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ROBUST SHELL PHENOTYPE IS A LOCAL RESPONSE TO STREAM SIZE IN THE GENUS *PLEUROCERA* (RAFINESQUE, 1818)

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ABSTRACT

Although local correlations between shell phenotype and stream size have often been documented in freshwater mollusks, the species- and even genus-level taxonomy of pleurocerid snails has historically been based almost entirely on aspects of the shell. Here I test the hypothesis that lightly shelled pleurocerid populations inhabiting smaller rivers in east Tennessee and north Georgia, variously assigned to the genus *Goniobasis* or *Elimia*, may be local variants of heavily shelled *Pleurocera* populations downstream. Populations of the nominal species *Goniobasis* ("*Elimia*") *acutocarinata*, *G. clavaeformis*, and *Pleurocera unciale* were sampled from the Powell, Little, and Hiwassee subdrainages of the Tennessee River, and populations nominally *Goniobasis carinifera* and *Pleurocera vestita* sampled from the Coahulla subdrainage of the Mobile Basin. A population of *Goniobasis simplex* was sampled from each of the four subdrainages to calibrate expected levels of genetic divergence. Gene frequencies at ten polymorphic allozyme-encoding loci (15 populations, 30 individuals per population) revealed that each population of *Pleurocera* was more closely related to its local populations of *Goniobasis* (or "*Elimia*") than to any other population of *Pleurocera*. All nine populations identified as *G. acutocarinata*, *G. clavaeformis*, and *P. unciale* appear to be conspecific, their minimum genetic identity of 0.771 much greater than the 0.356 minimum identity among the four *G. simplex* controls. The specific relationship between the nine Tennessee populations and populations of *G. carinifera* and *P. vestita* from the Mobile Basin is ambiguous, with identities ranging down to 0.284. This larger set of 11 populations is here referred to as the *carinifera* group. Evidence that intraspecific variation in shell morphology has risen to the level of the genus suggests that *Goniobasis*, *Elimia*, and several other generic nomina be subsumed under *Pleurocera* (Rafinesque, 1818).

Key words: Shell morphology, ecophenotypic plasticity, genetic divergence, allozyme electrophoresis, freshwater gastropods, Pleuroceridae, *Goniobasis*, *Elimia*.

INTRODUCTION

Correlations between stream size and the shell phenotype of the freshwater mollusk populations that inhabit them have been the object of research interest for almost 100 years (Adams, 1915; Ortmann, 1920). The phenomenon was well documented by Calvin Goodrich, who published a series of eight papers on phenotypic variation in pleurocerid snails between 1934 and 1941. Relationships between stream size, current, or substrata and shell phenotype have more recently been demonstrated in the pulmonate gastropod *Lymnaea* (Lam & Calow, 1988), unionid mussels (Watters, 1994), the pleurocerids *Semisulcospira* (Urabe, 1998, 2000) and *Lithasia* (Minton et al., 2008), and the pulmonate limpet *Ferrissia* (Dillon & Herman, 2009).

Goodrich (1934, 1935, 1937) observed that populations of pleurocerid snails often vary in shell shape and sculpture from slender and more angulate in smaller tributaries to more robust or "obese" in larger rivers. He attributed the greater frequency of broader, more robust shells in downstream populations to physical disturbance or water current. Goodrich was influenced by the work of Wiebe (1926) who suggested that the "relatively great obesity" of the pleurocerid *Goniobasis* (or "*Elimia*") *livescens* on exposed shores of Lake Erie originated from the adaptive value of a large foot in areas of heavy wave action. The broader shell might be a secondary consequence of a larger foot. Goodrich suggested that this phenomenon might generalize to rivers and streams, observing that "an ecological analogy would appear to

exist between the exposed situations of Lake Erie, inhabited by obese *G. livescens*, and the rapid and sometimes tumultuous southern creeks and river headwaters where other obese pleurocerids have their habitats."

More recently, significant research interest has been directed towards the effects of predation on freshwater gastropod shell morphology (DeWitt et al., 2000; Rundle et al., 2004; Holomuzki & Biggs, 2006; Lakowitz et al., 2008; Hoverman & Relyea, 2009). Krist (2002) reared *G. livescens* in effluent from crayfish feeding on conspecific snails and documented a significant narrowing of the body whorl compared to controls. Although such experiments have not typically been designed to test for the effects of stream size or current directly, any increase in the size or abundance of crushing predators in the downstream reaches of a river might be expected to translate into more robust shell morphology in their freshwater gastropod prey.

