The Opposite of Speciation: Genetic Relationships Among the Populations of *Pleurocera* (Gastropoda: Pleuroceridae) in Central Georgia

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The opposite of speciation: Genetic relationships among the populations of *Pleurocera* (Gastropoda: Pleuroceridae) in central Georgia*

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Abstract: The ranges of *Pleurocera* (formerly *Goniobasis* or *Elimia*) *catenaria* (Say, 1822) and *P. proxima* (Say, 1825) extend from Virginia south through the Carolinas into the piedmont and upper coastal plain of Georgia, where they intersect with populations of *P. floridensis* (Reeve, 1860). In contrast to the situation in surrounding states, however, Georgia populations of *P. catenaria* have been taxonomically subdivided and re-described under at least ten specific nomina, over a complex monographic history extending 150 years. To see if this increased nomenclatural diversity might signal higher levels of population divergence, we compared gene frequencies at 11 polymorphic allozyme-encoding loci among eight populations of *P. catenaria* and three populations of *P. floridensis* sampled from central Georgia region to three populations of *P. proxima*, which have never been taxonomically subdivided. Genetic variation was moderate within our 14 populations and high among them, as has been reported in many prior surveys of pleurocerid allozyme divergence conducted elsewhere. The pairwise genetic distances demonstrated among our populations of *P. catenaria* from Georgia were lower than those observed among control *P. proxima* populations, or among a comparable sample of *P. catenaria* populations from the Carolinas previously published. Central Georgia does not appear to be a region of pleurocerid endemicity, but rather of faunal suturing, the proliferation of specific nomina attributable to qualitatively higher levels of shell morphological variation, possibly ecophenotypic in origin. Junior synonyms of *P. catenaria* include *albanyensis, boykiniana, caelatura, darwini, mutabilis, postelli, suturalis* and *viennaensis*. Junior synonyms of *P. floridensis* include *inclinans, induta, exul*, and *nymphaea*. The Goodrich nomen *timidus* is retained as a subspecies of *P. floridensis* (new combination). We suggest that faunas demonstrating great evolutionary stasis, such as the pleurocerid populations of central Georgia, might profitably serve as the next "laboratories of speciation."

Key words: freshwater snails, divergence, allozyme electrophoresis, *Goniobasis*, *Elimia*

The evolution of North American pleurocerid gastropods has been a subject of research interest for many years (*e.g.*, Adams 1915, Goodrich 1935, Chambers 1978, Dillon 1989, 1991, Holznagel and Lydeard 2000, Dillon and Robinson 2009). Their wide distribution and great abundance in rivers and streams throughout the continent, together with their striking genetic and morphological diversity, have made pleurocerid populations ideal models for the study of gene flow (Dillon 1988a), natural selection (Dillon 1984, 1988b), hybridization (Bianchi *et al.* 1994), divergence (Goodrich 1922, 1936, Lydeard *et al.* 1997, Dillon and Lydeard 1998), speciation (Chambers 1980, 1982, Dillon and Ahlstedt 1997) and phenotypic plasticity (Dillon, in press). But the same attributes that today render pleurocerid populations so attractive as evolutionary models also led nineteenth-century biologists to describe hundreds of pleurocerid species throughout North America (Tryon 1873, Graf 2001), yielding great taxonomic confusion.

Recently we have reviewed diverse lines of genetic, biogeographic, and ecological evidence suggesting that pleurocerid populations inhabiting the piedmont and Blue Ridge provinces of the southern Appalachians may be extremely old—perhaps dating to the Appalachian orogeny 300 mybp (Dillon and Robinson 2009). We have reported double-digit mtDNA sequence variation both within and among three sets of conspecific pleurocerid populations, correlated neither with simple overland distance, nor with continental drainage patterns as the rivers currently flow.

The genus-level taxonomy we employed in our 2009 survey has more recently been revised, the pleurocerid genera *Goniobasis* and *Elimia* being subsumed under *Pleurocera* by Dillon (in press). But the species-level taxonomy of most of the 13 populations we surveyed was uncontroversial. *Pleurocera* (formerly *Goniobasis* or *Elimia*) *proxima* (Say, 1825) is easily recognized throughout its entire five-state range (Dillon 1984), and *Pleurocera* (formerly *Goniobasis* or *Elimia*) *catenaria* (Say, 1822) is well-characterized and taxonomically stable through Virginia and the Carolinas (Dillon and Reed 2002). In Georgia, however, populations morphologically indistinguishable from *P. catenaria* have been referred to at least ten specific nomina in the modern

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literature, including *albanyensis* (Lea, 1864), *darwini* (Mihalcik and Thompson, 2002), *mutabilis* (Lea, 1862), *postelli* (Lea, 1858), *suturalis* (Haldeman, 1840), *timidus* (Goodrich, 1942), and *viennaensis* (Lea, 1862). The two populations of *P. catenaria* we sampled in 2009 from the Georgia piedmont have, in recent publications, been identified as *Goniobasis* (or *Elimia*) *boykiniana* (Lea, 1840), *caelatura* (Conrad, 1849), or *lecontiana* (Lea, 1841). Further taxonomic complication is added by populations of *Pleurocera* (formerly *Goniobasis* or *Elimia*) *floridensis* (Reeve, 1860), a biologically distinct species ranging into Georgia from the south, sometimes nearly indistinguishable from *P. catenaria* (Chambers 1978). The purpose of the present research is to determine whether the great nominal diversity that has been attributed to the *Pleurocera* populations of central Georgia reflects *bona fide* genetic divergence, or whether such secondary factors as ecophenotypic plasticity of shell or taxonomic artifact may be responsible.

