Malacologia.

[Ann Arbor,Institute of Malacology]1962-
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v.20 (1980-1981): http://www.biodiversitylibrary.org/item/47357
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MALACOLOGIA, 1980, 20(1): 83–98

THE *GONIOBASIS* OF SOUTHERN VIRGINIA AND NORTHWESTERN NORTH CAROLINA: GENETIC AND SHELL MORPHOMETRIC RELATIONSHIPS[1]

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ABSTRACT

Three species of *Goniobasis* inhabiting the upper New River and surrounding drainages, *G. proxima*, *G. semicarinata*, and *G. simplex*, were found to be very distinct using starch gel electrophoresis. Heterozygosity (H) is low, averaging 0.0113 across 12 populations and 15 loci. The average genetic identity (Nei, 1972) between conspecific populations is 0.89, also very low compared to averages recorded in other animals. Much of the difference between conspecific populations is due to fixation of alternative alleles, suggesting that gene flow even among populations connected through water can be quite low, or that selection is high. Three races are provisionally recognized in both *G. proxima* and *G. semicarinata* on the basis of differences in isozyme frequencies. Compared to electrophoresis, species identification using shell morphology alone was found to be unreliable. A population indistinguishable from *G. simplex* after a multidimensional scaling based on seven shell characters was revealed to be *G. proxima* using electrophoresis. The unusual shell morphology of this population may result from the introduction of the normally softwater-dwelling *G. proxima* into a hard-water stream similar to those inhabited by *G. simplex*. If divergence is measured as euclidean distance to species centroid after multidimensional scaling, the amount of population divergence in shell character is correlated to the amount of genetic divergence at the .05 level.

INTRODUCTION

Pleurocerid snails (freshwater prosobranchs) have undergone an extensive endemic radiation in streams and rivers of southeastern U.S.A. A bewildering variety of shell phenotypes has been the basis for some 500 nominal species (review of Tryon, 1873; Goodrich, 1940, 1941, 1942, 1944). The description of so many phenotypes has posed tremendous problems to taxonomists working with these mollusks.

Centers of gastropod endemism have provided areas for the study of adaptive radiation, speciation, and divergence. Some examples of such studies involve the pulmonate land snail genera *Partula* (Murray & Clarke, 1968) and *Cerion* (Gould & Woodruff, 1978), and the freshwater prosobranch Triculinae (Davis, 1979). This study deals with some members of the pleurocerid genus *Goniobasis* in parts of four drainages on the northern edge of their area of endemism.

The upper New River drains a sparsely populated, mountainous region on the border of Virginia and North Carolina. In an earlier survey (Dillon, 1977), several species of the freshwater snail genus *Goniobasis* (Pleuroceridae) were found common and widespread in small streams and tributaries of the drainage but absent from the main bed of the New River (Fig. 1). The tendency for *Goniobasis* populations to diminish downstream is widespread and has been shown previously in *G. proxima* by Foin (1971) and Foin & Stiven (1970). In a survey of a single *G. proxima* population, Dillon (unpublished) found snail densities over 300 per square meter in a stream only one meter wide. Snail density gradually decreased to less than two per square meter by the time the stream was five meters wide, five kilometers downstream. Dispersal further downstream, between isolated populations, probably occurs only when snails are dislodged by flooding. Eggs are cemented firmly onto rocks, and individual snails generally move against the current (Crutchfield, 1966; Krieger & Burbanck, 1976).

Although the New River is quite ancient geologically (Ross, 1969), observed *Goniobasis* distributions probably date only from the

[1]This work was supported, in part, by a NSF Grant to G. M. Davis, No. DEB 78-01550.
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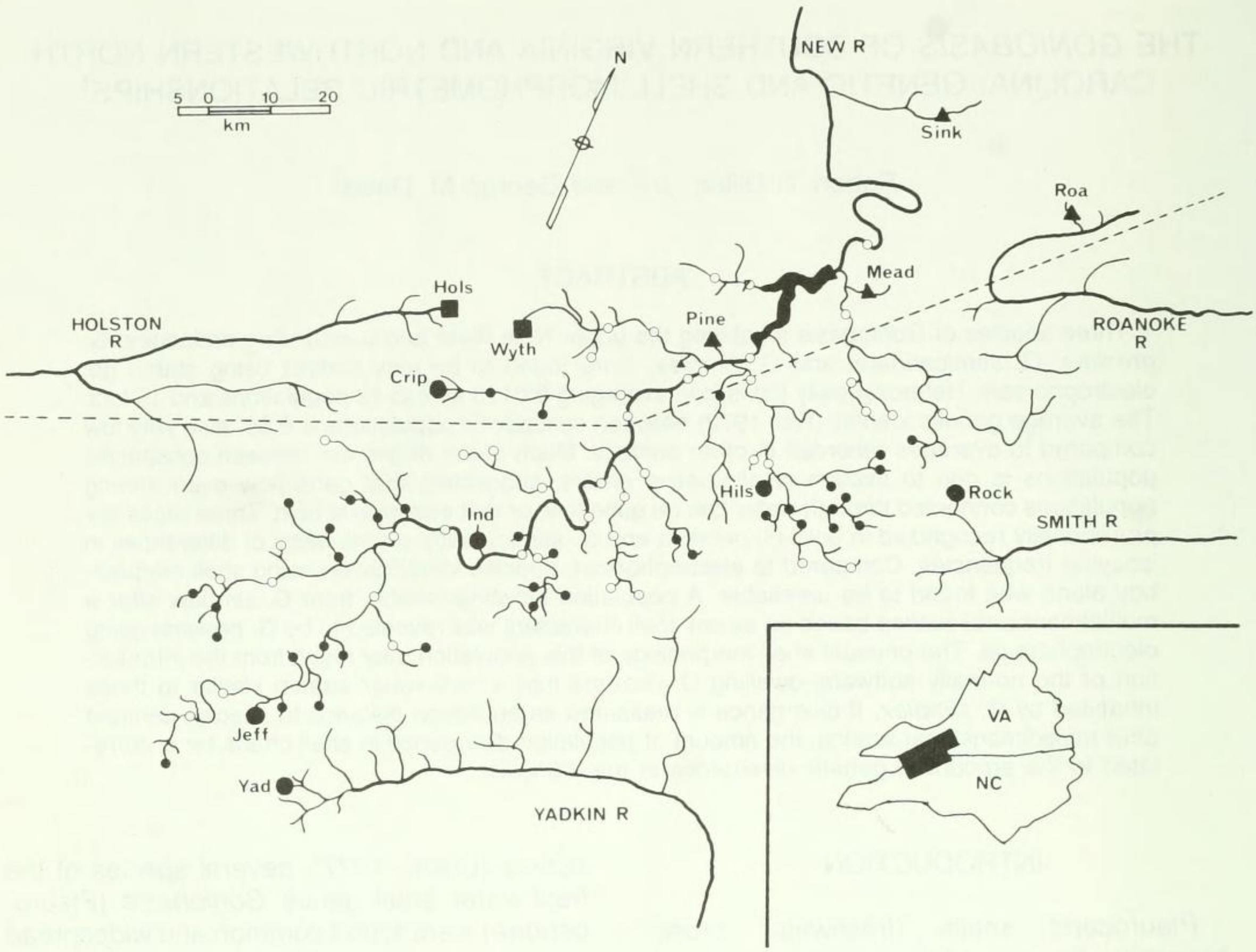

FIG. 1. Schematized map of the 5 drainage systems in the study area. Darkened symbols indicate presence of *Goniobasis:* circles, *G. proxima*; triangles, *G. semicarinata*; squares, *G. simplex*. Open circles are sites where no *Goniobasis* were collected by Dillon (1977). The 12 larger symbols designate the locations of population sampled for this study. The dashed line approximates the southeastern extent of limestone and dolomite.