In many of the situations examined by Goodrich, earlier taxonomists had described downstream morphological variants of pleurocerid populations as distinct species, which Goodrich subsequently synonymized. For example, *Goniobasis* (or "*Elimia*") *clavaeformis* (Lea, 1841) is a common inhabitant of mid-sized rivers in east Tennessee and southwest Virginia, typically bearing a moderately robust shell with rounded body whorls. Headwater streams in these same regions are inhabited by *Goniobasis* (or "*Elimia*") populations with thinner, more highly spired shells bearing carinae, previously referred to *Goniobasis acutocarinata* (Lea, 1841). Goodrich (1940) observed that populations in streams of intermediate size bore shells of intermediate robustness between the two types, synonymizing the nomen "*acutocarinata*" under *G. clavaeformis*.

Larger streams in southwest Virginia, such as the Clinch, Powell, and Holston rivers, are inhabited by pleurocerid populations that were identified by Goodrich (1940) as *Pleurocera unciale* (= *uncialis*, Haldeman 1841). These populations have shells that are heavier and more robust than typical *Goniobasis* populations, their whorls typically marked with prominent anterior angulation and their anterior apertures at least slightly flared. Dillon & Robinson (2007a, b) have reported evidence from gene frequencies at allozyme-encoding loci suggesting that the shell morphology demonstrated by populations identified as "*Pleurocera unciale*" in the upper Powell River might represent downstream morphological variants of *G. clavaeformis*, just as the *"acutocarinata*" morphology represents the upstream.

Through extensive application of over 30 years, the technique of allozyme electrophoresis has proven a valuable tool for measuring genetic diversity within and among populations of pleurocerid snails (Chambers, 1978; Dillon & Davis, 1980; Bianchi et al., 1994; Dillon & Lydeard, 1998). Levels of genetic divergence are best understood in *Goniobasis proxima*, from which dozens of populations have been studied in five states (Dillon, 1984; Stiven & Kreiser, 1994), with calibration against breeding data (Dillon, 1986, 1988a), cytogenetics (Dillon, 1991) and mitochondrial sequence divergence (Dillon & Frankis, 2004; Dillon & Robinson, 2009). Genetic divergence at allozyme-encoding loci has also been well studied among populations of *Goniobasis catenaria*, ranging from North Carolina through Georgia (Dillon & Reed, 2002; Dillon & Robinson, in press).

Here I extend the survey of Dillon & Robinson (2007a, b) from southwest Virginia through east Tennessee and into north Georgia. I select four subdrainages – the Powell, Little, Conasauga, and Cohutta rivers – and within each of these subdrainages sample populations displaying the *G. acutocarinata – G. clavaeformis – P. unciale* morphology along a gradient of stream size. Because of taxonomic uncertainty in populations bearing shells of this general form, I also sample reference populations of *G. simplex*, a widespread and easily recognized species, to calibrate expected levels of genetic divergence. I then estimate genetic divergence among all 15 populations at ten polymorphic loci using allozyme electrophoresis. If populations of the more robustly shelled *Pleurocera* type in the larger rivers are more genetically similar to their local populations of *Goniobasis* than they are to each other, the hypothesis of Dillon & Robinson (2007a, b) will be confirmed.

METHODS

Taxonomy

No consensus regarding the taxonomy of the Pleuroceridae has emerged at any time throughout the history of North American malacology. The taxonomy of Goodrich (1940) is used throughout the present work for consistency, deferring resolution of the issues raised to an Appendix.

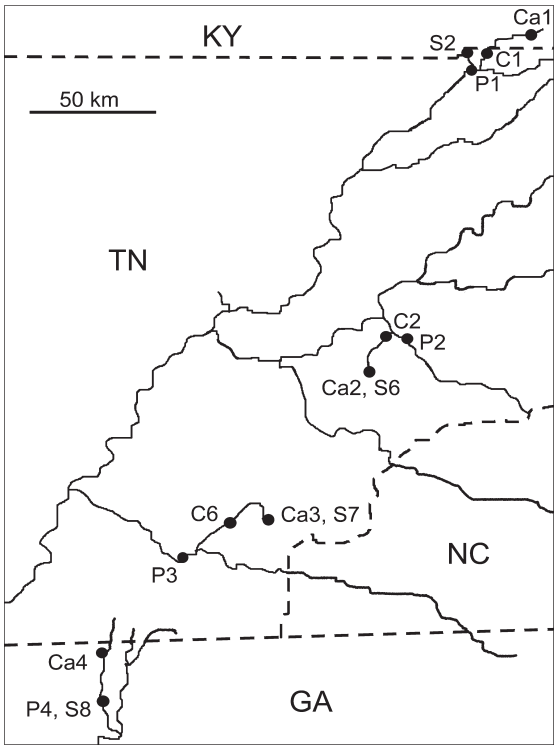


FIG. 1. River drainages in southwest Virginia, east Tennessee, and north Georgia, showing sample sites for all pleurocerid populations sampled in this study.