Through extensive application over 30 years, the technique of allozyme electrophoresis has proven to be a valuable tool both for quantifying genetic diversity, as well as for resolving the specific status of problematic pleurocerid populations. Levels of divergence are best understood in *Pleurocera proxima*, from which dozens of populations have been studied in four states, with calibration against breeding data (Dillon 1986, 1988b) and mitochondrial sequence divergence (Dillon and Frankis 2004). In the present survey we compare levels of divergence at allozyme-encoding loci among populations of *P. proxima* and *P. catenaria* from the Carolinas, whose conspecific status is uncontroversial, to the levels of divergence in a larger sample of Georgia *Pleurocera* populations variously identified under nine specific nomina, whose true status as biological species is far from clear.

MATERIALS AND METHODS

We sampled 11 populations of *Pleurocera* from ten rivers and streams in central Georgia, selected to represent the taxonomic diversity, geographic range, and morphological variability of the genus in the region. We also sampled three control populations from adjacent states: *P. proxima* and *P. catenaria* from South Carolina, and *P. floridensis* from Florida. The locations of these 14 populations are shown (Fig. 1), and detailed locality data are given in Appendix 1, along with sample sizes and notes regarding the specific identifications that have been accorded these populations by previous investigators. Sample sizes were in almost all cases greater than 30, but smaller for the populations previously sampled by Dillon and Reed (2002): **Prx1** ($N = 5$), **Catn** ($N = 5$), and **Mut** ($N = 22$). These three smaller data sets were combined with our 2002 data ($N = 29$, 38, and 40, respectively) before analysis. Voucher specimens have been deposited in the Academy of Natural Sciences of Philadelphia.

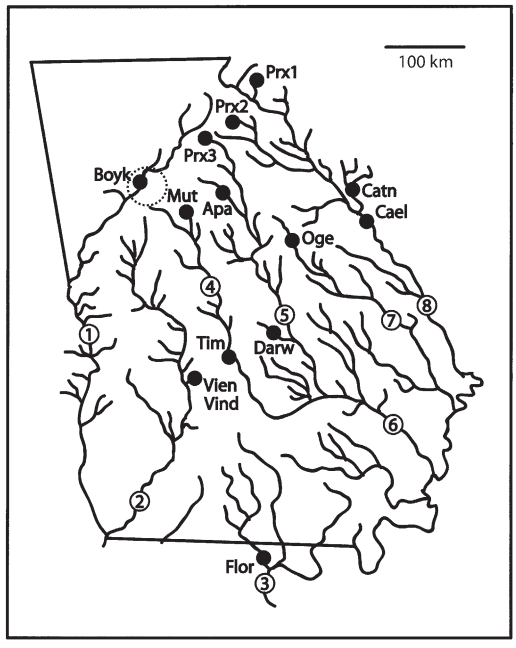


Figure 1. Map showing sample sites in Georgia and adjacent states (see Appendix 1 for a key to site names and detailed locality data). Major rivers are (1) Chattahoochee, (2) Flint, (3) Withlacoochee/Suwanee, (4) Ocmulgee, (5) Oconee, (6) Altamaha, (7) Ogeechee, and (8) Savannah. The Atlanta metropolitan area is indicated with a dashed circle.

Animals were returned alive to the laboratory, where they were cracked and frozen in tris tissue buffer until electrophoretic analysis. Our techniques and apparatus for horizontal starch gel electrophoretic resolution of allozyme variation in homogenates of molluscan tissues were detailed by Dillon (1992), along with recipes for all buffers and stains employed here. The Tris Cit 6 buffer (buffer XIII of Shaw and Prasad 1970) was used to resolve 6-phosphogluconate dehydrogenase (6PGD), glucose-phosphate isomerase (GPI), and isocitrate dehydrogenase (two loci, the cathodal IDHF and the anodal IDHS). A Poulik (1957) discontinuous buffer system was employed for phosphoglucomutase (PGM—the strong, fast locus only), sorbitol dehydrogenase (SDH), and octopine dehydrogenase (OPDH). The TEB8 buffer system (buffer III of Shaw and Prasad 1970) was used to analyze xanthine dehydrogenase (XDH), mannose phosphate isomerase (MPI), and PGM, the single locus resolved being identical with the strong fast locus resolved using the Poulik buffer. A TEB9.1 buffer (Dillon and Davis 1980) was used for octanol dehydrogenase (OLDH), esterases (EST1—the strong, slow locus only), and XDH (a second time).

Mendelian inheritance of allozyme phenotype has been confirmed for GPI, OPDH, and EST1 by Dillon (1986) and for 6PGD by Chambers (1980). Putative allelic designations

for each zone of allozyme activity were assigned using the system of Dillon and Reed (2002), setting the shared populations as standards. For example, since Dillon and Reed designated the OPDH allele for which population **Prx1** was fixed as "106," the new OPDH allele discovered in population **Tim**, with a gene product migrating 4 mm faster than that of OPDH106 in our standard conditions, was named "OPDH110."