Pleistocene. The heavy rains, high erosion rates, and lowered temperatures that accompanied worldwide climatic change probably affected the ranges of mountain stream-dwelling organisms greatly. Currently, the main body of the New River forms a barrier fragmenting the range of *Goniobasis* into numerous, isolated populations. To some extent these events must have been repeated in other rivers of the southeastern U.S.A., from which scores of pleurocerid species have been described. The erection of barriers to gene flow, while surely not the sole method of animal speciation (Bush, 1975; White, 1978; Futuyma, 1979), has probably played an important role in pleurocerid radiation. One objective of this study is to assess the impact of barriers to dispersal between *Goniobasis* populations.

Electrophoretic investigation of this interesting system has been initiated by Chambers (1977, 1978, 1980). He has found that genetic divergence among consubspecific populations of *Goniobasis* in scattered Florida rivers is greater than the divergence in *Drosophila* populations reported by Ayala et al. (1974). Chambers found heterozygosity quite low in the 18 populations he surveyed and conspecific populations occasionally fixed for alternative alleles at particular loci. This suggests that migration among *Goniobasis* populations may be rare. Does migration seem to be as uncommon among *Goniobasis* isolated within the same river system as it seems between rivers? The second objective of this study is to assess the amount of genetic similarity within and between populations of the three *Goniobasis* species found in the upper New River, *G. proxima* (Say), *G. semicarinata* (Say), and *G. simplex* (Say), and compare these populations to conspecific populations in adjacent drainages.

Species identities of North American pleurocerids have been based entirely on shell characters. Many authors (cf. Dazo, 1965) have observed great variability in the shells of pleurocerids and suggested that varying environments are responsible. A great deal of interpopulation variability is apparent in the shells of the three *Goniobasis* species examined in this study. How do shell characters compare with electrophoretic data in distinguishing between species and populations? The third objective of this study is to examine the relationship between electrophoretic and shell morphological data.

METHODS

Populations studied

Eight populations of *Goniobasis* were selected to represent the geographic and morphological range of the genus in the upper New River. Four populations were chosen from surrounding drainages for comparison (Fig. 2). These populations represent three species, *Goniobasis proxima*, *Goniobasis semicarinata* and *Goniobasis simplex*. Species identifications were based on conchological comparisons with Tryon (1873) and collections in the Academy of Natural Sciences of Philadelphia. As will be shown later, conchological comparisons are no sure method of determining species status. Collection sites are shown in Fig. 1. Populations of *G. proxima* were given the names CRIP, HILL, IND, JEF, ROCK, and YAD, populations of *G. semicarinata* were designated MEAD, PINE, ROA, and SINK, and those of *G. simplex* were designated HOLS and WYTH. Locality data for the 12 populations are presented in the Appendix.

At least 100 large individuals were randomly collected at each site in September, 1978. Snails were kept alive in cloth sacks submerged under water in a large bucket in order to clean the guts of contents; the water was changed periodically for 24 hours. They were then quickly frozen (around −20°C) and kept in the freezer during the course of the study. Voucher specimens have been deposited in the Academy of Natural Sciences of Philadelphia; voucher specimen catalog numbers are given in the Appendix.

Although the geology of the study area is complex, limestone and dolomite generally underlie regions northwest of the dashed line in Fig. 1. Southeast of the line, the surface

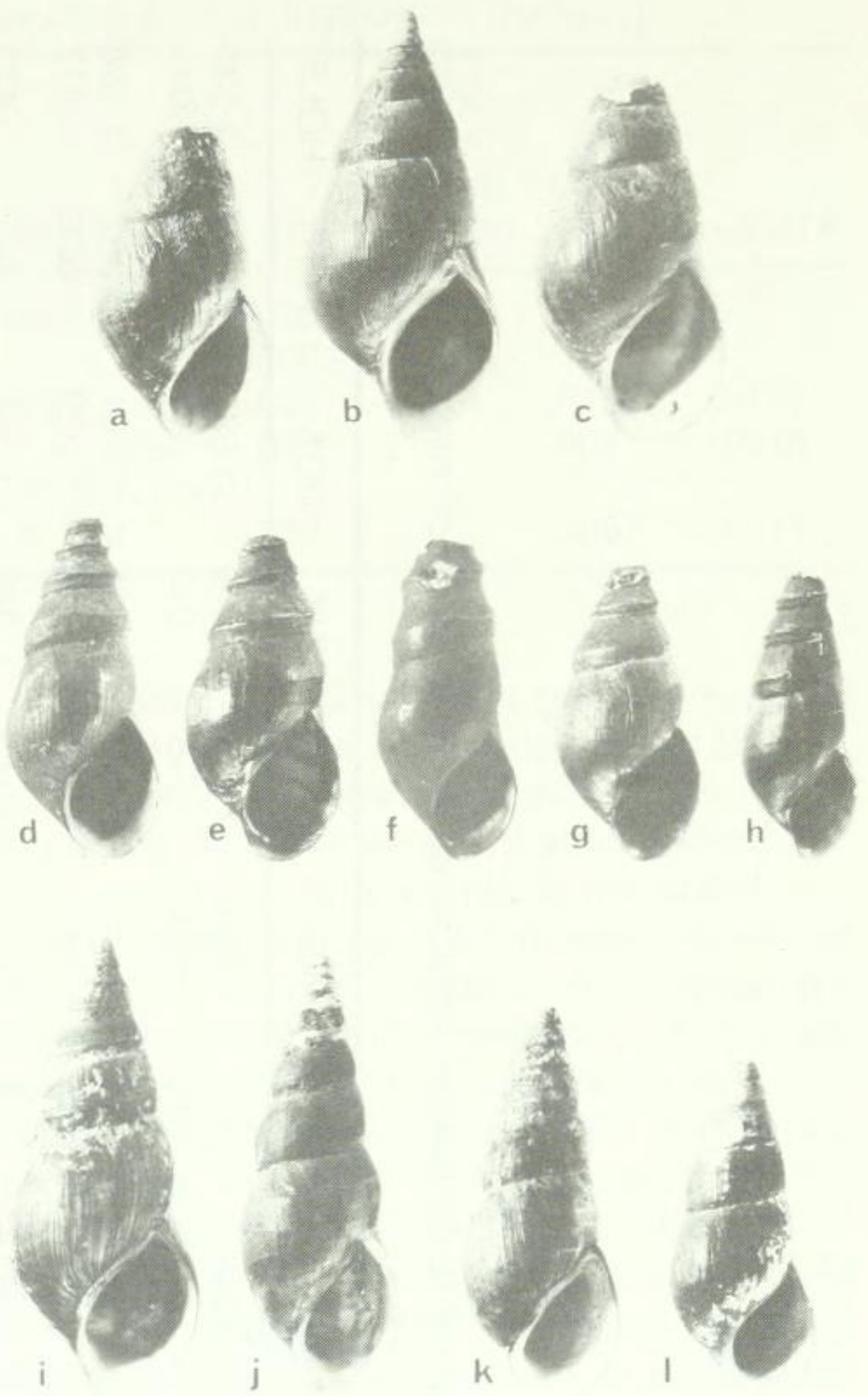

FIG. 2. Representative shells from the *Goniobasis* populations surveyed. a, b, *G. simplex*; c–h, *G. proxima*; i–l, *G. semicarinata*. a—HOLS, b—WYTH, c—CRIP, d—HILL, e—IND, f—YAD, g—JEFF, h—ROCK, i—MEAD, J—ROA, k—PINE, l—SINK. Length of shell "a" 1.65 cm; remaining shells to same scale. See Fig. 1.

