

# The *Goniobasis* (“*Elimia*”) of southwest Virginia,

## I. Population Genetic Survey

Robert T. Dillon, Jr.

John D. Robinson

Department of Biology

College of Charleston

Charleston, SC 29424

[dillonr@cofc.edu](mailto:dillonr@cofc.edu)

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## ABSTRACT

Four nominal species of the pleurocerid snail genus *Goniobasis* have been documented from tributaries of the Tennessee River in southwest Virginia. *Goniobasis arachnoidea* and *G. aterina* are listed as Tier II species in the state Wildlife Action Plan, with *G. arachnoidea* receiving state endangered status on July 1, 2006. *Goniobasis clavaeformis* and *G. simplex* are generally considered common and secure. But the shell characters by which the four species have been distinguished are so variable that field identification can be problematic. Here we report the results of a survey of 76 sites in southwest Virginia and adjacent Tennessee, 55 of which were positive for populations of *Goniobasis*. Protein electrophoresis was used to determine gene frequencies at 11 allozyme-encoding loci in 3 populations of *G. arachnoidea*, 4 populations of *G. clavaeformis*, and 5 populations of *G. simplex* inhabiting six of these sites. The population of nominal *G. aterina* inhabiting the type locality of the species was not genetically distinct from *G. simplex*, and is proposed for synonymy. We offer detailed observations on the shell and body color of the three other *Goniobasis* species sufficient to distinguish them in future studies without special genetic testing. Our field survey confirms both that *G. simplex* and *G. clavaeformis* are widespread and common in southwest Virginia and that *G. arachnoidea* is much more restricted, apparently limited to small tributaries of the Powell River in Lee and Wise Counties. The conservation status of *G. arachnoidea* is cause for some concern.

## INTRODUCTION

Specific relationships among diverse populations of the pleurocerid genus *Goniobasis* inhabiting tributaries of the upper Tennessee River system in Virginia, North Carolina, and Tennessee are far from clear. Tryon (1873) listed approximately 40 species with ranges that might reasonably include the upper Tennessee River drainage. Goodrich (1940) reduced this number to four, citing his previous works documenting extensive non-genetic shell variation in other pleurocerid species (e.g., Goodrich 1935). Goodrich's model was adopted by Burch (1989) for his influential "North American Freshwater Snails," the treatment that forms the basis of our present understanding of the fauna (Stewart & Dillon 2004).

Burch resurrected the genus name "*Elimia*" (H. & A. Adams) to replace the firmly-established *Goniobasis* citing the Pilsbry & Rhoads (1896) type designation, failing to note that Pilsbry subsequently reversed himself (Walker 1918). Although *Goniobasis* remains the proper generic name for the group (Dillon 1989), "*Elimia*" has entered the literature as a synonym. Further background on this taxonomic controversy is available from the Freshwater Gastropods of North America web site: <http://www.cofc.edu/~fwgna/archive/28Sept04.html>

The four nominal species of *Goniobasis* inhabiting tributaries of the upper Tennessee River as recognized by Goodrich (1940), Burch (1989), and Stewart & Dillon (2004), together with their ranges as currently understood, are as follows: *Goniobasis arachnoidea* (Anthony 1854) ranges through "small streams in Tennessee and southwestern Virginia." *Goniobasis aterina* (Lea 1863) is listed as "restricted to a few springs and small streams in Tennessee and southwest Virginia." *Goniobasis clavaeformis* (Lea 1841) is "restricted to streams and small rivers in the Tennessee River drainage." *Goniobasis simplex* (Say 1825) ranges through "the

Tennessee and New River drainages."

Recently the conservation status of *G. aterina* and *G. arachnoidea* has become a focus of concern. *Goniobasis aterina* is listed as a G1 species ("critically imperiled") by NatureServe, and *G. arachnoidea* as a G3 ("vulnerable") species. Both *G. arachnoidea* and *G. aterina* were listed in conservation Tier II in Virginia's 2005 Wildlife Action Plan (VDGIF 2005) and *G. arachnoidea* was listed state endangered by the Virginia Department of Game and Inland Fisheries (VDGIF) on July 1, 2006.

As is the case for all snails of the genus *Goniobasis*, the four southwest Virginia species were initially described on the basis of shell characteristics, and the shell remains the only criterion by which they can be distinguished today. But shell morphology has proven to be a poor guide for recognizing biological species of pleurocerid gastropods, showing extreme variability within and among conspecific populations and subject to ecophenotypic plasticity (Urabe 1998, 2000). *Goniobasis clavaeformis* is especially noted for intraspecific shell variability, individuals varying widely in the strength and position of the carination (or "shouldering") on the whorl periphery. Nor is the distinction between the nominally imperiled *G. aterina* and the much more common *G. simplex* clear from published figures and descriptions. Thus, the precise and current distribution of the four *Goniobasis* species of southwest Virginia is unknown, at least partly because the species are not unambiguously identifiable.