Gene frequencies and mean direct-count heterozygosities (the unbiased estimate of Nei 1978) were calculated using Biosys version 1.7 (Swofford and Selander 1981). Because large numbers of alleles were resolved at some loci, our sample sizes dictated that genotypes be pooled into three classes before testing for Hardy-Weinberg equilibrium: homozygotes for the most common allele, common/rare heterozygotes, and rare homozygotes together with other heterozygotes. Yates-corrected chi-square statistics were then employed for this purpose. We calculated matrices of Nei's (1978) unbiased genetic identities and distances, as well as Cavalli-Sforza and Edwards (1967) chord distance. As distances of the latter type are Pythagorean in Euclidean space, they were used as the basis for the construction of a neighbor-joining tree using Phylip v3.65 program NEIGHBOR (Felsenstein 2004).

An $N \times N$ symmetric matrix contains only N - 1 statistically independent entries, carefully chosen. So as a measure of genetic divergence within the Georgia populations of *Pleurocera catenaria*, we used their symmetric matrix of Nei's unbiased genetic distances to construct a minimum spanning network with the method of Prim (1957). We also extracted N - 1 independent segments from the symmetric matrices of genetic distances among the 7 populations of *P. catenaria* sampled from the Carolinas by Dillon and Reed (2002), and among the 6 populations of *P. proxima* sampled in the two studies combined (three unique from 2002, two unique from the present study, one shared). Then we used Mann-Whitney U statistics to test the one-tailed hypothesis that the genetic divergence among Georgia populations of *P. catenaria* might be greater than either the Carolina populations of *P. catenaria*, or the *P. proxima* populations sampled from across the three states combined.

RESULTS

Typical shells sampled from our control populations of *Pleurocera proxima*, *P. catenaria*, and *P. floridensis* have been figured previously (Chambers 1980, Dillon and Reed 2002). Our fresh samples from central Georgia displayed striking shell morphological variation, especially with regard to strength of shell sculpture (Fig. 2). Costation and carination demonstrated great variability both within and among populations, and did not suggest to us any taxonomic significance. We were impressed, however, by the systematic slenderness of the shells borne by snails of populations **Vind** and **Tim**. Although the shells we sampled from most central Georgia populations of *Pleurocera* were characterized by relatively long body whorls, as demonstrated by the two specimens at the left of Fig. 2, populations **Vind** and **Tim** displayed narrower shells with body whorls shorter in proportion to total shell length.

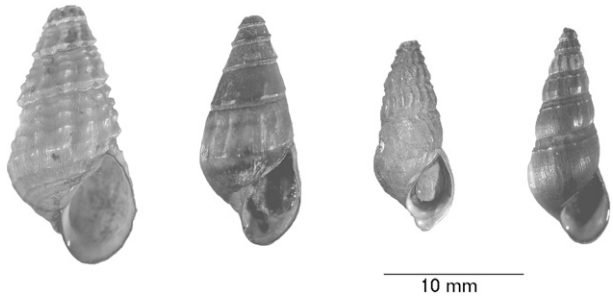


Figure 2. Example shells from populations **Vien**, **Darw**, **Vind**, and **Tim** (left to right). For others see Dillon and Reed (2002) and Chambers (1980, 1990).

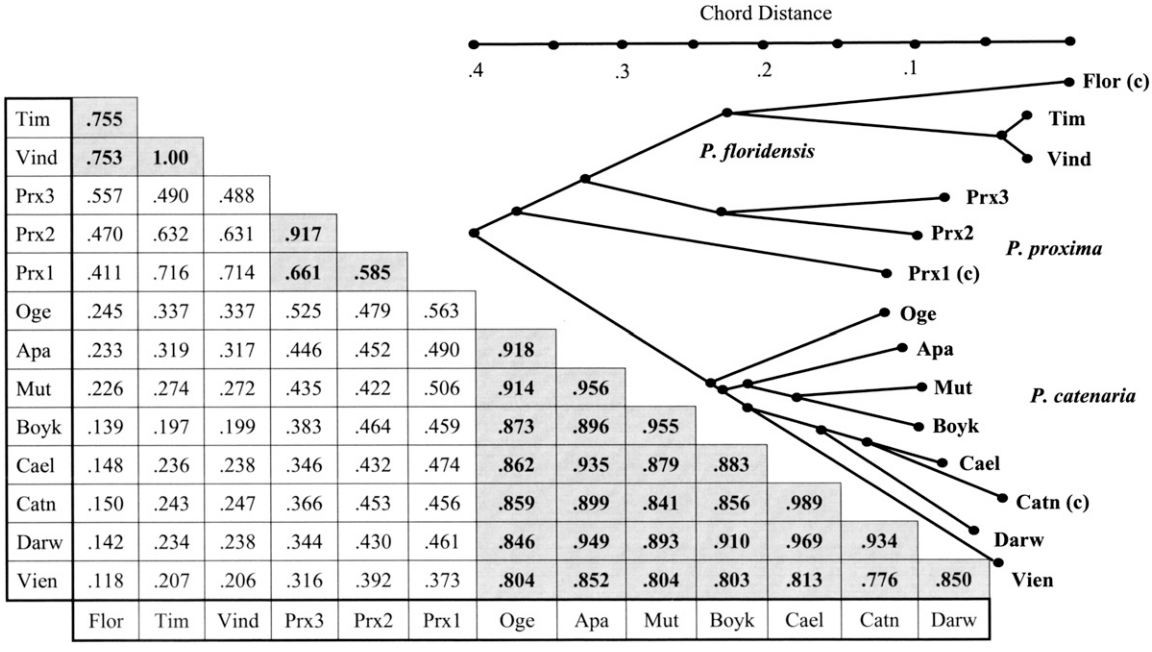
Putative gene frequencies at the 11 allozyme-encoding loci we examined are given in Table 1, together with mean direct-count heterozygosities. Intrapopulation genetic variation was moderate in our sample of 14 *Pleurocera* populations from three states, and interpopulation divergence high, as has often been reported in surveys of pleurocerid population genetics (Dillon and Davis 1980, Dillon 1984). Of the $11 \times 14 = 154$ loci examined, 37 were polymorphic by the 95% criterion. The lowest value of P returned by goodness-of-fit tests to Hardy-Weinberg expectation among any of these was $P = 0.015$ (PGM in population **Cael,** $\chi^2 = 5.89$), which is not significant with Bonferroni correction ($0.05/37 = 0.0014$). Thus our data contained no evidence that any assumption of Hardy-Weinberg equilibrium has been violated within populations.

