

ROSEMARY MACKAY FUND ARTICLE

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In this 5th article of the series, R. T. Dillon and J. D. Robinson propose that the distribution of some prosobranch snail species belonging to the Pleuroceridae might be a relict of the Mesozoic or, possibly, the Paleozoic Era. Robert T. Dillon, Jr. is Associate Professor of Biology at the College of Charleston where he teaches evolution and genetics. His research focuses on the genetics, evolution, and ecology of mollusks. John D. Robinson is a graduate student at the University of Georgia where he studies evolutionary biology and population genetics. His research focuses on conservation and genetic diversity of subdivided populations.

The snails the dinosaurs saw: are the pleurocerid populations of the Older Appalachians a relict of the Paleozoic Era?

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Abstract. Pleurocerid snails are a common element of the benthos in rivers and streams throughout the Appalachian highlands from Virginia to Georgia on both sides of the continental divide. Yet their dispersal capabilities are so limited today that significant gene frequency differences have been demonstrated over a scale of meters. We obtained mitochondrial cytochrome oxidase c subunit I (COI) sequence data from 3 individual snails sampled from each of 13 populations of pleurocerids representing 3 species—*Leptoxis carinata* (4 populations), *Goniobasis* (“*Elimia*”) *catenaria* (4 populations), and *Goniobasis* (“*Elimia*”) *proxima* (5 populations). To these data we added previously published COI sequences from 3 other *G. proxima* populations. Levels of intrapopulation sequence divergence were strikingly high, ranging up to 21.9% within populations and 22.6% between populations within species. A neighbor-joining analysis revealed 3 loose clusters corresponding to the species, but featured numerous extreme outliers. Wilcoxon rank-sum tests returned no evidence that the Continental Divide (as it presently stands) makes any contribution to mean levels of interpopulation sequence divergence, nor that simple geographic distance (regardless of modern drainage) has an effect. We suggest that populations of pleurocerid snails inhabiting the Older Appalachians might date to such an age that all geographic signal in the divergence of our test gene has been lost. We review additional lines of evidence from other genetic studies and from ecology, life history, continental biogeography, and the fossil record that suggest that our pleurocerid populations might be living fossils from the Paleozoic uplift of the Appalachians.

Key words: biogeography, drainage evolution, genetic divergence, gastropods, *Goniobasis*, *Elimia*, *Leptoxis*, mtDNA, COI.

Broad consensus holds that the present Appalachian highlands of North America developed from terrain accretion as Gondwana, Laurentia, and Baltica converged during the Paleozoic Era, and that uplift

proceeded in the region, with numerous spatial and temporal breaks, for >100 million y (Hatcher 1987, van der Voo 1988, Windley 1995). At the close of the Alleghanian orogeny (~300 million y before present) the elevation of the Appalachian Mountains is thought to have averaged ~1300 m (Manspeizer 1988). Then through the Mesozoic and Cenozoic eras, the conti-

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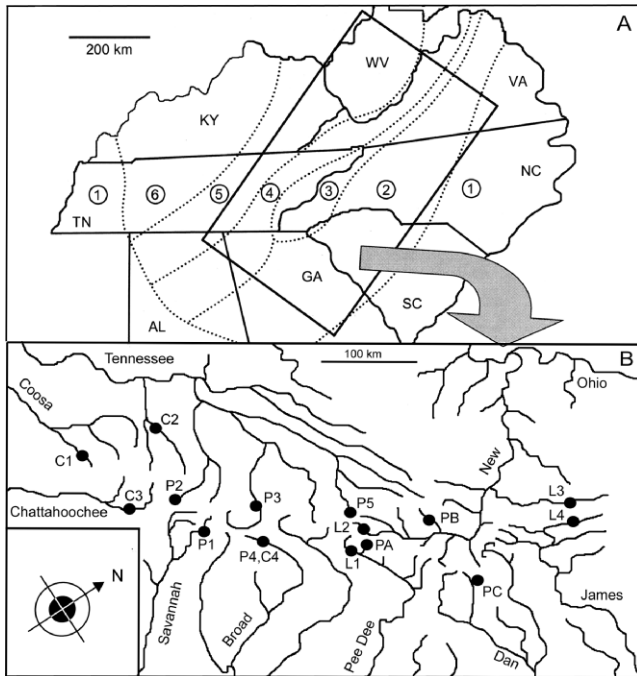


FIG. 1. Study area, showing physiographic provinces (A), and major rivers and sample populations (B). Physiographic provinces are delineated by dotted lines: 1 = Coastal Plain, 2 = Piedmont, 3 = Blue Ridge, 4 = Ridge and Valley, 5 = Appalachian Plateau, 6 = Interior Lowlands. AL = Alabama, GA = Georgia, KY = Kentucky, NC = North Carolina, SC = South Carolina, TN = Tennessee, VA = Virginia, WV = West Virginia. (See Table 1 for details of sampling sites.)

nents rifted, and the prevailing geological processes have been erosional (Olsen et al. 1991).