Populations Studied

At least 30 individual snails were sampled from 15 pleurocerid populations as follows. CA1 – *Goniobasis "acutocarinata"* from Indian Creek at Co. 724 bridge (Ewing), Lee County, Virginia (36.6374°N, 83.4316°W). This was site VG055 of Dillon & Robinson (2007a). C1 – *Goniobasis clavaeformis* from Indian Creek at Co. 684 bridge (Kesterson Mill), Lee County, Virginia (36.6283°N, 83.5019°W). This was Site 1 of Dillon & Robinson (2007a). P1 – *Pleurocera unciale* from the Powell River by River Road, 4 km S of Harrogate, Claiborne Co, Tennessee (36.5544°N, 83.6091°W). S2 – *Goniobasis simplex* from the head of Gap Creek in Cumberland Gap, Claiborne County, Tennessee (36.6001°N, 83.6681°W). This was Site 2 of Dillon & Robinson (2007a). CA2 – *Goniobasis "acutocarinata"* from Pistol Creek in the Courthouse Park, Maryville, Blount County, Tennessee (35.7535°N, 83.9711°W). S6 – *Goniobasis simplex* from the same location. C2 – *Goniobasis clavaeformis* from Pistol Creek below Williams Mill Dam, 5 km N of Maryville, Blount County, Tennessee (35.8147°N, 83.9424°W). P2 – *Pleurocera unciale* from Little River at the US 411 bridge, 6 km E of Maryville, Blount County, Tennessee (35.7856°N; 83.8841°W). CA3 – *Goniobasis "acutocarinata"* from Lick Creek at Shoal Creek Road, 3 km NW of Jalapa, Monroe County, Tennessee (35.3583°N, 84.3881°W). S7 – *Goniobasis simplex* from the same location. C6 – *Goniobasis clavaeformis* from Conasauga Creek at the Co. 879 bridge, 5 km E of Etowah, McMinn County, Tennessee (35.3236°N, 84.4791°W). P3 – *Pleurocera unciale* from the Hiwassee River at the boat ramp 4 km N of Benton, Polk County, Tennessee (35.2035°N, 84.6531°W). The robustness of the shells borne by this population may rise to fit *Pleurocera curtum*, in the concept of Goodrich

(1928). CA4 – *Goniobasis carinifera* from the spring at the Cohutta Fisheries Center Public Park, Whitfield County, Georgia (34.9731°N, 84.9505°W). P4 – *Pleurocera vestita* from Coahulla Creek at the GA 2 bridge (Prater Mill), Whitfield County, Georgia (34.8968°N, 84.9202°W). S8 – *Goniobasis simplex* from the same location. A map locating the collection sites for all populations is given in Figure 1, and example shells are shown in Figure 2. Voucher specimens have been deposited in the Academy of Natural Sciences of Philadelphia (pending).

Allozyme Electrophoresis

Snails were returned alive to the laboratory, where they were cracked and frozen in tris tissue buffer for electrophoretic analysis. Techniques and apparatus for horizontal starch gel electrophoretic resolution of allozyme variation in homogenates of molluscan tissues are detailed in Dillon (1992), along with recipes for all the buffers and stains employed here. Dillon & Robinson (2007a) initially screened five individuals from four nominal pleurocerid species for scorable polymorphisms on four buffer systems staining for 19 enzymes. The enzymes tested were alcohol dehydrogenases (ethanol, hexanol, octanol), sorbitol dehydrogenase, isocitrate dehydrogenase, 6-phosphogluconate dehydrogenase, glucose-6-phosphate dehydrogenase, xanthine dehydrogenase, octopine dehydrogenase, superoxide dismutase, aspartate aminotransferase, phosphoglucomutase, esterases (napthyl acetate, napthyl proprionate), phosphatases (acid, alkaline), leucine aminopeptidase, mannose phosphate isomerase, and glucose phosphate isomerase. Ultimately, bands interpretable as the products of codominant genes segregating in Mendelian fashion at the loci using nine enzyme stains were resolved as detailed below.

The Tris Cit 6 buffer (buffer XIII of Shaw & Prasad, 1970) was used to resolve 6-phosphogluconate dehydrogenase (6PGD), octopine dehydrogenase (OPDH), and isocitrate dehydrogenase (two loci, the cathodal IDHF and the anodal IDHS). A Poulik (1957) discontinuous buffer system was employed for glucose-phosphate isomerase (GPI), and octopine dehydrogenase (a second time). The TEB8 buffer system (buffer III of Shaw & Prasad, 1970) was used to analyze phosphoglucomutase (PGM – the strong, fast locus only), xanthine dehydrogenase (XDH), and mannose phosphate isomerase (MPI). A TEB9.1 buffer (Dillon & Davis, 1980) was used for octanol dehydrogenase (OLDH), esterases (EST1 – the strong, slow locus only) and xanthine dehydrogenase (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 by setting the population of *G. simplex* from its type locality as a standard (population S5 of Dillon & Robinson, 2007a). The putative allele encoding the most common allozyme at population S5 was designated “100”, and all other alleles were named by the mobility of their allozymes (in millimeters) relative to this standard.

