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MORPHOLOGICAL AND ALLOZYMIC VARIATION WITHIN AND BETWEEN POPULATIONS OF *BYTHINELLA* MOQUIN-TANDON, 1855 (GASTROPODA: PROSOBRANCHIA). II. PHENETIC ANALYSIS

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ABSTRACT: The paper describes morphometric and allozymic differences between 20 Central European populations of *Bythinella*. Morphometric differences were studied using principal component analysis, based on 40 biometrical characters in males and 42 in females, for each sex separately. The results showed a slight inter- and a wide intrapopulation morphometric variation, the variability ranges of the populations and postulated morphospecies overlapping. To visualize the pattern of interpopulation allozymic differences, studied at 9 loci, correspondence analysis of allele frequencies and multidimensional scaling based on genetic distances (Prevosti, unbiased Nei and Cavalli-Sforza and Edwards arc distance) were used. The values of Nei distances between populations (0.000–0.362) ranged from ones typical of conspecific populations (in the majority of populations) to ones characteristic rather of congeneric species. The patterns of interpopulation distinctness for molecular and morphological data were different and did not unequivocally confirm either the morphological or molecular distinctness of the distinguished morphospecies. All the genetic distances and Euclidean (morphology-based) distances were correlated with geographic distances, whereas no significant correlations were observed between the genetic and Euclidean distances.

KEY WORDS: spring snail, *Bythinella,* population, variation, morphology, allozymes, phenetics, multivariate analysis

INTRODUCTION

The genus *Bythinella* Moquin-Tandon, 1855, of the world-wide distributed Hydrobiidae sensu lato, comprises small (up to 3 mm in shell height), roller-shelled snails. These dioecious, oviparous snails inhabit springs (BOETERS 1979, 1982, GIUSTI & PEZZOLI 1980, FALNIOWSKI 1987) as well as subterranean waters in southern, western and central Europe, and in Asia Minor. They are especially abundant on mosses and other aquatic plants, but may also occur in low numbers among fallen leaves in spring-fed marshes.

The geographic range of *Bythinella* extends from Spain, through France, Benelux, southern Germany and Poland, Italy, Switzerland, Austria, the Czech Republic, Slovakia, Hungary and the Balkan countries to Asia Minor. Within such a wide range, the snails show little interspecific morphological differentiation and are highly varied in each locality. This makes the taxonomy of the genus still poorly understood (FALNIOWSKI 1987, 1992). A great deal of controversies have arisen as to the intrageneric systematics: from distinguishing a large number of species (e.g. RADOMAN 1976 – *Bythinella* of the Balkans and Asia Minor); or reducing it to a few species (e.g. BOETERS 1973 – *Bythinella* of western Europe), to the conclusion that all *Bythinella* represent a single superspecies (GIUSTI & PEZZOLI 1977, 1980). Many of the authors considered only the shell; in other cases anatomical characters were dealt with but the material examined was insufficiently large or the interpretation of characters was inconsistent (HERSHLER & PONDER 1998). In Poland, more detailed studies concerning

Bythinella are only those by FALNIOWSKI (1987). Based on anatomical characters, he distinguished six species of *Bythinella* living in Poland. These species show a wide variability and minor interspecific differences. Four of them are considered in this study.

This paper is part of a more extensive study concerning intra- and interpopulation variation in allozymes and morphology in *Bythinella* (FALNIOWSKI et al. 1998, 1999, SZAROWSKA et al. 1998, MAZAN 2000) and contains multidimensional phenetic analysis of morphological and molecular variation. The results should enable a more rigorous and formalized description of the morphological differences between the studied populations and ealier distinguished species of *Bythinella*, and a comparison of the patterns of morphological and molecular differentiation.

MATERIAL AND METHODS

LOCALITIES, COLLECTION AND MORPHOLOGICAL STUDY

During the summer of 1994 and 1995, *Bythinella* was sampled at 20 localities in Central Europe: 1. Bernecebarati, Borzsony Mountains, Hungary; 2–5. Slovakia: 2. Klenány, Krupinska Vrchovina; 3. Bansky Studenec, Stiavnicke Vrchy; 4. Klacno, Mala Vatra; 5. Telgart, Nizke Tatry; 6–20. Poland: 6. Zakopane, valley Dolina Strążyska, Tatra Mts; 7. Olszówka, Gorce Mountains; 8. Krowiarki pass, Beskid Wysoki mountains; 9. Zawoja-Składy, Beskid Wysoki mountains; 10. Węglówka, Beskid Wyspowy mountains; 11–19. Kraków-Częstochowa Upland: 11. spring Źródło Świętego Eliasza; 12. spring Źródło Bazana; 13. spring Źródło pod Grotą; 14. valley Dolina Sąspowska, Ojców National Park; 15. Młynnik spring, Ojców National Park; 16. Zimny Dół gorge; 17. Chechło near Wolbrom; 18. Kadłubek spring, Kwaśniów; 19. spring Źródło Zygmunta; 20. spring Źródło Romanowskie, Romanowo/Żelazno near Kłodzko, Sudetes.

All populations were preliminarily classified as "morphospecies": 1 – *Bythinella* sp. 1 (undescribed); 2 – *Bythinella* sp. 2 (undescribed); 3 – *Bythinella* sp. 3 (undescribed); 4, 11–16 and 19–20 – *Bythinella austriaca* (Frauenfeld, 1856), sensu FALNIOWSKI (1987); 5 – *Bythinella* sp. 4 (undescribed); 6 – *Bythinella* sp. (5) after FALNIOWSKI (1987); 7–10 – *B. cylindrica* (Frauenfeld, 1856), sensu FALNIOWSKI (1987); 17–18 – *B. zyvionteki* Falniowski, 1986 (FALNIOWSKI et al. 1999).

About 300 specimens were collected from each population. The morphological study was performed for 60 adults (30 males and 30 females) taken at random from each population; 40 characters in males and 42 in females were counted or measured. A more detailed description of the localities, a distribution map, the collection and preservation techniques applied and the methods of morphometrical studies are presented in MAZAN (2000).

ELECTROPHORESIS

The snails were transported in a car refrigerator and kept in aerated aquaria at 4–8°C. Electrophoresis was carried out within a few days from collection. Adult specimens were selected for electrophoresis immediately before each run. Each individual was briefly blotted on filter-paper and killed by freezing (–20°C, 10 min). The last whorl containing the foot, head and anterior part of the mantle was removed under a stereomicroscope. The remaining part of the body, comprising mostly the hepatopancreas, was homogenized on ice in a glass homogenizer in 20 µl of homogenizing solution (100 ml distilled water, 10 mg 2':3'-cyclic NADP, 10 mg NAD, 100 µl ß-mercaptoethanol). The homogenates were used immediately for celluose acetate electrophoresis, following the protocol of RICHARDSON et al. (1986).

Electrophoresis was carried out in a refrigerator, simultaneously in two 1000 BR Chemetron apparatuses, using "CELLOGEL" cellulose acetate gels (Malta, Chemetron Products, Milan, Italy) and a Shandon sample applicator. All other chemicals were from Sigma, St. Louis, MO, USA. The samples were loaded in cathodal or middle origin position and gels were run for 30–90 minutes, depending on the enzyme system. Pairs of individuals from different populations were run together, providing a comparison of alleles between and within gels. The stains were made according to the recipes in RICHARDSON et al. (1986). After application of the stain solution, the gels were blotted to remove excess stain using a sheet of filter paper, wrapped in plastic film wrap and incubated at 37°C. Gels were fixed in 10% formalin solution and photocopied to provide a permanent record. Phenotypes were scored directly from the gel and the photocopy. Only presumptive loci which were consistently scorable were reported. Common principles of scoring and interpretation of zymograms (RICHARDSON et al. 1986) were followed. Loci were numbered and alleles were designated with letters in order of decreasing mobility. Twenty six enzyme systems were studied, not less than 30 snails being electrophoresed for each.

NUMERICAL TECHNIQUES

Morphological data

Each measured or counted character was tested for significance of interpopulation differences, using ANOVA in STATISTICA/DOS (STATSOFT 1991); the descriptive statistics is given in MAZAN (2000). Only those characters which showed statistically significant differences between populations were used for further analysis. The data were processed with NTSYSpc (ROHLF 1994), using multivariate statistical techniques (BOOKSTEIN et al. 1985, JAJUGA 1993, SOKAL & ROHLF 1996, JOHNSON & WICHERN 1998), for males and females separately. Principal component analysis (PCA) was applied to visualize the structure of the data with no a priori assumption.

