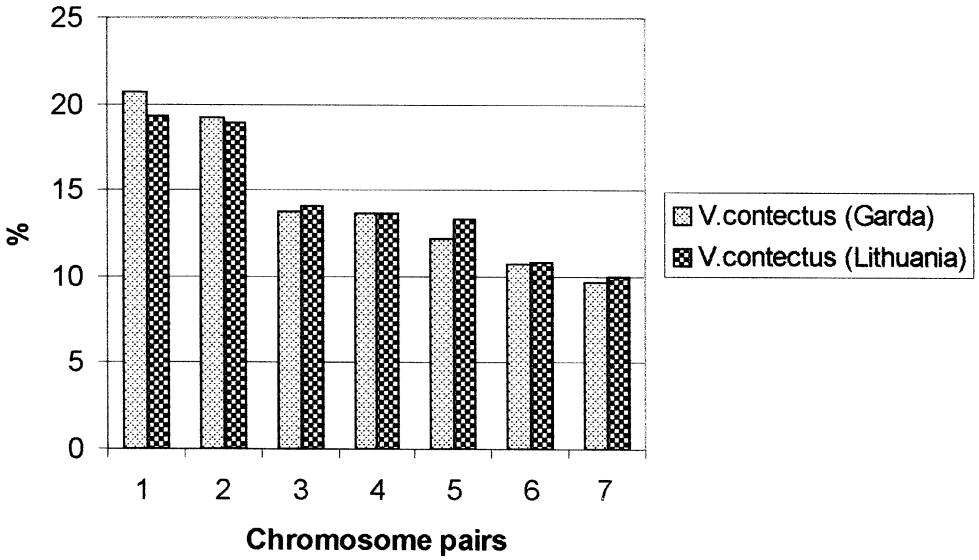

Figure 7. Ideograms of the relative length of *V. contectus* chromosomes.

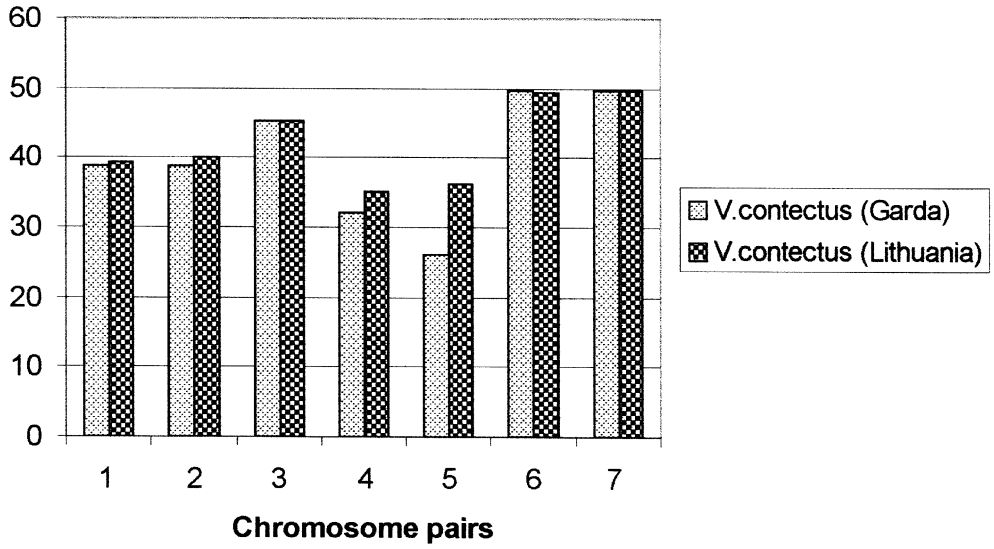

Figure 8. Ideograms of centromeric indices of *V. contectus* chromosomes.

types coexist (Vasiliev, 1985; Schmid *et al.*, 1991).

Intraspecific conservatism of karyotypes, unusual mechanism of sex-determination and wide variation of chromosome numbers within the family Viviparidae might be the main aspects for future karyological studies of viviparid snails.