Geomorphologists divide the Appalachian highlands into 4 provinces: Appalachian Plateau, Ridge and Valley, Piedmont, and Blue Ridge (Thornbury 1965; Fig. 1A). The first 2 have been termed the Newer Appalachians because they are underlain by sedimentary rocks, whereas the latter 2 are called the Older Appalachians because their geology is primarily crystalline.

As the mountains have evolved, so too have the rivers that drain them. The present asymmetry of the Blue Ridge escarpment suggests to some geomorphologists that the eastern Continental Divide might have migrated significantly westward through the Piedmont during the Mesozoic and Cenozoic eras, but this theory is controversial (Judson 1976, Hack 1982, Battiau-Queney 1988, Soller and Mills 1991). In any case, although a significant fraction of the Older Appalachian region drains westward toward the interior in the present day, the rivers through $\frac{1}{2}$ of the Blue Ridge and almost all of the Piedmont now flow southeast to the Atlantic.

Today, certain freshwater prosobranch snail species of the family Pleuroceridae are restricted to the rivers and streams of the divided Older Appalachians. The hypothesis we will advance here is that the distribution of these snail populations might be a relict of watersheds that date to the Mesozoic Era, perhaps even to the Paleozoic. Insofar as we are aware, no previous worker has suggested that any element of the eastern American freshwater biota might remain from such an age.

With few exceptions, the diverse vertebrate fauna inhabiting Appalachian rivers and streams today is Cenozoic in origin (Cavender 1986, Duellman and Trueb 1986). The fish and amphibian communities of such interior paleodrainages as the Teays/Mississippi, the Ohio/Cumberland, and the Appalachian River (the present Mobile Basin extending into east Tennessee) are hypothesized to have been strikingly distinct from those that inhabited the Tertiary drainages of the Appalachians eastward to the Atlantic (Hocutt et al. 1986, Swift et al. 1986, Berendzen et al. 2003, Kozak et al. 2006). In addition to redirecting the interior paleodrainages, Pleistocene climatic oscillations are hypothesized to have caused numerous stream piracies between interior tributaries and drainages of the Atlantic. A great deal of research has been directed toward identifying the sites of such piracies and evaluating their effects on the aquatic fauna of today (e.g., Ross 1969, 1971, Howard and Morgan 1993, Strange and Burr 1997, Jones et al. 2006).

Patterns in the modern distribution of the freshwater biota that might date to the Mesozoic or earlier will be difficult to identify against the clear geological and biogeographical evidence of great change in Appalachian drainage systems through the Cenozoic Era. Any group of organisms showing such a pattern must demonstrate great stasis both geographically (so as not to move) and evolutionarily (so as not to diverge).

Pleurocerid gastropods are among the more conspicuous elements of the freshwater macrobenthos in the southern and eastern US. The fossil record of the Pleuroceridae (broadly defined) dates to the Early Carboniferous of Scotland (Yen 1949), with a modern distribution across both the Old and the New Worlds implying a Pangaeian origin (Banarescu 1990). Their gross morphology is not strikingly different from marine cerithiaceans with fossil records dating to the Devonian (Houbrick 1988). Approximately 100 species are recognized in North America today, in ~ 7 genera (Dillon 1989, Strong 2005).

Pleurocerids are generalist grazers and shredders of detritus and the periphyton, and typically inhabit shallow lotic environments with firm substrate and good flow (Miller 1985, Dillon and Davis 1991,

Morales and Ward 2000, Stewart and Garcia 2002). Populations can reach densities sufficient to consume the entire net primary production of their habitat (Elwood and Nelson 1972), affecting algal community structure (McCormick and Stevenson 1989, 1991), biomass (McCormick 1990, Hill 1992, Rosemond et al. 1993), and nutrient cycling (Mulholland et al. 1985, 1991, Evans-White and Lamberti 2005).

Several species of pleurocerids range across the modern Appalachian divide. The best studied is *Goniobasis* ("Elimia") *proxima*, an inhabitant of creeks and small rivers from southern Virginia through eastern Tennessee and the Carolinas to northern Georgia. The dispersal capabilities of this snail are so limited that significant gene frequency differences have been documented across a 3-m culvert (Dillon 1988b). Yet today, thousands of *G. proxima* populations are scattered through a rugged and mountainous region of 80,000 km². Dillon (1984a) sampled 25 populations across 3 states and showed correlations among allozyme divergence, morphological divergence, and geographic distance. Pairs of populations were identified that shared no alleles at 6 of 7 polymorphic allozyme loci, but transplants and artificial introductions reveal no evidence of reproductive isolation (Dillon 1986, 1988a).

Levels of mitochondrial deoxyribonucleic acid (mtDNA) sequence divergence are also high within and among populations of pleurocerids inhabiting the Older Appalachians. Dillon and Frankis (2004) reported 16.9% sequence divergence in the cytochrome oxidase c subunit I (COI) gene and 14.1% in the ribosomal 16S gene within a single population of *G. proxima* sampled from a tributary of the Dan River in the Virginia piedmont. These values were not strikingly different from the sequence divergence observed among 3 populations of *G. proxima* or between *G. proxima* and 2 other species of *Goniobasis*. Dillon and Frankis (2004) sampled from the American Southeast as a part of the same study. Such results suggest that populations of pleurocerid snails inhabiting the Older Appalachians might be of great age.

