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

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ABSTRACT: Phylogenetic relationships among 20 Central European populations of the genus *Bythinella* have been reconstructed on the basis of morphological characters and allozymic variation presented in our previous papers. Fitch-Margoliash additive trees based on Euclidean distances were constructed for morphological data, and genetic distances (Prevosti, unbiased Nei, Cavalli-Sforza and Edwards arc distances) were computed. Of all the genetic trees which were fairly similar, those based on Cavalli-Sforza and Edwards arc distances seem to reflect the phylogeny best. 21 discrete morphological character states were used to reconstruct phylogenies by means of the parsimony method. Allele frequencies were used directly to compute a tree of interpopulation relationships by means of the frequency-parsimony method. The inferred morphological and allozymic phylogenies differ in their topology and the amount of evolution (measured as the number of changes averaged over all reconstructions) along the corresponding branches; the correlation between them is statistically insignificant. The species rank of most of the postulated morphospecies seems doubtful and requires a further study.

KEY WORDS: spring snail, *Bythinella*, population, variation, morphology, allozymes, systematics, phylogeny

INTRODUCTION

Members of the genus *Bythinella* are minute prosobranchs inhabiting springs and subterranean waters of South and Central Europe. Taxonomy within the European *Bythinella* has always caused a great deal of controversies and still remains unclear. BOETERS (1973) reduced the number of West European *Bythinella* to a few species showing conspicuous differences in anatomy. However, it seems that as a result of insufficient material, he overlooked the wide variation (FALNIOWSKI 1987, 1992). On the other hand, RADOMAN (1976) maintained that closely related species could not be differentiated anatomically and, consequently, distinguished dozens of species from the Balkans and Asia Minor. GIUSTI & PEZZOLI (1977, 1980) concluded that all the Italian, and probably all the European *Bythinella*, represented a single superspecies.

For a long time, most members of the genus *Bythinella* from Poland were recorded as *B. austriaca* (Frauenfeld, 1856). In some of the earlier papers, *B. cylindrica* (Frauenfeld, 1856) and *B. hungarica* (Hazay, 1881) were mentioned (FALNIOWSKI 1987). Later, based on morphological characters only, FALNIOWSKI (1987, 1992) distinguished six species: *B. austriaca*, *B. cylindrica*, *B. metarubra* Falniowski, 1987, *B. micherdzinskii* Falniowski, 1980, *B. zyvionteki* Falniowski, 1986 and *Bythinella* sp. and pointed to the wide ranges of morphological variation and minor interspecific differences between populations of *Bythinella*.

Results of the molecular study on the evolutionary processes in the studied populations of *Bythinella* have been published elsewhere (FALNIOWSKI et al. 1998, 1999, SZAROWSKA et al. 1998). Analysis of biometrical variation revealed considerable intrapopulation and slight interpopulation differences (MAZAN 2000, MAZAN & SZAROWSKA 2000). In this paper, the analysis

of morphological and allozymic differentiation within *Bythinella* was aimed at a comparison of the evolution of morphological and allozymic characters, and an attempt was made at a phylogeny reconstruction within the genus. The phylogenetic analysis has enabled us to make an attempt to determine not only their overall similarity reflected by the phenetic approach applied in MAZAN & SZAROWSKA (2000) but also the genealogical relationships between the studied populations.

MATERIAL AND METHODS

In 1994–95, *Bythinella* was sampled at 20 localities in Hungary (population 1), Slovakia (populations 2–5) and Poland (populations 6–20). A detailed description of the localities, a distribution map, collection and fixation techniques applied, methods of morphometric and allozymic studies, as well as multidimensional phenetic analysis of morphological and allozymic variation, have been described in MAZAN (2000) and MAZAN & SZAROWSKA (2000). About 300 specimens were collected from each population. For the morphometric study, wherever the material was sufficiently abundant, 30 males and 30 females were taken from each population, and 40 characters in males and 42 in females were counted or measured. Cellulose acetate-gel electrophoresis, as described by RICHARDSON et al. (1986), was applied. 26 enzyme systems were studied, at least 30 snails being electrophoresed for each. All populations were preliminarily classified as "morphospecies" (Table 1).

Multivariate techniques, although useful in visualization of internal and external relations of data, can hardly be applied to phylogeny reconstruction. Clustering reflects overall similarity and as such should not be applied to phylogeny reconstruction, since the data are usually not ultrametric (SWOFFORD & OLSEN 1990, WEIR 1990). Therefore, we used genetic distances (Prevosti, unbiased Nei, Cavalli-Sforza and Edwards arc distances), as well as Euclidean distances based on morphological characters (MAZAN & SZAROWSKA 2000) to compute additive phylogenetic trees by means of the Fitch-Margoliash method (FITCH & MARGOLIASH 1967), with FITCH of PHYLIP 3.5c package (FELSENSTEIN 1990). The option of EDWARDS & CAVALLI-SFORZA (1964) was used, which enabled us to apply the criterion in which the value of average percent standard deviation is a measure of goodness of fit of a tree. When comparing trees computed on the basis of different distances, the ones with the lowest values of average percent standard deviation are the best.

Twenty one discrete morphological character states (measurements, proportions, or qualitative descriptive characters) were used to reconstruct phylogeny by means of the parsimony method, to seek the simplest possible course of evolution (SWOFFORD & OLSEN 1990, WEIR 1990, MADDISON & MADDISON 1992). The characters were chosen from among those which showed statistically significant differences between populations, were biologically sound, and/or had earlier been taken into account in taxonomical studies on the genus. The evolution of the characters was analysed with MacCLADE (MADDISON & MADDISON 1992) and the shortest, most parsimonious tree was found by PAUP (SWOFFORD 1991). Since the characters had different weights and the knowledge of transformation series was insufficient in all cases, they were weighted during the analysis on the basis of biological premises and using Dynamic Weighting (WILLIAMS & FITCH 1989, 1990), performed with MacCLADE. However, in order to avoid circular reasoning, it was done manually, based on the parameters of evolution reconstruction of each character, every time for a new weight considering the biological soundness of the character. The tree statistics: consistency index (CI), retention index (RI), and rescaled consistency index (RC) were calculated for each tree (MADDISON & MADDISON 1992).

Allele frequencies were used directly to compute a tree by means of the frequency-parsimony method with FREQPARS (SWOFFORD & BERLOCHER 1987). The procedure uses a linear programming for finding the shortest tree, which joins the frequencies observed in the terminal taxa, and minimizes the amount of allele frequency change along each branch. The lengths of the branches on a tree are proportional to the amount of change in allele frequency.

The phylogenetic trees, based on either the distances or parsimony method, are unrooted. Their rooting was impossible because no information regarding outgroup and/or character polarization was available.

The resulting trees, constructed by different techniques on the basis of morphometry and allozyme data, were very different, thus constructing a consensus tree would cause too much information loss (DE QUEIROZ 1993). The rates of allozymic versus morphological evolution were compared using the method proposed by OMLAND (1994). The tree constructed with MacCLADE, based on morphological data, had the topology identical with the frequency-parsimony-based allozymic tree. The length of every branch, i.e. the amount of morphological change, was computed with two options: minimum

Table 1. Matrix of morphological characters and their states for parsimony analysis. Population No.: 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

