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THE GENETIC DIFFERENTIATION IN THREE SPECIES OF THE GENUS *HYDROBIA* AND SYSTEMATIC IMPLICATIONS (CAENOGASTROPODA, HYDROBIIDAE)

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ABSTRACT

In order to investigate whether the genus *Hydrobia* should be subdivided, three species representing the nominal genera involved (*Hydrobia, Ventrosia*, and *Peringia*) were compared on the basis of allozyme data. Based on genetic distances, anatomical and ecological data, as well as data on reproductive biology, it is argued that (1) there is no reason to split the genus *Hydrobia* into different genera, (2) *Hydrobia* can be subdivided into the subgenera *Hydrobia s. s.* and *Peringia*, and (3) *Ventrosia* has to be considered synonymous with *Hydrobia*.

The analysis of the genetic structure of the three populations investigated revealed heterozygote deficiencies in practically all polymorphic loci in one case, and low, respectively complete lack of variability in the remaining two populations. The deficiencies of heterozygotes are primarily attributed to selection, probably due to a high infection rate with parasites, whereas the reduced variability is explained by genetic drift following a bottleneck.

Key words: Allozymes, electrophoresis, genetics, systematics, Hydrobiidae, *Hydrobia, Peringia, Ventrosia*.

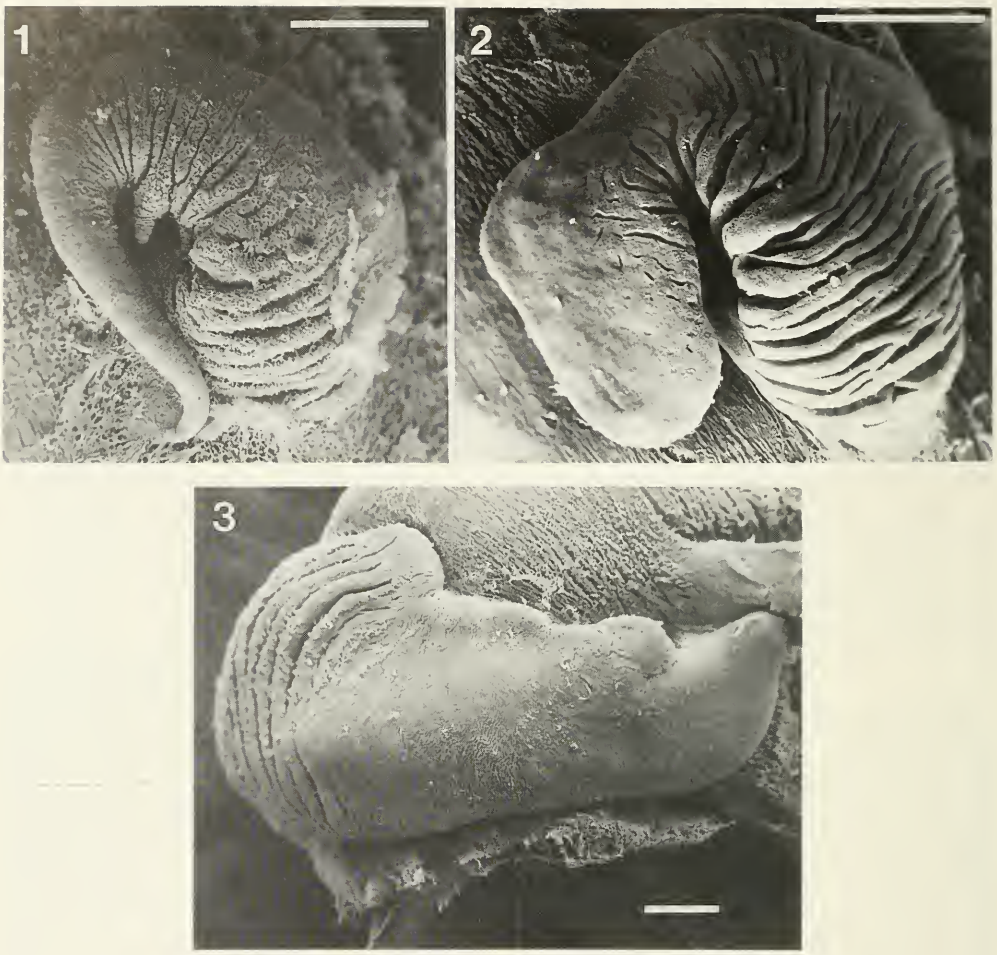
INTRODUCTION

The family Hydrobiidae is one of the largest among gastropods, and its systematics are one of the most confusing in malacology. A principal problem is the assessment of the systematic value of minor differences among these usually tiny snails, which are poor in characters and reveal a considerable degree of convergence. In many issues, there exist as many opinions as there are authors. Such a case is the debate whether the genus *Hydrobia* Hartmann, 1821, which defines the whole family, should be subdivided into subgenera or even split into several genera. In order to evaluate the status of the three nominal genera involved—*Hydrobia, Ventrosia* Radoman, 1977, and *Peringia* Paladilhe, 1874—their type species *H. acuta* (Draparnaud, 1805) (Fig. 2), *V. ventrosa* (Montagu, 1803) (Fig. 1), and *P. ulvae* (Pennant, 1777) (Fig. 3), respectively, were investigated genetically using standard methods of allozyme electrophoresis.