Here we extend the survey of Dillon and Frankis (2004) across the eastern continental divide from West Virginia to Georgia to include 5 additional populations of *G. proxima*, 4 populations of *Goniobasis catenaria*, and 4 populations of *Leptoxis carinata*. We test 2 hypotheses—whether the observed levels of interpopulation sequence divergence are a function of simple geographic distance (regardless of modern drainage patterns) or whether the Continental Divide (as it presently stands) affects divergence levels. In so doing, we indirectly evaluate the hypothesis that these

populations might be so ancient that any biogeographical signal in our test gene could have been lost.

Methods

Sample sites were selected such that conspecific nearest neighbors were reciprocally paired and separated by the eastern Continental Divide. Five populations of *G. proxima* were sampled: P1 (from a tributary of the Savannah River), P2 (Little Tennessee), P3 (French Broad), P4 (Broad/Santee), and P5 (Watauga/Holston). Data from the 3 populations of *G. proxima* previously reported by Dillon and Frankis (2004) also were included in the present study: PA (from a tributary of the Yadkin/Pee Dee River), PB (New/Kanawha), and PC (Dan/Roanoke). Samples of 3 snails from each of these populations, analyzed using techniques comparable to those used in the present study, returned single COI sequences designated A and B from populations PA and PB, respectively, and 2 copies of a sequence designated C1 and a single copy of a sequence designated C2 from population PC.

Four populations of *G. catenaria* were sampled: C1 (from the Coosa/Alabama drainage), C2 (Hiwassee/Tennessee), C3 (Chattahoochee), and C4 (Broad/Santee). Populations of these snails inhabiting the Chattahoochee River have been referred to *Goniobasis boykiniana* (Chambers 1990), those inhabiting the Hiwassee River to *Goniobasis interrupta* or *Goniobasis christyi* (Minton et al. 2004), and those inhabiting tributaries of the Coosa River to *Goniobasis caelatura* or *Goniobasis lecontiana* (Thompson 2000). But no consistent differences, morphological or otherwise, have been reported to distinguish any of these nominal taxa from their Atlantic drainage cognate *G. catenaria* earlier described.

Four populations of *Leptoxis carinata* were sampled: 2 from Atlantic drainages (L1 from the Yadkin/Pee Dee system and L4 from the James) and 2 from interior drainages (L2 from the New/Kanawha and L3 from the Greenbrier/Kanawha). Populations of *Leptoxis* inhabiting the Kanawha River and its tributaries, such as L2 and L3, conventionally have been referred to as *Leptoxis dilatata*. But again, no consistent difference, morphological or otherwise, has been reported to distinguish *L. dilatata* from its Atlantic drainage cognate *L. carinata*.

Samples of the 13 populations collected for this study were taken in absolute ethanol in July 2006 and returned to the laboratory for sequencing. These populations and the 3 populations from Dillon and Frankis (2004) are mapped in Fig. 1B, and locality data are given in Table 1. Example shells are shown in Fig.

TABLE 1. Locality data for populations of pleurocerid snails and GenBank accession numbers for all haplotypes isolated. Co. = county, Rd. = road, GA = Georgia, NC = North Carolina, SC = South Carolina, TN = Tennessee, VA = Virginia, WV = West Virginia.

Species	Population	Locality	Latitude (°N)	Longitude (°W)	GenBank accession no.
<i>Goniobasis proxima</i>	P1	West Village Creek at SSR-196, Oconee Co., SC	34.8656	83.1630	EU414634-6
	P2	Betty Creek at O.V. Justus Rd., Rabun Co., GA	34.9742	83.4218	EU414637-9
	P3	Bent Creek at NC 191, Buncombe Co., NC	35.5020	82.5940	EU414640-2
	P4	Green River at Green River Cove Rd., Polk Co., NC	35.2744	82.3054	EU414643-5
	P5	Watauga River at NC 194, Watauga Co., NC	36.2168	81.7861	EU414646-8
	PA	Naked Creek at NC 1154, Wilkes Co., NC	36.1451	81.3596	AY063464
	PB	Cripple Creek at VA 671, Wythe Co., VA	36.8303	81.2922	AY063465
	PC	Nicholas Creek at VA 623, Franklin Co., VA	36.8916	80.0589	AY063466-7
<i>Goniobasis catenaria</i>	C1	Etowah River at GA 372, Cherokee Co., GA	34.3182	84.3432	EU414649-51
	C2	Hiwassee River at TN 315, Polk Co., TN	35.1885	84.5023	EU414652-4
	C3	Chattahoochee River at GA 384, White Co., GA	34.5408	83.6227	EU414655-7
	C4	Green River at Green River Cove Rd., Polk Co., NC	35.3019	82.2650	EU414658-60
<i>Leptoxis carinata</i>	L1	Yadkin River at Grandin Rd., Caldwell Co., NC	36.0579	81.4318	EU414661-3
	L2	South Fork New River at US 421, Watauga Co., NC	36.2216	81.6406	EU414664-6
	L3	Knapp Creek at WV 39, Pocahontas Co., WV	38.2128	80.0694	EU414667-9
	L4	Jackson River at VA 39, Bath Co., VA	38.0777	79.8454	EU414670-2