All the data were logarithmically transformed (ln+1) and standarized (SUBYBAR+DIVSTD). Euclidean distances between the specimens and correlations between the variables (ROHLF 1994) were computed. The original data together with minimum spanning trees (MST) showing local distorsions in the data (situations where the closest specimens in two-dimensional space were not closed in n-dimensional space) were projected into PC space. In PCA, where possible, those eigenvectors were used, which explained more variability than the variability explained by chance under the broken-stick model (ROHLF 1994). The first eigenvectors were usually not used, as they mainly reflected size differences, polymorphism and sexual dimorphism. The second and third eigenvectors, explaining also much of the variability and representing mainly shape differences (GOULD 1977), were used instead. The first eigenvectors were used only when the second and/or third eigenvectors explained less variability than expected under the broken-stick model.

The number of the studied specimens was so large (527 males and 564 females) that it was impossible to explicitly illustrate all of them in one figure. Thus, PCA was preliminarily performed for the mean values of each character for each population, to visualize the relationships between all the populations. PCA was also performed for specimens; then, for the legibility of projection, up to 4 or 5 populations were compared. For each projection, populations were chosen so as to illustrate the differences between the populations of one as well as of different morphospecies. Since non-linear multidimensional scaling (MDS) gave results very similar to those of PCA, they are not presented.

Molecular data

Allozymic data were analyzed with the NTSYSpc package (ROHLF 1994). In the majority of such studies, populations are compared by computing Nei distance (NEI 1972, 1978), and then the UPGMA clustering technique is applied. However, Nei distance is seriously influenced by numerous assumptions that are commonly violated (WRIGHT 1978, FALNIOWSKI et al. 1993). Nei distance was originally intended to measure the number of codon substitutions per locus that had occurred after divergence between two populations. However, the rate of gene substitutions per locus has to be uniform at the locus in all the populations. Moreover, Nei distance is based on Kimura's infinite isoallele model of selectively neutral mutation, with each mutation resulting in a completely new allele and the mutation rate being constant for all loci (COOK 1991, KIMURA 1991). Nei distance is also heavily influenced by intrapopulation heterozygosity (FELSENSTEIN 1985, 1990, SWOFFORD & OLSEN 1990). Therefore, its application, even if generally useful, is dubious in most cases; in particular, it should not be applied to species of little known biology, genetics, mutation rate, mutations' selective value, etc.

It has to be stressed that, in fact, no genetic distance would behave correctly in all cases. Thus, although Nei distance was computed for comparison with other species, Cavalli-Sforza and Edwards arc distance (CAVALLI-SFORZA & EDWARDS 1967) and Prevosti distance (ROHLF 1994) were also calculated. In the studied situation, with the populations which are closely related and diverged so recently that mutations could not be the source of their genetic variability, Cavalli-Sforza and Edwards arc distance seems to be most useful. It is not affected by intrapopulation heterozygosity, assumes genetic drift as the only source of variability (WRIGHT 1978), and reflects small differences between populations better than Nei distance (DAVIS 1994). Prevosti distance is a common Euclidean distance calibrated by the number of loci, without genetic assumptions.

To visualize the interpopulation variation pattern expressed by allozymes, a multidimensional scaling combined with minimum spanning tree, both based on values of genetic distances, and a correspondence analysis of allele frequencies were computed (ROHLF 1994).

Comparison between the morphological and molecular differences

In order to describe the relationships of morphological to molecular variation as well as the relationships of morphological and molecular differentiation to the geographical distance, Mantel test for matrix correlations was computed, with NTSYSpc (ROHLF 1994). Geographic distances between populations were calculated from the map. The test assumes that two matrices have been obtained independently, and the degree of relationship between them is measured by the Mantel statistic Z. If two matrices show similar relationships then Z should be large in comparison to what one would expect by chance.

RESULTS

MORPHOLOGICAL INTERPOPULATION VARIATION

To visualize relationships between all the populations, principal component analysis (PCA) was performed for the mean values of each character for each population. In the males, the first and second eigenvalues explained together 35.27% and 26.06% of the total variability, respectively; the third eigenvalue explained 8.38%. In the females, the first and second eigenvalues explained 20.55% and 16.67% of the total variability, respectively; the third eigenvalue explained 10.75% (Table 1). PCA showed a different picture for each sex (Figs 1, 2).

The projection of males in the second and third PC (Fig. 1) showed populations 3, 5 and 6 being the closest to each other (each of them represents a different morphospecies). All the populations of *B. austriaca* were grouped marginally along the second axis, except for population 4, representing *B. austriaca* from Slovakia and situated on the other side of the axis. However, the minimum spanning trees (MST) joined population 4 with the populations of *B. austriaca*, as well as all the populations of this morphospecies between each other. Also populations 7, 8, 9 and 10, classified as *B. cylindrica*, were joined by MST in one group. Populations 17 and 18 (*B. zyvionteki*) were placed near those belonging to *B. cylindrica*.

In PCA for the females (Fig. 2), like in that for the males, MST joined together all the populations representing *B. austriaca*, except population 20 which was the closest to *B. cylindrica*. The populations of *B. zyvionteki* (17 and 18) were joined together by MST and situated within the group comprising *B. austriaca*. The females of population 3, the males of which were the closest to population 5, were near those of populations 1 and 2 (each classified as a different morphospecies). The females of population 10 (*B. cylindrica*) were more similar to *B. austriaca* than to the remaining populations of *B. cylindrica*.

Tables 2–8 show the eigenvalues, the percent of the total variability explained by the first twelve eigenvalues (the remaining eigenvalues were lower than 1.00), and the percent of variability explained by chance under the broken-stick model computed for specimens from selected groups of populations. Four populations of *B. austriaca* are presented in Figures 3 and 4. The males (Fig. 3) of all the populations overlap and are mixed along the second and third axes. The specimens from population 12 are most scattered and include some outliers, whose projections in the PC space are situated far from the centroid of the group. For the females (Fig. 4), the analysis is based on the first and second PC, 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 not reflect any true relationships between the variables. The picture is very similar to that for the males. However, for the females, in nearly all the populations the intrapopulation differentiation was wider than for the males. Only in population 19 the females were distributed more closely.

For the other four populations of *B. austriaca*, the third PC was not used for either sex, since it explained less variability than was expected under the broken-stick model (Table 3). The projection in the first and second PC showed the same picture for both sexes (Figs 5, 6). Two distinct groups were distinguished.

Table 1. Principal component analysis for mean values for every character in all populations of *Bythinella*; eigenvalues and percent of variability explained. Proportions of variance expected under broken-stick model

PC no	MALES				FEMALES			
	Eigenvalue	Percent of variability explained	Cumulative percent	Expected percent	Eigenvalue	Percent of variability explained	Cumulative percent	Expected percent
1	14.1063	35.2658	35.2658	10.6964	12.8312	30.5504	30.5504	10.3018
2	10.4232	26.0581	61.3239	8.1964	7.0027	16.6731	47.2235	7.9208
3	3.3533	8.3833	69.7072	6.9464	4.5139	10.7474	57.9709	6.7303
4	3.0031	7.5078	77.2150	6.1130	3.2761	7.8002	65.7710	5.9367
5	2.1212	5.3031	82.5181	5.4880	2.5112	5.9790	71.7501	5.3415
6	1.5835	3.9587	86.4768	4.9880	2.4202	5.7625	77.5125	4.8653
7	1.1957	2.9892	89.4661	4.5714	1.6016	3.8132	81.3258	4.4684
8	0.9950	2.4875	91.9536	4.2142	1.4126	3.3632	84.6890	4.1283
9	0.6694	1.6734	93.6271	3.9017	1.2242	2.9147	87.6037	3.8307
10	0.5474	1.3684	94.9955	3.6239	1.0599	2.5235	90.1272	3.5661

Fig. 1. Principal component analysis for mean values of each character for each population – males. For population numbers see: Localities, collection and morphological study

Fig. 2. Principal component analysis for mean values of each character for each population – females. For population numbers see: Localities, collection and morphological study