The matrix of pairwise Nei (1978) unbiased genetic identities among populations is shown (Fig. 3), together with the results of our neighbor-joining analysis based on Cavalli-Sforza and Edwards chord distances. Seven Georgia populations clustered in a group together with our control population of *Pleurocera catenaria* from South Carolina (**Catn**), and two Georgia populations clustered with our control *P. floridensis* (**Flor**). Our three populations of *P. proxima* were depicted as central in the network, more loosely clustered than either *P. catenaria* or *P. floridensis*.

Values of Nei's (1978) unbiased genetic distances are compared from the survey of Dillon and Reed (2002) in North Carolina and South Carolina to values obtained in the present study (Fig. 4). For this analysis population **Catn** was grouped with the 2002 Carolina data, and population **Mut**

Table 1. Gene frequencies and average (direct count) heterozygosity over eleven polymorphic enzyme loci in 14 populations of *Pleurocera* from Georgia and surrounding states.

	P. floridensis			*P. proxima*			*P. catenaria*							
allele	Flor	Tim	Vind	Prx1	Prx2	Prx3	Apa	Boyk	Cael	Catn	Darw	Mut	Oge	Vien
GPI														
100	.000	.000	.000	.000	.000	.000	1.000	1.000	.500	.202	1.000	1.000	.567	1.000
102	1.000	1.000	1.000	1.000	1.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000
105	.000	.000	.000	.000	.000	.000	.000	.000	.500	.750	.000	.000	.433	.000
110	.000	.000	.000	.000	.000	.000	.000	.000	.000	.048	.000	.000	.000	.000
MPI														
95	.097	1.000	1.000	.026	1.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000
98	.903	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
100	.000	.000	.000	.974	.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
6PGD														
100	.000	1.000	1.000	.897	1.000	.967	.973	.597	.935	1.000	1.000	.515	1.000	1.000
103	.258	.000	.000	.000	.000	.033	.000	.000	.000	.000	.000	.000	.000	.000
105	.000	.000	.000	.103	.000	.000	.027	.403	.065	.000	.000	.394	.000	.000
107	.742	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
110	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.091	.000	.000
EST1														
100	1.000	1.000	1.000	.218	1.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000
103	.000	.000	.000	.769	.000	.000	.886	.532	1.000	.814	1.000	.581	1.000	.897
106	.000	.000	.000	.013	.000	.000	.000	.000	.000	.186	.000	.008	.000	.000
107	.000	.000	.000	.000	.000	.000	.114	.468	.000	.000	.000	.411	.000	.103
OPDH														
105	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
106	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
110	.000	1.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
111	.000	.000	.000	.000	1.000	1.000	.203	.677	.000	.105	.000	.457	.821	.000
114	.000	.000	.000	.000	.000	.000	.797	.290	.983	.895	.839	.543	.054	.015
118	1.000	.000	.000	.000	.000	.000	.000	.032	.017	.000	.161	.000	.125	.985
IDHF														
97	.000	.000	.000	.000	.000	.000	.000	.032	.000	.000	.177	.000	.000	.000
100	1.000	1.000	.989	1.000	1.000	.800	.000	.000	.000	.000	.000	.000	.000	.000
105	.000	.000	.011	.000	.000	.000	1.000	.968	.952	1.000	.823	1.000	1.000	1.000
108	.000	.000	.000	.000	.000	.000	.000	.000	.048	.000	.000	.000	.000	.000
110	.000	.000	.000	.000	.000	.200	.000	.000	.000	.000	.000	.000	.000	.000
PGM														
104	.000	.000	.076	.000	.097	.000	.014	.371	.371	.500	.550	.000	.069	.029
102	.984	.968	.924	.000	.903	1.000	.986	.274	.500	.500	.450	.625	.741	.971
100	.016	.032	.000	.900	.000	.000	.000	.355	.129	.000	.000	.275	.190	.000
98	.000	.000	.000	.100	.000	.000	.000	.000	.000	.000	.000	.100	.000	.000
IDHS														
100	.000	.000	.000	1.000	1.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000
102	1.000	1.000	1.000	.000	.000	.000	.000	.000	.016	.000	.000	.000	.000	.000
104	.000	.000	.000	.000	.000	.000	1.000	1.000	.984	1.000	1.000	1.000	1.000	1.000
OLDH														
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.968	1.000	1.000	.977	1.000	.265
104	.000	.000	.000	.000	.000	.000	.000	.000	.032	.000	.000	.023	.000	.735
SDH														
100	.000	.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
104	1.000	1.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
XDH														
97	1.000	1.000	1.000	1.000	.000	.917	.392	.048	.000	.000	.000	.508	.683	.000
98	.000	.000	.000	.000	1.000	.083	.608	.952	1.000	1.000	1.000	.492	.317	1.000
Het	0.050	0.006	0.016	0.069	0.018	0.052	0.102	0.211	0.150	0.099	0.113	0.198	0.146	0.053