2. Vouchers have been deposited in the Academy of Natural Sciences of Philadelphia.

Three individuals, designated a, b, and c, were sequenced from each population. DNA was isolated from an anterior portion of the foot muscle using either Puregene DNA Isolation Kits (Gentra Systems, Minneapolis, Minnesota) or a cetyl trimethylammonium bromide isolation protocol (based on Doyle and Doyle 1987). A ~700 base-pair (bp) fragment of COI was amplified, using the universal primers of Folmer et al. (1994), via the polymerase chain reaction (PCR) in a final volume of 25 μ L. Reaction solutions consisted of 5 μ L Promega GoTAQ PCR buffer (5 \times), 2 mM MgCl₂, 0.8 mM of the 4 deoxynucleotide triphosphates, 0.5 mM of each primer, and 0.75 active units of Promega GoTAQ.

All amplifications were done with the following cycling protocol: initial denaturing at 94°C for 2 min 30 s, followed by 40 cycles of 94°C for 30 s, 45°C for 30 s, and 72°C for 45 s. A final elongation step at 72°C for 5 min was followed by a hold at 10°C. PCR products were electrophoresed on a 1% agarose gel containing 10 \times GelRed (Biotium Inc., Hayward, California), and amplified products were visualized on a High Performance Ultraviolet Illuminator (Ultra-Violet Products, LLC, Upland, California). Successful amplifications were subjected to an Exo/SAP cleanup (10 μ L product, 1 μ L exonuclease I, 1 μ L Antarctic phosphatase; New England Biolabs, Ipswich, Massachusetts) and cycle-sequenced using half-reactions of ABI BigDye Terminator sequencing (Applied Biosystems, Foster City,

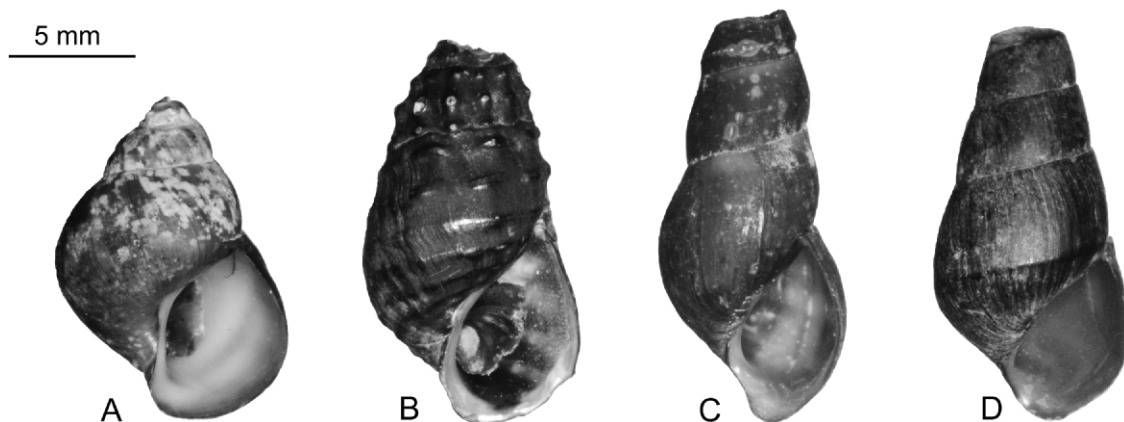


FIG. 2. Example shells of *Leptoxis carinata* (population L3) (A), *Goniobasis catenaria* (population C1) (B), *Goniobasis proxima* (population P2) (C), and *Potadoma ignobilis* from Stanleyville, the Congo (present-day Kisangani, Democratic Republic of Congo) (ANSP 132146) (D).