As to the specific (not generic) designation of the populations used in this study—topotypes were not available—I have followed Giusti & Pezzoli (1984) and their suggestion that within *Hydrobia* populations with identical anatomy belong to a single species despite slight, mainly conchological differences (in contrast to the views of Radoman, 1973, and Boeters, 1988). This assumption is corroborated by the morphological, anatomical and genetic studies of Davis et al. (1988, 1989), who compared six populations of *H. truncata* (Vanatta, 1924) from Massachusetts, New York and Maryland. But even if this assumption turned out to be unwarranted, the purpose of this paper would not be affected, because each population can unambiguously be attributed to one of these nominal genera. Until further notice, the genus *Hydrobia* is used for all three species for reasons of simplicity and clarity.

Nomenclatural History

The genus *Hydrobia* was introduced by Hartmann (1821), who included *Cyclostoma acutum* Draparnaud, 1805, which later was designated as type species by Gray (1847). Radoman (1977) was the first who anatomically described a *Hydrobia* from southern France, the presumptive origin of Draparnaud's specimens, with males possessing a distally lobed penis (Fig. 2). He ascribed this anatomy to *H. acuta* and restricted the type locality to Palavas, Etang du Prévost. Previously, Radoman (1974) had introduced the genus *Obrovia* Radoman, 1974, for two taxa with this type of penis from the Adriatic coast. So, after having identified *H. acuta, Obrovia*



FIGS. 1–3. Penis. 1. *Hydrobia ventrosa*; 2. *H. acuta*; 3. *H. ulvae* (scale bars = 100 μm).

became a synonym of *Hydrobia* (Radoman, 1977). In the same paper, Radoman described the new genus *Ventrosia* Radoman, 1977, for the species with a slender penis bearing a pointed lobe on the left side (Fig. 1). This type of verge has always been associated with the taxon *H. ventrosa* (Montagu, 1803) (Robson, 1922; Krull, 1935; Muus, 1963; Bank & Butot, 1984; Giusti & Pezzoli, 1984; Falniowski, 1987). [Radoman (1977) erroneously used the name *Ventrosia stagnorum* (Gmelin, 1791), which is a *Heleobia* Stimpson, 1865, and considered *Hydrobia ventrosa* a junior synonym (c.f. Bank & Butot, 1984).]

Boeters (1984) found species with both penial types at Radoman's restricted type locality of *H. acuta* and claimed that Draparnaud's original material of *H. acuta* also contained both species. This assumption is based on the comparison of two syntypes deposited at the Muséum d'Histoire Naturelle in Paris. One of these shells has significantly deeper sutures, like, according to Boeters (1984), the species with males possessing the pointed penis. In order to save the traditional view of *Hydrobia*, Boeters (1984) designated this shell as lectotype of *H. acuta* and attributed to it the anatomy of what all authors cited above thought was *H. ventrosa*. Thus, *Ventrosia* would have to be considered a junior synonym of *Hydrobia* and *H. acuta* a junior syn-

TABLE 1. Conditions for electrophoresis.