geology is principally gneiss and schist. Since it has been demonstrated that the distributions of freshwater mollusks can be highly influenced by the effects of limestone on water quality (Shoup, 1943; McKillop & Harrison, 1972; Dussart, 1976), alkalinity was measured at each of the 12 sites. Sepkoski & Rex (1974) have found a correlation between alkalinity ("bicarbonate ion concentration" and calcium concentration, hardness, pH, and total dissolved solids at the .01 level. All of these variables tend to increase in value as a drainage area includes more limestone and dolomite. Alkalinity measurements were made at the riverbank by staining 50 ml of water first with phenolphthalein and then with methyl purple, and titrating with .02N sulfuric acid (American Public Health Association, 1976).

TABLE 1. Means for shell characters measured on 20 individuals from each of 12 *Goniobasis* populations. Directly below each mean is the standard error of the mean.

	HILL	IND	JEFF	YAD	ROCK	CRIP	PINE	MEAD	SINK	ROA	WYTH	HOLS
Shell length (cm)	1.515	1.488	1.358	1.439	1.293	1.707	1.613	1.535	1.409	1.586	1.759	1.532
	.023	.023	.019	.018	.017	.016	.024	.018	.015	.018	.029	.015
Body whorl length (cm)	1.142	1.128	1.015	1.038	.942	1.310	1.179	1.113	1.022	1.161	1.352	1.188
	.016	.015	.014	.011	.012	.015	.017	.014	.012	.023	.020	.012
Aperture width (cm)	.382	.361	.338	.320	.281	.468	.377	.348	.340	.367	.442	.423
	.009	.015	.008	.004	.004	.006	.006	.004	.011	.008	.004	.006
Spire angle (degrees)	22.8	20.5	19.7	17.9	15.6	25.6	20.9	17.0	20.9	17.4	27.7	25.5
	.50	.63	.76	.60	.58	.53	.86	.49	.59	.77	.77	1.04
Shell length/Shell width	2.145	2.146	2.118	2.248	2.255	2.075	2.178	2.289	2.170	2.359	2.067	2.015
	.020	.017	.023	.018	.027	.013	.021	.016	.012	.024	.021	.017
Third whorl width/Shell width	.535	.570	.546	.600	.594	.494	.565	.582	.564	.585	.495	.450
	.077	.013	.011	.008	.011	.008	.007	.008	.006	.007	.011	.009
Aperture length/Shell length	.428	.427	.412	.378	.376	.454	.386	.401	.412	.408	.439	.460
	.004	.006	.006	.003	.004	.004	.005	.005	.005	.006	.004	.005

Shell morphometrics

Twenty large snails were chosen from the collection made at each site for shell morphology measurements. The seven measurements made on each shell are listed in Table 1. Spire angle, aperture length, shell width, and length of body whorl were measured with calipers using the methods of Davis (1969). Shell length was measured as the length of the last three whorls only, since shell apices were often eroded. Aperture width was the maximum distance across the aperture perpendicular to the measure of aperture length. The width of the third whorl up from the aperture was measured. Shell width, aperture length, and third whorl width were converted into ratios for an analysis of shape.

Electrophoresis

Horizontal starch gel electrophoresis was performed using the procedure described by Ayala et al. (1973). The shells of individual snails were crushed using a pair of pliers and picked from the body with forceps. Then each body was placed in a centrifuge tube with 0.5 ml deionized water and homogenized by sonication. Small tabs of Whatman No. 3 filter paper were dipped in the crude homogenate, blotted, and applied to the gel. Gels were prepared using 35 g of Electrostarch and 250 ml of one of 3 gel buffers. Table 2 shows the compositions of both the gel buffers and the buffers used in the electrode trays. Gels were run at 35 milliamps or 350 volts, but not exceeding either.

TABLE 2. Buffers used in gels and electrode trays. Concentration of ingredients (Molarity).

Buffer, pH	Tris	Citric acid (monohydrate)	Boric acid	Na_2EDTA
Tris-Cit 6 Tray	.237	.085		
Gel	.0083	.0030		
TEB 8 Tray	.500		.645	.0179
Gel	.050		.097	.0018
TEB 9.1 Tray & Gel	.087		.0087	.0011

After electrophoresis, the gels were sliced and stained for one of 15 enzymes (Table 3). Gel scoring methods were those of Ayala et al. (1973). The following are stain buffers and other standard components of the stains employed along with an abbreviated name for each. "0.1 Tris HCl buffer"—0.1 molar tris (hydroxymethyl) aminomethane ("Tris") adjusted to pH 7.95 with concentrated HCl. "0.2 Tris HCl buffer"—the same but 0.2M Tris. "DH buffer"—0.102M Tris, adjusted to pH 8.4 with concentrated HCl. "Tris maleate A"—0.2M Tris, 0.2M maleic acid. "Tris maleate B"—0.2M NaOH. "NAD"—0.05 ml of a 3% solution. "NADP"—0.05 ml of a 2.5% solution. "$MgCl_2$"—1 drop of a 1% solution. "MTT"—0.05 ml of a 40 mg/ml suspension of 3-(4,5 dimethylthiazolyl-2)-2,5 diphenyl tetrazolium bromide. "PMS"—about 0.1 mg of phenazine methosulfate. "Agar"—10 ml of a 2 g/100 ml solution. "G6PdH" and "PGI"—5 μl of a 1000 units/ml solution of glucose-6-

TABLE 3. Enzymes surveyed in *Goniobasis* populations.

Enzyme	Abbreviation	Buffer system	Run time (hr.)
Acid phosphatase	Acph	TEB 9.1	3
Aspartate aminotransferase	Aat	TEB 9.1	3
Glucose-6-phosphate dehydrogenase	G6pd	TEB 9.1	4
Glucose phosphate isomerase	Gpi	Tris-Cit 6	3
Hexanol dehydrogenase	Hexdh	TEB 9.1	4
Isocitrate dehydrogenase	Isdh	TEB 8	3
Leucine aminopeptidase	Lap	TEB 9.1	3
Malate dehydrogenase	Mdh	Tris-Cit 6	2
Mannose-6-phosphate isomerase	Mpi	Tris-Cit 6	3
Octopine dehydrogenase	Odh	Tris-Cit 6	3
6-Phosphogluconate dehydrogenase	6Pgd	Tris-Cit 6	2
Phosphoglucomutase	Pgm	Tris-Cit 6	2
Sorbitol dehydrogenase	Sdh	TEB 9.1	4
Superoxide dismutase	Sod	TEB 9.1	4
Xanthine dehydrogenase	Xdh	TEB 8	3

phosphate dehydrogenase and phosphoglucose isomerase, respectively.