Analysis

Gene frequencies and mean direct-count heterozygosities (the unbiased estimate of Nei, 1978) were calculated using Biosys version 1.7 (Swofford & Selander, 1981). Because large numbers of alleles were resolved at some loci, 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. I calculated matrices of Nei’s (1978) unbiased genetic identity and distance, as well as Cavalli-Sforza & Edwards (1967) chord distance. As chord distances are Pythagorean in Euclidean space (Wright, 1978), they were used as the basis for a neighbor-joining tree (Phylip v3.65 program NEIGHBOR, Felsenstein, 2004).

RESULTS

Putative gene frequencies at the ten allozyme-encoding loci examined are reported for 15 pleurocerid populations in Table 1, together with mean direct-count heterozygosities. Of the 10 x 15 = 150 loci examined, 47 were polymorphic by the 95% criterion. Genotype frequencies at three of these 47 loci were significantly different from Hardy-Weinberg expectation by chi-square tests at the nominal 0.05 level, a result clearly attributable to type I statistical error.

The matrix of pairwise Nei (1978) unbiased genetic identities among populations is shown in Figure 3, together with the results of a neigh-

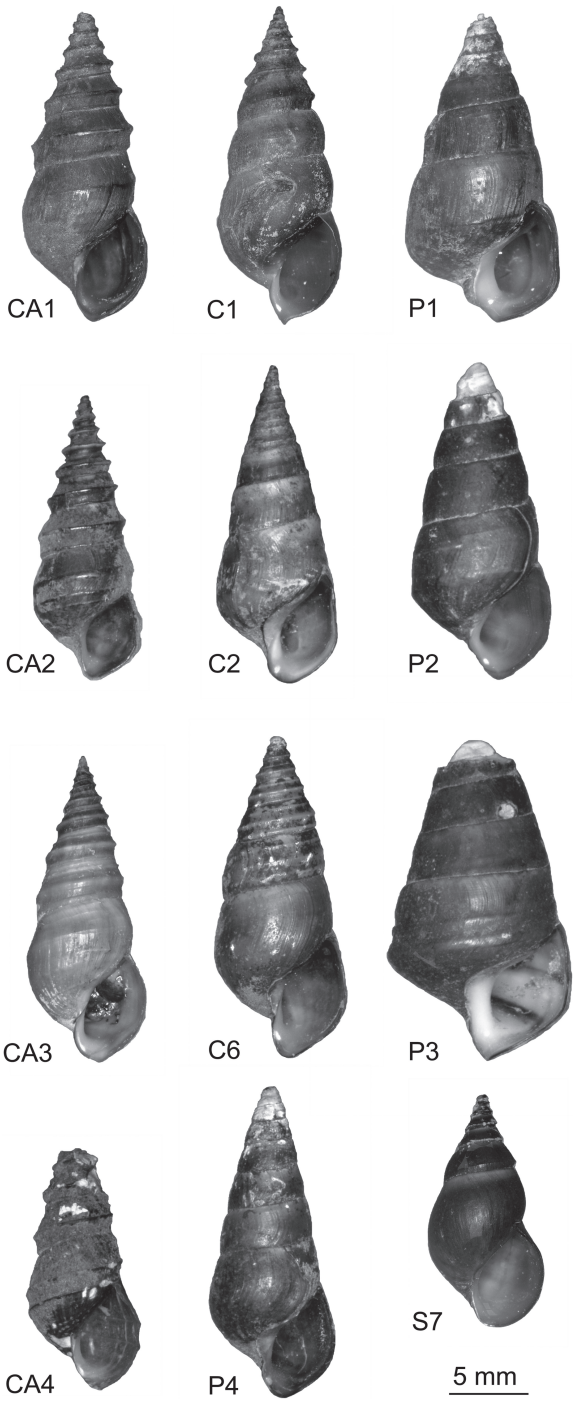


FIG. 2. Example shells from most of the pleurocerid populations studied. Only a single representative shell of *G. simplex* is figured (S7); shells from the other three *simplex* populations were similar.

TABLE 1. Gene frequencies and average (direct count) heterozygosity (H) over ten polymorphic enzyme loci in 15 pleurocerid populations from 4 subdrainages in the southern Appalachians.