Tim	**.755**												
Vind	**.753**	**1.00**											
Prx3	.557	.490	.488										
Prx2	.470	.632	.631	**.917**									
Prx1	.411	.716	.714	**.661**	**.585**								
Oge	.245	.337	.337	.525	.479	.563							
Apa	.233	.319	.317	.446	.452	.490	**.918**						
Mut	.226	.274	.272	.435	.422	.506	**.914**	**.956**					
Boyk	.139	.197	.199	.383	.464	.459	**.873**	**.896**	**.955**				
Cael	.148	.236	.238	.346	.432	.474	**.862**	**.935**	**.879**	**.883**			
Catn	.150	.243	.247	.366	.453	.456	**.859**	**.899**	**.841**	**.856**	**.989**		
Darw	.142	.234	.238	.344	.430	.461	**.846**	**.949**	**.893**	**.910**	**.969**	**.934**	
Vien	.118	.207	.206	.316	.392	.373	**.804**	**.852**	**.804**	**.803**	**.813**	**.776**	**.850**
	Flor	Tim	Vind	Prx3	Prx2	Prx1	Oge	Apa	Mut	Boyk	Cael	Catn	Darw

Figure 3. Nei's (1978) unbiased genetic identities among 14 populations of *Pleurocera* are shown below the diagonal, with conspecific comparisons shaded. Above the diagonal is the neighbor-joining network based on Cavalli-Sforza and Edwards (1967) chord distances. Control populations are designated (c): *P. floridensis* (**Flor**), *P. proxima* (**Prx1**), and *P. catenaria* (**Catn**).

with the Georgia. Then the central tendency of the 21 pairwise comparisons among the seven *Pleurocera catenaria* populations sampled from Georgia (median = 0.125, range 0.032 - 0.219) was slightly lower than the sample of 21 pairwise comparisons among the seven *P. catenaria* sampled from the Carolinas (median = 0.131, range 0.000 - 0.330), and much below the 15 comparisons among populations of *P. proxima* (median = 0.273, range 0.091 - 0.619). Our Mann-Whitney *U*-statistic testing for a difference between the $N - 1 = 6$ segments of the Prim network extracted from the Georgia matrix and the $N - 1 = 6$ segments from the Carolinas was $U = 20$, not significant. Our Mann-Whitney test for a difference in central tendency between the 6 Georgia *P. catenaria* comparisons and the $N - 1 = 5$ comparisons of *P. proxima* populations yielded $U = 3$, a very significant value in the direction opposite of our prediction. Thus there is no evidence that Georgia populations of *P. catenaria* are more genetically variable than pleurocerid populations sampled from similar areas.

DISCUSSION

There is some evidence of a geographic component to the divergence shown among the eight populations identified as *Pleurocera catenaria* in Fig. 3, as has been documented previously for *P. proxima* by Dillon (1984). The population most geographically removed (**Vien**) was also the most genetically divergent of the *catenaria* samples, and the pair of populations collected nearest each other (**Catn** and **Cael**) showed the highest genetic identity (I = 0.99). The group of **Boyk**, **Mut**, and **Apa** was also both geographically close and genetically similar. It is interesting to note that the *catenaria* population designated "Yel" by Dillon and Reed was both the most genetically distinctive and the most geographically removed of the eight populations surveyed in 2002. In the present survey, where that same *catenaria* population was renamed **Mut** and its geographic position rendered internal rather than peripheral, its genetic distinctiveness disappeared.

Our results do not, however, support the hypothesis of endemicity by drainage advanced by previous authors (Mihalcik and Thompson 2002). The divergence among the seven Georgia populations we identify here as *Pleurocera catenaria* is not greater than a sample of seven *P. catenaria* from the Carolinas, despite the fact that both sets of populations were drawn from areas of comparable geographic extent, and significantly less than a sample of six populations of *P. proxima* (Fig. 4).