	Character																				
Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1. Bernecebarati	*3*	*3*	*6*	*7*	*2*	*4*	*3*	*2*	*4*	*4*	*3*	*0*	*4*	*2*	*0*	*1*	*1*	*0*	*3*	*1*	*1*
2. Klenany	*2*	*4*	*5*	*6*	*2*	*3*	*3*	*2*	*3*	*3*	*3*	*0*	*6*	*1*	*0*	*2*	*1*	*0*	*2*	*2*	*0*
3. Bansky Studenec	*4*	*2*	*7*	*3*	*5*	*3*	*2*	*2*	*1*	*2*	*1*	*1*	*6*	*1*	*1*	*1*	*0*	*3*	*4*	*1*	*2*
4. Klacno	*1*	*2*	*4*	*2*	*0*	*3*	*1*	*1*	*2*	*4*	*3*	*1*	*0*	*2*	*0*	*1*	*0*	*2*	*4*	*3*	*3*
5. Spring of Hron	*2*	*0*	*5*	*5*	*3*	*4*	*3*	*2*	*4*	*3*	*1*	*0*	*3*	*1*	*1*	*2*	*1*	*2*	*2*	*0*	*1*
6. Strążyska Valley	*2*	*3*	*2*	*2*	*3*	*4*	*3*	*4*	*3*	*4*	*1*	*1*	*4*	*1*	*0*	*1*	*1*	*0*	*3*	*2*	*1*
7. Olszówka Stream	*2*	*2*	*4*	*5*	*3*	*3*	*1*	*1*	*1*	*2*	*3*	*1*	*2*	*0*	*1*	*2*	*0*	*1*	*4*	*2*	*2*
8. Krowiarki Pass	*2*	*2*	*4*	*5*	*2*	*4*	*2*	*2*	*1*	*1*	*2*	*2*	*2*	*0*	*1*	*1*	*0*	*1*	*3*	*3*	*2*
9. Zawoja-Składy	*1*	*1*	*2*	*4*	*4*	*3*	*2*	*2*	*2*	*2*	*3*	*2*	*0*	*0*	*1*	*1*	*0*	*1*	*3*	*0*	*1*
10. Węglówka	*0*	*1*	*3*	*1*	*1*	*4*	*1*	*1*	*1*	*3*	*0*	*0*	*2*	*1*	*1*	*0*	*0*	*3*	*4*	*3*	*3*
11. St. Elias' Spring	*5*	*2*	*3*	*1*	*2*	*2*	*2*	*1*	*0*	*4*	*4*	*2*	*3*	*1*	*0*	*1*	*0*	*5*	*5*	*0*	*5*
12. Bazan's Spring	*4*	*1*	*4*	*4*	*1*	*1*	*1*	*1*	*1*	*3*	*3*	*0*	*3*	*1*	*0*	*2*	*0*	*3*	*5*	*1*	*4*
13. Będkowska Valley	*2*	*1*	*4*	*1*	*1*	*3*	*2*	*2*	*2*	*3*	*0*	*1*	*2*	*1*	*0*	*2*	*0*	*4*	*6*	*0*	*3*
14. Sąspowska Valley	*3*	*1*	*4*	*2*	*2*	*4*	*2*	*1*	*1*	*3*	*4*	*2*	*3*	*1*	*0*	*2*	*0*	*4*	*0*	*0*	*4*
15. Młynnik Spring	*2*	*1*	*0*	*3*	*0*	*0*	*1*	*0*	*1*	*0*	*2*	*0*	*2*	*2*	*0*	*2*	*0*	*3*	*6*	*0*	*3*
16. Zimny Dół Valley	*3*	*1*	*3*	*1*	*3*	*4*	*2*	*1*	*1*	*3*	*3*	*2*	*2*	*2*	*0*	*2*	*0*	*3*	*5*	*0*	*3*
17. Chechło	*2*	*1*	*3*	*1*	*0*	*2*	*0*	*1*	*3*	*4*	*0*	*1*	*2*	*0*	*1*	*2*	*0*	*4*	*5*	*0*	*3*
18. Kadłubek spring	*2*	*1*	*2*	*0*	*5*	*2*	*0*	*1*	*1*	*4*	*2*	*2*	*6*	*0*	*1*	*2*	*0*	*5*	*7*	*0*	*5*
19. Zygmunt's springs	*3*	*0*	*4*	*5*	*2*	*3*	*2*	*2*	*2*	*5*	*3*	*1*	*2*	*1*	*0*	*2*	*0*	*3*	*1*	*2*	*4*
20. Romanowskie spring	*5*	*4*	*4*	*3*	*1*	*4*	*1*	*1*	*0*	*1*	*5*	*3*	*5*	*2*	*0*	*1*	*0*	*1*	*3*	*3*	*4*
Weight	0.7	0.7	1.0	0.7	0.5	0.7	1.0	1.0	0.5	0.7	0.7	0.5	0.2	1.0	1.0	0.5	1.0	0.7	0.5	0.5	1.0
Character type	O	O	O	O	O	O	O	O	O	O	O	O	O	U	U	U	U	O	O	O	O

Character No.: **1** – shell height (*0* – 2.300–2.399, *1* – 2.400–2.499, *2* – 2.500–2.599, *3* – 2.600–2.699, *4* – 2.700–2.799, *5* – 2.800–2.899); **2** – shell height:breadth ratio (*0* – 1.550–1.599, *1* – 1.600–1.649, *2* – 1.650–1.699, *3* – 1.700–1.749, *4* – 1.750–1.799); **3** – nucleus diameter (*0* – 0.140–0.149, *1* – 0.150–0.159, *2* – 0.160–0.169, *3* – 0.170–0.179, *4* – 0.180–0.189, *5* – 0.190–0.199, *6* – 0.200–0.209, *7* – 0.210–0.219); **4** – shell height to radula length ratio (*0* – 1.200–1.299, *1* – 1.300–1.399, *2* – 1.400–1.499, *3* – 1.500–1.599, *4* – 1.600–1.699, *5* – 1.700–1.799, *6* – 1.800–1.899, *7* – 1.900–1.999); **5** – radula transverse row number (*0* – 95.0–99.9, *1* – 100.0–104.9, *2* – 105.0–109.9, *3* – 110.0–114.9, *4* – 115.0–119.9, *5* – 120.0–124.9); **6** – central tooth plate cusp number (*0* – 3.500–3.999, *1* – 4.000–4.499, *2* – 4.500–4.999, *3* – 5.000–5.499, *4* – 5.500–5.999); **7** – lateral tooth inner cusp number (*0* – 3.000–3.499, *1* – 3.500–3.999, *2* – 4.000–4.499, *3* – 4.500–4.999); **8** – lateral tooth outer cusp number (*0* – 3.500–3.999, *1* – 4.000–4.499, *2* – 4.500–4.999, *3* – 5.000–5.499, *4* – 5.500–5.999); **9** – inner marginal tooth cusp number (*0* – 22.000–22.999, *1* – 23.000–23.999, *2* – 24.000–24.999, *3* – 25.000–25.999, *4* – 26.000–26.999); **10** – outer marginal tooth cusp number (*0* – 25.000–25.999, *1* – 26.000–26.999, *2* – 27.000–27.999, *3* – 28.000–28.999, *4* – 29.000–29.999, *5* – 30.000–30.999); **11** – ctenidium lamellae number (*0* – 18.000–18.999, *1* – 19.000–19.999, *2* – 20.000–20.999, *3* – 21.000–21.999, *4* – 22.000–22.999, *5* – 23.000–23.999); **12** – ctenidium length (*0* – 0.900–0.999, *1* – 1.000–1.099, *2* – 1.100–1.199, *3* – 1.200–1.299); **13** – flagellum to penis length ratio (*0* – 0.900–0.999, *1* – 1.000–1.099, *2* – 1.100–1.199, *3* – 1.200–1.299, *4* – 1.300–1.399, *5* – 1.400–1.499, *6* – 1.500–1.599); **14** – penis habitus (*0* – massive, *1* – intermediate, *2* – slender); **15** – penis left arm breadth (*0* – similar as the right arm, *1* – much more slender); **16** – bursa copulatrix shape (*0* – slender, *1* – j-shaped, *2* – u-shaped); **17** – bursa copulatrix proportions (*0* – slender, *1* – bulky); **18** – bursa to its duct length proportion (*0* – 1.500–1.999, *1* – 2.000–2.499, *2* – 2.500–2.999, *3* – 3.000–3.499, *4* – 3.500–3.999, *5* – 4.000–4.499); **19** – bursa copulatrix to receptaculum seminis length ratio (*0* – 1.000–1.999, *1* – 2.000–2.999, *2* – 3.000–3.999, *3* – 4.000–4.999, *4* – 5.000–5.999, *5* – 6.000–6.999, *6* – 7.000–7.999, *7* – 8.000–8.999); **20** – receptaculum seminis length:breadth ratio (*0* – 1.600–1.699, *1* – 1.700–1.799, *2* – 1.800–1.899, *3* – 1.900–1.999); **21** – bursa copulatrix length (*0* – 0.400–0.499, *1* – 0.500–0.599, *2* – 0.600–0.699, *3* – 0.700–0.799, *4* – 0.800–0.899, *5* – 0.900–0.999); Character type: ordered (O), unordered (U).

amount of change and nearly all possible changes. The values for the corresponding branches of the morphological and allozymic trees of the same topologies, were used to compare the rate of allozymic and morphological evolution by means of Pearson's correlation coefficient (SOKAL & ROHLF 1996).