Table 2. Principal component analysis for all specimens of *Bythinella austriaca* (populations 4, 12, 15 and 19); eigenvalues and percent of variability explained. Proportions of variance expected under broken-stick model

PC no	MALES				FEMALES			
	Eigenvalue	Percent of variability explained	Cumulative percent	Expected percent	Eigenvalue	Percent of variability explained	Cumulative percent	Expected percent
1	7.9610	19.9026	19.9026	10.6964	9.4392	22.4744	22.4744	10.3018
2	4.3932	10.9831	30.8856	8.1964	5.3155	12.6560	35.1303	7.9208
3	3.0231	7.5578	38.4435	6.9464	2.5333	6.0316	41.1619	6.7303
4	2.2916	5.7291	44.1726	6.1130	2.2455	5.3463	46.5082	5.9367
5	1.9401	4.8502	49.0228	5.4880	2.0983	4.9958	51.5040	5.3415
6	1.7971	4.4928	53.5156	4.9880	1.5106	3.5968	55.1008	4.8653
7	1.5109	3.7773	57.2929	4.5714	1.3883	3.3055	58.4063	4.4684
8	1.3207	3.3017	60.5946	4.2142	1.3596	3.2372	61.6435	4.1283
9	1.2636	3.1591	63.7537	3.9017	1.2726	3.0300	64.6736	3.8307
10	1.2249	3.0623	66.8160	3.6239	1.1794	2.8080	67.4816	3.5661
11	1.1743	2.9357	69.7516	3.3739	1.0591	2.5216	70.0032	3.3280
12	1.0436	2.6089	72.3605	3.1467	1.0361	2.4669	72.4701	3.1116

One consisted of completely mixed populations 13, 14 and 16. The other group comprised all the specimens of population 20. In all the populations the females were more scattered than the males and often situated far from the centroid of the group (Fig. 6).

The fractions of variability of the males of the four populations of *B. cylindrica*, explained by the second and third PC, were lower than the ones expected by chance (Table 4), thus the first, instead of the second PC, was used. For both sexes of *B. cylindrica* all the

Fig. 3. Principal component analysis for all male specimens in four populations of *Bythinella austriaca*. For population numbers see: Localities, collection and morphological study

Fig. 4. Principal component analysis for all female specimens in four populations of *Bythinella austriaca*. For population numbers see: Localities, collection and morphological study

populations were mixed together and placed closely about the centroid (Figs 7, 8). However, there were also some outliers, more frequent in males.

In PCA for *B. zyvionteki* compared with *B. austriaca* the third PC was not used in either sex, since it explained less variability than was expected under the broken stick model (Table 5). For the males (Fig. 9), along the first axis, two groups of populations, although to some extent overlapping, were distinguished: one comprising the specimens of two popu-

Table 3. Principal component analysis for all specimens of *Bythinella austriaca* (populations 13, 14, 16 and 20); eigenvalues and percent of variability explained. Proportions of variance expected under broken-stick model

PC no	MALES				FEMALES			
	Eigenvalue	Percent of variability explained	Cumulative percent	Expected percent	Eigenvalue	Percent of variability explained	Cumulative percent	Expected percent
1	10.2314	25.5786	25.5786	10.6964	12.6046	30.0108	30.0108	10.3018
2	4.1283	10.3208	35.8993	8.1964	3.7894	9.0224	39.0332	7.9208
3	2.6914	6.7284	42.6278	6.9464	2.5884	6.1629	45.1961	6.7303
4	2.4990	6.2474	48.8752	6.1130	1.9004	4.5247	49.7208	5.9367
5	2.0277	5.0693	53.9445	5.4880	1.7657	4.2041	53.9249	5.3415
6	1.7160	4.2900	58.2345	4.9880	1.6752	3.9886	57.9135	4.8653
7	1.5163	3.7908	62.0253	4.5714	1.4655	3.4893	61.4027	4.4684
8	1.3131	3.2828	65.3081	4.2142	1.2526	2.9824	64.3851	4.1283
9	1.1875	2.9689	68.2769	3.9017	1.1913	2.8364	67.2215	3.8307
10	1.0686	2.6715	70.9484	3.6239	1.1107	2.6445	69.8659	3.5661
11	1.0659	2.6646	73.6131	3.3739	1.0356	2.4658	72.3318	3.3280
12	0.8485	2.1212	75.7343	3.1467	0.9669	2.3021	74.6338	3.1116

Fig. 5. Principal component analysis for all male specimens in four populations of *Bythinella austriaca*. For population numbers see: Localities, collection and morphological study

Fig. 6. Principal component analysis for all female specimens in four populations of *Bythinella austriaca*. For population numbers see: Localities, collection and morphological study

Table 4. Principal component analysis for all specimens of *Bythinella cylindrica* (populations 7, 8, 9 and 10); eigenvalues and percent of variability explained. Proportions of variance expected under broken-stick model

PC no	MALES				FEMALES			
	Eigenvalue	Percent of variability explained	Cumulative percent	Expected percent	Eigenvalue	Percent of variability explained	Cumulative percent	Expected percent
1	10.1490	25.3725	25.3725	10.6964	9.2849	22.1069	22.1069	10.3018
2	3.1455	7.8637	33.2362	8.1964	4.0205	9.5727	31.6796	7.9208
3	2.7358	6.8394	40.0756	6.9464	2.9255	6.9655	38.6451	6.7303
4	2.3215	5.8037	45.8793	6.1130	2.3526	5.6015	44.2466	5.9367
5	1.7155	4.2887	50.1679	5.4880	1.7933	4.2698	48.5164	5.3415
6	1.6327	4.0818	54.2497	4.9880	1.6809	4.0020	52.5184	4.8653
7	1.5001	3.7502	57.9999	4.5714	1.5438	3.6758	56.1942	4.4684
8	1.4049	3.5123	61.5122	4.2142	1.4235	3.3892	59.5834	4.1283
9	1.2522	3.1306	64.6428	3.9017	1.2326	2.9348	62.5183	3.8307
10	1.1916	2.9789	67.6217	3.6239	1.1528	2.7448	65.2631	3.5661
11	1.0560	2.6401	70.2618	3.3739	1.0936	2.6038	67.8670	3.3280
12	1.0451	2.6127	72.8745	3.1467	1.0775	2.5656	70.4325	3.1116

lations of *B. zyvionteki* and the other representing *B. austriaca*. Outliers were sporadic. With respect to the females, all the populations were more overlapping, and population 16 was scattered along the whole range of values of the first axis (Fig. 10).

B. zyvionteki was more distinct from *B. cylindrica* than from *B. austriaca*. In PCA for the males, the first and third PC`s were used (Table 6). The first principal component axis grouped the specimens according to the classification to morphospecies: popula-

Fig. 7. Principal component analysis for all male specimens in all populations of *Bythinella cylindrica*. For population numbers see: Localities, collection and morphological study

Fig. 8. Principal component analysis for all female specimens in all populations of *Bythinella cylindrica*. For population numbers see: Localities, collection and morphological study

Fig. 9. Principal component analysis for all male specimens of *Bythinella austriaca* (populations 11, 16) and *B. zyvionteki* (populations 17, 18). For population numbers see: Localities, collection and morphological study

Table 5. Principal component analysis for all specimens of *Bythinella austriaca* (populations 11, 16) and *B. zyvionteki* (populations 17, 18); eigenvalues and percent of variability explained. Proportions of variance expected under broken-stick model

PC no	MALES				FEMALES			
	Eigenvalue	Percent of variability explained	Cumulative percent	Expected percent	Eigenvalue	Percent of variability explained	Cumulative percent	Expected percent
1	11.5324	28.8311	28.8311	10.6964	10.9715	26.1226	26.1226	10.3018
2	3.4984	8.7460	37.5771	8.1964	3.3434	7.9604	34.0830	7.9208
3	2.5783	6.4456	44.0228	6.9464	2.4117	5.7423	39.8253	6.7303
4	2.3954	5.9886	50.0113	6.1130	2.3151	5.5121	45.3374	5.9367
5	2.2034	5.5085	55.5198	5.4880	1.8257	4.3468	49.6842	5.3415
6	1.5544	3.8860	59.4058	4.9880	1.6306	3.8823	53.5665	4.8653
7	1.4126	3.5315	62.9373	4.5714	1.5281	3.6383	57.2048	4.4684
8	1.2591	3.1478	66.0851	4.2142	1.3906	3.3111	60.5159	4.1283
9	1.1505	2.8763	68.9613	3.9017	1.3168	3.1353	63.6512	3.8307
10	1.0776	2.6939	71.6552	3.6239	1.1786	2.8061	66.4573	3.5661
11	0.9473	2.3681	74.0234	3.3739	1.0594	2.5223	68.9795	3.3280
12	0.8815	2.2038	76.2272	3.1467	1.0232	2.4362	71.4157	3.1116

tions 7 and 10 (*B. cylindrica*) lay marginally along the axis, whereas on the other side there were populations 17 and 18 representing *B. zyvionteki* (Fig. 11). The second component axis distinguished the morphospecies in females, clearly separating population 17 from 7, and less distinctly 10 from 18. MST joined specimens from population 7 with 10, and 17 with 18 (Fig. 12).