ACKNOWLEDGEMENTS

This research was supported by a grant of the University of Zurich to Janina Baršiene. We wish to express our grateful thanks to Dr Catherine Thiriot-Quievreux for helpful comments and improvement of our paper. We also thank F. Statzler and H. Krattli for assistance in preparing slides and microphotos; and Dr D.A. Lovejoy for the supervision of the English.

REFERENCES

AHMED, M. 1976. Chromosome cytology of marine pelecypod mollusc. *Journal of Sciences*. (Karachi), **4**: 77-94.

BARŠIENE, J. 1978. Ontogenetic variability of the chromosome number of Atlantic salmon (*Salmo salar* L.). *Genetica*, **24**: 2029-2036, (in Russian).

BARŠIENE, J. 1991. Cytogenetic changes of molluscs. In: *Nemunas Water Pollution and Biological Effects in Ecosystem* (R. Lekevičius *et al*., eds), 100-106, Vilnius, Academia, (in Lithuanian).

BARŠIENE, J. 1994. Chromosome set changes in molluscs from highly polluted habitat. In: *Genetics and Evolution of Aquatic Organisms* (A.R. Beaumont, ed.), 434-447. Chapman and Hall, London.

BARŠIENE, J. & GRABDA-KAZUBSKA, B. 1988. A comparative study on chromosomes in plagiorchiid trematodes. I. Karyotypes of *Opisthioglyphe ranae* (Frolich, 1791), *Haplometra cylindracea* (Zeder, 1800) and *Leptophallus nigrovenosus* (Bellingham, 1844). *Acta Parasitologica Polonica*, **33**: 249-257.

BARŠIENE, J., BARŠYTE, D. & VIRBICKAS, T. 1996. Environmental genotoxicity *in vivo*: Vilnia River. *Ekologija*, **1**: 64-73.

BARŠIENE, J. & RIBI, G. 1998. Karyological studies of *Viviparus ater* (Gastropoda: Prosobranchia) from different populations in Switzerland. *Malacological Review*, **31** (2): (in press).

BURCH, J.B. 1960. Chromosome studies of aquatic pulmonate snails. *Nucleus*, **3**: 177-208.

BUTOT, L.J.M. & KIAUTA, B. 1969. Cytotaxonomic observation in the stylommatophoran family helicidae, with consideration on the affinities within family. *Malacologia*, **9**: 261-262.

FALNIOWSKI, A., KOZIK, A. & SZAROWSKA, M. 1993. Two common European viviparid species hybridize. *American Malacological Bulletin*, **10**: 161-164.

FALNIOWSKI, A., KOZIK, A., SZAROWSKA, M., FIALKOWSKI, W. & MAZAN, K. 1996. Allozyme and morphology evolution in European Viviparidae (Mollusca: Gastropoda: Architaenioglossa). *Journal of Zoology, Systematics and Evolutionary Research*, **34**: 49-62.

FECHTER, R. & FAKNER, G. 1990. *Weichtiere, Europaische Meeres- und Binnenmollusken*. Mosaik Verlag, Munchen.

FRANZ, V. 1932. *Viviparus*: Morphometrie, Phylogenie und Geographie der europaischen, fossilen und rezenten Paludinen. *Denkschriften der Medizinisch-Naturwissenschaftlichen Gesellschaft zu Jena (Gustav Fisher)*, **18**: 1-160.

FRETTER, V. & GRAHAM, A. 1994. *British prosobranch molluscs: their functional anatomy and ecology*, 2nd edition, Ray Society, London.

HINEGARDNER, R. 1974. Cellular DNA content of the Mollusca. *Comparative Biochemistry and Physiology*, **47 A**: 447-460.

JACOB, J. 1959. Sex chromosomes in melaniid snails. I. *Paludomus transhaurica* (Gmelin) (Prosobranchia: Gastropoda). *Journal of the Zoological Society of India*, **11**: 17-25.

INABA, A. & TANAKA, H. 1953. Studies on the chromosome numbers of some freshwater gastropods. *Journal of Sciences of Hirosima University*, Ser. B, div. 1, **14**: 213-220.

KATOH, M. & RIBI, G. 1996. Genetic evidence for natural hybridization and apparent introgression between freshwater snail species (*Viviparus ater* and *V. contectus*). *Journal of Evolutionary Biology*, **9**: 67-82.