RESULTS

MORPHOLOGICAL PHYLOGENY

The additive trees based on Euclidean distances for morphological data, generated with Fitch-Margoliash's least-square technique do not show any conspicuous distinctness of any of the purported morphospecies (Figs 1, 2). The trees for males and females are similar in topology, the distant position of the males of population 4 (Fig. 1) is due to the extremely small size of this sample. For both sexes, all the populations belonging to *B. austriaca* (4, 11–16, 19 and 20) lie close to each other, with populations 17 and 18, representing *B. zyvionteki*, among them. Females of *B. cylindrica* (populations 7–10) are grouped close to each other (those of population 10 are somewhat farther), whereas the males of population 9 are connected with population 10, population 7 is somewhat more remote from the latter two, and population 8 is included in the *B. austriaca* clade. The morphospecies from Hungary (population 1), Slovakia (populations 2, 3 and 5), and *Bythinella* sp. (population 6) lie together and near *B. cylindrica*, the branches terminating with these populations in both trees are not longer than the ones terminating with the remaining populations. The averge percent standard deviation is 8.341 (21,942 trees analysed) for the male tree, and 8.306 (23,612 trees analysed) for the female tree.

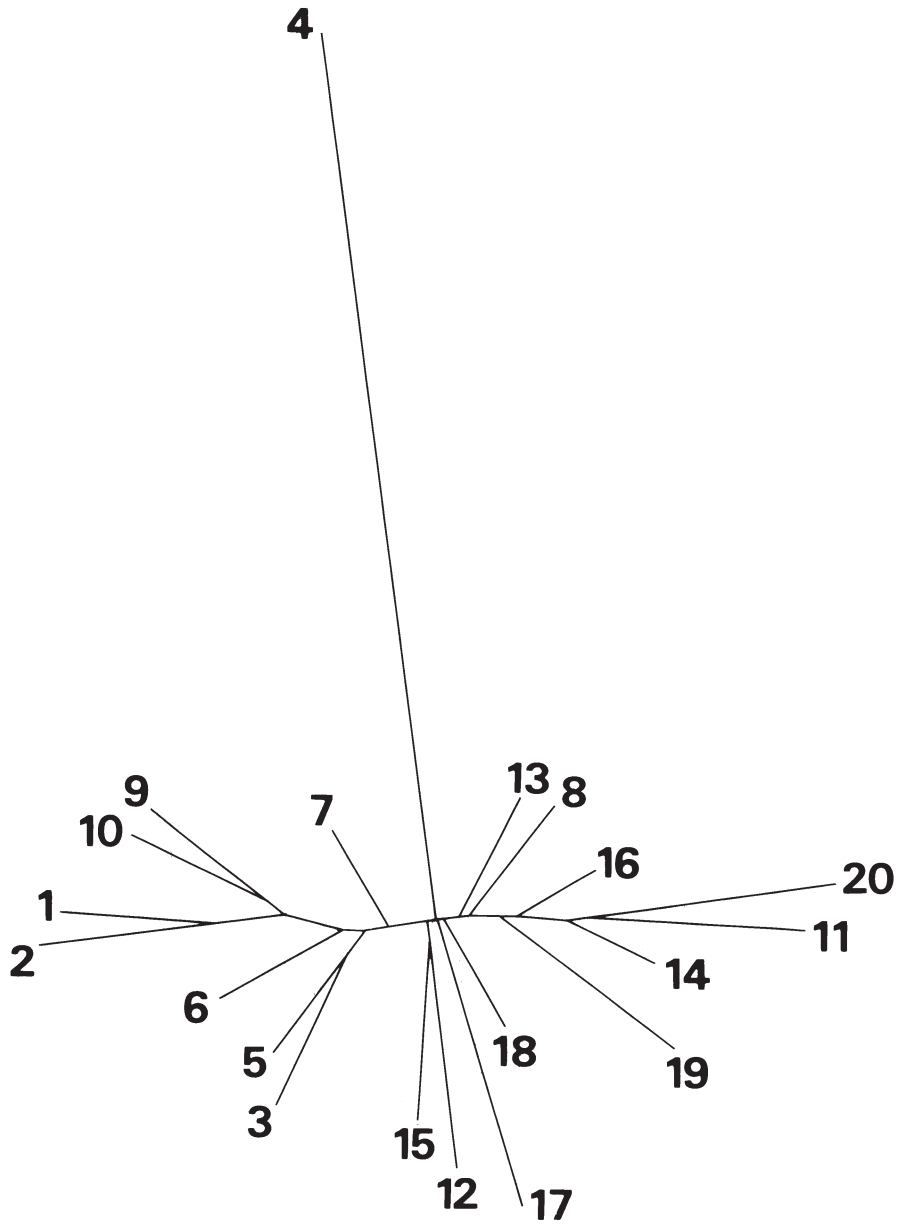

Fig. 1. Fitch-Margoliash additive tree, Euclidean distances, male morphological characters; sum of squares = 2.62956, average percent standard deviation = 8.34057, examined 21,942 trees. For population numbers see Table 1

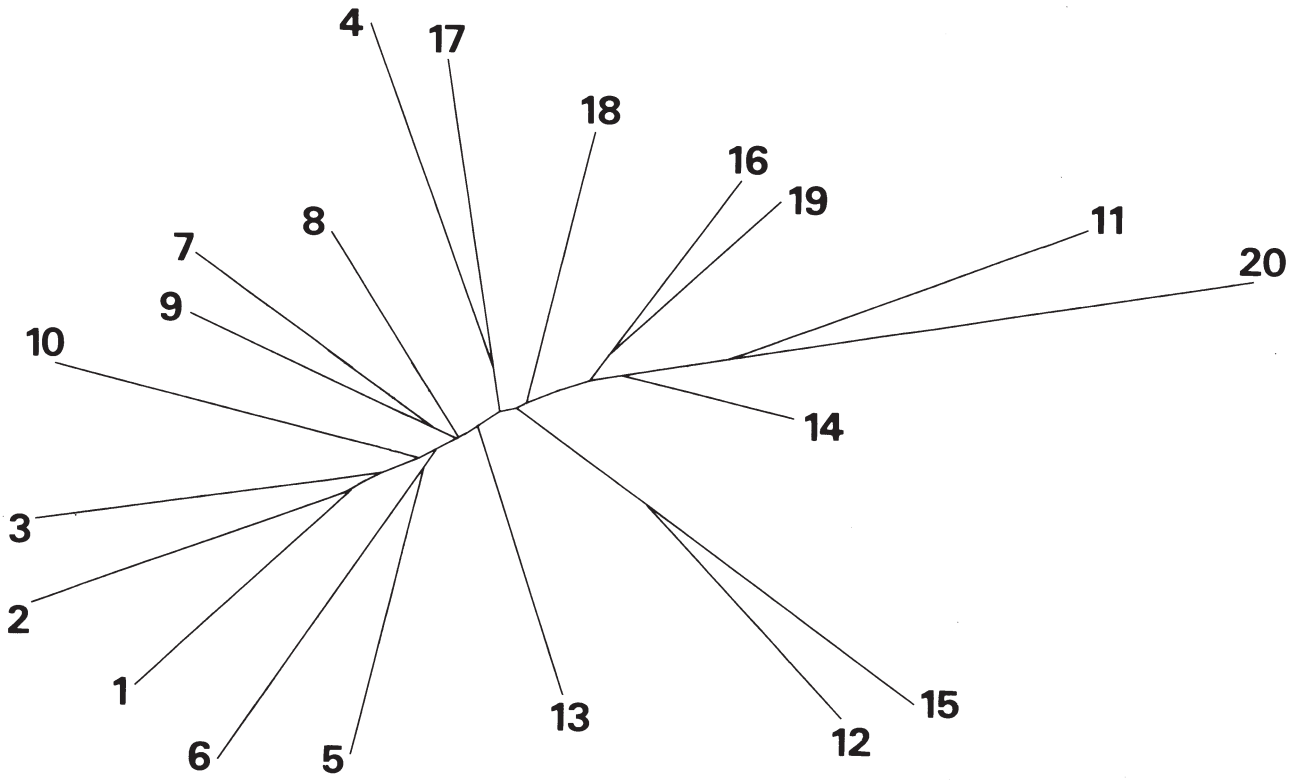

Fig. 2. Fitch-Margoliash additive tree, Euclidean distances, female morphological characters; sum of squares = 2.60797, average percent standard deviation = 8.30626, examined 23,612 trees. For population numbers see Table 1