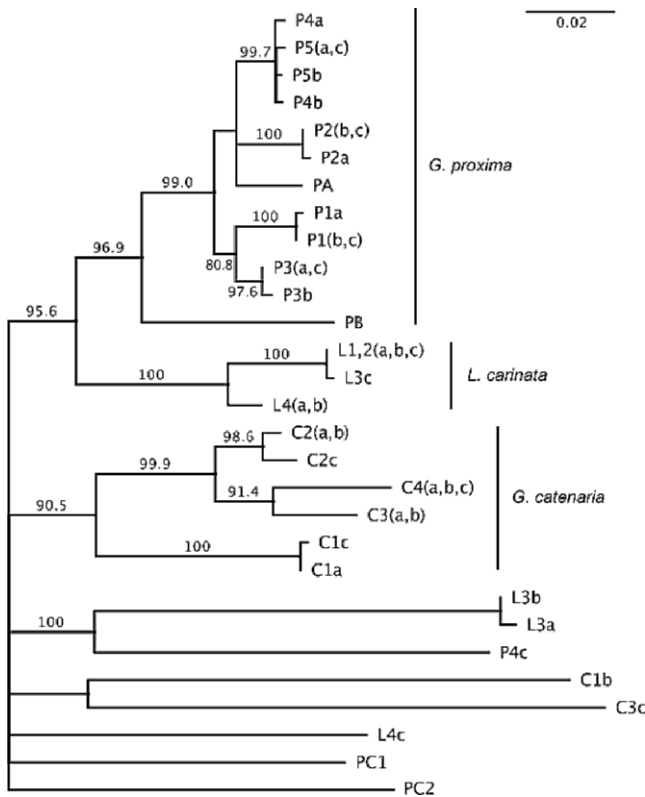


FIG. 3. Neighbor-joining tree of cytochrome oxidase c subunit I (COI) sequences from 16 populations of pleurocerid snails (Table 1, Fig. 1). Numbers at the nodes are percentages of 2000 bootstrap replicates (values <70% not shown). Sequences are labeled by species P = *Goniobasis proxima*, C = *Goniobasis catenaria*, L = *Leptoxis carinata*, population (numbers or uppercase letters), and individual (lowercase letters).

California). DNA sequencing was done on an ABI 3730 automated sequencer (Pratt laboratory, University of Georgia, Athens, Georgia). Each individual was sequenced in the reverse direction, and multiple reads were obtained for all but 2 individuals.

All sequence alignments were done with Codon-Code Aligner (CodonCode Corporation, Dedham, Massachusetts). Sequences were initially aligned within the 3 species, including the sequences of Dillon and Frankis (2004) for *G. proxima*, then combined to produce an alignment across all 16 populations. A matrix of pairwise % differences was calculated among all sampled individuals for each of the 3 species, under the Hasegawa–Kishino–Yano model of sequence evolution (Hasegawa et al. 1985). A bootstrapped neighbor-joining (NJ) tree, connecting the unique haplotypes identified in the present study with the 4 of Dillon and Frankis (2004), was constructed using Geneious v. 3.0.6 (Biomatters Ltd., Auckland, New Zealand) set to 2000 replicates. For topological simplicity, identical haplo-

types were represented by a single tip. Bootstrap values <70% were considered nonsignificant and are not reported on the tree.

Three matrices of pairwise interpopulation sequence divergence were calculated, 1 for each species, with 6 entries in the *L. carinata* matrix, 6 entries in the *G. catenaria* matrix, and 28 entries in the *G. proxima* matrix. Each of these 40 values was calculated as the simple average of the % differences between the 3 haplotypes identified in each pair of populations.

Seven of the 40 were classified as *trans* values (i.e., across the Appalachian divide) and represented the mean sequence divergence between pairs of nearest conspecific neighbors: L1 × L2, L3 × L4, P1 × P2, P3 × P4, P5 × PA, PB × PC, and C2 × C3. The Appalachian divide separates the 2 members of each of these 7 pairs. A Wilcoxon rank-sum test (equivalent to the Mann–Whitney *U*) was used to test the hypothesis that the central tendency in these 7 values of interpopulation divergence might be lower than the 33 other values remaining in the background. This analysis was done with JMP 7.0 (SAS Institute, Cary, North Carolina).

In our 2nd analysis, 10 of the 40 values of mean interpopulation sequence divergence were reclassified as *cis* values and represented divergence between adjacent pairs of conspecific populations that were not separated by the Appalachian divide: L1 × L4, L2 × L3, P1 × P4, P4 × PA, PA × PC, P2 × P3, P3 × P5, P5 × PB, C1 × C2, and C1 × C3. (Note that the rivers inhabited by populations C1, C2, and C3 are thought to have been joined in the pre-Pleistocene Appalachian River drainage, whereas C4 would have remained separate.) A Wilcoxon rank-sum test was used to test the hypothesis that this set of 10 values might be lower in central tendency than the other 30 values remaining in the background (JMP).

Results

Our sample of $13 \times 3 = 39$ snails yielded 25 unique COI haplotypes (GenBank Accession numbers EU414634–EU414672). All but 1 of these sequences were >500 bp; unsequenced leading and trailing portions of the gene were scored as missing data in the analyses. Analysis of these haplotypes together with the 4 COI haplotypes obtained by Dillon and Frankis (2004) for *G. proxima* populations PA, PB, and PC, yielded the NJ tree shown in Fig. 3.