The first modern review of the Georgia Pleuroceridae was offered by Goodrich (1942), who preferred the generic nomen "*Goniobasis*." He recognized two species in Georgia Atlantic drainages, *Goniobasis catenaria postelli* (Lea, 1858) and *G. mutabilis timidus* (newly described by him),

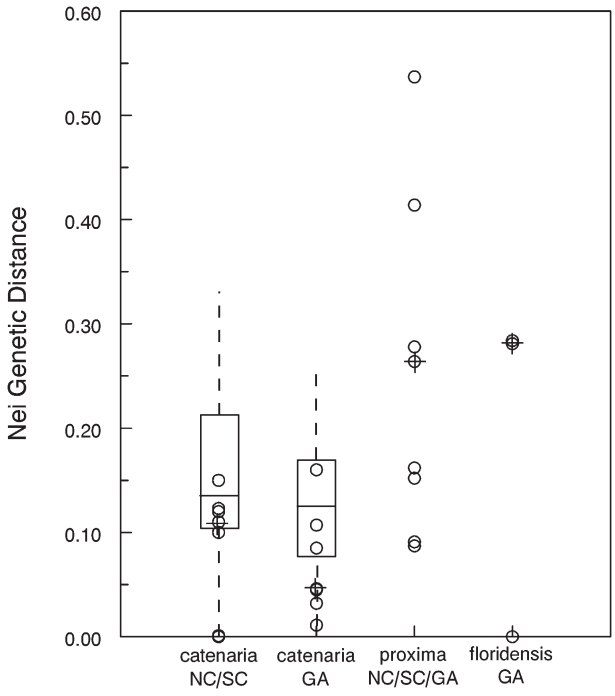


Figure 4. Nei's (1978) genetic distances among all Georgia pairs of *Pleurocera catenaria* from the present study (21 comparisons), *P. catenaria* from the Dillon and Reed (2002) survey of NC/SC (21 comparisons), and *P. proxima* from the 2002 study and the present study combined (15 comparisons). Boxes show medians and quartiles, dashed lines show ranges, and the individual data plotted are the *N* - 1 segments of the minimum-spanning network.

incorrectly assuming that the range of *G. proxima* extended no further south than the Carolinas. From the Gulf drainages of western Georgia Goodrich recognized seven taxa: *G. catenaria inclinans* (Lea, 1862), *G. catenaria cancellata* (Say, 1829, of which he considered *floridensis* a synonym), *G. boykiniana viennaensis* (Lea, 1862), *G. boykiniana albanyensis* (Lea, 1864), *G. mutabilis* (s.s., Lea, 1862), *G. induta* (Lea, 1862), and *G. curvicostata* (Reeve, 1861). *Goniobasis curvicostata* is a well-characterized species, primarily Floridian in its distribution, not treated in the present work.

Clench and Turner (1956) devoted substantial attention to the Georgia Pleuroceridae in their survey of the freshwater molluscan fauna of American Gulf drainages, recognizing six species: *boykinana* (s.s., Lea, 1840) and *catenoides* (Lea, 1842, extinct), as well as *albanyensis*, *floridensis*, *viennaensis* and *curvicostata*. The dissertation of Krieger (1977) focused on the Atlantic populations of Georgia, nominally *G. postelli*, *G. boykiniana viennaensis*, and *G. suturalis* (Haldeman, 1840). Chambers (1990) simplified the taxonomy of the pleurocerids inhabiting Georgia's Gulf drainages substantially, preferring *Elimia* over *Goniobasis*. He synonymized *inclinans* under *Elimia floridensis*, *albanyensis* under *E. boykiniana*, and *viennaensis* and *induta* under *E. curvicostata*. Chambers also noted the similarity of *E. boykiniana* from Georgia Gulf drainages to *E. catenaria* populations inhabiting Atlantic drainages of the Carolinas, admitting the possibility that the former might ultimately prove a junior synonym of the latter.

Most recently, Mihalcik and Thompson (2002) have recognized 12 nominal pleurocerid species from central Georgia, with no overlap between the Atlantic and Gulf faunas. From Atlantic drainages they listed *Elimia mutabilis* and *E. timida timida*, as well as *E. caelatura* (Conrad, 1849) and three newly-described taxa: *E. timida exul*, *E. timida nymphaea*, and *E. darwini*. From Gulf drainages they recognized *E. boykiniana, E. floridana, E. induta, E. albanyensis, E. viennaensis,* and *E. curvicostata*.

No worker since Goodrich (1942) has proposed that any similarity might exist between the virtually indistinguishable pleurocerid populations of the Gulf and Atlantic drainages of central Georgia, or even between Georgia and South Carolina immediately to the east. But against the apparently ancient evolutionary history of the fauna, the presumption of narrow endemism is difficult to rationalize. Approximately 20 km of low hills separate the main Chattahoochee River (draining toward the Gulf) and tributaries of the upper Ocmulgee River (draining toward the Atlantic) in the vicinity of Atlanta (Fig. 1). Further downstream, the main Ocmulgee River and the main Flint River (draining toward the Gulf) are separated by as little as 40 km of flat topography. A more plausible model would invoke bidirectional biotic interchange or suturing of widespread species in Georgia, *P. catenaria* from the north and *P. floridensis* from the south.