Fig. 10. Principal component analysis for all female specimens of *Bythinella austriaca* (populations 11, 16) and *B. zyvionteki* (populations 17, 18). For population numbers see: Localities, collection and morphological study

Table 6. Principal component analysis for all specimens of *Bythinella cylindrica* (populations 7, 10) and *B. zyvionteki* (populations 17, 18); eigenvalues and percent of variability explained. Proportions of variance expected under broken-stick model

PC no	MALES				FEMALES			
	Eigenvalue	Percent of variability explained	Cumulative percent	Expected percent	Eigenvalue	Percent of variability explained	Cumulative percent	Expected percent
1	9.4526	23.6314	23.6314	10.6964	8.0319	19.1235	19.1235	10.3018
2	3.0418	7.6046	31.2361	8.1964	4.7634	11.3415	30.4650	7.9208
3	2.71335	6.7832	38.0192	6.9464	2.8921	6.8860	37.3510	6.7303
4	2.2226	5.5564	43.5756	6.1130	2.3091	5.4979	42.8489	5.9367
5	2.1679	5.4197	48.9953	5.4880	1.7741	4.2241	47.0730	5.3415
6	1.7324	4.3309	53.3262	4.9880	1.6668	3.9686	51.0415	4.8653
7	1.5872	3.9679	57.2941	4.5714	1.5571	3.7075	54.7490	4.4684
8	1.3665	3.4164	60.7104	4.2142	1.4202	3.3814	58.1304	4.1283
9	1.2509	3.1272	63.8376	3.9017	1.3103	3.1198	61.2501	3.8307
10	1.1211	2.8029	66.6405	3.6239	1.1641	2.7717	64.0218	3.5661
11	1.0497	2.6243	69.2647	3.3739	1.1217	2.6706	66.6925	3.3280
12	1.0152	2.5379	71.8027	3.1467	1.0394	2.4748	69.1673	3.1116

In order to show differences between all the morphospecies, PCA was performed on selected 4 or 5 populations, so that each population represented a different morphospecies. The results for the Polish morphospecies studied, represented by populations 19 *(B. austriaca)*, 7 *(B. cylindrica)*, 17 *(B. zyvionteki)* and 6 *(Bythinella* sp. (5)) are shown in Table 7 and Figures 13 and 14. For the males, all the populations were dis-

Fig. 11. Principal component analysis for all male specimens of *Bythinella cylindrica* (populations 7, 10) and *B. zyvionteki* (populations 17, 18). For population numbers see: Localities, collection and morphological study

Fig. 12. Principal component analysis for all female specimens of *Bythinella cylindrica* (populations 7, 10) and *B. zyvionteki* (populations 17, 18). For population numbers see: Localities, collection and morphological study

Fig. 13. Principal component analysis for all male specimens of *Bythinella* sp. (5) (population 6), *B. cylindrica* (population 7), *B. zyvionteki* (population 17) and *B. austriaca* (population 19). For population numbers see: Localities, collection and morphological study

Fig. 14. Principal component analysis for all female specimens of *Bythinella* sp. (5) (population 6), *B. cylindrica* (population 7), *B. zyvionteki* (population 17) and *B. austriaca* (population 19). For population numbers see: Localities, collection and morphological study

tinct (Fig. 13). Specimens of *B. austriaca* were scattered along the centroid and slightly mixed with the other populations. PCA for the females (Fig. 14) separated *B. cylindrica* well (no individual from this population was placed among those belonging to the other populations) and *Bythinella* sp. (5) rather well (this group comprises a few specimens of *B. austriaca*). Contrary to the males, the females of population 17

Table 7. Principal component analysis for all specimens of *Bythinella austriaca* (population 19), *B. cylindrica* (population 7), *B. zyvionteki* (population 17), *Bythinella* sp. (5) (population 6); eigenvalues and percent of variability explained. Proportions of variance expected under broken-stick model

PC no	MALES				FEMALES			
	Eigenvalue	Percent of variability explained	Cumulative percent	Expected percent	Eigenvalue	Percent of variability explained	Cumulative percent	Expected percent
1	8.7977	21.9942	21.9942	10.6964	6.0971	14.5168	14.5168	10.3018
2	4.4417	11.1043	33.0985	8.1964	5.5482	13.2100	27.7269	7.9208
3	3.0097	7.5242	40.6227	6.9464	3.9344	9.3677	37.0945	6.7303
4	2.4719	6.1796	46.8024	6.1130	2.7982	6.6624	43.7570	5.9367
5	1.9783	4.9459	51.7482	5.4880	2.0896	4.9751	48.7321	5.3415
6	1.7483	4.3707	56.1189	4.9880	1.6422	3.9100	52.6421	4.8653
7	1.4959	3.7397	59.8586	4.5714	1.5581	3.7098	56.3519	4.4684
8	1.3427	3.3568	63.2153	4.2142	1.3536	3.2229	59.5748	4.1283
9	1.2327	3.0818	66.2971	3.9017	1.2631	3.0073	62.5821	3.8307
10	1.1940	2.9849	69.2820	3.6239	1.1985	2.8536	65.4356	3.5661
11	1.1233	2.8082	72.0902	3.3739	1.0994	2.6177	68.0533	3.3280
12	1.0001	2.5003	74.5905	3.1467	1.0892	2.5932	70.6466	3.1116

Table 8. Principal component analysis for all specimens of *Bythinella* populations from Slovakia (populations 2, 3, 5), Hungary (population 1) and *B. austriaca* from Poland (population 11); eigenvalues and percent of variability explained. Proportions of variance expected under broken-stick model

PC no	MALES				FEMALES			
	Eigenvalue	Percent of variability explained	Cumulative percent	Expected percent	Eigenvalue	Percent of variability explained	Cumulative percent	Expected percent
1	14.2357	35.5892	35.5892	10.6964	11.2288	26.7352	26.7352	10.3018
2	3.2078	8.0195	43.6087	8.1964	3.5236	8.3895	35.1247	7.9208
3	2.6174	6.5436	50.1524	6.9464	2.8960	6.8952	42.0199	6.7303
4	2.1073	5.2683	55.4207	6.1130	2.2470	5.3501	47.3700	5.9367
5	1.8133	4.5332	59.9539	5.4880	1.8933	4.5079	51.8779	5.3415
6	1.3720	3.4300	63.3839	4.9880	1.5713	3.7412	55.6191	4.8653
7	1.1174	2.7936	66.1775	4.5714	1.4622	3.4814	59.1006	4.4684
8	1.0190	2.5474	68.7249	4.2142	1.2662	3.0146	62.1152	4.1283
9	0.9471	2.3678	71.0927	3.9017	1.1844	2.8199	64.9351	3.8307
10	0.8969	2.2421	73.3349	3.6239	1.0504	2.5009	67.4360	3.5661
11	0.8735	2.1837	75.5186	3.3739	1.0190	2.4260	69.8620	3.3280
12	0.8070	2.0174	77.5359	3.1467	0.9230	2.1973	72.0593	3.1116

(*B. zyvionteki*) were completely mixed with *B. austriaca*.