	Enzyme	Buffer System	Current/ Voltage	Run time (hrs)	Loci[1]
AAT	Aspartate Amino Transferase	TEB 9.1[2]/TEB 8 (gel/tray)	35 MA	2	2
ACPH	Acid Phosphatase	TC[3]7	35 MA	2	1
AK	Adenylate Kinase	TC 7	35 MA	2	1
AO	Aldehyde Oxidase	TEB 8	35 MA	3.5	1
APH	Alkaline Phosphatase	TEB 9.1	350 V	4.5	1
CK	Creatine Kinase	TEB 8	35 MA	3.5	1
EST	Carboxyl Esterase	TC 8 & TEB 9.1/TEB 8	40 MA/35 MA	3.25/2	0
GDH	Glutamate Dehydrogenase	TEB 8	35 MA	3.5	1
G6PD	Glucose-6-Phosphate Dehydrogenase	TEB 9.1	350 V	4.5	1
GPI	Glucose-6-Phosphate Isomerase	Poulik	350 V	2	1
ISDH	Isocitrate Dehydrogenase	TEB 8	35 MA	3.5	2
LAP	Leucine Aminopeptidase (= Cytosol Aminopeptidase)	TC 7	35 MA	2	0
LDH	Lactate Dehydrogenase	TEB 8	35 MA	3.5	1
MDH	Malate Dehydrogenase	TC 8	40 MA	3.25	1
ME	Malic Enzyme	TC 8	40 MA	3.25	0
MPI	Mannose-6-Phosphate Isomerase	Poulik	350 V	2	1
NADD	Nicotinamide Adenosine Dinucleotide Dehydrogenase	TEB 8	35 MA	3.5	1
OCT	Octopine Dehydrogenase	TEB 8	35 MA	3.5	1
6PGD	6-Phosphogluconic Dehydrogenase	TEB 8	35 MA	3.5	2
PGM	Phosphoglucomutase	Poulik & TEB 9.1/TEB 8	350 V/35 MA	2/2	2
SDH	Sorbitol Dehydrogenase	TEB 9.1	350 V	4.5	1
SOD	Superoxide Dismutase	see text			2
XDH	Xanthine Dehydrogenase	TEB 8	35 MA	3.5	1

[1]Number of loci included in the analysis
[2]Tris-EDTA-Borate, pH 9.1
[3]Tris-Citrate

onym of *H. ventrosa.* The latter synonymy is not mentioned by Boeters. He refrained from discussing any consequences, left the other species unnamed and did not state its generic allocation (Boeters, 1984).

Subsequent authors explicitly (Giusti & Pezzoli, 1984) or implicitly (Davis *et al.*, 1989) rejected Boeters' view. To avoid the consequences and further systematic confusion arising from Boeters' article, and because there is no biological reason for Boeters' purely taxonomic action, as is demonstrated in this paper, Boeters' type designation should be suppressed by the International Commission of Zoological Nomenclature, and I am preparing a petition to this effect.

Peringia Paladilhe, 1874, is occasionally used as a full genus (Kennard & Woodward, 1926; Wenz, 1938–1944; Nordsieck, 1982) or as a subgenus (Zilch & Jaeckel, 1956; Fretter & Graham, 1978; Boeters, 1988) for *Hydrobia ulvae* (Pennant, 1777) (Fig. 3), although most authors consider *Peringia* as a synonym of *Hydrobia* (Ehrmann, 1933; Giusti & Pezzoli, 1984; Falniowski, 1987).

MATERIALS AND METHODS

Hydrobia ventrosa and *H. ulvae* were collected on the German Baltic island Fehmarn in August 1991, *H. ventrosa* from the west bank of the Burger Binnensee, where it lives on mud, and *H. ulvae* from the sandy Südstrand. The salinity in both localities was 12‰. *Hydrobia acuta* was found in a muddy marsh (22‰) on Torcello, an island in the Gulf of Venice/Italy, in July 1991. The animals were taken alive to the University of Vienna. The specific identity of the samples was determined by investigating the male copulatory organ in living specimens under the stereo microscope. In each sample, only one type of penis was found, indicating the presence of only one species per sample. Most of the animals were deep frozen at −70°C in tissue buffer. The frozen material was carried in liquid nitrogen to the Academy of Natural Sciences in Philadelphia, where electrophoresis was done. Parts of the samples were fixed in 70% ethanol or BOUIN's fixative and deposited at the Museum of Natural History

TABLE 2. Allele frequencies. N, number of specimens.