Descriptions of the staining procedures employed follow, with standard recipe components referenced by their abbreviated names above. Most recipes have been modified from Shaw & Prasad (1970) and Bush & Huettel (1972).

Acph—α-napthyl acid phosphate 16 mg, Fast Blue BB salt 20 mg, $MgCl_2$. Tris maleate A 2.5 ml, Tris maleate B 1.3 ml, water 6.2 ml, Agar.

Aat—L-aspartic acid 400 mg, α-ketoglutaric acid 200 mg. Fast Blue BB salt 300 mg, pyridoxal-5′-phosphate 0.5 mg, 0.2 Tris HCl buffer 100 ml.

G6pd—D-glucose-6-phosphate 20mg, NADP, $MgCl_2$, MTT, PMS, 0.1 TrisHCl buffer 10 ml, Agar.

Gpi—D-fructose-6-phosphate 20 mg, G6PdH, NADP, $MgCl_2$, MTT, PMS, 0.1 TrisHCl buffer 10 ml, Agar.

Hexdh—1-hexanol 5 ml, NAD 25 mg, Nitro blue tetrazolium 20 mg, PMS 0.5 mg, DH buffer 100 ml.

Isdh—DL-isocitric acid 20 mg, NADP, $MgCl_2$, MTT, PMS, 0.1 TrisHCl buffer 10 ml, Agar.

Lap—L-leucyl-β-napthylamide HCl 20 mg, Black K salt 50 mg, Tris maleate A 12.5 ml, Tris maleate B 6.5 ml, water 81 ml.

Mdh—Substrate solution 1.5 ml (containing L-malic acid 13.4 g, 2M Na_2CO_3 49 ml, water to 100 ml, adjusted to pH 7 with Na_2CO_3). NAD, MTT, PMS. 0.1 TrisHCl buffer 10 ml, Agar.

Mpi—D-mannose-6-phosphate 10mg, G6PdH, PGI, NADP, MTT, PMS, 0.1 TrisHCl buffer 10 ml, Agar.

Odh—(+)octopine 8 mg, NAD, MTT, PMS, 0.1 TrisHCl buffer 10 ml, Agar.

6Pgd—6-phosphogluconic acid 10mg, NADP, MTT, PMS, 0.1 TrisHCl buffer 10 ml, Agar.

Pgm—α-D-glucose-1-phosphate 30 mg, α-D-glucose-1,6-diphosphate 0.5 mg, G6PdH, NADP, MTT, PMS, $MgCl_2$, 0.1 TrisHCl buffer 10 ml, Agar.

Sdh—D-sorbitol 250 mg, NAD, MTT, PMS, DH buffer 10 ml, Agar.

Sod—lightly colored bands representing this enzyme were most apparent on the darkly stained Hexdh gel.

Xdh—Hypoxanthine 10 mg, 0.05M TrisHCl pH 7.5 10 ml (boiled to dissolve Hypoxanthine then cooled to room temperature), KCl 15 mg, NAD, MTT, PMS, Agar.

Isozyme bands are named according to their mobilities compared to the most common allele in WYTH, the reference population. Methods and assumptions are those in Ayala et al. (1973).

Analytical methods

The program NT-SYS of Rohlf et al. (1972) was used to explore the electrophoretic and morphological relationships among the *Goniobasis* populations. For the shell morphometric study, a principal component analysis was performed on the correlation matrix of standardized means for the seven characters. The factor scores on the first 3 principal components were used as the initial configuration for multidimensional scaling. The scaling was done to maximize goodness-of-fit to the monotonic regression of the distance between populations in three-dimensional space and their true distances in seven-dimensional space. Here taxonomic distance was calculated as the square root of the average squared difference between populations across the morphological variables (Sokal, 1961). Finally, principal component analysis was reapplied to the covariance matrix of the new three-dimensional scaled distances (three factors account for 100% of the variance).

The analysis of the electrophoretic data was similar to that applied to the shell characters. Genetic distances were calculated according to Nei (1972). Then multidimensional scaling was performed to maximize goodness-of-fit to the regression of genetic distance and distance in three-dimensional space. The initial configuration of the points in the analysis of electrophoretic data was random. Three-dimensional scaling was followed by principal component analysis as described above. More complete discussion of these methods is given by Sneath & Sokal (1973).

Here we introduce a method of quantifying population divergence. The amount of divergence necessary to explain variation in the phenotypes of n populations is minimized by joining them in a Wagner or Steiner network including $2n-3$ segments and $n-2$ branching points or nodes (Farris, 1970). Thus if evolution has occurred parsimoniously, the Wagner network best reconstructs the evolutionary relationships among a group of populations. But a problem may arise using this method if the object is to compare divergence measured by several criteria, for small differences

in the measures to be compared may alter the order of linkage in the network. Thus we propose an alternate method of examining divergence that may, for small numbers of populations, approximate the Wagner network.

The species centroid for some group of characters is the point corresponding to the average of all character measurements over some set of conspecific populations. In the case where the number of populations, *n*, equals three, a Wagner network is formed by connecting the populations to their centroid. The centroid can be seen as a hypothetical population, and the minimum amount of divergence necessary to explain observed population variation can be measured as the length of the three segments radiating from the centroid. It is not necessary to designate the centroid or one of the surrounding points as the ancestral population if only the amount of divergence is of interest, and its direction is immaterial.

When *n* is greater than three, the centroid connected to its surrounding populations is no longer the minimum estimate of population divergence, but it remains a (successively worsening) approximation. In this study we estimate the divergence of each population *i* as its euclidean distance *D* to the species centroid:

$$D = (\sum_{k} (\bar{x} - x_i)^2)^{1/2}$$

where x_i is the coordinate of the population on some axis *x*, $\bar{x}$ is the mean coordinate over *n* conspecific populations, and *k* is the number of characters or axes. We employ this technique to cases where $n = 4$ and $n = 6$.

RESULTS

Electrophoresis

Allelic frequencies at each of the ten polymorphic loci are presented in Table 4. No variability was found at five loci, *Acph*, *Hexdh*, *Mdh*, *6Pgd*, and *Sdh*. Table 4 also shows mean heterozygosities (H) of 15 loci for the 12 populations. Some enzyme assays, particularly that for *Xdh*, were not included until later in the study and thus have small sample sizes. Small samples may also result from reduced enzymatic activity in some populations. *Lap* activity in particular was occasionally absent from individuals in the IND and ROCK populations and was entirely absent in the HILL population. But 12 snails collected from the HILL population in July, 1979, all showed strong *Lap* activity. They were homozygous for the common *G. proxima* allele, *Lap 94*. It thus seems possible that synthesis of this enzyme is under environmental control, and that all individuals from the HILL population may have the capacity to synthesize *Lap 94*. No "null" allele of *Lap* is believed to be segregating.