Levels of sequence divergence, both within and among conspecific populations, were exceptionally high. Haplotypes C1b and C3c differed from each other by 21.3% and together differed from all other *G. catenaria* sequences by $\geq 17.4\%$. Haplotype P4c differed

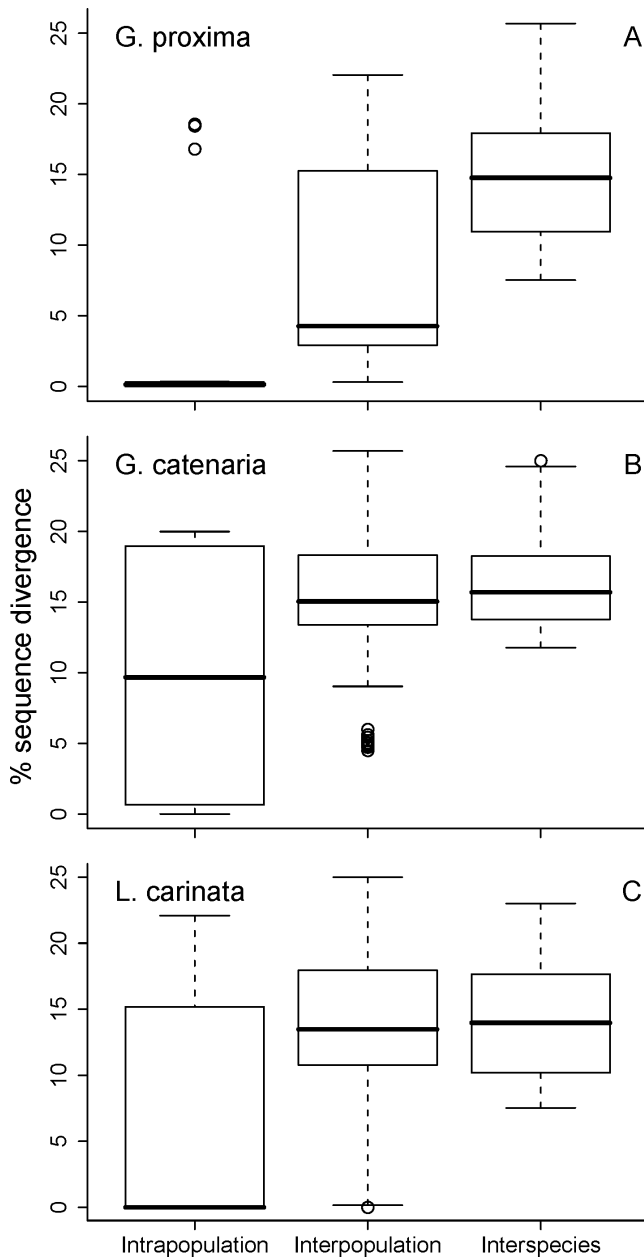


FIG. 4. Box-plots showing sequence divergence within populations, between conspecific populations, and between species for *Goniobasis proxima* (A), *Goniobasis catenaria* (B), and *Leptoxis carinata* (C). Boxes delineate upper and lower quartiles, dark lines show medians, and dashed lines extend to the last observations within 1.5× interquartile range of the boxes (Benjamini 1988).

from the most similar conspecific haplotype (of any population) by 19.0%, and haplotype L4c was 15.1% different from its nearest conspecific neighbor. Levels of intrapopulation sequence divergence exceeded levels of interpopulation and even interspecific divergence in many cases. Figure 4A–C compares sequence

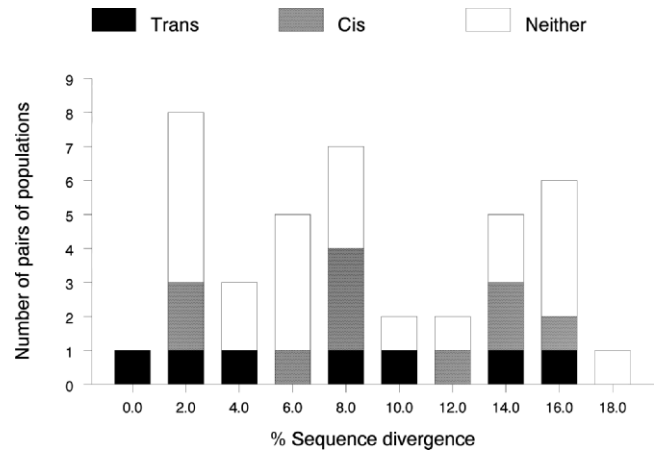


FIG. 5. Mean % sequence divergence between all conspecific pairs of pleurocerid populations. *Trans* pairs are nearest neighbors, separated by the Appalachian Divide. *Cis* pairs are adjacent populations on the same side of the divide. *x*-axis labels are class minima.

divergence within conspecific populations (24 pairs for P, 12 for C and L) to divergence between conspecific populations (252 pairs for P, 54 for C and L) and to values calculated among species (576 pairs for P, 432 values for C and L).

The 40 values of mean sequence divergence among conspecific populations are shown in Fig. 5. Wilcoxon rank-sum tests returned no evidence to support either our *cis* hypothesis or our *trans* hypothesis. Our test of the 1-tailed hypothesis that the 7 *trans* values might be lower than the background level of mean intrapopulation sequence divergence yielded an $\sim\chi^2 = 0.168$ ($p = 0.68$). Our analogous test of the hypothesis that the 10 *cis* values might be lower than background yielded an $\sim\chi^2 = 0.141$ ($p = 0.71$).