KOMATSU, S. & INABA, A. 1982. Chromosome numbers of 14 species in the Neritidae (Gastropoda, Archaeogastropoda). *Venus*, **41**: 47-60.

LEVAN, A., FREDGA, K. & SANDBERG, A. 1964. Nomenclature for centromere position on chromosomes. *Hereditas*, **52**: 201-220.

NAKAMURA, H.K. 1986. Chromosomes of Archaeogastropoda (Mollusca: Prosobranchia), with some remarks on their cytotaxonomy and phylogeny. *Publications of the Seto Marine Biological Laboratory*, **31**: No 3/6: 191-267.

NATARAJAN, R. 1969. Cytological studies on Indian molluscs (Archaeogastropoda: Neritidae). *Malacologia*, **9**: 279-281.

OLMO, E., ODIERNA, G. & CAPRIGLIONE, T. 1987. Evolution of sex-chromosomes in lacertid lizards. *Chromosoma* (Berlin), **96**: 33-38.

PATTERSON, C.M. 1965. The chromosomes of *Tulotoma angulata* (Streptoneura: Viviparidae). *Malacologia*, **2**: 259-265.

PATTERSON, C.M. 1967. Chromosome numbers and systematics in streptoneuran snails. *Malacologia*, **5**: 111-125.

PATTERSON, C.M. 1969. Chromosomes of molluscs. *Proceedings of the 2nd Symposium on Mollusca, Ernakulam, Cochin, India*. Marine Biological Association, Bangalore, India, **2**: 635-686.

POLLISTER, A. W. & POLLISTER, P.F. 1940. Distribution of males in the genus *Campeloma*, with a note on the chromosome numbers in Viviparidae. *Anatomical Record*, **78**: 83-84.

POLLISTER, A.W. & POLLISTER, P.F. 1943. The relation between centriole and centromere in atypical spermatogenesis of viviparid snails. *Annals of New York Academy of Sciences*, **45**: 1-48.

RAINER, M. 1963. Vergleichende Chromosomenmessungen an *Viviparus*—Arten (Prosobranchia). *Dreiundzwanzigster Jahresbericht der Schweizerischen Gesellschaft fur Vererbungsforschung, S.S.G.; Archiv der Julius Klaus-Stiftung fur Vererbungsforschung, Soziallanthropologie und Rassenhygiene*, **38** (3/4): 61-68.

RAMAMOORTHI, K. 1958. Chromosomes of *Viviparus dissimilis* (Müller) and *Viviparus bengalensis* (Lamarck) (Prosobranchia-Gastropoda). *Journal of the Zoological Society of India*, **10**: 33-38.

ROTH, W. 1906. Uber die Herkunft und das Alter der Zurichseepaludina. *Blatter fur Aquarien und Terrarienkunde*, Stuttgart, **17**: 175.

SCHMID, M., STEINLEIN, C., FEICHTINGER, W., DE ALMEIDA, C.G. & DUELLMAN, W.E. 1988. Chromosome banding in Amphibia. XIII. Sex chromosomes, heterochromatin and meiosis in marsupial frogs (Anura, Hylidae). *Chromosoma* (Berlin), **97**: 33-42.

SCHMID, M., NANDA, I., STEILEIN, C., KAUSCH, K. & HAAF, T. 1991. Sex-determining mechanisms and sex chromosomes in Amphibia. In: *Amphibian Cytogenetics and Evolution*, (D.M. Green and S.K. Sessions, eds), 393-430. Academic Press, New York.

STERN, E.M. 1975. The chromosomes of *Viviparus subpurpureus* (Say) (Streptoneura: Viviparidae). *Malacological Review*, **8**: 107-108.

VASILIEV, V.P. 1985. *Evolutionary karyology of fish*. Moscow, Nauka, 300p. (in Russian).

VITTURI, R., RASOTTO, M.B. & FARINELLA-FERRUZA, N. 1982. The chromosomes of 16 molluscan species. *Bolletin de Zoologia*, **49**: 61-71.

VITTURI, R. & CATALANO, E. 1984. Spermatocyte chromosomes in 7 species of the sub-class Prosobranchia (Mollusca, Gastropoda). *Biologisches Zentrablatt*, **103**: 69-76.