Locus	Alleles	*H. ventrosa*	*H. acuta*	*H. ulvae*
AAT 1	N	38	27	20
	A	0.684	1	0
	B	0.316	0	1
AAT 2	N	22	27	10
	A	1	0	0
	B	0	1	1
ACPH	N	43	27	30
	A	1	1	0
	B	0	0	1
AK	N	20	27	20
	A	0.600	0	0.700
	B	0.400	1	0
	C	0	0	0.300
AO	N	35	10	25
	A	1	1	0
	B	0	0	1
APH	N	20	26	10
	A	0.625	0.981	0
	B	0.100	0	0
	C	0.250	0	0
	D	0.025	0.019	0
	E	0	0	1
CK	N	25	40	10
	A	1	1	0
	B	0	0	1
GDH	N	35	30	15
	A	1	1	0
	B	0	0	1
G6PD	N	20	26	10
	A	1	1	0.700
	B	0	0	0.300
GPI	N	38	27	20
	A	0.987	1	0
	B	0.013	0	1
ISDH 1	N	40	44	25
	A	1	1	0
	B	0	0	1
ISDH 2	N	30	40	25
	A	1	1	0
	B	0	0	1
LDH	N	30	30	15
	A	1	1	0
	B	0	0	1
MDH	N	25	27	20
	A	0.780	1	0.500
	B	0.220	0	0.500
MPI	N	38	27	20
	A	0.605	1	0
	B	0.395	0	1
NADD	N	35	10	15
	A	1	1	0
	B	0	0	1
OCT	N	35	30	25
	A	1	1	0
	B	0	0	1

TABLE 2. (*Continued*)

Locus	Alleles	*H. ventrosa*	*H. acuta*	*H. ulvae*
6PGD 1	N	40	30	15
	A	1	1	1
6PGD 2	N	35	30	15
	A	1	1	1
PGM 1	N	39	27	20
	A	0.744	0	0
	B	0.256	1	0
	C	0	0	0.925
	D	0	0	0.075
PGM 2	N	28	27	10
	A	0.482	1	0
	B	0	0	1
	C	0.143	0	0
	D	0.286	0	0
	E	0.089	0	0
SDH	N	20	26	10
	A	1	1	0
	B	0	0	1
SOD 1	N	15	10	15
	A	1	1	0
	B	0	0	1
SOD 2	N	5	40	15
	A	1	1	0
	B	0	0	1
XDH	N	40	30	25
	A	1	1	0
	B	0	0	1

(NHMW) under the following collection numbers: *H. ventrosa* (NHMW 86801), *H. acuta* (NHMW 86802), *H. ulvae* (NHMW 86803).

Horizontal starch-gel electrophoresis was carried out following Davis *et al.* (1988). Instead of tris-citrate (TC) buffer with pH 6, TC pH 7, was used (Shaw & Prasad, 1970). Table 1 lists the 22 enzymes stained for and the conditions for electrophoresis. Superoxide dismutase was scored on gel slices stained for a dehydrogenase. The data were analyzed using the computer program BIOSYS-1 release 1.7 by Swofford & Selander (1981). Nei's standard genetic distance (Nei, 1972) and unbiased genetic distance (Nei, 1978) and Cavalli-Sforza & Edwards's arc and chord distances (Cavalli-Sforza & Edwards, 1967) were calculated, and cluster analysis based on Nei's unbiased distance and Cavalli-Sforza & Edwards's arc distance using UPGMA were performed.

RESULTS

The enzymes LAP and ME were hardly detectable. The esterases were extremely polymorphic and therefore not interpretable. Thus, these enzymes had to be excluded from the analysis. Allele frequencies for the remaining 25 loci with 57 alleles are given in Table 2. *Hydrobia ulvae* is characterized by 19 and *H. ventrosa* by seven unique alleles. *Hydrobia acuta* shares all alleles with at least one of the other two species. The genetic variability of the three populations is summarized in Table 3. In *H. ventrosa*, eight loci are polymorphic; seven of these are not in Hardy-Weinberg equilibrium (Table 4). *Hydrobia acuta* is remarkably uniform, with only one polymorphic locus (Table 5). The variability of *H. ulvae* lies between the other two species, but is still very low. Only four loci have more than one allele (Table 6). The MDH is 100% heterozygous. Tables 7 and 8 give the genetic distances between the three species. *Hydrobia ventrosa* and *H. acuta* are obviously very closely related. The remarkably and unexpectedly large distance of *H. ulvae* from the other two species is also depicted in the phenograms of Figures 4 and 5. The cophenetic correlation is 0.998 for the cluster analysis based on Nei's unbiased distance and 0.999

TABLE 3. Genetic variability. Standard errors in parentheses.