Through extensive application over almost 30 years, the technique of allozyme electrophoresis has proven to be a valuable tool for resolving the specific status of problematic pleurocerid populations (Chambers 1978, Dillon & Davis 1980, Bianchi et al 1994, Dillon & Lydeard 1998). Levels of genetic divergence are best understood in *G. proxima*, from which dozens of populations have been studied in four states (Dillon 1984, Dillon & Reed 2002), with

calibration against breeding data (Dillon 1986, 1988), cytogenetics (Dillon 1991) and mitochondrial sequence divergence (Dillon and Frankis 2004).

The purpose of this project was to survey the diverse *Goniobasis* populations inhabiting Virginia tributaries of the Tennessee River system, with special attention directed toward the rare *G. aterina* and endangered *G. arachnoidea*. From a large sample of diverse populations we selected a subset for detailed genetic analysis using allozyme electrophoresis. In addition to confirming the specific status of all four nominal taxa, we aimed to determine what shell characteristics or other morphological criteria might exist, if any, useful for distinguishing the species in the field.

## METHODS

We surveyed 65 sites in the Virginia portions of two major subdrainages - the Clinch River (35 sites) and the Powell River (27 sites) – with 3 sites located in the Holston subdrainage to the east for comparative purposes and 11 sites collected in Tennessee near the Virginia border. From the total of 76 sites, we selected six as locations for detailed population genetic analysis. We sampled *G. arachnoidea*, *G. clavaeformis*, and *G. simplex* together from Indian Creek, a tributary of the Powell River at Kesterson Mill (Site 1). From Gap Creek, the Powell River tributary in Cumberland Gap given as the type locality of *G. aterina*, we sampled nominal *G. aterina* and *G. arachnoidea* (Site 2). We sampled *G. simplex* and *G. clavaeformis* from Big Spring Run, a tributary of the Powell River 2 km SE of East Stone Gap (Site 3) and also from Cove Creek, a tributary of the Clinch River at Kerns (Site 4). From the type locality of *G. simplex*, the North Fork Holston River at Saltville (Site 5), we sampled both *G. simplex* and *G. clavaeformis*, and from Shafer Creek, a tributary of the Powell River 2 km W of Collier Mill, we

sampled *G. arachnoidea* (Site 6). Sample sizes were at least  $N = 31$  for all populations, with a grand mean sample size per locus of 34.9. Sample sites are shown in Figure 1, and detailed locality data are available in Appendix 1. Voucher specimens have been forwarded to the VDGIF.

Animals 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 buffers and stains employed here. We initially used five individuals from the four nominal species to screen 19 enzymes for scorable polymorphism on four buffer systems. 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 (naphthyl acetate, naphthyl proprionate), phosphatases (acid, alkaline), leucine aminopeptidase, mannose phosphate isomerase, and glucose phosphate isomerase. Ultimately we were able to resolve bands interpretable as the products of codominant genes segregating in Mendelian fashion at 11 loci using 10 enzyme stains, as detailed below.

The Tris Cit 6 buffer (buffer XIII of Shaw and 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), sorbitol dehydrogenase (SDH), and octopine dehydrogenase (a second time). The TEB8 buffer system (buffer III of Shaw and 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 and 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 type locality *G. simplex* population (S5) as a standard. 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.

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 identity and 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 an UPGMA cluster analysis (Wright 1978).

## RESULTS

We found field identification of *G. arachnoidea*, *G. clavaeformis*, and *G. simplex* to be unambiguous. *Goniobasis simplex* bears an uncarinated shell with rounded (or "shouldered") whorls and a low spire; its body whorl typically greater than 55% of total shell length. The body

color of *G. simplex* is generally dark, often black. Both *G. clavaeformis* and *G. arachnoidea* have higher spires and unshouldered whorls, with body whorls less than 55% of their total shell length. The shell of *G. arachnoidea* is always uncarinate and narrow, width 35% of the shell length or less. The shell of *G. clavaeformis* is often carinate and broader, width greater than 35% of its shell length. The body color of *G. arachnoidea* is slightly orange and that of *G. clavaeformis* strikingly orange, with black flecks. We were unable, however, to distinguish *G. aterina* in the field. Specimens collected from the type locality of the species in Cumberland Gap gave the impression of small (perhaps stunted) *G. simplex*, consistent with the apparent low-nutrient status of Gap Creek.