Table 5 presents values of Nei's (1972) genetic identity *I* and distance *D* for all pairs of populations, and Fig. 3 depicts these relationships graphically. Stress in the multidimensional scaling afer 50 iterations was 0.015, and the correlation between taxonomic distance and three-dimensional scaled distance was 0.975. The first two factors account for 53.4% and 45.3% of the variance, respectively. The third factor (ungraphed) accounts for only 1.3% of the variance, but ROCK (Rc) is separated by a high factor III score and ROA (Ra) is distinguished by a low factor III score. Several segments of the minimum-spanning tree (cf. Sneath & Sokal, 1973) are shown connecting the major clusters. For the sake of clarity the entire tree is not shown, but since the two dimensions graphed account for nearly 99% of the variance, the tree's structure is generally obvious. Details of the two major species clusters are shown in a pair of magnified insets to Fig. 3. In the inset each population is connected to its species centroid. The length of these segments is a measure of population divergence in the two dimensions graphed.

Shell morphometrics

The population means for the seven shell characters measured are presented in Table 1, along with their standard errors. The results of the three-dimensional scaling of these data are shown in Fig. 4. Once again the third factor accounts for a small portion of the variance (5.6%) and is ungraphed. The YAD (Y) and PINE (P) populations both had exceptionally low factor III scores and are thus more distinct from conspecific populations than Fig. 4 indicates. The two graphed factors accounted for 77.1% and 17.2% of variance, respectively. After 50 iterations, stress was 0.003 and the correlation between taxonomic distance and three-dimensional scaled distance was 0.999.

Fig. 5 shows the relationship between two measures of intraspecific divergence, genetic and shell morphometric. Divergence has been

TABLE 4. Allelic frequencies at polymorphic loci in 12 populations of *Goniobasis*.

Enzyme allele	HILL	IND	JEF	YAD	ROCK	CRIP	PINE	MEAD	SINK	ROA	WYTH	HOLS
Aat 100											1.00	1.00
102	1.00	1.00	1.00	1.00	1.00	1.00						
98							1.00	1.00	1.00	1.00		
No.	66	36	71	103	49	58	44	75	36	46	123	50
G6pd 100	1.00	1.00	1.00	1.00	0.84	1.00	1.00	1.00	1.00	1.00	1.00	1.00
105					0.16							
No.	36	42	71	67	47	52	44	61	34	40	90	44
Gpi 100											0.98	1.00
99											0.02	
97	.02	.03			.21		1.00	0.99	1.00	0.99		
95										0.01		
102	.98	.97	1.00	1.00	0.79	1.00		0.01				
No.	65	60	64	108	63	52	44	75	43	46	122	50
Isdh 100	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00
91		0.01								0.01		
No.	72	60	29	78	40	58	44	70	43	37	87	50
Lap 100											1.00	1.00
99							1.00	1.00	1.00			
98										1.00		
96		1.00	1.00	1.00	1.00							
94						1.00						
No.	0	6	71	46	13	58	28	75	17	41	113	54

Mpi 100				0.21							1.00	0.95
97							1.00	1.00	1.00	1.00		
95	1.00	1.00	1.00	0.79	1.00	1.00						0.05
No.	73	60	71	97	60	58	40	75	43	46	136	55
Odh 100											1.00	0.98
103												0.02
106				0.64			1.00	0.95				
108		0.94		0.21								
109	1.00		1.00	0.01		0.99						
110					1.00			0.05		1.00		
112		0.06		0.14		0.01						
117									1.00			
No.	27	24	71	45	59	58	26	75	43	46	56	54
Pgm 100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		1.00	1.00	1.00
95									1.00			
No.	41	60	71	97	60	38	44	75	43	46	133	50
Sod 100											1.00	1.00
102	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
No.	24	48	57	31	22	58	36	75	36	46	128	50
Xdh 100											1.00	1.00
98	1.00	1.00	1.00	1.00			1.00	1.00	1.00	1.00		
96					1.00	1.00						
No.	14	15	6	12	13	64	12	47	36	46	35	50
Heterozygosity (H)	.0026	.0127	0.00	.0572	.0400	.0013	0.00	.0076	0.00	.0026	.0026	.0089

No. = number of individuals.

estimated using euclidean distance to the species centroid as outlined in the methods section. All three dimensions were considered, although only the first two have been depicted in Figs. 3 and 4. Since the pair of *G. simplex* populations sampled were electrophoretically indistinguishable, *G. simplex* was omitted from this analysis. The Spearman rank correlation coefficient is .70, significant at the .05 level. Rank correlation is appropriate regardless of whether the segment lengths in the morphometric analysis are de-