	Mean Sample Size Per Locus	Mean No of Alleles Per Locus	Percentage of Loci Polymorphic[1]	Mean Heterozygosity	
				Direct Count	HdyWbg Expected[2]
H. ventrosa	30.0	1.5	32.0	0.043	0.136
	(1.9)	(0.2)		(0.016)	(0.045)
H. acuta	27.8	1.0	4.0	0.002	0.002
	(1.7)	(0.0)		(0.002)	(0.002)
H. ulvae	17.2	1.2	16.0	0.070	0.062
	(1.2)	(0.1)		(0.043)	(0.031)

[1]A locus is considered polymorphic if more than one allele was detected.
[2]Unbiased estimate (see NEI, 1978).

TABLE 4. Chi-square test for deviations from Hardy-Weinberg equilibrium in *H. ventrosa*.

Locus	Genotype	Observed Frequency	Expected Frequency	χ^2	DF	P
AAT 1	A-A	25	17.680			
	A-B	2	16.640			
	B-B	11	3.680	30.471	1	0
AK	A-A	11	7.077			
	A-B	2	9.846			
	B-B	7	3.077	13.429	1	0
APH	A-A	12	7.692			
	A-B	0	2.564			
	A-C	0	6.410			
	A-D	1	0.641			
	B-B	0	0.154			
	B-C	4	1.026			
	B-D	0	0.103			
	C-C	3	1.154			
	C-D	0	0.256			
	D-D	0	0.000	23.680	6	0.001
GPI	A-A	37	37.000			
	A-B	1	1.000			
	B-B	0	0.000	0	1	1
MDH	A-A	18	15.122			
	A-B	3	8.755			
	B-B	4	1.122	11.708	1	0.001
MPI	A-A	18	13.800			
	A-B	10	18.400			
	B-B	10	5.800	8.154	1	0.004
PGM 1	A-A	26	21.468			
	A-B	6	15.065			
	B-B	7	2.468	14.737	1	0
PGM 2	A-A	13	6.382			
	A-B	0	3.927			
	A-C	0	7.855			
	A-D	1	2.455			
	B-B	3	0.509			
	B-C	0	2.327			
	B-D	2	0.727			
	C-C	8	2.182			
	C-D	0	1.455			
	D-D	1	0.182	56.901	6	0

TABLE 5. Chi-square test for deviation from Hardy-Weinberg equilibrium in *H. acuta*.

Locus	Geno-type	Observed Frequency	Expected Frequency	χ^2	DF	P
APH	A-A	25	25.000			
	A-D	1	1.000			
	D-D	0	0.000	0	1	1

for the analysis based on Cavalli-Sforza & Edwards's arc distance, respectively.

DISCUSSION

All but one polymorphic loci of *H. ventrosa* significantly lack heterozygotes. That one, GPI, is polymorphic due only to a rare allele. Under the frequently applied 95% criterion (a locus is considered polymorphic if the frequency of the most common allele does not exceed 95%), the GPI locus would be considered monomorphic. The theoretically possible reasons for heterozygote deficiencies are: (1) inbreeding, (2) the Wahlund effect, (3) biased sampling of homozygotes due to genetic patchiness caused by ecological or behavioural factors across a population's habitat, (4) scoring bias for homozygotes, (5) differential survival of homozygotes following collection, (6) location of the locus on a sex chromosome, (7) assortative mating, (8) presence of null alleles, and (9) selection against heterozygotes (Crouau-Roy, 1988; Staub et al., 1990).