Populations 2, 3 and 5 from Slovakia, population 1 from Hungary, each of them representing a different, yet undescribed, morphospecies, and *B. austriaca* from Poland (population 11) were analysed together (Table 8, Figs 15, 16). The projection of males in the first and second PC space (Fig. 15) showed the dis-

Fig. 15. Principal component analysis for all male specimens of *Bythinella* sp. 1 (population 1), *Bythinella* sp. 2 (population 2), *Bythinella* sp. 3 (population 3), *Bythinella* sp. 4 (population 5) and *B. austriaca* (population 11). For population numbers see: Localities, collection and morphological study

Fig. 16. Principal component analysis for all female specimens of *Bythinella* sp. 1 (population 1), *Bythinella* sp. 2 (population 2), *Bythinella* sp. 3 (population 3), *Bythinella* sp. 4 (population 5) and *B. austriaca* (population 11). For population numbers see: Localities, collection and morphological study

tinctness of population 11 along the first component axis, all the remaining populations overlapping each other, with some outliers in each population. The females (Fig. 16) were more distinct, the specimens of population 3 were grouped marginally along the second axis, whereas on the other side of the axis populations 1 and 5 were situated and separated along the third axis. Along the centre the specimens of population 11 were mixed with 2.

ALLOZYMIC INTERPOPULATION VARIATION

Out of the 26 enzyme systems checked initially (acid phosphatase (ACP) E.C.3.1.3.2, aconitate hydratase (ACOH) E.C.4.2.1.3, adenylate kinase (AK) E.C.2.7.4.2, alcohol dehydrogenase (ADH) E.C.1.1.1.1, aldehyde oxidase (AO) E.C.1.2.3.1, alkaline phosphatase (ALP) E.C.3.1.3.1, aspartate aminotransferase (AAT) E.C.2.6.1.1, esterases (EST) E.C.3.1.1.1, fumarate hydratase (FUMH) E.C.4.2.1.2, glucose dehydrogenase (GCDH) E.C.1.1.1.118, glucose-6-phosphate dehydrogenase (G6PDH) E.C.1.1.1.49, glucose-6-phosphate isomerase (GPI) E.C.5.3.1.9, glutamate dehydrogenase (GTDH) E.C.1.4.1.2, glycerol-3-phosphate dehydrogenase (G3PDH) E.C.1.1.1.8, hexokinase (HK) E.C.2.7.1.1, beta-hydroxybutyrate dehydrogenase (HBDH) E.C.1.1.1.30, L-iditol dehydrogenase (IDDH) E.C.1.1.1.14, isocitrate dehydrogenase (IDH) E.C.1.1.1.42, lactate dehydrogenase (LDH) E.C.1.1.1.27, malate dehydrogenase (MDH) E.C.1.1.1.37, malic enzyme (MDHP) E.C.1.1.1.40, mannose phosphate isomerase (MPI) E.C.5.3.1.8, phosphoglucomutase (PGM) E.C.5.4.2.2, 6-phosphogluconate dehydrogenase (PGDH) E.C.1.1.1.44, superoxide dismutase (SOD) E.C.1.15.1.1, xanthine oxidase (XO) E.C.1.2.3.22), well resolved and always genetically interpretable zymograms were found for eight systems represented by nine loci (GPI, MDH, MDHP, IDDH-1, IDDH-2, PGDH, AAT, PGM and ALP). The allele frequencies data and the results of the molecular study on the evolutionary processes in populations of *Bythinella* are presented in FALNIOWSKI et al. (1998, 1999) and SZAROWSKA et al. (1998).

The lowest Prevosti distance values (Table 9) were those between populations 11 and 17, between 11 and 14, as well as 14 and 17. The highest value (0.356) was found between populations 1 and 13. The values exceeding 0.3 were also those between populations 1 and 12, 18, 19; 5 and 13, 18; 20 and 13, 18 as well as 2 and 6, 13. Multidimensional scaling (MDS) placed populations 5, 20 and 12 farthest from the centroid on one side, and populations 3 and 13 on the opposite side of the same axis (Fig. 17). On the axis transverse to the one definied above, population 1 was situ-

Table 9. Above diagonal: Prevosti genetic distances, below diagonal: unbiased Nei genetic distances

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	*****	0.255	0.293	0.208	0.216	0.285	0.260	0.221	0.232	0.288	0.273	0.314	0.356	0.254	0.210	0.242	0.272	0.347	0.306	0.238
2	0.191	*****	0.276	0.217	0.227	0.305	0.213	0.198	0.207	0.199	0.207	0.258	0.312	0.198	0.235	0.216	0.200	0.259	0.245	0.251
3	0.291	0.219	*****	0.160	0.251	0.233	0.188	0.133	0.147	0.200	0.172	0.260	0.285	0.163	0.207	0.204	0.181	0.262	0.249	0.269
4	0.167	0.131	0.110	*****	0.160	0.117	0.113	0.041	0.057	0.090	0.068	0.115	0.181	0.053	0.050	0.047	0.076	0.158	0.106	0.178
5	0.190	0.142	0.210	0.135	*****	0.229	0.218	0.175	0.188	0.243	0.225	0.265	0.320	0.201	0.168	0.191	0.218	0.307	0.249	0.036
6	0.250	0.217	0.184	0.071	0.210	*****	0.185	0.115	0.118	0.181	0.152	0.219	0.246	0.143	0.141	0.157	0.160	0.210	0.206	0.232
7	0.198	0.100	0.124	0.040	0.155	0.111	*****	0.091	0.075	0.108	0.083	0.201	0.183	0.075	0.123	0.118	0.081	0.135	0.165	0.236
8	0.172	0.130	0.100	0.006	0.153	0.072	0.027	*****	0.022	0.073	0.056	0.141	0.163	0.039	0.088	0.081	0.057	0.137	0.128	0.195
9	0.180	0.132	0.103	0.015	0.171	0.080	0.024	0.001	*****	0.089	0.063	0.157	0.145	0.053	0.104	0.096	0.057	0.119	0.146	0.208
10	0.237	0.137	0.159	0.042	0.211	0.123	0.042	0.045	0.048	*****	0.034	0.136	0.200	0.042	0.090	0.054	0.039	0.078	0.057	0.261
11	0.219	0.125	0.126	0.021	0.185	0.095	0.024	0.018	0.019	0.005	*****	0.137	0.170	0.023	0.067	0.038	0.011	0.091	0.082	0.243
12	0.302	0.175	0.233	0.097	0.261	0.183	0.124	0.110	0.119	0.080	0.082	*****	0.279	0.136	0.139	0.121	0.147	0.147	0.125	0.283
13	0.323	0.255	0.233	0.128	0.318	0.201	0.128	0.108	0.102	0.142	0.116	0.232	*****	0.159	0.205	0.203	0.160	0.213	0.252	0.337
14	0.194	0.122	0.115	0.010	0.166	0.083	0.023	0.008	0.010	0.013	0.001	0.085	0.112	*****	0.052	0.045	0.025	0.110	0.094	0.224
15	0.174	0.141	0.141	0.009	0.140	0.089	0.062	0.030	0.046	0.034	0.027	0.086	0.159	0.020	*****	0.041	0.076	0.158	0.105	0.180
16	0.192	0.131	0.139	0.014	0.160	0.093	0.045	0.027	0.038	0.011	0.009	0.075	0.145	0.008	0.005	*****	0.044	0.122	0.069	0.214
17	0.208	0.124	0.126	0.021	0.186	0.096	0.024	0.017	0.018	0.006	0.000	0.084	0.114	0.001	0.029	0.010	*****	0.089	0.093	0.239
18	0.311	0.158	0.194	0.095	0.296	0.172	0.057	0.078	0.069	0.021	0.028	0.088	0.152	0.042	0.102	0.059	0.027	*****	0.110	0.325
19	0.294	0.176	0.221	0.084	0.251	0.166	0.089	0.101	0.108	0.012	0.034	0.093	0.202	0.049	0.054	0.027	0.036	0.044	*****	0.281
20	0.192	0.166	0.240	0.150	0.007	0.237	0.193	0.182	0.207	0.239	0.216	0.275	0.362	0.194	0.142	0.173	0.218	0.350	0.272	*****

ated on one side, and 18 on the other. Populations 6, 7 and 19 were placed moderately far from the centroid, whereas all the remaining populations grouped around the centroid.