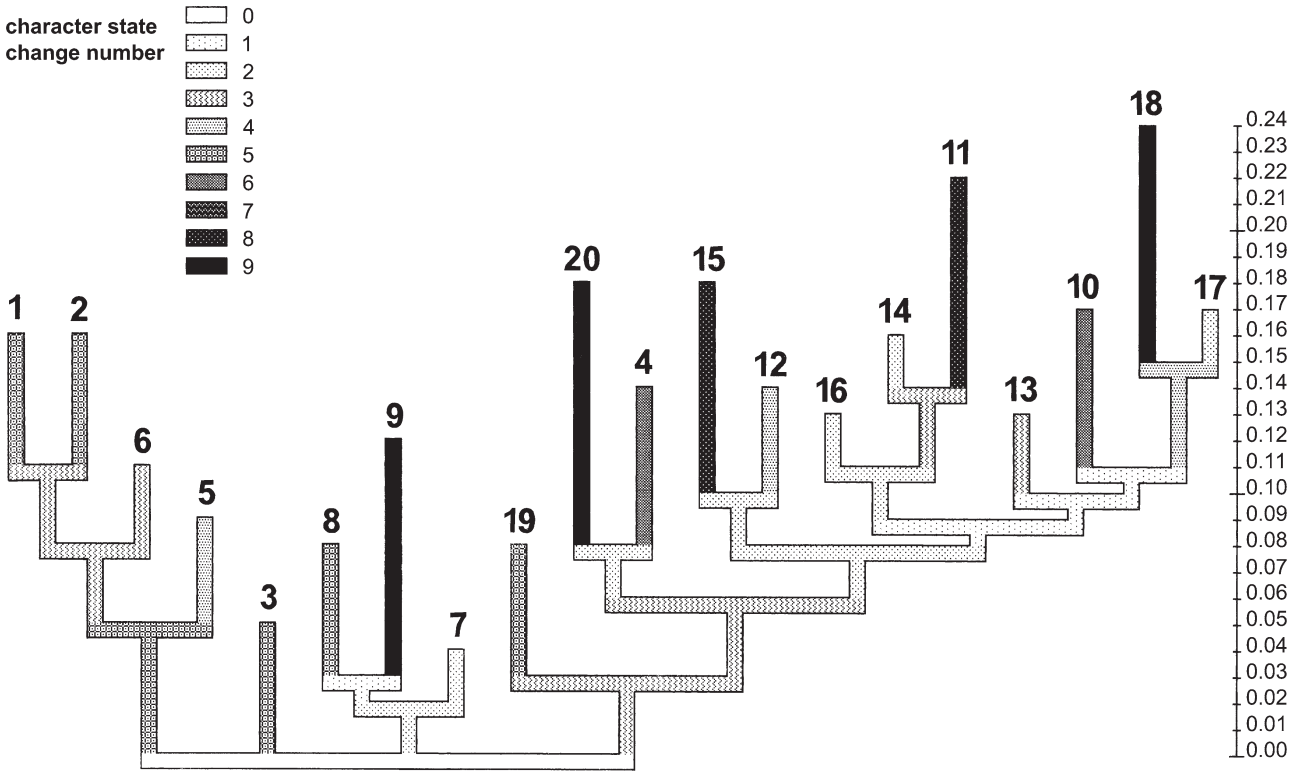

Fig. 3. Most parsimonious phylogeny based on morphological characters; 159 steps, CI = 0.380, RI = 0.495, RC = 0.188, 177–189 changes. Branch-and-bound option. For population numbers see Table 1

The tree (Fig. 3) found using the parsimony method based on discrete morphological characters (Table 1), with the branch lengths reflecting the amount of change, is 159 steps long, CI = 0.380, RI = 0.495 and RC = 0.188. The rather low values of the above indices reflect the relatively numerous parallelisms and reversals in the character evolution during the inferred phylogenetic process. The tree presents unresolved soft polytomies, and thus it is impossible to define unequivocally the relationships between the four distinguished clades. The first clade consists of populations 1, 2, 5 and 6. Each represents a distinct morphospecies. Populations 1 and 2 form one clade, like in the Fitch-Margoliash trees, and the amount of anagenetic change along the respective branches is not larger than the amount of change corresponding to the populations of *B. austriaca*. The second clade is represented by the branch terminating with population 3, postulated as a distinct morphospecies. The third clade includes three from among the four studied populations of *B. cylindrica* (populations 7–9). In this clade, the geographically close populations 8 and 9 are surprisingly distinct. The fourth clade includes all the studied populations of *B. austriaca* (population 4 from Slovakia is here closest to population 20), and *B. zyvionteki* (populations 17 and 18) to which population 10 of *B. cylindrica* is joined. The largest amount of change characterizes the branches terminating with populations 9, 11, 15, 18 and 20.

ALLOZYMIC PHYLOGENY

The Fitch-Margoliash additive trees, based on genetic distances (Prevosti, unbiased Nei, Cavalli-Sforza and Edwards arc distances) do not differ much among each other and the populations are clustered in a similar way (Figs 4–6). In all the trees, populations 5 and 20 are the closest to each other and both are situated far from the others. Populations 1, 2, 3, 6, 12 and 13 lie always far from each other and from all the remaining populations. Populations 17 and 18, representing *B. zyvionteki,* are rather distant from each other and mixed with those of *B. austriaca*. The trees based on various genetic distances differ between each other only in the branches with a low amount of anagenetic change.

The Fitch-Margoliash additive tree technique based on the Prevosti distance results in a tree (Fig. 4) of average percent standard deviation of 12.115 (26,128 trees analysed). For the unbiased Nei distance (Fig. 5) the value was higher: 24.377 (45,672 trees analysed), and for the Cavalli-Sforza and Edwards arc distance (Fig. 6) lower: 9.035 (21,652 trees analysed). Thus, the most reliable phylogeny reconstruction is the one based on Cavalli-Sforza and Ed-

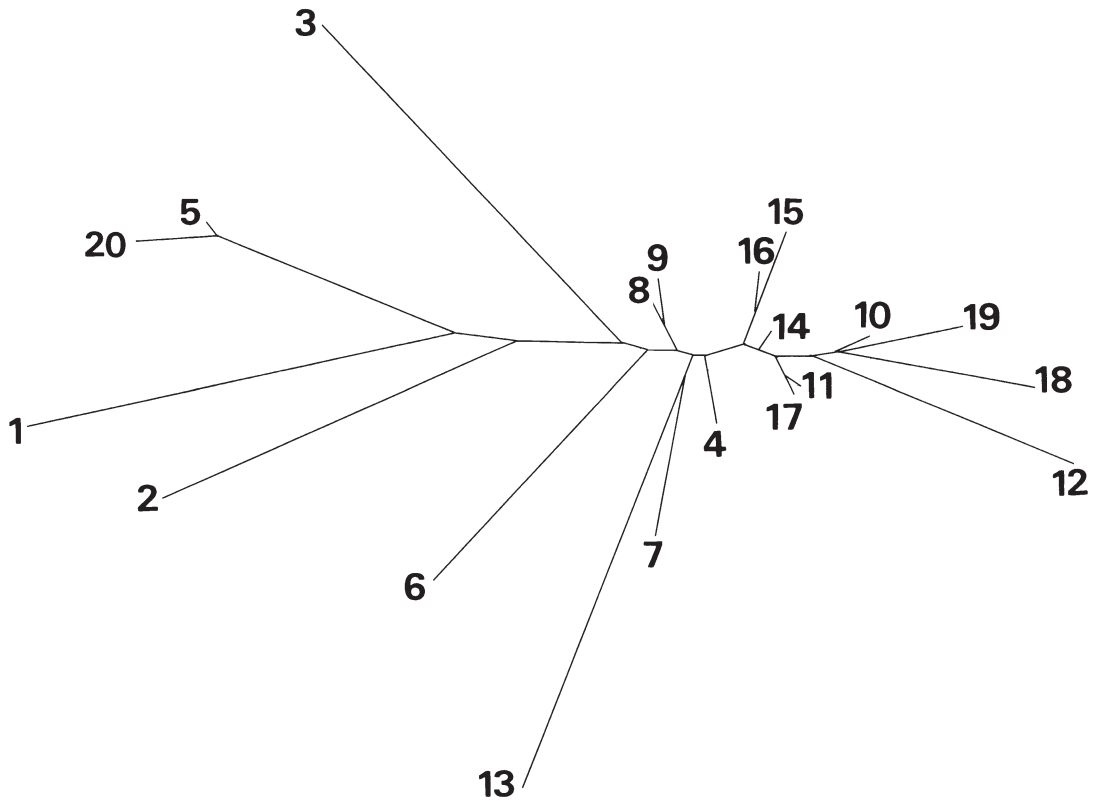

Fig. 4. Fitch-Margoliash additive tree, Prevosti distances; sum of squares = 5.54784, average percent standard deviation = 12.11480, examined 26,128 trees. For population numbers see Table 1