Because practically all polymorphic loci are deficient in heterozygotes, it is tempting to assume a single explanation. Inbreeding or the Wahlund effect would affect the allele frequencies of all loci. Both hypotheses, however, are rejected for the following reasons. The population is very big and the habitat very uniform, so that there are no constraints for inbreeding. The Wahlund effect can be excluded, because the sample stems from a homogeneous area of less than ½ m^2, so it seems very unlikely that the sample contained members of two or more subpopulations. The remaining causes are more likely to affect a single locus rather than the whole genome. Thus, probably a combination of factors accounts for the heterozygote deficiencies. However, three more of the above-listed points can be excluded. The habitat of the population is too homogeneous to establish genetic patchiness, so that there is certainly no sampling bias. The staining patterns were easily and unambiguously interpretable. Thus, a scoring bias can be excluded, as can the differential survival of homozygotes following collection, because the sample was frozen less than one week after collection, and few snails had died during that time. It cannot be estimated to which degree location of polymorphic loci on a sex chromosome and assortative mating are involved, because nothing is known about the determination of sex and the choice of mates in *Hydrobia*. The presence of null alleles cannot be excluded. The most probable explanation is selection against heterozygotes. The population is highly infected with trematode sporocysts and rediae, which might cause a considerable selective pressure. Four alleles each were detected in APH and PGM 2. For these two loci, the small sample sizes (20 and 28, respec-

TABLE 6. Chi-square test for deviation from Hardy-Weinberg equilibrium in *H. ulvae*.

Locus	Genotype	Observed Frequency	Expected Frequency	χ^2	DF	P
AK	A-A	5	4.789			
	A-C	4	4.421			
	C-C	1	0.789	0.105	1	0.745
G6PD	A-A	6	4.789			
	A-B	2	4.421			
	B-B	2	0.789	3.488	1	0.062
MDH	A-A	0	4.872			
	A-B	20	10.256			
	B-B	0	4.872	19.000	1	0
PGM 1	C-C	17	17.077			
	C-D	3	2.846			
	D-D	0	0.077	0.086	1	0.770

TABLE 7. Matrix of Nei's genetic distances. Above the diagonal: Nei's (1972) standard distance; below: Nei's (1978) unbiased distance.

	H. ventrosa	*H. acuta*	*H. ulvae*
H. ventrosa	—	0.111	1.648
H. acuta	0.110	—	1.753
H. ulvae	1.645	1.751	—

TABLE 8. Matrix of Cavalli-Sforza & Edwards's (1967) distances. Above the diagonal: chord distance; below: arc distance.

	H. ventrosa	*H. acuta*	*H. ulvae*
H. ventrosa	—	0.306	0.790
H. acuta	0.323	—	0.814
H. ulvae	0.873	0.903	—

tively) alone might account for the deviations from Hardy-Weinberg equilibrium.

The 100% heterozygosity of the MDH in *H. ulvae* is probably due to selection against homozygotes, which means the remarkable loss of 50% of the offspring.

Lack of genetic variation as in *H. acuta*, which has no polymorphic locus under the 95% criterion (the polymorphism of the APH locus is again due to a rare allele), is usually explained by the assumption of genetic drift following a bottleneck in the population's past (Nei et al., 1975).

Nei's commonly used distances were chosen for reasons of comparability, although these measures are nonmetric (Wright, 1978) and the constant substitution of amino-acids, on which Nei based his model (Nei, 1972), is hardly, if ever, met (Hillis, 1984). Cavalli-Sforza & Edwards's arc distance is, according to Wright (1978), superior to all other distance coefficients due to its geometrical clarity. But the validity of Cavalli-Sforza & Edwards's distances is restricted in that only random genetic drift and selection are considered causes for divergence between populations (Cavalli-Sforza & Edwards, 1967). More comprehensive presentations of the strengths and limitations of the various distance measures can be found in Wright (1978), Davis et al. (1988), and Swofford & Olsen (1990). However, the cophenetic correlations (cc) of the phenograms of Figures 4 and 5 (cc = 0.998 and 0.999, respectively) indicate that in the present case both distance measures applied yield equivalent results.