Our data suggest that the following nomina variously applied to central Georgia populations of *Pleurocera* should be considered junior synonyms of *P. catenaria* (Say, 1822): *albanyensis* (Lea, 1864), *boykiniana* (Lea, 1840), *caelatura* (Conrad, 1849), *darwini* (Mihalcik and Thompson, 2002), *mutabilis* (Lea, 1862), *postelli* (Lea, 1858), *suturalis* (Hald, 1840), and *viennaensis* (Lea, 1862). In addition, the following should be considered synonyms of *P. floridensis* (Reeve, 1860): *inclinans* (Lea, 1862), *induta* (Lea, 1862), *exul* (Mihalcik and Thompson, 2002) and *nymphaea* (Mihalcik and Thompson, 2002). Much of this synonymy has previously been suggested by Goodrich (1942) or by Chambers (1990). It is interesting that *P. boykiniana* was considered a "probable synonym" of *P. catenaria* by Pilsbry as early as 1891.

Although populations **Flor**, **Vind**, and **Tim** are genetically similar, the shell morphology of the latter two populations does not match that of the former. The shells borne by snails of population **Flor** are marked with strong sculpture, showing both radial costae and spiral cords as is typical for *P. floridensis* (figured by Chambers 1980, 1990). The shells of **Vind** and **Tim** are weakly costate, largely lacking spiral cords.

This is quite reminiscent of the situation in *P. catenaria*, where typical shells sampled from the central part of the range in Georgia and the Carolinas are strongly sculptured with costae and cords (*e.g.*, population **Vien**, Fig. 2), while populations from the eastern edge of the range bear shells that are nearly smooth (figured by Dillon and Reed 2002).

The extent to which such variation in shell morphology may reflect genetic relationships is not clear. Dillon and Reed (2002) reported that the loss of sculpture in two populations of *Pleurocera catenaria* sampled from the eastern edge of the range in South Carolina appeared to be independent, and possibly due to ecophenotypic plasticity (Urabe 2000). In any case, Goodrich (1942) referred such weakly-sculptured populations to the subspecies *Goniobasis catenaria dislocata* (Ravenel, 1834), applying the nomen *Goniobasis catenaria catenaria* to populations bearing the typical shells with strong costae and spiral cords. By analogy, we suggest that weakly-sculptured populations of *P. floridensis* such as **Vind** and **Tim** should be accorded the subspecific designation *Pleurocera floridensis timidus* (new combination), reserving *P. floridensis floridensis* for populations with strong costae and spiral cords as demonstrated in population **Flor**.

The nomen *timidus*, which Goodrich (1942) originally proposed as a subspecies of *Goniobasis mutabilis*, appears to be the earliest name applied by any author to populations we here recognize as a weakly-sculptured form of *Pleurocera floridensis*. Neither Goodrich nor Mihalcik and Thompson (2002) reported *timidus* populations elsewhere beyond tributaries of the Ocmulgee River in the vicinity of Hawkinsville. Thus our inclusion of *Pleurocera* populations from Flint River tributaries (previously referred to the nomen "*induta*") in a broadened concept of *P. floridensis timidus* represents a substantial broadening of the range of this taxon.

Mihalcik and Thompson sequenced a 385 bp fragment of the mitochondrial CO1 gene amplified from 36 individuals representing 17 nominal species and subspecies of pleurocerids, obtaining 26 unique sequences. Although such data must be interpreted with care (Dillon and Frankis 2004, Dillon and Robinson 2009), the general outlines of the maximum-parsimony tree derived by Mihalcik and Thompson agree with the results of the allozyme analysis we report here. Three broad clusters emerged from their analysis, corresponding to our *P. catenaria*, our *P. floridensis*, and *P. curvicostata* (excluded from the present study). The *catenaria* cluster of Mihalcik and Thompson included *darwini* and *viennaensis*, and the *floridensis* cluster included *timida* and *induta*, as might have been predicted from the similarities of these same populations at allozyme-encoding loci.

North of Atlanta, no more than 15 km of low hills separate the upper Chattahoochee River from the Etowah River, which drains west through the Coosa River of Alabama into the Mobile Basin. The Mobile Basin is home to the greatest diversity of Pleuroceridae in North America, approx. 77 nominal species in four genera (Dillon and Lydeard 1998). Although this fauna has conventionally been considered almost entirely endemic (Lydeard and Mayden 1995), the sequence data of Dillon and Robinson (2009) confirmed that the range of *Pleurocera catenaria* extends from the Chattahoochee into the Etowah River, just as it extends from the upper Ocmulgee to the Chattahoochee (Fig. 1). The development of a better model for the origin of the Mobile Basin Pleuroceridae, recognizing its affinities both with the Atlantic fauna here untangled and with the interior fauna of the Tennessee River to its west (Dillon, in press), will be fertile ground for future inquiry.

Coyne and Orr (2004: 123) considered the phenomenon of allopatric speciation "so plausible that it hardly seems worth documenting. Given enough time, and barring extinction, any pair of geographically isolated populations is likely to evolve reproductive barriers." Against this background, the great wonder is not that there are so many species of freshwater molluscs on earth, but that there are so few. We attribute the "opposite of speciation" in the *Pleurocera* populations of central Georgia, despite apparently great antiquity and extreme geographic isolation, to stabilizing selection.