	Powell			Little			Hiwassee			Coahulla		*G. simplex*			
Allele	Ca1	C1	P1	Ca2	C2	P2	Ca3	C6	P3	Ca4	P4	S2	S6	S7	S8
GPI															
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	0.065
99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000
98	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000
97	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.935
MPI															
103	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.000
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.950	1.000
97	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000
98	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.933	0.984	0.000	0.000	0.000	0.000
95	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.067	0.016	0.000	0.000	0.000	0.000
93	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
90	0.984	0.991	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000
87	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
EST1															
103	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.067	0.032
101	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.083	0.000	0.000	0.000	0.000
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.933	0.968
99	0.000	0.000	0.000	0.015	0.015	0.000	0.603	0.258	0.017	0.323	0.917	0.000	0.000	0.000	0.000
98	1.000	1.000	1.000	0.985	0.971	0.941	0.397	0.742	0.983	0.677	0.000	0.000	0.000	0.000	0.000
95	0.000	0.000	0.000	0.000	0.015	0.044	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6PGD															
106	0.000	0.000	0.107	0.000	0.000	0.059	0.000	0.017	0.067	0.000	0.034	0.000	0.000	0.000	0.000
103	0.732	0.973	0.571	0.530	0.576	0.809	0.692	0.883	0.667	0.183	0.172	0.000	0.000	0.000	0.000
100	0.268	0.027	0.321	0.470	0.424	0.132	0.308	0.100	0.267	0.817	0.793	1.000	1.000	1.000	1.000
PGM															
104	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.403
102	0.887	0.945	1.000	0.029	0.074	0.559	0.000	0.000	0.383	0.952	0.783	0.094	0.054	0.000	0.597
101	0.000	0.000	0.000	0.000	0.000	0.000	0.362	0.733	0.000	0.048	0.217	0.000	0.000	0.000	0.000
100	0.113	0.055	0.000	0.971	0.926	0.441	0.638	0.267	0.517	0.000	0.000	0.906	0.000	1.000	0.000
96	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.946	0.000	0.000
OPDH															
120	0.000	0.000	0.145	0.000	0.044	0.206	0.103	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000
119	0.000	0.000	0.000	0.000	0.000	0.147	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
118	0.774	0.675	0.677	1.000	0.956	0.574	0.776	0.516	0.867	0.000	0.000	0.000	0.000	0.000	0.000
116	0.226	0.325	0.113	0.000	0.000	0.074	0.121	0.242	0.067	0.000	0.000	0.000	0.000	0.000	0.000
112	0.000	0.000	0.065	0.000	0.000	0.000	0.000	0.242	0.017	0.484	1.000	0.000	0.000	0.000	0.000
110	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
107	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.516	0.000	0.000	0.000	0.000	0.000

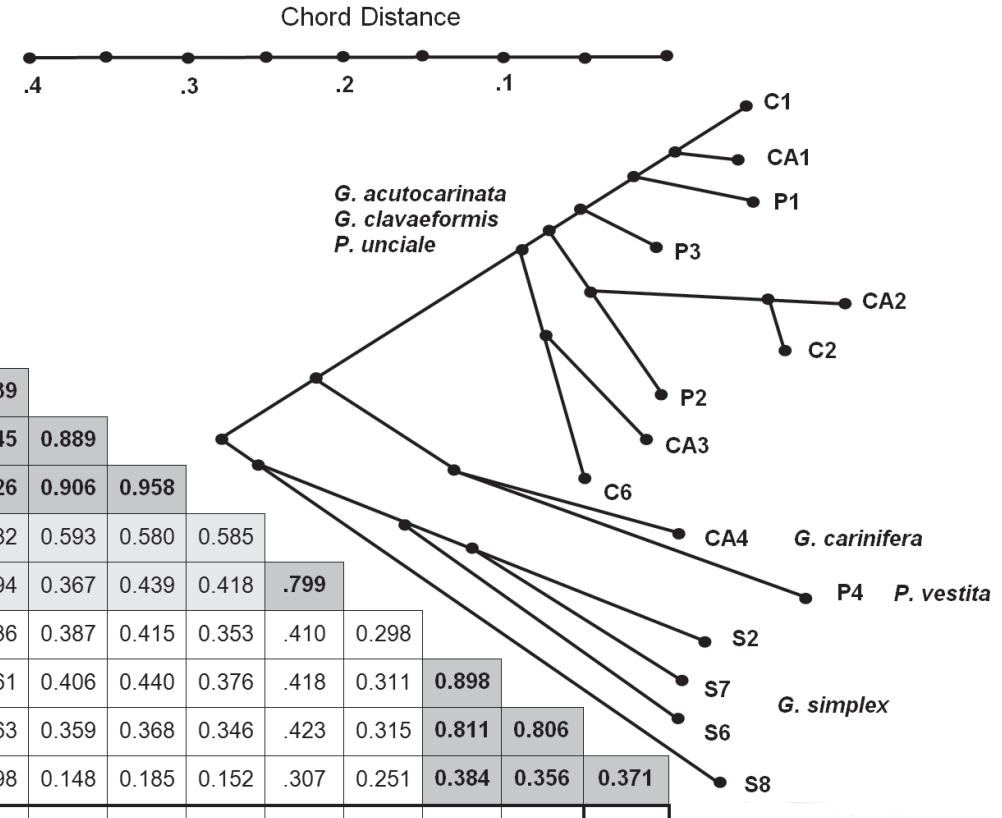
(continues)