Nei's (1972) genetic distance D between congeneric species of molluscs is typically in the range from 0.20–0.60 (Woodruff et al., 1988). In a survey on distance data, Thorpe (1983) found D values larger than 1.05 in only 15% of approximately 900 estimates of interspecific distances of congeners of various eukaryotes. This value was exceeded in 80% of about 160 comparisons between confamilial genera. Davis et al. (1989) compared six populations of the North American *H. truncata* (Vanatta, 1924). The highest distance value (Nei's unbiased distance, 1978) was 0.018. However, one has to be careful drawing taxonomic conclusions from distance data only. Certain ranges of genetic distance do not have simple correspondence to taxonomic levels (Hoagland & Davis, 1987). Based on the genetic distances in Tables 7 and 8, one could conclude that *H. ventrosa* and *H. acuta* were conspecific populations or very closely related species, whereas *H. ulvae* belonged to another genus. Taking anatomical (Krull, 1935; Giusti & Pezzoli, 1984; Falniowski, 1987; personal observations) and cytological (Butot & Kiauta, 1966) data into account, it becomes clear that *H. ventrosa* and *H. acuta* are distinct species and that there is no character that would separate *H. ulvae* from the other two species on a higher level. [The duct connecting the prostate with the mantle cavity described by Johansson (1948) for *H. ulvae* has also been found in *H. acuta* and *H. ventrosa* (personal observations).] However, the large distance values between *H. ulvae* and the other two species correspond with ecological differences and differences in reproductive biology. *Hydrobia ventrosa* and *H. acuta* prefer sheltered bays, whereas *H. ulvae* also tolerates higher water movement (Fretter & Graham, 1978; Falniowski, 1987; personal observations). *Hydrobia ulvae* has free swimming veligers (Fish & Fish, 1977), whereas in *H. ventrosa* the whole veliger stage is intracapsular (Thorson, 1946). For *H. acuta* there is only indirect evidence for the same mode of reproduction as in *H. ventrosa*. The animals reproduced in an aquarium equipped with pump and filter (personal observations). Planktonic larvae would not have survived these conditions.

In this study, only a single population of each species could be investigated, and the following systematic conclusions should be taken with some reservation. However, because the genetic distances correspond with ecological and developmental data, it can well be assumed that the results obtained from

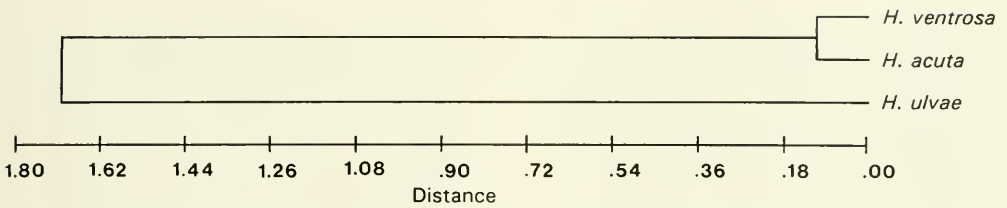


FIG. 4. UPGMA phenogram based on Nei's (1978) unbiased genetic distance.

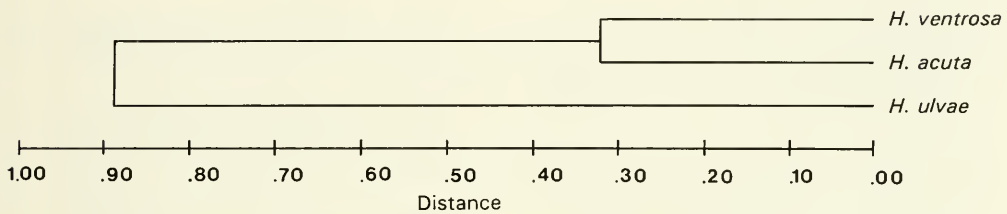


FIG. 5. UPGMA phenogram based on Cavalli-Sforza & Edwards's (1967) arc distance.

these three populations reflect the true relationships between the three species. Thus, a separation of *H. ulvae* from the other two species based on allozymes, ecological and developmental data can well be justified. Because the general anatomical organization of all three species is practically identical, a separation beyond the subgenus level would be unwarranted. Consequently, the genus *Hydrobia* Hartmann, 1821, can be subdivided into the subgenera *Hydrobia s. s.* and *Peringia* Paladilhe, 1874, and *Ventrosia* Radoman, 1977, has to be considered synonymous with *Hydrobia*. This synonymy is based on natural arguments, which demonstrate that Boeters's (1984) purely taxonomic attempt discussed in the introduction is unnecessary and also therefore to be rejected.

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