Unbiased Nei distance values (Table 9) ranged from 0.000 between populations 11 and 17 (0.001 between 8 and 9, as well as 17 and 14) to 0.362 between 13 and 20. High Nei distances were those between populations 1, 2, 3, 5, 20 and each of the other populations, except that between 5 and 20. MDS (Fig. 18) placed populations 5 and 20 together and farthest from the centroid on one side, and population 13 on

Fig. 17. Minimum spanning tree projected on multidimensional scaling (first three factors), based on Prevosti distance. For population numbers see: Localities, collection and morphological study

Fig. 18. Minimum spanning tree projected on multidimensional scaling (first three factors), based on Nei unbiased distance. For population numbers see: Localities, collection and morphological study

the opposite side; on the transverse axis, populations 3 and 6 were placed on one side, and 2 on the other. Populations 1, 18 and 19 were located moderately far from the centroid. The remaining populations were closely associated with the centroid.

Cavalli-Sforza and Edwards arc distances (Table 10) were the lowest between populations 11 and 14, between 17 and 14, 11, as well as 8 and 9; the highest values were those between populations 20 and 18, between 12 and 13, as well as 1 and 12. High

Table 10. Above diagonal: Cavalli-Sforza and Edwards arc genetic distances, below diagonal: geographic distances

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	*****	0.358	0.442	0.389	0.413	0.494	0.396	0.357	0.358	0.406	0.421	0.510	0.491	0.410	0.397	0.409	0.402	0.469	0.462	0.428
2	16.0	*****	0.386	0.354	0.339	0.464	0.366	0.339	0.340	0.378	0.369	0.445	0.441	0.359	0.368	0.369	0.359	0.423	0.412	0.377
3	43.0	40.0	*****	0.288	0.375	0.413	0.333	0.283	0.286	0.339	0.313	0.436	0.411	0.304	0.318	0.323	0.317	0.396	0.387	0.406
4	100.0	95.0	57.0	*****	0.357	0.317	0.185	0.096	0.117	0.152	0.110	0.334	0.308	0.092	0.088	0.109	0.125	0.280	0.219	0.377
5	122.0	111.0	100.0	115.0	*****	0.466	0.393	0.359	0.374	0.412	0.393	0.486	0.474	0.379	0.369	0.374	0.389	0.489	0.431	0.128
6	157.0	143.0	118.0	112.0	50.0	*****	0.356	0.311	0.310	0.363	0.338	0.456	0.425	0.330	0.339	0.347	0.342	0.392	0.403	0.476
7	186.0	175.0	147.0	120.0	83.0	35.0	*****	0.188	0.182	0.174	0.162	0.376	0.327	0.165	0.205	0.190	0.164	0.242	0.240	0.431
8	178.0	165.0	134.0	100.0	93.0	40.0	33.0	*****	0.062	0.195	0.156	0.341	0.302	0.137	0.164	0.170	0.153	0.282	0.270	0.395
9	185.0	172.0	140.0	108.0	100.0	50.0	38.0	7.5	*****	0.197	0.159	0.347	0.302	0.133	0.184	0.182	0.152	0.268	0.283	0.416
10	205.0	192.0	165.0	139.0	95.0	50.0	15.0	40.0	38.0	*****	0.063	0.338	0.315	0.094	0.128	0.091	0.076	0.188	0.114	0.439
11	240.0	225.0	192.0	155.0	148.0	97.0	68.0	63.0	59.0	58.0	*****	0.337	0.298	0.051	0.110	0.079	0.060	0.201	0.142	0.425
12	240.0	225.0	192.0	155.0	148.0	97.0	68.4	65.0	60.0	56.0	2.8	*****	0.451	0.338	0.339	0.337	0.343	0.295	0.342	0.502
13	240.0	225.0	195.0	160.0	142.0	92.0	66.2	66.0	61.0	52.0	9.0	5.7	*****	0.293	0.321	0.314	0.286	0.354	0.356	0.510
14	249.0	234.0	203.0	167.0	151.0	101.0	69.4	70.4	66.0	56.0	13.0	10.0	4.7	*****	0.105	0.069	0.057	0.222	0.167	0.412
15	249.0	234.0	203.0	167.0	151.0	101.0	71.4	73.0	69.0	58.0	15.0	11.9	7.5	3.8	*****	0.076	0.127	0.285	0.172	0.376
16	235.0	218.0	185.0	155.0	135.0	85.0	57.0	53.0	47.0	45.4	11.0	11.7	13.1	18.1	20.6	*****	0.088	0.247	0.122	0.401
17	262.0	248.0	214.0	173.0	173.0	122.0	92.4	86.0	79.0	80.0	25.0	25.3	28.1	28.1	26.6	35.4	*****	0.201	0.157	0.423
18	263.0	249.0	217.0	174.0	173.0	121.0	91.2	88.0	80.0	78.0	24.0	22.5	24.0	23.1	20.6	33.8	5.5	*****	0.229	0.538
19	300.0	285.0	253.0	208.0	210.0	162.0	128.0	123.8	116.8	115.0	60.0	61.3	60.9	59.0	57.0	69.4	34.8	37.5	*****	0.462
20	305.0	302.0	265.0	212.0	298.0	259.0	253.0	220.0	215.0	252.0	205.0	208.0	220.0	220.0	220.0	215.0	200.0	203.0	200.0	*****

Fig. 19. Minimum spanning tree projected on multidimensional scaling (first three factors), based on Cavalli-Sforza and Edwards distance. For population numbers see: Localities, collection and morphological study

Cavalli-Sforza and Edwards distances were found between populations 1, 2, 5, 6, 20 and each of the other studied populations, except that between 5 and 20. MDS (Fig. 19) placed populations 5 and 20 together along one axis and marginally on one side of the centroid, and population 6 on the other side. Along the transverse axis, populations 1 and 3 were situated on one side, and populations 13 and 18 on the other.

Table 11. Correspondence analysis – eigenvalues and percent of variability explained

Factor no	Eigenvalue	Percent of variability explained	Cumulative percent
1	0.15622	18.39	18.39
2	0.11812	13.91	32.30
3	0.11188	13.17	45.47
4	0.11089	13.06	58.52
5	0.09579	11.28	69.80
6	0.08414	9.91	79.71
7	0.07733	9.10	88.81
8	0.04400	5.18	93.99
9	0.02402	2.83	96.82
10	0.01460	1.72	98.54

Populations 2 and 12 were located moderately far from the centroid, while the other populations were closely associated with it.

The phenetic analysis of allozymic differentiation included also correspondence analysis of allele frequencies. The cumulative variability explained by the first ten dimensions covered 98.54% of the total variability (Table 11). The plot of the column factors in the first two-dimension space (Fig. 20) showed populations 6, 3, 5, 20, 1 and 2 scattered along the first axis, populations 13, 12 and 18 scattered along the second axis and all the remaining populations grouped close to the centre.

INTERPOPULATION VARIATION AND GEOGRAPHIC DISTANCES

Mantel test (Table 12) showed no significant correlation between each of the genetic distances (Tables 9, 10) and the Euclidean distances based on morphology (Table 13) for the males and females alike. However, a statistically significant positive correlation was observed between all the genetic distances and the geographic distances. Also Euclidean distances were significantly and positively correlated with geographic distances; for males the correlation was weaker yet statistically significant.