(continued)

	Powell			Little			Hiwassee			Coahulla		*G. simplex*			
Allele	Ca1	C1	P1	Ca2	C2	P2	Ca3	C6	P3	Ca4	P4	S2	S6	S7	S8
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.000	1.000	0.000
98	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.054	0.000	0.000
97	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.953	0.000	0.000	0.000
96	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.946	0.000	0.000
94	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000
IDHF															
107	0.000	0.000	0.000	1.000	0.941	0.545	0.000	0.083	0.017	0.000	0.000	0.000	0.000	0.000	0.000
102	1.000	1.000	1.000	0.000	0.059	0.455	1.000	0.917	0.950	1.000	0.645	0.000	0.000	0.000	0.903
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	0.000
98	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.097
97	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.355	0.000	0.000	0.000	0.000
IDHS															
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.048	1.000	1.000	1.000	0.033
98	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.952	0.000	0.000	0.000	0.000
97	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.967
OLDH															
106	0.083	0.053	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000
104	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.683
100	0.917	0.947	1.000	1.000	1.000	1.000	1.000	1.000	0.983	1.000	1.000	0.969	1.000	1.000	0.317
XDH															
101	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.781	1.000	1.000	0.000
95	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.219	0.000	0.000	0.000
H	0.100	0.065	0.102	0.066	0.102	0.181	0.168	0.147	0.140	0.104	0.106	0.059	0.022	0.023	0.098

bor-joining analysis based on Cavalli-Sforza & Edwards Chord distances. The four control populations of *G. simplex* clustered together, as expected, with genetic identities ranging from 0.898 to 0.356. Within the larger group of 11 study populations, clustering was primarily by region, independent of putative taxonomy. The three populations from the Powell River subdrainage (C1, CA1 and P1) were most similar to each other genetically, as were the three populations from the Little River subdrainage (C2, CA2, and P2) and the two populations from the Coahulla (CA4 and P4). Population P3 was somewhat separated from the other two populations of the Hiwassee subdrainage (C6 and CA3), being intermediate between the Powell subdrainage group and the Little subdrainage group, but again, independent of putative taxonomy.

Calibrated against the *G. simplex* standard, all populations previously assigned to *G. acutocarinata*, *G. clavaeformis*, and *P. unciale* are clearly attributable to a single species of highly variable shell morphology. The genetic identities among this set of nine populations ranged down only to 0.771, as against the 0.356 among the *G. simplex* controls. The two north Georgia populations, previously assigned to *G. carinifera* and *P. vestita*, showed genetic identities ranging from 0.667 down to 0.284 with the larger set of nine Tennessee populations, leaving their specific relationships ambiguous. The label "*carinifera* group" would be most appropriate to describe this set of 11 populations together, since *"Melania" carinifera* (Lamarck 1822) is the oldest of the many names available.



CA1	**0.994**												
P1	**0.983**	**0.995**											
P3	**0.957**	**0.975**	**0.961**										
CA2	**0.771**	**0.801**	**0.780**	**0.875**									
C2	**0.794**	**0.821**	**0.801**	**0.891**	**1.00**								
P2	**0.942**	**0.946**	**0.938**	**0.958**	**0.923**	**0.939**							
CA3	**0.871**	**0.889**	**0.869**	**0.947**	**0.830**	**0.845**	**0.889**						
C6	**0.905**	**0.905**	**0.886**	**0.943**	**0.808**	**0.826**	**0.906**	**0.958**					
CA4	0.644	0.667	0.691	0.625	0.467	0.482	0.593	0.580	0.585				
P4	0.401	0.418	0.446	0.391	0.284	0.294	0.367	0.439	0.418	**.799**			
S2	0.308	0.344	0.347	0.393	0.438	0.436	0.387	0.415	0.353	.410	0.298		
S7	0.318	0.354	0.356	0.413	0.463	0.461	0.406	0.440	0.376	.418	0.311	**0.898**	
S6	0.317	0.347	0.361	0.358	0.362	0.363	0.359	0.368	0.346	.423	0.315	**0.811**	**0.806**
S8	0.204	0.231	0.247	0.198	.093	0.098	0.148	0.185	0.152	.307	0.251	**0.384**	**0.356**	**0.371**
	C1	CA1	P1	P3	CA2	C2	P2	CA3	C6	CA4	P4	S2	S7	S6

FIG. 3. Nei's (1978) unbiased genetic identities are shown below the diagonal, with conspecific values shaded. Paler shading marks values comparing *clavaeformis* with the other members of the *carinifera* complex. Above the diagonal is a neighbor-joining tree based on Cavalli-Sforza & Edwards (1967) genetic distances.