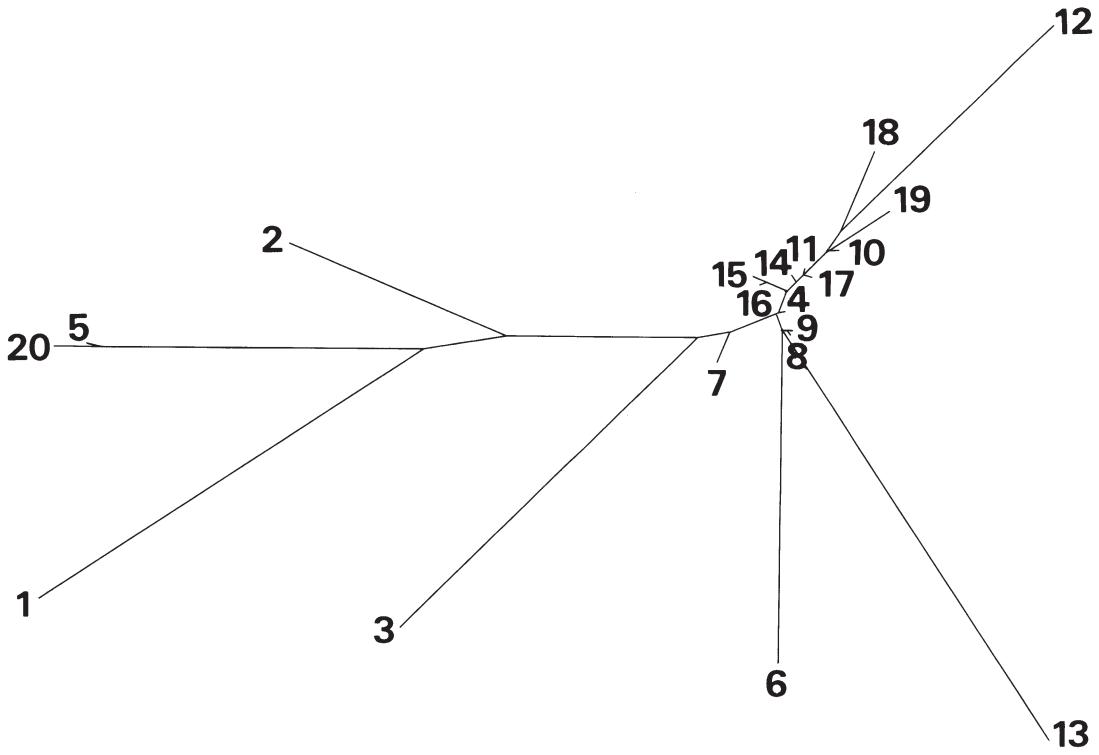

Fig. 5. Fitch-Margoliash additive tree, unbiased Nei distances; sum of squares = 22.46271, average percent standard deviation = 24.37727, examined 45,672 trees. For population numbers see Table 1

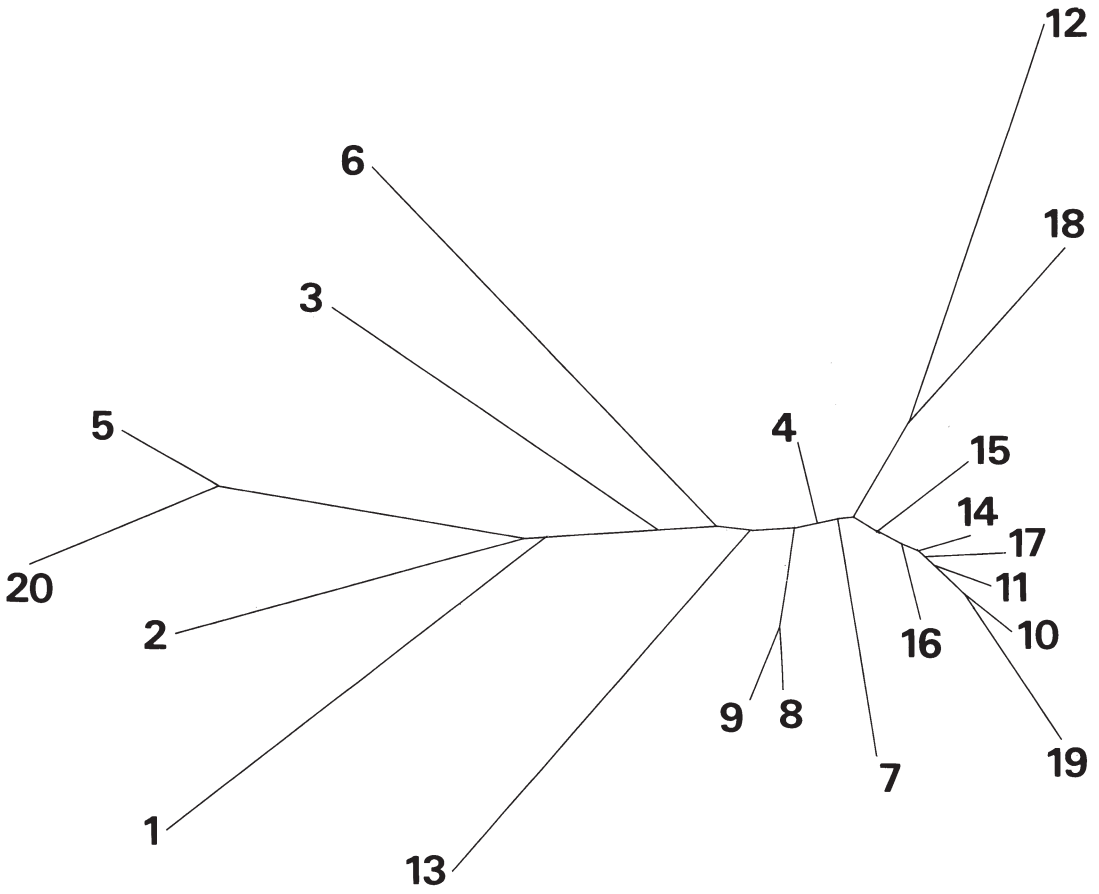

Fig. 6. Fitch-Margoliash additive tree, Cavalli-Sforza and Edwards arc distances; sum of squares = 3.08540, average percent standard deviation = 9.03462, examined 21,652 trees. For population numbers see Table 1

wards arc distance. This implies that the underlying model of evolution, assuming genetic drift as the only source of variability, is the most appropriate for the studied populations. From among all the trees, the one based on the unbiased Nei distance (Fig. 5) shows the largest differences between the distant populations, and the minutest differences between the close ones. This results from the fact that the Nei distance poorly reflects small distances, being more compact than Cavalli-Sforza and Edwards arc distance. In the tree based on the latter distance (Fig. 6) the differences between close populations are reflected best.

The tree computed by means of the frequency-parsimony method based on allele frequencies (Fig. 7) shows an unresolved trichotomy. One of these three clades terminates with population 3. The second clade comprises populations 7, 8, 9 of *B. cylindrica*, 13, 14 of *B. austriaca*, and 17 of *B. zyvionteki*; populations 8 and 9 lie together, like in the trees based on the genetic distances but contrary to those trees, populations 14 and 17 form one clade, to which populations 7 and 13 are joined. The third clade consists of populations 1, 2, 5, 6, each of them represening a distinct morphospecies, plus the remaining populations of *B. austriaca*; population 10 of *B. cylindrica* and 18 of *B. zyvionteki*. In this clade populations 5 and 20 lie together. The largest amount of anagenetic change characterizes the branches terminating with populations 1, 2, 3, 6, 7, 12 and 13.

COMPARISON OF MORPHOLOGICAL AND ALLOZYMIC PHYLOGENY

The additive trees generated with Fitch-Margoliash's technique for the morphological data (Figs 1, 2), compared with the ones for the allozymic data (Figs 4–6), show both similarities and differences. Populations 5 and 20 are very close to each other according to the allozymic data, but morphologically they are distant. Conversely, population 1 is close to population 2 according to the morphological data, but these two populations are distant, based on the allozymic data. Populations 3 and 6 are both morphologically and allozymically distinct. Populations 11 and 17, as well as 8 and 9 are rather distinct based on the morphological data but close to each other according to the allozymic data. Populations 12 and 13 differ conspicuously with respect to the allozymic data but the morphological differences between them are not large.