Fig. 20. Correspondence analysis, first and second dimension. For population numbers see: Localities, collection and morphological study

Table 12. Matrix correlation (top row: matrix correlation r, mid row: approximate Mantel statistic Z, bottom row: probability p that random Z<obs. Z), N = 190; values not significant are given in italics, *indicates 0.05 significance level, the other 0.01. Euclid.m. – Euclidean distance based on all morphological characters of males; Euclid.f. – Euclidean distance based on all morphological characters of females; C-S&E – Cavalli-Sforza and Edwards arc distance; Prevosti - Prevosti distance; Nei un. – Nei unbiased distance; geog. – geographic distance

	Euclid.m.	Euclid.f.	C-S&E	Prevosti	Nei un.	geog.
Euclid.m.	-.——	0.3903	*-0.1088*	*-0.1104*	*-0.0966*	0.3342
	-.——	2.231*	*-0.569*	*-0.573*	*-0.506*	2.036*
	-.——	0.9872	*0.2847*	*0.2835*	*0.3066*	0.9791
Euclid.f.	0.3903	-.——	*0.2233*	*0.2005*	*0.2205*	0.4796
	2.231*	-.——	*1.335*	*1.189*	*1.320*	3.309
	0.9872	-.——	*0.9090*	*0.8828*	0.9066	0.9995
C-S&E	*-0.1088*	*0.2233*	-.——	0.9413	0.9260	0.5376
	-0.569	*1.335*	-.——	5.116	5.079	3.420
	0.2847	*0.9090*	-.——	1.0000	1.0000	0.9997
Prevosti	*-0.1104*	*0.2005*	0.9413	-.——	0.9681	0.5814
	-0.573	*1.189*	5.116	-.——	5.267	3.671
	0.2835	*0.8828*	1.0000	-.——	1.0000	0.9999
Nei	*-0.0966*	*0.2205*	0.9260	0.9681	-.——	0.5765
	-0.506	*1.320*	5.079	5.267	-.——	3.671
	0.3066	*0.9066*	1.0000	1.0000	-.——	0.9999
geog.	0.3342	0.4796	0.5376	0.5814	0.5765	-.——
	2.036*	3.309	3.420	3.671	3.671	-.——
	0.9791	0.9995	0.9997	0.9999	0.9999	-.——

Table 13. Euclidean distances between population of *Bythinella*, based on all morphological characters: above diagonal – males, below diagonal – females

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	*****	4.789	7.003	18.161	6.094	5.896	6.940	8.517	6.651	6.893	11.824	7.714	7.892	9.582	8.109	8.643	10.172	8.009	9.535	11.482
2	6.898	*****	7.812	18.534	6.596	6.339	7.266	9.082	5.053	6.207	12.863	9.036	8.666	10.696	8.502	9.953	10.228	8.433	11.533	11.907
3	8.230	7.427	*****	16.780	4.476	5.294	5.350	5.728	7.667	7.446	9.327	7.971	6.497	7.051	7.669	6.247	8.801	5.421	7.675	9.196
4	9.742	9.794	7.699	*****	17.051	17.065	16.952	17.170	17.718	18.084	17.149	16.769	16.924	17.124	16.541	16.483	17.414	16.304	16.751	17.772
5	7.319	7.576	8.208	9.167	*****	4.954	4.567	5.565	5.968	5.854	9.078	7.353	5.698	6.637	6.657	5.779	7.798	5.279	7.493	9.257
6	8.393	8.430	9.260	10.108	6.631	*****	4.795	5.213	5.468	6.341	9.047	7.272	5.396	6.864	6.613	6.152	8.722	5.207	8.046	8.991
7	7.753	7.855	8.798	9.884	7.976	7.442	*****	4.117	5.607	4.727	8.603	7.289	4.688	6.168	4.836	4.942	6.825	4.341	7.576	8.365
8	7.979	8.778	8.235	8.104	5.972	6.570	6.191	*****	7.622	6.959	6.975	8.045	4.393	4.503	6.968	4.614	7.138	5.008	7.024	6.707
9	8.229	8.007	7.686	8.668	7.233	7.439	6.568	6.118	*****	4.497	11.539	8.345	6.799	9.384	6.360	8.228	8.661	7.038	10.129	11.590
10	9.207	7.592	8.768	8.507	7.837	8.247	8.736	7.639	8.418	*****	12.137	9.498	6.602	9.607	6.931	8.195	8.063	6.725	10.067	11.468
11	11.621	14.575	12.919	12.699	11.403	11.773	10.432	10.253	10.782	14.678	*****	7.829	7.390	4.647	8.810	5.803	9.281	7.489	7.413	6.837
12	9.181	10.159	9.312	9.515	10.216	11.050	8.926	9.659	9.381	11.552	8.800	*****	6.364	6.567	5.827	6.280	9.203	6.215	6.658	9.299
13	7.255	8.446	8.909	8.764	7.126	8.059	7.474	7.445	6.946	6.663	10.765	9.271	*****	5.135	5.813	4.064	6.857	4.236	5.160	8.123
14	7.921	9.854	8.898	8.046	7.268	8.165	7.204	6.109	6.782	9.759	6.997	7.445	7.082	*****	7.033	3.347	7.807	5.187	5.506	6.222
15	10.417	10.359	9.215	8.915	11.093	11.853	10.313	10.122	9.150	10.468	11.419	6.772	8.718	9.050	*****	5.192	7.805	4.727	7.123	9.506
16	8.229	11.189	9.941	8.999	7.902	8.881	7.977	6.366	7.205	10.024	7.130	8.934	7.158	4.979	9.614	*****	7.272	3.743	4.606	7.304
17	9.032	9.450	9.500	7.458	7.893	9.337	9.267	7.460	8.448	7.473	11.239	9.261	7.042	7.119	8.606	7.954	*****	6.963	8.697	9.581
18	9.593	9.765	8.437	7.661	8.185	8.287	6.903	6.985	7.221	8.096	9.841	8.391	6.596	6.075	8.638	6.258	6.574	*****	5.667	7.492
19	8.235	10.661	9.832	8.604	7.228	7.842	8.450	6.732	7.462	9.539	8.669	9.100	6.945	5.411	10.228	4.719	7.893	6.504	*****	8.842
20	13.439	15.641	15.127	12.985	12.531	12.446	11.549	8.918	12.749	14.712	9.846	12.637	12.901	9.321	14.740	9.532	12.438	11.636	10.743	*****

DISCUSSION

MORPHOLOGICAL DIFFERENCES

Principal component analysis showed, for both sexes, little morphological interpopulation differentiation and numerous outliers in almost all the populations. This confirms FALNIOWSKI's (1987) observations concerning the weak interspecific differentiation and overlapping variability ranges in *Bythinella*. The morphological separateness of the preliminarily distinguished morphospecies is also poorly marked, and the picture differs between sexes. There are minor morphological differences between the postulated morphospecies from Hungary and Slovakia. On the other hand, principal component analysis has confirmed the morphological separateness of the Polish morphospecies distinguished by FALNIOWSKI (1987, 1992), although not unequivocally, simultaneously indicating relatively large interpopulation differences within *B. austriaca*. This method demonstrates that the Polish morphospecies, although distinguishable, differ little between each other. Another observation in agreement with the earlier data (FALNIOWSKI 1987) is the fact that within each morphospecies there are specimens that differ from the typical ones so much that it may be impossible to determine them correctly.

The widest intrapopulation variation, coupled with the largest differentiation between populations, was observed in *B. austriaca*. Despite this, the variability range of this morphospecies overlaps the ranges of the remaining studied morphospecies only slightly. In the group of *B. austriaca* populations, the most distinct is population 20 of the spring Źródło Romanowskie near Kłodzko. This is the farthest and westernmost locality of *Bythinella* in Poland. The spring is situated at the distribution border of the genus and is the only *Bythinella* locality in Lower Silesia (WIKTOR 1964). It is the type locality of *B. austriaca* ssp. *ehrmanni* (Pax, 1938). As noted by FALNIOWSKI (1987), the snails of this population have exceptionally large shells, and their penes and female reproductive organs differ in shape from those of *B. austriaca* from the Kraków-Częstochowa Upland. The difference may be due to the geographic isolation of this population, or an adaptation to somewhat different environmental conditions.

The high interpopulation differentiation among the remaining populations of *B. austriaca* may result from different habitat conditions at particular localities. PONDER et al. (1996) suggest that the high morphological variation in the genus *Dalhousia* inhabiting artesian springs of Australia may be caused by some environmental, physico-chemical and/or biotic factors. It has to be stressed that in this study the most numerous populations were those of *B. austriaca,* and it is difficult to compare its differentiation with species represented by fewer populations. Furthermore, the populations of *B. austriaca* distributed practically all over the studied territory probably display the largest differentiation, and although the environmental conditions seem almost the same in all the springs inhabited by the snails, the stability of the conditions in each spring may be somewhat different. HYLLEBERG (1975, 1976) found that such seemingly unimportant ecological differences are essential to *Hydrobia*.