DISCUSSION

The results depicted in Figure 3 confirm and generalize the observations of Dillon & Robinson (2007a, b). Independently, in four different subdrainages, populations of pleurocerid snails apparently demonstrate a more robust shell phenotype as stream size increases. In headwater streams of the Tennessee system, such populations have been identified as *G. acutocarinata*, in middle reaches they have been called *G. clavaeformis*, and in larger rivers they have been called *Pleurocera unciale,* or perhaps by some authors *P. curtum*. In headwaters of the Alabama/Coosa system, populations of this group have been identified as *G. carinifera*, and in middle reaches as *Pleurocera vestita*.

Populations of pleurocerids bearing shells in the *acutocarinata – clavaeformis – unciale* gradient of morphology are the most common freshwater gastropods in east Tennessee, certainly ranging from the Clinch and Holston subdrainages of southwest Virginia through the western French Broad and Little Tennessee drainages to the vicinity of Chattanooga, probably further west. The names *clavaeformis* (Lea, March 1841: 12) and *acutocarinata* (Lea, March 1841: 14) and would have priority over *unciale* (Haldeman, Oct. 1841) to refer to this single, highly variable species, and I here select the first name as first reviser.

It should be emphasized that stream size *per se*, as it might be measured by catchment area or discharge volume, is almost certainly not the variable to which these pleurocerid populations are responding. Water chemistry, temperature, depth, current, substrate, primary and secondary productivity, allochthanous input, and the entire communities of producers, herbivores and carnivores are all expected to vary significantly down a river gradient. To determine which of these many environmental and ecological factors might most directly affect the shell morphology of resident pleurocerid populations would require extensive experimentation.

The proportion of the variation here documented in shell phenotype that might have an additive genetic basis also cannot be determined at present. *Goniobasis* populations may not be panmictic, even at a scale of meters (Dillon, 1988b). Populations CA3 and C6, for example, separated by approximately 12 km, showed significant differences at three loci (EST1, PGM and OPDH), and populations C1 and CA1, separated by approximately 6 km, showed a significant difference at 6PGD (Table 1).

However, a great many controlled studies involving other freshwater gastropods have returned evidence that the additive genetic component of shell robustness may be low. The literature on inducible shell defenses in pulmonate snails is especially rich, with striking and often rapid ecophenotypic responses to predation in shell thickness, shell width, and aperture shape documented in physids (DeWitt et al., 2000; Langerhans & DeWitt, 2002), lymnaeids (Rundle et al., 2004; Lakowitz et al., 2008) and planorbids (Hoverman & Relyea, 2007, 2009). The laboratory experiments of Urabe (1998, 2000) returned evidence that both shell shape and shell sculpture are ecophenotypically plastic responses to current and substrate in the Japanese pleurocerid *Semisulcospira*.

The pleurocerid genera *Pleurocera* and *Goniobasis* or *Elimia* have historically been distinguished by a flare or notch in the anterior aperture of the former, a weak and unreliable character. Thorough anatomical investigations have returned no evidence of any significant difference between the two genera (Dazo, 1965; Strong, 2005), nor have mtDNA sequence studies (Sides, 2005). The evidence offered here, that interpopulation variation in shell morphology can rise to the level of the genus, suggests that *Pleurocera*, *Goniobasis* and *Elimia* be combined. See also the Appendix.