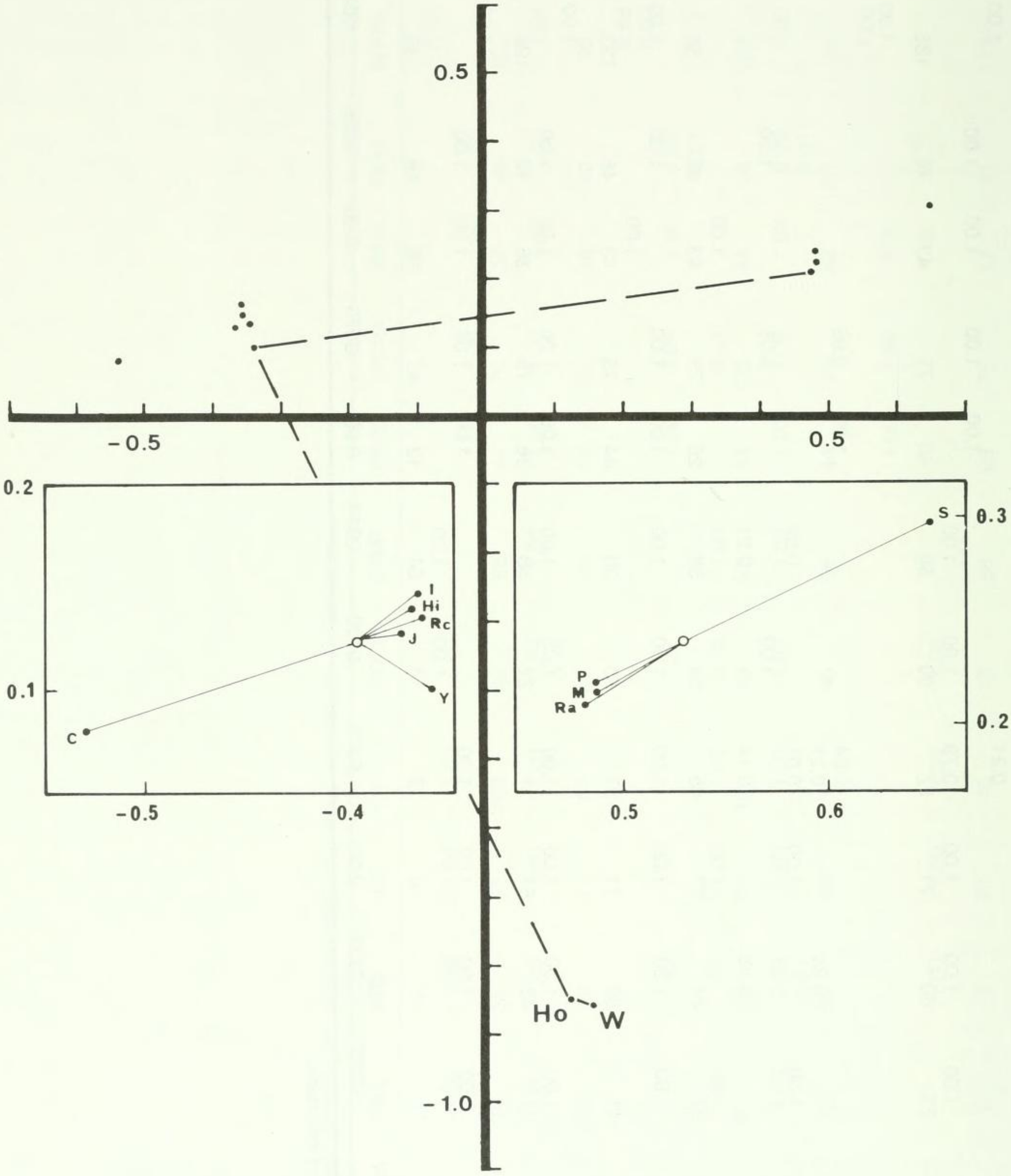

FIG. 3. Multidimensional scaling of *Goniobasis* populations based on Nei's (1972) genetic distances. The dashed lines are segments from the minimum-spanning tree. The magnified inset on the left shows details of the *Goniobasis proxima* cluster, and the inset on the right shows *G. semicarinata*. Centroids for the two common species are graphed as open circles. Population names are abbreviated as follows: C—CRIP, Hi—HILL, Ho—HOLS, I—IND, J—JEFF, M—MEAD, P—PINE, Ra—ROA, Rc—ROCK, S—SINK, W—WYTH, Y—YAD.

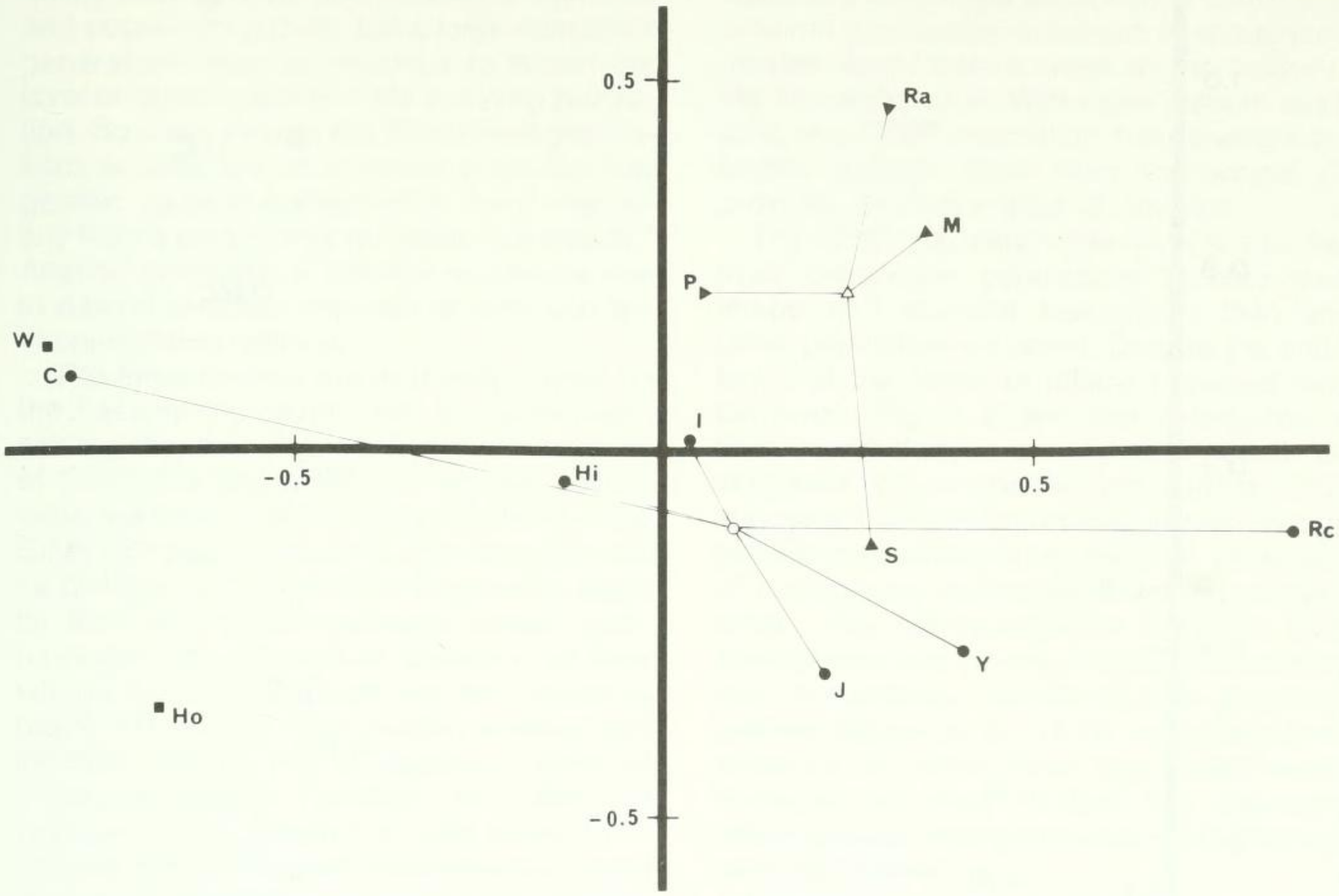

FIG. 4. Multidimensional scaling of *Goniobasis* populations based on shell morphology and Sokal's (1961) measure of taxonomic distance. Circles are *G. proxima* populations, triangles are *G. semicarinata,* and squares are *G. simplex.* Centroids are graphed as open circles. Abbreviations as in Fig. 3.

TABLE 5. Nei's (1972) genetic identities (below diagonal) and distances (above diagonal) between *Goniobasis* populations.

	HILL	IND	JEF	YAD	ROCK	CRIP	PINE	MEAD	SINK	ROA	WYTH	HOLS
HILL	—	.065	0.00	.051	.151	.144	.402	.398	.508	.402	.627	.615
IND	.937	—	.065	.033	.147	.219	.397	.392	.502	.396	.622	.610
JEF	1.00	.937	—	.050	.152	.143	.405	.401	.511	.405	.627	.616
YAD	.950	.968	.951	—	.134	.204	.326	.324	.493	.387	.609	.598
ROCK	.860	.863	.859	.875	—	.150	.485	.475	.602	.380	.627	.616
CRIP	.866	.803	.867	.816	.861	—	.510	.505	.628	.509	.627	.617
PINE	.669	.673	.667	.722	.616	.600	—	0.00	.143	.143	.627	.623
MEAD	.672	.676	.670	.723	.622	.603	1.00	—	.140	.136	.623	.620
SINK	.602	.605	.600	.611	.548	.534	.867	.869	—	.223	.761	.757
ROA	.669	.673	.667	.679	.684	.601	.866	.872	.800	—	.627	.623
WYTH	.534	.537	.534	.544	.534	.534	.534	.536	.467	.534	—	0.00
HOLS	.541	.543	.540	.550	.540	.540	.536	.538	.469	.536	1.00	—

termined by the additive effects of numerous genes or slight allometric differences.