The topology of the tree found using parsimony, based on discrete morphological characters (Fig. 3) is quite different from that of the allozymic tree. The values of Pearson's correlation coefficients for the lengths of the respective branches of the morphological (Figs 8, 9) and allozymic (Fig. 7) trees of the same topologies are insignificant for both options: r = -0.0667 for the minimum amount of change (Fig. 8) and r = 0.1917 for nearly all possible changes (Fig. 9).

Fig. 7. Relationships between populations, frequency-parsimony method based on allele frequencies. Lengths of branches proportional to the amount of allele frequency change. For population numbers see Table 1

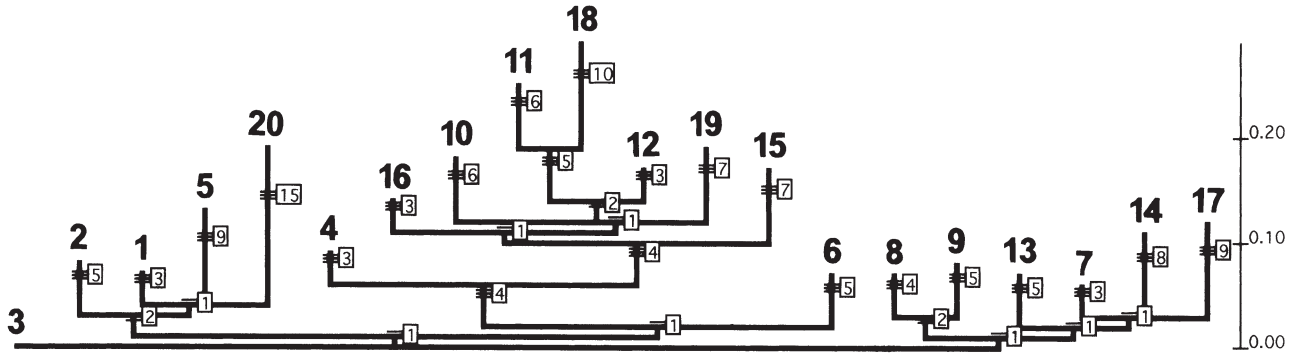

Fig. 8. MPR phyllogram with branch lengths proportional to the amount of change, morphological characters; with the same topology as in Fig. 7. Option: minimum amount of change. Each bar equals one change (unambiguous events only). For population numbers see Table 1

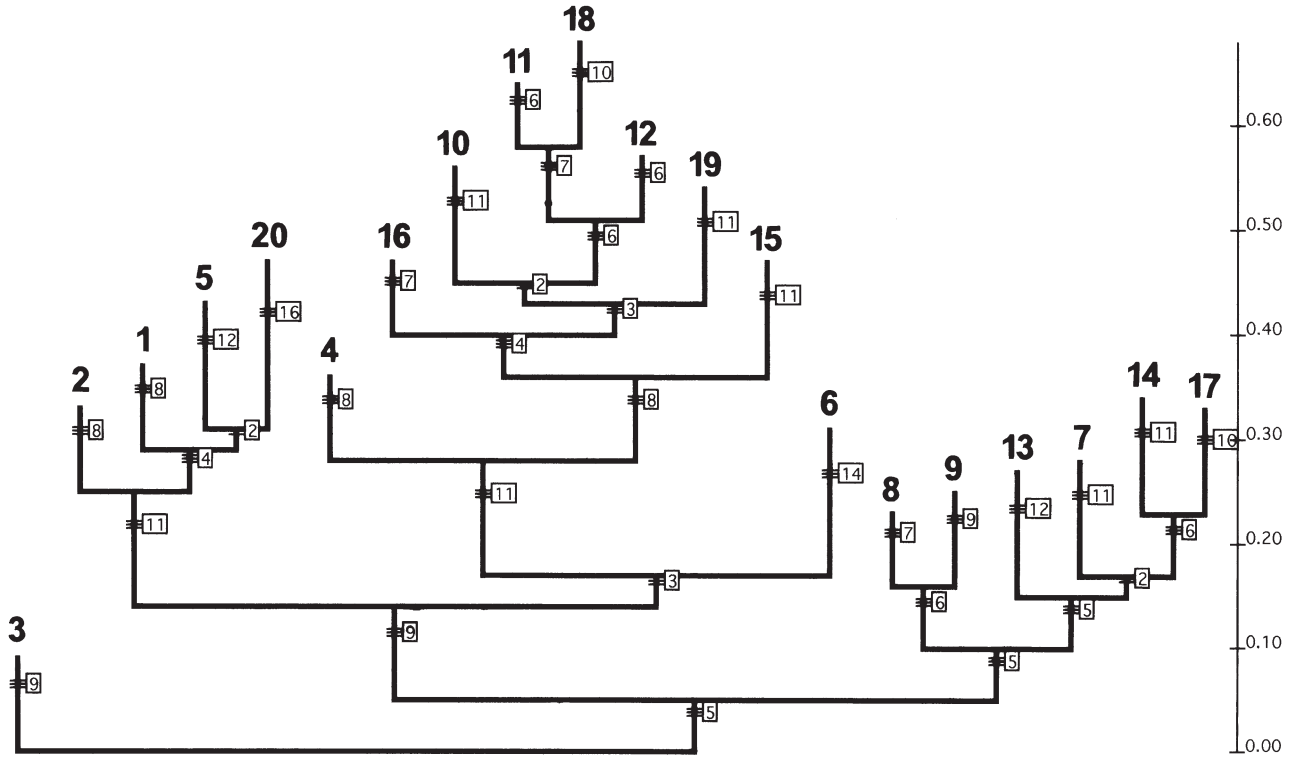

Fig. 9. MPR phyllogram with branch lengths proportional to the amount of change, morphological characters, with the same topology as in Fig. 7. Option: nearly all possible changes. Each bar equals one change (unambiguous events only). For population numbers see Table 1

DISCUSSION

Each of the methods of both phenetic (MAZAN & SZAROWSKA 2000) and phylogenetic analysis used in this study for morphological data, showed a generally similar picture of interpopulation differentiation. Also for the allozymic data, the results of all the applied techniques of phenetic analysis (MAZAN & SZAROWSKA 2000) and phylogenetic analysis were similar. However, the patterns of population grouping based on the allozymic and morphological data sets were different and none of them unequivocally confirmed the distinctness of any of the purported morphospecies. Populations 5 and 20, allozymically very close to each other, were morphologically distant. Conversely, populations 1 and 2, as well as 12 and 13, close to each other morphologically, were distant according to the allozymic data. Only populations 3 and 6 were consistently morphologically and allozymically distinct. Population 20, representing *B. austriaca* ssp. *ehrmanni* at the only locality of *Bythinella* in the Polish Sudetes (WIKTOR 1964), mor-

phologically distinct from all of the examined populations (including the other populations of *B. austriaca*), based on the allozymic data was also separated from all the other populations, except 5.

Congruence of morphological and molecular species distinctness is often reported. PONDER et al. (1995) found it in the spring Hydrobiidae of the genera *Fonscochlea* and *Trochidrobia*. BORSA & BENZIE (1993) observed a similar phenomenon in the marine snails *Trochus* and *Tectus*, and NELSON et al. (1993) in the bivalves of the genus *Donax*. On the other hand, incongruence of morphological and molecular data is also not rare, since the evolution of these two sets of characters may take a different course (WILEY 1981, CHEVERUD 1988, LEWONTIN 1991, DAVIS 1994, OMLAND 1994). Thus, it is not surprising that the taxonomic inferences based on morphology may depart from those based on allozymes (JOHNSON et al. 1977, 1986, HILLIS et al. 1987, HOAGLAND & DAVIS 1987, WOODRUFF & SOLEM 1990, MURRAY et al. 1991, PONDER & COLGAN 1992, FALNIOWSKI et al. 1993, HAASE 1994).

In our study, both morphological and allozymic data consistently confirm the distinctness of populations 3 and 6, each of them representing a different morphospecies found only at one locality. Contrary to the allozymic data, the morphological data do not confirm the distinctness of the other two purported morphospecies (populations 1 and 2). The morphospecies represented by population 5 is allozymically more distinct than morphologically; on the other hand, according to the allozymic data, it was almost identical with population 20. The latter population was morphologically and allozymically distinct from the remaining populations.