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APPENDIX

The taxonomy of the Pleuroceridae was entirely unstable throughout the nineteenth century, hundreds of species being described and dozens of genera being proposed to contain them (Tryon, 1873). Modern workers have generally recognized seven or eight North American genera, but have often selected different nineteenth-century names to call them. Walker (1918) preferred *Io*, *Lithasia*, *Gyrotoma*, *Eurycaelon*, *Anculosa*, *Goniobasis* and *Pleurocera*. Goodrich (1924, 1940) split *Nitocris* from *Anculosa* but otherwise followed closely: *Io, Lithasia, Gyrotoma, Eurycaelon, Anculosa, Nitocris, Goniobasis* and *Pleurocera*. Morrison (1954) considered that Rafinesque's nomen "*Pleurocera*" was more correctly applied to the group that Walker termed "*Lithasia*," and that the Rafinesque nomen "*Oxytrema*" was better applied to a combination of Walker's *Pleurocera* and *Goniobasis*. He also preferred *Leptoxis* over *Anculosa*, and split the genus referred to as "*Nitocris*" by Goodrich into *Mudalia* and *Anaplocamus*. The result again yielded eight genera: *Io, Pleurocera, Gyrotoma, Eurycaelon, Leptoxis, Mudalia, Anaplocamus*, and *Oxytrema*. Burch & Tottenham (1980) returned to the Walker/Goodrich concept of *Pleurocera*, anticipating Opinion 1195 of the ICZN (Melville, 1981). Burch subsumed *Eurycaelon*, *Mudalia*,and *Anaplocamus* of Morrison under the single genus *Leptoxis*, preferred *Elimia* over *Goniobasis*, and split out the western *Juga*, yielding*: Io, Lithasia, Gyrotoma, Leptoxis, Juga, Elimia*, and *Pleurocera*.

The research results presented in the main body of the present work demonstrate that the shell characters by which Walker, Goodrich, and Burch distinguished *Pleurocera* from either *Goniobasis* or *Elimia* do not rise to the level of the genus. Hence, these two groups should be combined, as preferred by Morrison. But following Opinion 1195, the oldest available name of the combined group is not *Oxytrema*, but rather *Pleurocera*, as follows:

Family Pleuroceridae Fischer, 1885
Genus *Pleurocera* Rafinesque, 1818

Pleurocera Rafinesque, 1818: 355. Type species, *Pleurocerus acutus* Rafinesque in Blainville, 1824. Opinion 1195 of the ICZN (Melville, 1981).

Oxytrema Rafinesque, 1819: 423, genus without species. Type species, *P. acutus* Rafinesque, by subsequent monotypy by Blainville (Morrison, 1954: 360).

Ceriphasia Swainson, 1840: 204. Type species, *C. sulcata* Swainson, 1840, by monotypy.

Telescopella Gray, 1847: 153. Type species, *Melania undulata* Say, 1829, by original designation.

Elimia H. Adams & A. Adams, 1854: 300. Type species *Melania acutocarinata* Lea, 1841, by subsequent designation of Pilsbry & Rhoads (1896).

Melasma H. Adams & A. Adams, 1854: 300. Type species *Melania laqueata* Say, 1829, by subsequent designation of Baker (1963).

Strephobasis Lea, 1861: 96. Type species *Melania plena* Anthony, 1854, by subsequent designation of Pilsbry & Rhoads (1896).

Trypanostoma Lea, 1862: 169. Type species *Melania canaliculata* Say, 1821, by original designation.

Goniobasis Lea, 1862: 262. Type species, *G. osculata* Lea, 1862, = *Melania olivula* Conrad, 1834, by subsequent designation of Hannibal, 1912.

Macrolimen Lea, 1863: 220. Type species *Melania showalterii* Lea, 1862, by monotypy.

Strepoma Haldeman, 1863: 274, *ex* Rafinesque ms. Type species, *Melania canaliculata* Say, 1821.

Diagnosis: A genus in the freshwater Cerithiacean family Pleuroceridae with elongate-conic or cylindrical shells, often carinate or costate but not spinose or (ordinarily) pustulate. Aperture may be notched anteriorly, but never posteriorly. Dioecious, sexually reproducing. Differring from the similar *Juga* by the absence of a seminal receptacle (Strong & Frest, 2007).

Description: See Strong (2005) for a thorough description of *P. acuta* and *E. livescens*. All differences noted between those two taxa are here considered significant only at the specific level, not at the level of genus.

Distribution and Habitat: Found in freshwater habitats through much of eastern North America, from northern Florida to southern Ontario, west to Texas and Minnesota. Primarily an inhabitant of rivers and streams, but may also be abundant in cooler, well-oxygenated lentic environments.

Phylogenetic Relationships: Dillon & Robinson (2009) suggested that populations of *Pleurocera* (as here more broadly defined) inhabiting the southern Appalachians may have undergone little morphological evolution since the Mesozoic Era, or possibly even the Paleozoic. Molecular evolution seems to have continued unabated, however, yielding intrapopulation sequence divergence up to 21.9% for some mitochondrial genes. It is perhaps unsurprising, therefore, that larger molecular phylogenetic surveys of the North American Pleuroceridae published thus far have returned mixed and contradictory results (Holznagel & Lydeard, 2000; Lydeard et al., 2002; Ó Foighil et al., 2009). Molecular confirmation of the genus *Pleurocera* as proposed here may await data from some more slowly-evolving markers, as yet unidentified.