DISCUSSION

On the basis of isozyme frequencies, the three *Goniobasis* species are quite distinct. Fig. 3 illustrates that populations of the three species form clusters easily distinguishable from one another. Within species, however, a great deal of genetic divergence has taken place. The mean Nei identity between conspecific populations, .89 ± .06 (one standard deviation), is quite similar to the value of .86 ±

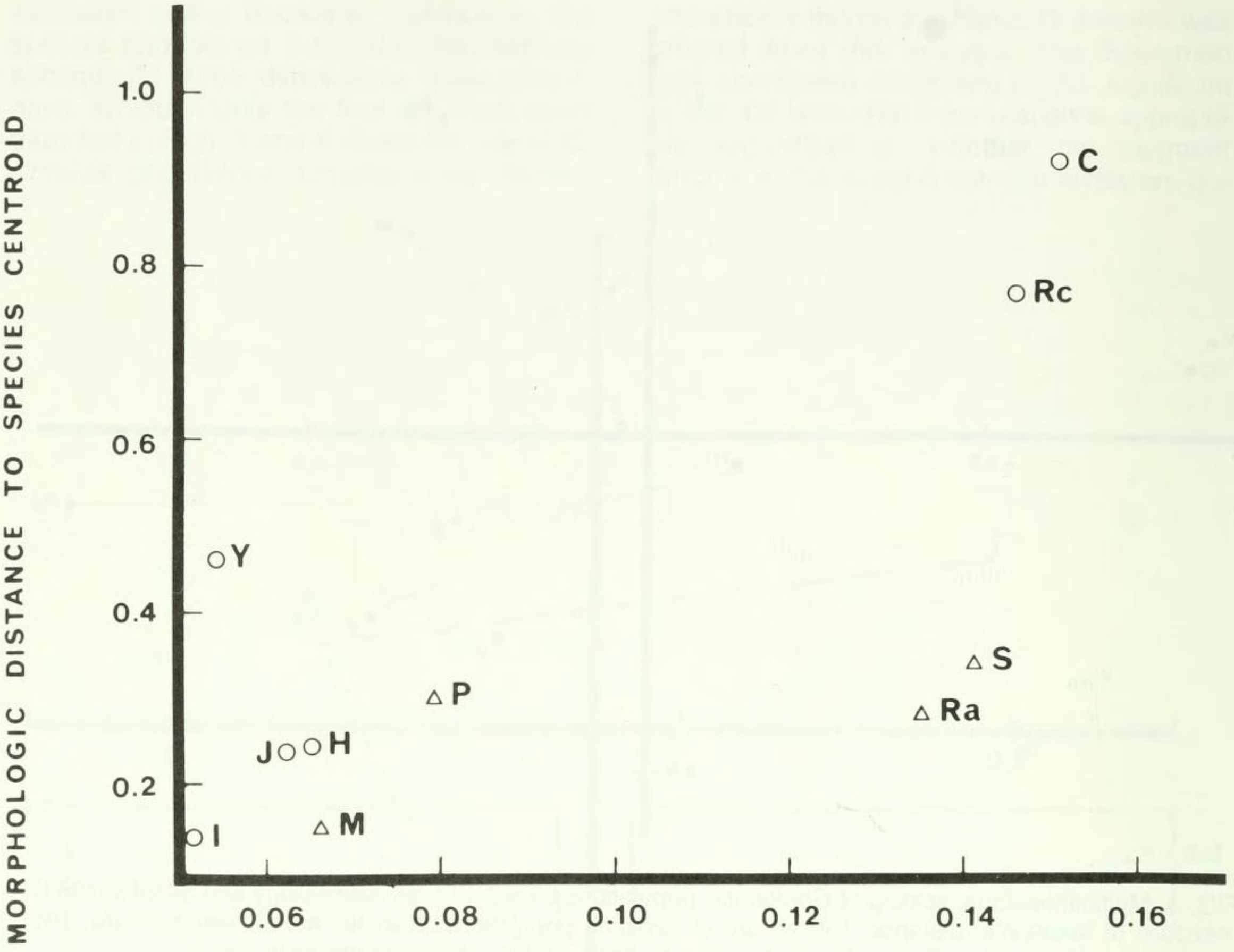

FIG. 5. Comparisons of two measures of population divergence, electrophoretic and morphometric. Symbols as in Fig. 4; abbreviations as in Fig. 3.

.09 obtained by Chambers (1977) for Florida *Goniobasis* and strikingly less than the average identities of conspecific animal populations compiled by Avise (1976), generally .95 to .99. The degree of genetic identity of conspecific *Goniobasis* populations seems more comparable to that of subspecies in other animals. The fixation of alternative alleles is also quite unusual in conspecifics. It seems probable that gene flow between populations of the same species or species-complex can be extremely low, even within a single drainage system.

A widely recognised shortcoming of electrophoresis is the problem of hidden variation. Recently workers have varied gel pH and concentration and employed techniques such as heat denaturation and isoelectric focusing to show that the amount of genetic variation visible using more conventional electrophoretic techniques is a small fraction of the actual amount (Coyne, 1976; Singh et al., 1976; Johnson, 1977). It is probable, therefore, that the values we report here for genetic similarity are systematically overestimated. However, the genetic similarities compiled by Avise (1976) were generally based on electrophoretic techniques comparable to ours, so the comparison is valid.

The mean heterozygosity (H) across the 12 populations was 0.0113, a very low value compared to those of other animal populations (Selander, 1976). Although many factors can influence the amount of genetic variation within populations, one likely explanation for this extremely low heterozygosity is that many of these *Goniobasis* populations have experienced a severe "bottleneck effect" (Nei et al., 1975). If populations are founded by small numbers of individuals and immigration remains low, the genetic variability of the founding population tends to decrease. There is a high probability that alleles will be lost from small populations through chance alone. Vari-

ability increases by new mutations with time and population growth, but a large number of generations may be required to regain the level of heterozygosity of the founding population. So even though the *Goniobasis* populations studied are all currently large, the low genetic variability observed in them may result from a long history of "bottleneck effects." Another possibility is that the results are due to natural selection imposed by particular environmental conditions.

The three species are also fairly distinct on the basis of the seven shell characters considered together (Fig. 4). With the exceptions of SINK (S) and CRIP (C) species occupy separate regions of the figure. Shells from the SINK (S) population of *G. semicarinata* can be distinguished easily from *G. proxima* shells by their light brown exteriors, whitish apertures and the absence of carination on their whorls. *G. proxima* shells are dark brown to black, with a dark, often banded aperture and carinate whorls. Fig. 2 illustrates some of these distinctions. However, the CRIP (C) population of *G. proxima* is quite similar to *G. simplex* both in the shell characters measured (Fig. 4) and in color and lack of ornamentation (Fig. 2). The CRIP population was in fact confused with *G. simplex* in initial field surveys.

A likely explanation for the convergence in shell characters of the CRIP population is that CRIP is the only *G. proxima* population collected from a limestone-draining stream. Fig. 1 shows that throughout the study area, *G. proxima* inhabits only gneiss/schist areas and other species are found only in limestone areas. Table 6 shows that alkalinities were high where *G. simplex* and *G. semicarinata* were collected and low where *G. proxima* was collected. The single exception is CRIP, a *G. proxima* population collected in moderately alkaline water from a creek on the border of the limestone area. With more calcium available, the CRIP population has developed a larger, heavier shell than the typical *G. proxima*, and resembles *G. simplex*.

TABLE 6. Methyl orange alkalinity at 12 *Goniobasis* stations (phenolphthalein alkalinity in parenthesis).