On the other hand, the morphological differences between the postulated morphospecies 1 and 2 from Hungary and Slovakia seem weak. PONDER et al. (1989) reported a resemblance between the shells of three species of *Fonscochlea* in Kewson Hill springs, pointing to the similar ecotypic reaction to the same environmental stress in the springs inhabited by these snails. They also noted that such evident differences between the springs, as their size or substrate type, were not correlated with any conspicuous differences in the phenotype of the inhabiting snails. This may also concern the studied morphospecies of *Bythinella*.

PCA placed the females of *B. zyvionteki* among *B. austriaca* whereas the males were farther from *B. austriaca* and closer to *B. cylindrica*. *B. zyvionteki* is characterized by its penis shape, which is completely different from that of *B. austriaca*. With respect to their female reproductive organs, both morphospecies are highly variable and their variability ranges almost overlap. This sex-dependent morphological distinctness of *B. zyvionteki* agrees with the characters, based on which FALNIOWSKI (1987) distinguished this species.

ALLOZYME DIFFERENCES

Despite all the criticism concerning Nei distance, the latter is most commonly applied, therefore it was used in this study for comparison with the results of other similar investigations. It must be stressed, however, that the mere fact that a computed Nei value is within a specified range is not a sufficient criterion of intra- versus interspecific, or else intergeneric differences. In his survey of over 7,000 comparisons of conspecific populations of plants and animals, THORPE (1983) found that only 2% of the intraspecific estimates exceeded 0.1. In a cephalopod *Nautilus pompilius* the value found did not exceed 0.06 (WOODRUFF et al. 1987), and was 0.002–0.108 in another cephalopod, *Loligo forbesi* (BRIERLEY et al. 1993); in a bivalve *Mytilus galloprovincialis* it was 0.001–0.018 (KARAKOUSIS et al. 1993); 0.08–0.29 in *Teredo* (HOAGLAND 1986); 0.000–0.373 in *Chamelea gallina* (BACKELJAU et al. 1994); 0.023–0.137 in *Elliptio complanata* (DAVIS 1984).

The highest intraspecific Nei value for gastropods (0.701) was reported in parthenogenic *Melanoides tuberculata* (LIVSHITS et al. 1984) and the highest value for sexually reproducing gastropods (0.63) in *Cepaea nemoralis* (JOHNSON et al. 1984). However, usually the values are lower. For land snails, the intraspecific Nei distance values were as follows: 0.017–0.282 in *Bradybaena fruticum* (FALNIOWSKI et al. 1993), 0.001–0.340 in *Helix aspersa* (GUILLER et al. 1994; LAZARIDOU-DIMITRIADOU et al. 1994); for marine species: 0.001–0.051 in *Trochus* and *Tectus* (BORSA & BENZIE 1993), 0.002–0.016 in *Crepidula fornicata* (HOAGLAND 1985) and 0.000–0.007 in *Haliotis* (BROWN 1993).

Among freshwater snails, Nei values range from 0.000 to 0.057 in different *Viviparus* species (KATOH & FOLTZ 1994, FALNIOWSKI et al. 1996); 0.000–0.180 in *Biomphalaria glabrata* (MULVEY et al. 1988); 0.051–0.191 in various species of *Oncomelania* (DAVIS et al. 1994). For Hydrobiidae the following values were found: 0.000–0.012 in *Hydrobia* (DAVIS et al. 1988), 0.000–0.118 in *Graziana* (HAASE 1994) and 0.065–0.428 in the artesian spring inhabitant *Fonscochlea zeidleri* (PONDER et al. 1995). In the present study, the Nei distances among the populations of *Bythinella* ranged from 0.000 to 0.362, and in most cases they were within the range of intraspecific variation. However, as was already mentioned and shown by the above examples, it is impossible to specify the exact values delimiting intra- from interspecific differences, even when the results are compared with those obtained for Hydrobiidae only.

WOODRUFF et al. (1988) report that interspecific Nei distances for congeners are within the range of 0.2–0.6. THORPE (1983) reviewed 900 comparisons of interspecific Nei values for congeners in plants and animals and reported a mean value of about 0.40 (from 0.03 to more than 1). WOODRUFF et al. (1987) found values 0.072–0.834 for *Nautilus*; HOAGLAND (1986) reported 0.63–0.88 for a bivalve *Teredo*; KAT (1983) – 0.373–1.32 for *Anodonta*; BACKELJAU et al. (1994) – 0.772–1.509 for *Chamelea*. The highest values of interspecific Nei distance between snail species were noted for marine prosobranchs: 5.383 in *Haliotis* (BROWN 1993), 1.726 in *Trochus* (BORSA & BENZIE 1993), but they equalled only 0.37 between the sympatric *Littorina mariae* and *L. obtusata* (ROLÁN-ALVAREZ et al. 1995). BACKELJAU et al. (1992) listed mean Nei values for congeneric species of land gastropods; they report the range from 0.039 in *Cristilabrum* to 0.482 in *Arion*. In *Samoana* interspecific Nei distances varied from 0.004 to 0.602 (JOHNSON et al. 1986). The value for species of *Partula*, which were ca. 8,000 km apart, was 0.125 (JOHNSON et al. 1977); for *Cerion*: 0.27 (WOODRUFF & GOULD 1987). BUTH & SULOWAY (1983) reported 0.45 for *Physa*, KATOH & FOLTZ (1994) and FALNIOWSKI et al. (1996) – 0.230–0.989 for *Viviparus*, but RUDOLPH & BURCH (1989) only 0.077 for *Stagnicola*.

In Hydrobiidae the values of interspecific Nei distance have also wide ranges, e.g. 0.111–1.735 in *Hydrobia* (HAASE 1993), 0.577–0.655 in *Belgrandiella* and 0.000–0.803 in *Graziana* (HAASE 1994), or 0.432–0.573 in *Fonscochlea* and 0.277–1.798 in *Trochidrobia* (PONDER et al. 1995). The above data clearly indicate that there is no general rule concerning the Nei distance. For many congeneric species its interspecific values are lower than distances between populations within one species, e.g. in *Partula*

(MURRAY et al. 1993) or *Graziana* (HAASE 1994). In *Bythinella* Nei distances (0.000–0.362) were never high. The values ranged from ones characteristic of apparently conspecific populations to ones characteristic rather of congeneric species. In some morphospecies the distances between populations were higher than those between the morphospecies, like in *Partula* and *Graziana*.

The values of Nei distance between the populations of *B. cylindrica* (0.001–0.048) were low, and relatively low, though varied, in *B. austriaca*. The highest values, approximating interspecific comparisons (near 0.2) were those between population 20 (*B. austriaca* ssp. *ehrmanni*) and each of the remaining populations (except population 5). Like the morphological data, this confirms the distinctness of population 20, as shown by multidimensional scaling. It is difficult to explain the observed high genetic similarity (Nei distance: 0.007) of populations 20 and 5, which is contrary to the large morphological differences between them. This situation is similar to that described in *Graziana* (HAASE 1994). Perhaps the loci considered for these two populations are not sufficiently representative.

The high values (often exceeding 0.2) of Nei distances are characteristic of the Hungarian population 1, Slovakian populations 2, 3 and 5 (all these populations are purported morphospecies) as well as for population 6 from the Polish Tatra Mountains, distinguished by FALNIOWSKI (1987) as *Bythinella* sp.

The other computed genetic distances and the correspondence analysis of allele frequencies revealed a pattern of interpopulation variation in allozymes similar to that resulting from Nei distances. Cavalli-Sforza and Edwards arc distance reflects small differences between populations better then the other coefficients.

INTERPOPULATION DIFFERENCES AND GEOGRAPHIC DISTANCES

No significant correlation was found between the genetic distances and Euclidean distances based on morphology. The patterns of interpopulation distinctness shown by phenetic multidimensional analysis are different for the molecular and morphological data sets and do not unequivocally confirm either the morphological or molecular distinctness of the purported morphospecies. However, to draw any taxonomic conclusions, phylogenetic analysis is needed, which will be presented in a separate paper.

On the other hand, all the genetic distances as well as Euclidean distances are correlated with geographic distances. Moreover, some of the morphological characters show a geographic variation of a clinal character (FALNIOWSKI 1987, 1992, MAZAN 2000). This, evidently geographic pattern of interpopulation variation in both morphological and allozymic character sets in *Bythinella*, seems to point to the stepping-stone model of geographic subdivision rather than being typical for groups of isolated populations (FALNIOWSKI et al. 1998).

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