The springs and streams of the Georgia piedmont, as elsewhere throughout the southern Appalachians, are primarily fed by groundwater of relatively constant temperature and chemical composition. The pleurocerids inhabiting these environments are slow-growing, long-lived, perennial generalists, able to graze organic particles over a great range of size and quality, from living single-celled algae to entire leaves dehiscent from vascular plants (Dillon 2000). They are thus insulated to an unusual degree from climatic fluctuations and short-term global catastrophes. And changes in the longer term, such as continental drift, montane erosion, evolution of seed plants, and diversification of benthic insect competitors have been felt uniformly across the pleurocerid populations of the southern Appalachians, imposing stabilizing selection on their morphology, reproductive biology, life history, and other fitness traits, even as molecular clocks have continued to tick.

Throughout most of the history of evolutionary science, researchers have been drawn to centers of great biotic diversity as "laboratories of speciation." We suggest that the future laboratories of speciation should be centers of great evolutionary stasis, such as we describe here.

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Appendix 1. Locality data and taxonomic notes for the 14 populations of *Pleurocera* in this study. Sample sizes given in parentheses. Catalog numbers listed are for dry-lot voucher specimens deposited in the Academy of Natural Sciences of Philadelphia (ANSP).

Apa – (*N* = 37). Apalachee River at Ga183, North High Shoals, Oconee County, Georgia. This was site APAL of Krieger (1977), identified by him as *Goniobasis boykiniana viennaensis*. 33.8176°N; 83.5036°W. ANSP 422170

Boyk – (*N* = 31). Chattahoochee River, about 1 km N of I-285 bridge, Atlanta, Fulton/Cobb County line, Georgia. This site is called "Cochran Shoals," and is presently in the Chattahoochee River National Recreation Area. Chambers (1990) identified a museum lot from this area as *Elimia boykiniana*. Our collections were made at (approximately) the site designated CHAT by Krieger (1977), identified by him as *Goniobasis postelli*. 33.9048°N; 84.4446°W. ANSP 422171

Cael – (*N* = 31). Savannah River at "Savannah Rapids Pavilion" lock and dam, 4 km N of Augusta, Richmond County, Georgia. Populations of *Pleurocera* from this region have been identified as *Elimia caelatura* by Thompson (2000). 33.5500°N; 82.0391°W. ANSP 422172

Catn – *Pleurocera catenaria catenaria* (*N* = 5). Stevens Creek at SC 23 bridge, 24.6 km WSW of Edgefield, Edgefield County, South Carolina. Same population as 4c of Dillon and Keferl (2000) and McC of Dillon and Reed (2002). 33.7292°N; 82.1826°W. ANSP 422173

Darw – (*N* = 31). Rocky Creek at Lord Road, 7 km SW of Dudley, Laurens County, Georgia. This is the type locality of *Elimia darwini* (Mihalcik and Thompson 2002). 32.4889°N; 83.1206°W. ANSP 422174

Flor – *Pleurocera floridensis* (*N* = 31). Blue Spring by the Withlacoochee River at FL6, 15 km E of Madison, Madison County, Florida. Site 8 of Chambers (1980). This population was also sampled by F. G. Thompson for the karyotype studies of Dillon (1989, 1991). 30.4806°N; 83.2448°W. ANSP 422175

Mut – (*N* = 22). Yellow River below dam at Porterdale, Newton County, Georgia. This is population "Yel" of Dillon and Reed (2002), which we identified as *Goniobasis catenaria postelli*. Krieger (1977) designated this population "YELD" and identified it as *Goniobasis boykiniana viennaensis*. Mihalcik and Thompson (2002: 44) identified this population as *Elimia mutabilis*. 33.5683°N; 83.8910°W. ANSP 422176

Oge – (*N* = 30). Ogeechee River at Ga16 bridge, Jewells Mill, Warren County, Georgia. 33.2956°N; 82.7811°W. ANSP 422177

Prx1 – *Pleurocera proxima* (*N* = 5). West Village Creek at SC 196 bridge, 1 km W of Mountain Rest, Oconee County, South Carolina. This was population "West" of Dillon and Reed (2002) and population P1 of Dillon and Robinson (2009). 34.8604°N; 83.1676°W. ANSP 422178

Prx2 – *Pleurocera proxima* (*N* = 31). Small tributary of Nancytown Creek at Forest Service 591 bridge, 2 km SE of Mount Airy, Habersham County, Georgia. 34.5040°N; 83.4809°W. ANSP 422179

Prx3 – *Pleurocera proxima* (*N* = 30). North Oconee River at White Hall Road, 6 km SW of Lula, Hall County, Georgia. 34.3650°N; 83.7317°W. ANSP 422180

Tim – (*N* = 31). Mile Creek at municipal park by US 129, on the south edge of Hawkinsville, Pulaski County, Georgia. This is the type locality of *Elimia timida nymphaea* (Mihalcik and Thompson 2002). 32.2705°N; 83.4658°W. ANSP 422181

Vien – (*N* = 34). Limestone Creek at McCay Rd., 12 km SW of Vienna, Dooly County, Georgia. This site is inhabited by three biological species of *Pleurocera*. Mihalcik and Thompson (2002) identified the population with a broader shell (matching their figures 125–128 and 133–144) as *Elimia viennensis*. 32.0312°N; 83.9076°W. ANSP 422182

Vind – (*N* = 46). Locality data given above. Mihalcik and Thompson (2002) identified the population with a more slender shell and body whorl proximally smoothed (matching their figs. 29–31 and 34–43) as *Elimia induta*. ANSP 422183