Station	Alkalinity (ppm)
HILL	11
IND	14
JEF	8
YAD	10
ROCK	6
CRIP	36
PINE	84 (10)
MEAD	79
SINK	87
ROA	92
WYTH	80
HOLS	54

The CRIP population has diverged further from conspecific populations in both shell shape and isozyme frequencies than any other population surveyed. Despite the problem that the shells of different species may converge, Fig. 5 shows that divergence in shell morphology is correlated to genetic divergence in conspecific populations. This suggests that shell morphology has enough genetic component to be useful as a measure of evolutionary relationships among conspecifics. The correspondence between morphological and allozyme data has been examined in numerous studies on other groups of animals (Schell et al., 1978). In general, little evidence of relationship has been found. However, no study to date has examined within-species divergence and employed this centroid method.

Notice also in Fig. 5 that the data seem bimodally distributed on the electrophoretic axis, with a group of four "outliers." These four populations, CRIP (C), ROA (Ra), ROCK (Rc), and SINK (S), are fixed or nearly fixed at two of ten polymorphic loci for alleles not present in other conspecific populations. Without a much larger sample of population and a much better understanding of mating systems in these snails it is impossible to know whether the four distinctive populations are different species, subspecies, or geographical variants. Patton & Yang (1977), in discussing similar levels of electrophoretic dissimilarity in a species of pocket gopher, noted that divergence in structural gene loci does not necessarily imply reproductive isolation. But since the electrophoretic differences are substantial, the populations will be provisionally referred to as races. Race A includes HILL, IND, JEF, and YAD in *Goniobasis proxima*. CRIP is race B of *G. proxima*, and ROCK is race C. In *Goniobasis semicarinata*, PINE and MEAD are race A, SINK is race B, and ROA is race C.

In this limited sample of *Goniobasis* populations there seems to be little evidence that geographic distance between populations influences divergence. Two of the populations from outside the upper New River can be considered distinct races, and two are quite

similar to populations found in the New River drainage. Within the New River, the pair of *G. proxima* populations separated by the greatest distance, HILL and JEF, are nearly indistinguishable. The pair of *G. proxima* populations geographically closest, IND and CRIP, represent different races. Perhaps the correlation between geographic distance and genetic divergence would be evident on a smaller geographic scale.

In summary, it has been demonstrated that there is great variability in the amount of divergence between conspecific *Goniobasis* populations judged by either electrophoretic or morphometric criteria. The observed fixation of alternative alleles and the low heterozygosities are both consistent with what one might expect if interpopulation gene flow is very low, even between populations isolated only by short distances through water. However, occasionally populations of *Goniobasis* isolated by great distances remain quite similar, while those in close proximity diverge greatly. In one case an environmental variable, the limestone and dolomite in a drainage, has been implicated in morphometic divergence. Doubtless restricted gene flow and selection both play roles in promoting radiation and eventual speciation in pleurocerids.

A rigorous investigation into the influence of the various agents of pleurocerid evolution will require a large sample of populations, more detailed knowledge of their environments, and a more thorough familiarity with their biology. The anatomy and cytology of *Goniobasis* are virtually unknown. Our implicit assumptions that each population of *Goniobasis* within a single creek is randomly breeding, and that each population experiences uniform environmental pressures should be tested. Work on *Goniobasis* currently in progress addresses many of these problems. From a thorough understanding of divergence in this genus of snails inhabiting a restricted geographic area, insight may be gained regarding the process of evolution in isolated populations generally.

ACKNOWLEDGEMENTS

Special appreciation is due to Shary Dillon, who selflessly volunteered to rise at 5:00 a.m., grind dead snails, and wash the dirty dishes afterward. Appreciation is also expressed to Larry Hornick and Robert Dillon, Sr., for their help with the field work. The University of Pennsylvania Biology Department provided some of the computer time and other research support. Dr. T. Uzzell furnished the program to calculate Nei statistics. All electrophoretic analyses were performed in the laboratory of molecular genetics of the Department of Malacology, ANSP. Figs. 3–5 were prepared by Caryl Hesterman. This study was completed while the senior author held a National Science Foundation predoctoral fellowship. We thank Professor A. J. Cain and Dr. S. M. Chambers for reading and criticizing the paper.

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APPENDIX

The following are locality data for the 12 *Goniobasis* populations studied. "Quad" refers to United States Geological Survey topographic maps, 7.5

minute series. Map coodinates are approximate. The species of *Goniobasis* collected at each site is followed by the catalogue number of voucher specimens deposited in the Academy of Natural Sciences of Philadelphia (ANSP).

CRIP—*G. proxima,* ANSP 349362. Cripple Creek at Va. 749 bridge, 8 km E of Cedar Springs, Wythe County, VA. Cedar Springs Quad. 36°55′N; 81°19′W.

HILL—*G. proxima,* ANSP 349363. Tiny creek at U.S. 58 bridge, near junction with Va. 820. 4 km E of Hillsville, Carroll Co., VA. Hillsville Quad. 36°46′N; 80°44′W.

HOLS—*G. simplex,* ANSP 349364. Dry Run at Va. 617 bridge, 1.6 km NW of Groseclose, Smythe Co., VA. Rural Retreat Quad. 36°56′N; 81°24′W.

IND—*G. proxima,* ANSP 349365. Brush Creek at crossroads of U.S. 21 and Va. 701, 4.8 km S of Independence, Grayson, Co., VA. Sparta West Quad. 36°38′N; 81°11′W.

JEF—*G. proxima,* ANSP 349366. Cranberry Creek by U.S. 221 at Co. 1145 bridge, 11 km N of Deep Gap, Ashe Co., NC. Todd Quad. 36°14′N; 81°32′W.

MEAD—*G. semicarinata,* ANSP 349367. Meadow Creek at Va. 787 bridge, 3.2 km N of Grayson-town, Montgomery Co., VA. Radford South Quad. 37°05′N; 80°33′W.

PINE—*G. semicarinata,* ANSP 349368. Little Pine Run at Va. 100 bridge, near Pine Run Church. Pulaski Co., VA. Fosters Falls Quad. 37°01′N; 80°33′W.

ROA—*G. semicarinata,* ANSP 349369. Mill Creek, .8 km upstream from mouth, .3 km of Bennetts Mill, Montgomery Co., VA. McDonalds Mill Quad. 37°15′N; 80°19′W.

ROCK—*G. proxima,* ANSP 349370. Tiny branch of Rock Castle Creek at Va. 8 bridge, 4 km NW of Woolwine, Patrick Co., VA. Woolwine Quad. 36°39′N; 80°22′W.

SINK—*G. semicarinata,* ANSP 349371. Sinking Creek at Newport Park, Newport, Giles Co., VA. Newport Quad. 37°20′N; 80°29′W.

WYTH—*G. simplex,* ANSP 349372. Mill Creek downstream from confluence with Huddle Branch by Va. 680, 4.7 km N of Rural Retreat, Wythe Co., VA. Rural Retreat Quad. 36°54′N; 81°18′W.

YAD—*G. proxima,* ANSP 349373. Small creek at Lewis Fork Road Bridge (at crossroads of Co. 1155 and 1156), 3.2 km W of Mount Pleasant, Wilkes Co., NC. Purlear Quad. 36°04′N; 81°29′W.