Discussion

Although ranging widely, the values of mtDNA sequence divergence reported within mollusk species typically do not exceed 5%. The review of Dillon and Frankis (2004) returned 13 studies supporting this generalization, across all environments and molluscan taxa, and the more recent literature has tended to be supportive (e.g., Wise et al. 2004, Robinson and Dillon 2008). However, double-digit values of % sequence divergence within molluscan populations, such as those shown in Figs 3 and 4, are not entirely unprecedented. The phenomenon has been reported within numerous populations of land snails (Thomaz et al. 1996, Goodacre and Wade 2001, Guiller et al. 2001, Haase et al. 2003) and in the special case of doubly-uniparental inheritance in bivalves (Rawson

and Hilbish 1995, Hoeh et al. 1996). Within populations of freshwater snails, strikingly high sequence divergence has been reported in the Asian pomatiopids *Tricula* and *Oncomelania* (Davis et al. 1998, Wilke et al. 2006), the parthenogenetic thiarid *Melanoides tuberculata* (Facon et al. 2003), and the American pulmonate limpet *Laevapex* (Walther et al. 2006).

Lee et al. (2007) reported evolutionary patterns similar to those we describe here in populations of the East Asian pleurocerid *Semisulcospira*. Their analysis of 16S mitochondrial and 28S nuclear ribosomal DNA sequences in 82 individuals from 12 populations of 7 nominal species suggested a single genetically heterogeneous species with population-level admixtures of nonmonophyletic genotypes up to 8.9% divergent. They suggested 4 possible explanations for such genetic diversity: paralogous mitochondrial markers, cryptic species, introgression, and ancestral polymorphism.

Lee et al. (2007) considered paralogous mitochondrial markers unlikely in East Asian pleurocerids, and we agree in our American pleurocerid populations for similar reasons. We found no evidence of heteroplasmy. Nuclear sequences of mitochondrial origin are typically nonfunctional, and the relaxation of selective constraint leads to rapid evolution (Vanin 1985). However, translation of every unique sequence we obtained from our snails (McClade version 4.07; Sinauer, Sunderland, Massachusetts) yielded open reading frames and remarkable amino acid sequence conservation.

The likelihood that our populations contain mixtures of cryptic species is nil. Extensive studies of gene frequencies at allozyme-encoding loci, typically involving large sample sizes of individuals and populations, have not uncovered deviation from Hardy-Weinberg expectation in pleurocerid populations of the southern US (e.g., Chambers 1980, Dillon 1984a, Stiven and Kreiser 1994, Dillon and Lydeard 1998). Introgression of highly divergent haplotypes from other species also is not at all likely. Most pleurocerid populations in the Older Appalachians are monotypic. In those unusual situations where pairs of pleurocerid species co-occur in our study area, allozyme data return no evidence of hybridization (Dillon and Reed 2002). The present survey included 1 sample where *G. proxima* and *G. catenaria* co-occur, P4/C4 in the Green River, North Carolina. The haplotypes recovered from these 2 populations were strikingly different (Fig. 3).

We favor long-term accumulation and retention of ancestral polymorphism as an explanation for the extensive mitochondrial diversity documented in our samples. Great antiquity would explain our observa-

tion that the sequence divergence among our 16 populations of snails does not seem to correspond to the geography of the region today. Closely neighboring populations do not tend to be more genetically similar than distant populations, regardless of whether drainage pattern is considered in the measurement of distance. We suggest that high levels of intrapopulation genetic divergence, accumulated over long expanses of time, might have confounded any simple correlation between interpopulation divergence and geographic distance in our data set.

The results of the NJ analysis depicted in Fig. 3 do not support the older taxonomy that divided our *G. catenaria* populations into 4 different nominal species and separated the *Leptoxis* populations of the Older Appalachians into species by drainage (Atlantic and interior). Indeed, neither the species relationships nor even the generic relationships among the populations are entirely recovered by our NJ analysis. Fig. 3 does appear to demonstrate central clusters of haplotypes corresponding to *G. proxima*, *G. catenaria*, and *L. carinata*. However, we also isolated multiple haplotypes lying well outside the main haplotype cluster from each species.

The probability that a gene tree will be concordant with a species tree increases with internodal time and decreases with population size (Hudson 1992, Maddison 1997, Rosenberg 2002). We attribute the persistence of ancient polymorphisms in our case to large effective population sizes. Snail densities of 100/m² for linear distances in the scores of kilometers are not unusual (Foin and Stiven 1970, Foin 1971, Dillon 1988a). We also note that most theoretical models do not anticipate the origin of new polymorphisms within isolated populations that, over great expanses of time, might converge independently on the same adaptive peak or diverge to the extent that they are collected outside their species clusters by long-branch attraction (Bergsten 2005).