Typical shells borne by *G. arachnoidea*, *G. clavaeformis*, and *G. simplex*, are shown in Figure 2. The Freshwater Gastropods of Virginia web site, currently under construction, features color photos of the living animals and a dichotomous key to all 12 species and subspecies of pleurocerid snails inhabiting the waters of the Commonwealth of Virginia, together with notes on their distribution, habitat, ecology, taxonomy, and systematics:

<http://www.cofc.edu/~fwgna/FWGVA/>

*Goniobasis* populations were discovered at 55 of the 76 sites we sampled in our general survey, with 21 sites inhabited by more than one species. *Goniobasis clavaeformis* (33 sites) and *G. simplex* (37 sites) were common in all three of the subdrainages surveyed - those of the Powell, Clinch, and Holston Rivers. *Goniobasis simplex* populations seemed to reach greatest densities in small streams, while *G. clavaeformis* populations reached maximum densities in the medium-sized streams and small rivers. Populations of *G. arachnoidea* were discovered at only 13 sites. In Virginia, the snail appears to be restricted to tributaries of the Powell River in Lee and western Wise Counties. We did document one population of *G. arachnoidea* in a tributary



of the Clinch River in Tennessee, about 15 km south of the Virginia border, but were unable to confirm the snail in Clinch River tributaries further upstream. Nor were we able to confirm the report of Goodrich (1913) of *G. arachnoidea* in tributaries of the Holston River near Gate City.

Putative gene frequencies at the 11 allozyme-encoding loci we examined in our sample of 12 *Goniobasis* populations are given in Table 1, together with mean direct-count heterozygosities. Of the  $11 \times 12 = 132$  loci examined, 32 were polymorphic by the 95% criterion. Genotype frequencies at none of these 32 loci were significantly different from Hardy-Weinberg expectation by chi-square tests.

The matrix of pairwise Nei (1978) unbiased genetic identities among populations is shown in Figure 3, together with the results of our UPGMA cluster analysis based on Cavalli-Sforza & Edwards Chord distances. The (Fitch & Margoliash 1967) percent standard deviation for this result was 6.94, and the cophenetic correlation was 0.973 (Sneath & Sokal 1973: 278-280). Three clusters are apparent, corresponding to *G. arachnoidea*, *G. clavaeformis*, and *G. simplex*. Intraspecific divergence was greatest in the *simplex* cluster, with an unbiased Nei genetic identity as low as 0.552 observed between population S1 of Indian Creek and population S3 of the upper Powell River.

The population of nominal *G. aterina* we sampled from its type locality at Cumberland Gap (S2) was not genetically distinct from the much more widely-distributed *G. simplex*. Figure 2 shows that the minimum genetic identity between population S2 and any other *G. simplex* population was 0.634 (with population S1), higher than the identity displayed between S1 and S3. Thus, UPGMA analysis grouped population S2 within the larger *simplex* cluster.

## DISCUSSION

The primary finding of this study is that tributaries of the Tennessee River in southwest Virginia are inhabited by three genetically distinct species of *Goniobasis*. Table 1 shows fixed differences at three allozyme loci between *arachnoidea* and *clavaeformis* (EST1, XDH, SDH), four fixed differences between *arachnoidea* and *simplex* (MPI, OPDH, IDHF, OLDH) and five fixed differences between *clavaeformis* and *simplex* (GPI, MPI, EST1, OPDH, IDHF). All three of these species co-occur in Indian Creek at Site 1, and elsewhere in smaller tributaries of the Powell River, with no evidence of hybridization. Thus, their status as distinct biological species is not in question. The population of *Goniobasis* inhabiting the type locality of *G. aterina* at Cumberland Gap was not genetically distinct from *G. simplex* at any of the 11 loci we examined. *Goniobasis aterina* (Lea 1863) should be considered a junior synonym of *Goniobasis simplex* (Say 1825).

Within the three biological species of *Goniobasis* inhabiting southwest Virginia, levels of interpopulation divergence at allozyme-encoding loci were high. Unbiased genetic identities range from 0.63 to 0.86 among the three populations of *G. arachnoidea*, from 0.83 to 0.99 among the four populations of *G. clavaeformis*, and from 0.55 to 0.99 among the five populations of *G. simplex*. Such results are consistent with the generally high levels of intraspecific divergence that has been reported in surveys of *Goniobasis* population genetics conducted elsewhere (Dillon & Davis 1980, Dillon 1984, Dillon & Reed 2002).

Given the high levels of intraspecific genetic divergence, it is perhaps not surprising that a great deal of shell morphological variation was apparent among the *Goniobasis* populations of southwest Virginia. We found the shells of *G. clavaeformis* to be especially variable with regard

to carination - some populations uncarinate, others bearing a single carina at mid-whorl, and yet other populations bearing strong carination at the whorl anterior. Individuals bearing strong mid-whorl carination were originally described as *G. acutocarinata*, the nominal type species of the genus *Elimia* (Goodrich 1940, Dillon 1989). Individuals bearing shells with strong anterior carination can be mistaken for an entirely distinct pleurocerid genus, *Pleurocera*. A report detailing our supplementary allozyme survey on the diverse morphs of *G. clavaeformis* inhabiting Indian Creek follows as Part II of this series.