Systematics has mostly been based on morphological characters and probably it will, to a large degree, remain so in the future (MADDISON & MADDISON 1992); insufficiency of morphological characters creates a serious problem for systematists. However, such a situation is common, since changes in morphology do not have to be simultaneous with speciation (FALNIOWSKI 1987, 1992, LARSON 1989, GIUSTI & MANGANELLI 1992); they may follow speciation or remain insignificant. Morphological and molecular differences between taxa may increase independent of improvement in reproductive isolation mechanisms. Small morphological differences between molecularly distinct species are not rare among snails. Examples are the European *Viviparus* (FALNIOWSKI et al. 1996) or the Australian Hydrobiidae: *Tatea rufilabris* and *T. huonensis* (PONDER et al. 1991). The same may be the case of populations 1, 2 and 5 in *Bythinella*.

Completing speciation, and thus acquiring specific status, does not have to be related with considerable changes in allele presence or allele frequencies in most loci. It is quite easy to include in a study most of the morphological characters, whereas the number of examined loci cannot be large and must, of necessity, be chosen by chance. Thus, it is not surprising that the cases of morphologically distinct species which allozymically differ weakly, are not rare among snails. For example, the reproductively isolated and morphologically differentiated species of *Partula* (JOHNSON et al. 1977) and *Samoana* (JOHNSON et al. 1986), were allozymically highly varied within populations while the molecular differences between these species were slight. HAASE (1994) found two species of *Graziana*, which morphologically and anatomically differed enough to justify their specific distinctness, to be genetically identical at all of the 27 loci examined. Likewise, three reproductively isolated species of *Stagnicola* were molecularly undifferentiated (RUDOLPH & BURCH 1989). DAVIS (1994) lists a number of examples for molluscs, where molecular data do not provide a solution to taxonomic doubts.

However, snail's morphology, especially the shell, is very variable intraspecifically. It is enough to mention ROSZKOWSKI's (1914) classical study on a deep-water *Radix* in the Leman Lake, where conspecific individuals differed in shell habitus depending on whether they lived in deep or in shallow water; the anatomy of both was identical. In some species habitat-dependent morphs are found. According to DAVIS et al. (1988) the great morphological variation of North American brackish-water populations of *Hydrobia* is due to environmental conditions, and all those populations represent the same species. On the other hand, in the Australian Hydrobiidae, PONDER et al. (1991) found numerous sibling species and wide morphological variation. In general, the morphological and genetic variation were incongruent.

According to the preliminary classification proposed by FALNIOWSKI (1987), populations 4, 11–16, 19 and 20 represent *B. austriaca*, populations 7–10 – *B. cylindrica* and populations 17–18 – *B. zyvionteki*. These three species, although poorly distinct morphologically, are distinguishable. It is noteworthy that both phenetic (MAZAN & SZAROWSKA 2000) and phylogenetic techniques resulted in a pattern resembling that based merely on the poorly marked qualitative characters that served as a basis in FALNIOWSKI's (1987) study. The allozymic data, however, do not correspond to the morphological distinctness of these species. At the same time, populations 12 and 13 of *B. austriaca* differ allozymically from the other populations of this species so much that it is not unlikely that they represent sibling species other than *B. austriaca*. This rises the question of the distinctness of *B. austriaca*, *B. cylindrica* and *B. zyvionteki*. Theoretically, they may represent one distinct species, or they may be slightly allozymically different species, or the loci considered are not representative and they do not show interspecific differences. To solve these doubts, a

larger number of loci, as well as other populations, should be studied, especially ones representing other parts of the *Bythinella* range, in order to see the scale of genotypic differentiation within the genus.

Considering all the data presented in this paper we can merely speculate about the distinctness of *B. austriaca*, *B. cylindrica* and *B. zyvionteki*. Considering the fairly young age of the Polish populations (the oldest fossil record is dated at 7,750±130 years BP) and the geographic pattern of their morphological and allozymic differentiation (FALNIOWSKI et al. 1998), they may not represent distinct species. Their present differentiation may be due to stochastic factors, since the time available seems too short for mutation to contribute significantly. It seems that in the past, the territory of southern Poland was inhabited by a widely varied species resembling the present *B. austriaca* (this does not concern population 6 from the Tatra Mountains); it gave rise to *B. cylindrica* and *B. zyvionteki*. The distinctness of *B. zyvionteki* is poorly marked. Ecologically, it resembles *B. austriaca* and it may be its geographical race, whose range is confined to the Kraków–Czestochowa Upland. *B. cylindrica* is morphologically, as well as ecologically different from *B. austriaca*: it occurs in trickling outflows among dead leaves, whereas *B. austriaca* inhabits bigger springs. Thus, *B. cylindrica* may be regarded as an ecotype of *B. austriaca*. On the other hand, if this is true, according to the cohesion concept of species (TEMPLETON 1989), the two should be able to replace each other ecologically. This, however, is doubtful. Thus, the best approach, at least until more data are obtained, is to treat *B. cylindrica* as a distinct species.

The next taxonomic problem is population 20, representing *B. austriaca ehrmanni*. It is distinct from the other studied populations. The distribution centre of *B. austriaca* is in the Alps and the description of this subspecies is based on the differences between this population and the Alpine *B. austriaca*. Hence, we are not able to tell if population 20 is intermediate (between the Alpine *B. austriaca* and the remaining Polish populations) or atypical (the Polish and Alpine populations are thus more similar). If the former is true, the question will arise as to whether or not the Polish (and one Slovakian) populations regarded as *B. austriaca* really belong to this species.

To sum up, in *Bythinella*, slight interspecific differences in morphology are accompanied by moderate allozymic differences. The two sets of differences are usually not correlated, which resembles the case of two Australian Hydrobiidae (PONDER et al. 1991). For this reason distinguishing species in *Bythinella* is questionable. Morphologically and allozymically distinct populations are: 3 (morphospecies 3) and 6, distinguished by FALNIOWSKI (1987) as *Bythinella* sp. Based on the allozymic data, distinct populations are: 1 (morphospecies 1) and 2 (morphospecies 2). Also population 5 (morphospecies 4) represents a separate species. Similarly, there are no sufficient grounds for not distinguishing *B. austriaca* and *B. cylindrica*. On the other hand, the species rank of *B. zyvionteki*, *B. austriaca ehrmanni* (it may turn out to be a distinct species) as well as populations 12 and 13 (both preliminarily classified as *B. austriaca*) seems doubtful and requires a further study.

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REFERENCES

BOETERS H. D. 1973. Die Gattung *Bythinella* und die Gattung *Marstoniopsis* in Westeuropa, 1. Westeuropäische Hydrobiidae, 4. (Prosobranchia). Proc. Fourth Europ. Malac. Congr., Malacologia 14: 271–285.

BORSA P., BENZIE J. A. H. 1993. Genetic relationships among the topshells *Trochus* and *Tectus* (Prosobranchia: Trochidae) from the Great Barrier Reef. J. Moll. Stud. 59: 275–284.

CHEVERUD J. M. 1988. A comparison of genetic and phenotypic correlations. Evolution 42: 958–968.

DAVIS G. M. 1994. Molecular genetics and taxonomic discrimination. The Nautilus 2 Suppl.: 3–23.

DAVIS G. M., FORBES V., LOPEZ G. 1988. Species status of Northeastern American *Hydrobia* (Gastropoda: Prosobranchia): ecology, morphology and molecular genetics. Proc. Acad. Nat. Sci. Philad. 140: 191–246.

EDWARDS A. W. F., CAVALLI-SFORZA L. L. 1964. Reconstruction of evolutionary trees. In: Phenetic and Phylogenetic Classification. (HEYWOOD V. H., MCNEIL J., eds), Systematics Association Volume No. 6, Systematics Association, London.

FALNIOWSKI A. 1987. Hydrobioidea of Poland (Prosobranchia: Gastropoda). Folia Malacologica 1: 1–122.

FALNIOWSKI A. 1992. The genus *Bythinella* Moquin-Tandon, 1855, in Poland (Gastropoda, Prosobranchia, Hydrobii-

dae). Proc. Ninth Int. Malac. Congr. Edinburgh; Leiden: 135–138.

FALNIOWSKI A., KOZIK A., SZAROWSKA M. 1993. Biometrical and esterases pattern differences between local populations in *Bradybaena fruticum* (O.F. Müller, 1777) (Gastropoda: Stylommatophora: Helicoidea). Malak. Abh. 16: 147–164.