The diversity of pleurocerid gastropods in North America today is not centered in the Older Appalachians, but rather in the Tennessee and Mobile basins draining the Ridge and Valley and the Appalachian Plateau. The Older Appalachian species seem to number just 5: *Goniobasis semicarinata* of Virginia; *Goniobasis georgiana* of North Carolina, Georgia, and Tennessee; and the 3 surveyed here. However, in the Alabama/Coosa River system to the south of the present study area, >70 species of pleurocerids have been recognized in 4 genera: *Goniobasis*, *Leptoxis*, *Pleurocera*, and *Gyrotoma*, the last now extinct. Levels of mtDNA sequence divergence seem to be much lower among pleurocerid populations sampled from such present-day centers of taxonomic diversity

(Lydeard et al. 1997, Holznagel and Lydeard 2000, Minton and Lydeard 2003) than in the Older Appalachians. This pattern suggests both physical and temporal separation between the origin of the pleurocerids and their subsequent diversification.

Beyond the high levels of genetic diversity displayed by these populations, and their present dispersion across great distances they cannot today traverse, several indirect lines of evidence also support a hypothesis of antiquity for the pleurocerids of the Older Appalachians. Their generation times are long, by comparison with other aquatic invertebrates, and their growth is slow. Pleurocerids typically require 2 y to mature and reproduce iteroparously over many years of adulthood. The strikingly low allocation of energetic resources to reproduction (relative to growth and maintenance) led Dillon (2000:126–135) to characterize *Goniobasis* populations as stress tolerant—adapted to poor but stable environments. If indeed the ticking of the molecular clock is a function of body size and temperature (Gillooly et al. 2005), evolutionary time in such populations of slow-growing inhabitants of cool environments must nearly stand still.

Levels of genetic divergence at allozyme-encoding loci are exceptionally high among *G. proxima* populations compared to values tabulated for other organisms (Dillon and Davis 1980). In addition, the distribution of allozyme alleles is suggestive of antiquity. The most polymorphic locus examined by Dillon (1984a) encoded octopine dehydrogenase, with 16 alleles. One of these alleles appeared to be distributed across the study area from the Virginia piedmont to the mountains of Tennessee and was present in 17 of the 25 populations surveyed. Most of the remaining 15 alleles were private or nearly so and were present in single populations or pairs of neighboring populations. This pattern suggested to Dillon (1984a) that a period during which dispersal across the present Appalachian divide was possible has been followed by a long period during which dispersal has been negligible. Did pleurocerid snails evolve from marine ancestors and initially diversify on the interior side of the towering ancient Appalachians, then remain in evolutionary stasis for 300 million y as the eastern Continental Divide eroded westward to bisect their range?

The 25 populations surveyed by Dillon (1984a) were sampled from 4 major drainages: the Roanoke, Yadkin, New, and Tennessee River systems. But across the entire 25 × 25 symmetric matrix of pairwise genetic distances, the 2 lowest values were recorded between pairs of populations not presently in the same drainage. Population REED in the New was genetical-

ly indistinguishable from population MOFD in the Yadkin, and population CRIP of the New was indistinguishable from population SUGR of the Tennessee. These 2 pairs of populations occur at the sites of inferred stream piracies and have been isolated since the Pleistocene. If divergence since the Pleistocene has been negligible, what length of time might be required to fix 6 of 7 loci for alternative alleles, as Dillon (1984a) demonstrated in other pairs of *G. proxima* populations?

Prior to Triassic/Jurassic rifting, the mountains of our study area would have abutted present-day Morocco (Manspeizer 1988). That region is arid today, but forested rivers and streams draining to the Atlantic further south are inhabited by thiarid or pachychilid gastropods strikingly similar to the pleurocerids of the Older Appalachians (Kohler et al. 2004). Approximately 20 species of the West African genus *Potadoma* inhabit lotic environments from Liberia to the Democratic Republic of Congo (Brown 1994). The habitat of *Potadoma moerchi*, for example, would seem identical to that of *G. proxima*: “fast flowing rivers and streams, heavily shaded with gravelly or rocky substrates” (Ndifon and Ukoli 1989; p. 249). Recent authors generally have attributed these remarkable morphological and ecological similarities to convergence (Houbrick 1988, Lydeard et al. 2002). But might *Potadoma* and other tropical or Old World thiarid or pachychilid gastropods have diverged from pleurocerid ancestors inhabiting the Older Appalachians today?

The genetic diversity manifested by the pleurocerid populations of the Older Appalachians has not been completely unaccompanied by morphological divergence. Dillon and Davis (1980) recognized 3 races of *G. proxima* separable in minor aspects of shell shape and color and in allozyme gene frequency. Dillon (1984a, b) measured 21 morphological variables on 10 individuals from each of his 25 *G. proxima* populations and found only 4 entries in the entire 25 × 25 symmetric matrix of Mahalanobis D^2 statistics that were nominally nonsignificant. Nevertheless, the overall impression one takes from casual inspection of all the pleurocerids of the Older Appalachians is one of morphological uniformity across great geographic ranges.

The term *living fossil* often is used to describe an organism that has displayed striking morphological stasis over a prolonged period of time. Application of such a label to pleurocerid snails is complicated by their poor fossil record, which, given the erosional nature of their environment, seems unlikely to improve. Nevertheless, we suggest that “living fossil” might describe

the pleurocerid populations inhabiting the rivers and streams of the Older Appalachians today.

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