FALNIOWSKI A., KOZIK A., SZAROWSKA M., FIAŁKOWSKI W., MAZAN K. 1996. Allozyme and morphology evolution in European Viviparidae (Mollusca: Gastropoda: Architaenioglossa). J. Zool. Syst. Evol. Res. 34: 49–62.

FALNIOWSKI A., MAZAN K., SZAROWSKA M., 1999. Homozygote excess and gene flow in the spring snail *Bythinella* (Gastropoda: Prosobranchia). J. Zool. Syst. Evol. Res. 37: 165–175.

FALNIOWSKI A., SZAROWSKA M., FIAŁKOWSKI W., MAZAN K. 1998. Unusual geographic pattern of interpopulation variation in a spring snail *Bythinella* (Gastropoda: Prosobranchia). J. Nat. Hist. 32: 605–616.

FELSENSTEIN J. 1990. PHYLIP Manual Version 3.3. University Herbarium, University of California, Berkeley, California.

FITCH W. M., MARGOLIASH E. 1967. Construction of phylogenetic trees. Science 155: 270–284.

GIUSTI F., MANGANELLI G. 1992. The problem of the species in malacology after clear evidence of the limits of morphological systematics. Proc. Ninth Int. Malac. Congr. Edinburgh; Leiden: 153–172.

GIUSTI F., PEZZOLI E. 1977. Primo contributo alla revisione del genere *Bythinella* in Italia. Natura Bresciana, Annali Musei Civici di Storia Naturale di Brescia 14: 3–80.

GIUSTI F., PEZZOLI E. 1980. Gasteropodi, 2. (Gastropoda: Prosobranchia: Hydrobioidea, Pyrguloidea). Consiglio Nazionale delle Ricerche AQ/1/47, Guide per il Riconoscimento delle Specie Animali delle Acque Interne Italiane 8: 1–67.

HAASE M. 1994. Differentiation of selected species of *Belgrandiella* and the redefined genus *Graziana* (Gastropoda: Hydrobiidae). Zool. J. Linn. Soc. 111: 219–246.

HILLIS D. M., ROSENFELD D. S., SANCHEZ M. JR. 1987. Allozymic variability and heterozygote deficiency within and among morphologically polymorphic populations of *Liguus fasciatus* (Mollusca: Pulmonata: Bulimulidae). Amer. Malac. Bull. 5: 153–157.

HOAGLAND K. E., DAVIS G. M. 1987. The succineid snail fauna of Chittenango Falls, New York: taxonomic status with comparisons to the other relevant taxa. Proc. Acad. Nat. Sci. Philad. 139: 465–526.

JOHNSON M. S., CLARKE B., MURRAY J. 1977. Genetic variation and reproductive isolation in *Partula*. Evolution 31: 116–126.

JOHNSON M. J., MURRAY J., CLARKE B. 1986. High genetic similarities and low heterozygosities in land snails of the genus *Samoana* from the Society Islands. Malacologia 27: 97–106.

LARSON A. 1989. The relationship between speciation and morphological evolution. In: Speciation and its consequences (OTTE D., ENDLER J. A., eds), pp. 579–598, Sinauer Associates Inc., Sunderland, Massachusetts.

LEWONTIN R. C. 1991. Twenty–five years ago in genetics: Electrophoresis in the development of evolutionary genetics: Milestone or millstone? Genetics 128: 657–662.

MADDISON W. P., MADDISON D. R. 1992. MacClade, version 3. Analysis of phylogeny and character evolution. Sinauer Associates Inc., Sunderland, Massachusetts.

MAZAN K. 2000. Morphological and allozymic variation within and between populations of *Bythinella* Moquin-Tandon, 1855 (Gastropoda: Prosobranchia). I. Morphological characters. Folia Malacologica 8: 107–139.

MAZAN K., SZAROWSKA M. 2000. Morphological and allozymic variation within and between populations of *Bythinella* Moquin-Tandon, 1855 (Gastropoda: Prosobranchia). II. Phenetic analysis. Folia Malacologica 8: 189–213.

MURRAY J., STINE O. C., JOHNSON M. J. 1991. The evolution of mitochondrial DNA in *Partula*. Heredity 66: 93–104.

NELSON W. G., BONSDORFF E., ADAMKEWICZ L. 1993. Ecological, morphological, and genetic differences between the sympatric bivalves *Donax variabilis* Say, 1922, and *Donax parvula* Philippi, 1849. The Veliger 36: 317–322.

OMLAND K. E. 1994. Character congruence between a molecular and a morphological phylogeny for dabbling ducks (*Anas*). Syst. Biol. 43: 369–386.

PONDER W. F., COLGAN D. J. 1992. The tyranny of distanse – genetic differentiation of hydrobiid snails in streams and springs. Abstracts of the Eleventh Int. Malac. Congr., Siena: 219.

PONDER W. F., COLGAN D. J., CLARK G. A. 1991. The morphology, taxonomy and genetic structure of *Tatea* (Mollusca: Gastropoda: Hydrobiidae), estuarine snails from temperate Australia. Austr. J. Zool. 39: 447–497.

PONDER W. F., EGGLER P., COLGAN D. J. 1995. Genetic differentiation of aquatic snails (Gastropoda: Hydrobiidae) from artesian springs in arid Australia. Biol. J. Linn. Soc. 56: 552–596.

QUEIROZ A. DE 1993. For consensus (sometimes). Syst. Zool. 42: 368–372.

RADOMAN P. 1976. Speciation within the family Bythinellidae on the Balkans and Asia Minor. Zeischr. Zool. Syst. u. Evol.-forsch. 14: 130–152.

RICHARDSON B. J., BAVERSTOCK P. R., ADAMS M. 1986. Allozyme electrophoresis: a handbook for animal systematics and population studies. Academic Press, Sydney.

ROSZKOWSKI W. 1914. Contribution á l'étude des Limnées du lac Léman. Rev. suisse Zool. 22: 453–539.

RUDOLPH P. H., BURCH J. B. 1989. Electrophoretic analysis of enzymes in three species of *Stagnicola* (Pulmonata: Lymnaeidae). J. Med. Appl. Malac. 1: 57–64.

SOKAL R. R., ROHLF F. J. 1996. Biometry. The principles and practice of statistics in biological research. Third edition, W. H. Freeman and Company, New York.

SWOFFORD D. L. 1991. Phylogenetic Analysis Using Parsimony (PAUP), version 3.0s. Illinois Natural History Survey, Champaign.

SWOFFORD D. L., BERLOCHER S. H. 1987. Inferring evolutionary trees from gene frequency data under the principle of maximum parsimony. Syst. Zool. 36: 293–325.

SWOFFORD D. L., OLSEN G. J. 1990. Phylogeny reconstruction. In: Molecular Systematics (HILLIS D. M., MORITZ

C., eds), pp. 411–501, Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts.

SZAROWSKA M., FALNIOWSKI A., MAZAN K., FIAŁKOWSKI W. 1998. Adaptative significance of glucose phosphate isomerase (GPI) allozymes in spring snail *Bythinella*? J. Moll. Stud. 64: 289–293.

TEMPLETON, A. R. 1989. The meaning of species and speciation: a genetic perspective. In: Speciation and its consequences (OTTE D., ENDLER J. A., eds), pp. 3–27, Sinauer Associates Inc., Sunderland, Massachusetts.

WEIR B. S. 1990. Genetic Data Analysis. Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts.

WIKTOR A. 1964. Mięczaki Ziemi Kłodzkiej i gór przyległych. Studium faunistyczno-zoogeograficzne. Prace Komisji Biologicznej PTPN 29: 1–132.

WILEY E. O. 1981. Phylogenetics. The Theory and Practice of Phylogenetic Systematics. Wiley & Sons, New York.

WILLIAMS P. L., FITCH W. M. 1989. Finding the minimum change in a given tree. In: The hierarchy of life (FERNHOLM B., BREMER K., JÖRNVALL H., eds), pp. 453–470, Elsevier Press, Amsterdam.

WILLIAMS P. L., FITCH W. M. 1990. Phylogeny determination using dynamically weighted parsimony method. Methods in Enzymology 183: 615–626.

WOODRUFF D. S., SOLEM A. 1990. Allozyme variation in the Australian camaenid land snail *Cristilabrum primum*. A prolegomenon for a molecular phylogeny of an extraordinary radiation in an isolated habitat. The Veliger 33: 129–139.

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