In spite of the variation in shell morphology, however, unambiguous identification of the three species is possible in the field. Ratios of shell width and body whorl height to total shell height offer fairly reliable guides, as well as whorl shouldering and body color. Thus, the *Goniobasis* populations of southwest Virginia can be mapped and monitored without further recourse to time-consuming and expensive genetic techniques.

The results of our field survey confirm that *G. simplex* and *G. clavaeformis* are both common and widespread in Virginia tributaries of the Tennessee River system, *simplex* in the smaller springs and creeks and *clavaeformis* in the larger streams and small rivers. The conservation status of these two species would seem secure. *Goniobasis arachnoidea* is apparently more restricted, being found only in small tributaries of the Powell River in Lee and Wise counties, as well as further into the state of Tennessee. The future of this species is more problematic.

A great deal of development has been ongoing in Lee County in recent years, associated with the re-routing and widening of U.S. 58. The small streams with good water quality currently inhabited by populations of *Goniobasis arachnoidea* may be especially vulnerable to siltation from earth-moving activities and pollution from run-off. The recent placement of *G.*

*arachnoidea* on the Virginia endangered species list would seem well-warranted.

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TABLE 1 continued.

allele	<i>G. arachnoidea</i>			<i>G. clavaeformis</i>				<i>G. simplex</i>				
	A1	A2	A6	C1	C3	C4	C5	S1	S2	S3	S4	S5
OPDH												
120	.000	.000	.000	.000	.214	.162	.000	.000	.000	.000	.000	.000
119	.016	.000	.096	.000	.029	.088	.000	.000	.000	.000	.000	.000
118	.758	.000	.883	.633	.643	.750	.000	.000	.000	.000	.000	.000
116	.000	.000	.000	.367	.071	.000	.912	.000	.000	.000	.000	.000
115	.000	.000	.000	.000	.043	.000	.088	.000	.000	.000	.000	.000
114	.032	.837	.021	.000	.000	.000	.000	.000	.000	.000	.000	.000
108	.194	.163	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
100	.000	.000	.000	.000	.000	.000	.000	.000	.031	1.000	1.000	1.000
97	.000	.000	.000	.000	.000	.000	.000	.014	.953	.000	.000	.000
94	.000	.000	.000	.000	.000	.000	.000	.986	.016	.000	.000	.000
IDHF												
104	.000	.000	.000	.000	.000	.000	.000	.365	.000	.000	.000	.015
102	1.000	.984	1.000	1.000	1.000	1.000	1.000	.000	.000	.000	.000	.000
100	.000	.000	.000	.000	.000	.000	.000	.635	1.000	1.000	1.000	.985
98	.000	.016	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
IDHS												
100	1.000	1.000	.968	1.000	1.000	1.000	1.000	1.000	1.000	.629	1.000	1.000
97	.000	.000	.032	.000	.000	.000	.000	.000	.000	.371	.000	.000
OLDH												
110	.000	.000	.161	.000	.000	.000	.000	.000	.000	.000	.000	.000
106	1.000	1.000	.839	.067	.000	.000	.000	.000	.000	.000	.000	.000
104	.000	.000	.000	.000	.000	.000	.000	.986	.031	.000	.000	.000
100	.000	.000	.000	.933	1.000	1.000	1.000	.014	.969	1.000	1.000	1.000

TABLE 1, continued.

allele	<i>G. arachnoidea</i>			<i>G. clavaeformis</i>				<i>G. simplex</i>				
	A1	A2	A6	C1	C3	C4	C5	S1	S2	S3	S4	S5
XDH												
100	.000	.000	.000	1.000	1.000	1.000	1.000	.135	.781	1.000	1.000	1.000
95	1.000	.000	1.000	.000	.000	.000	.000	.865	.219	.000	.000	.000
102	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
SDH												
104	.468	1.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
102	.000	.000	.000	1.000	1.000	1.000	1.000	.028	.000	.971	.971	.000
101	.000	.000	.000	.000	.000	.000	.000	.139	1.000	.029	.029	.088
100	.532	.000	.000	.000	.000	.000	.000	.833	.000	.000	.000	.912
H	0.109	0.170	0.187	0.059	0.093	0.078	0.053	0.119	0.054	0.060	0.008	0.021

Figure 1. River drainages in southwest Virginia and northeastern Tennessee, showing sites in the general distributional survey as small circles and sites from which *Goniobasis* was sampled for population genetic analysis as larger, enumerated circles.

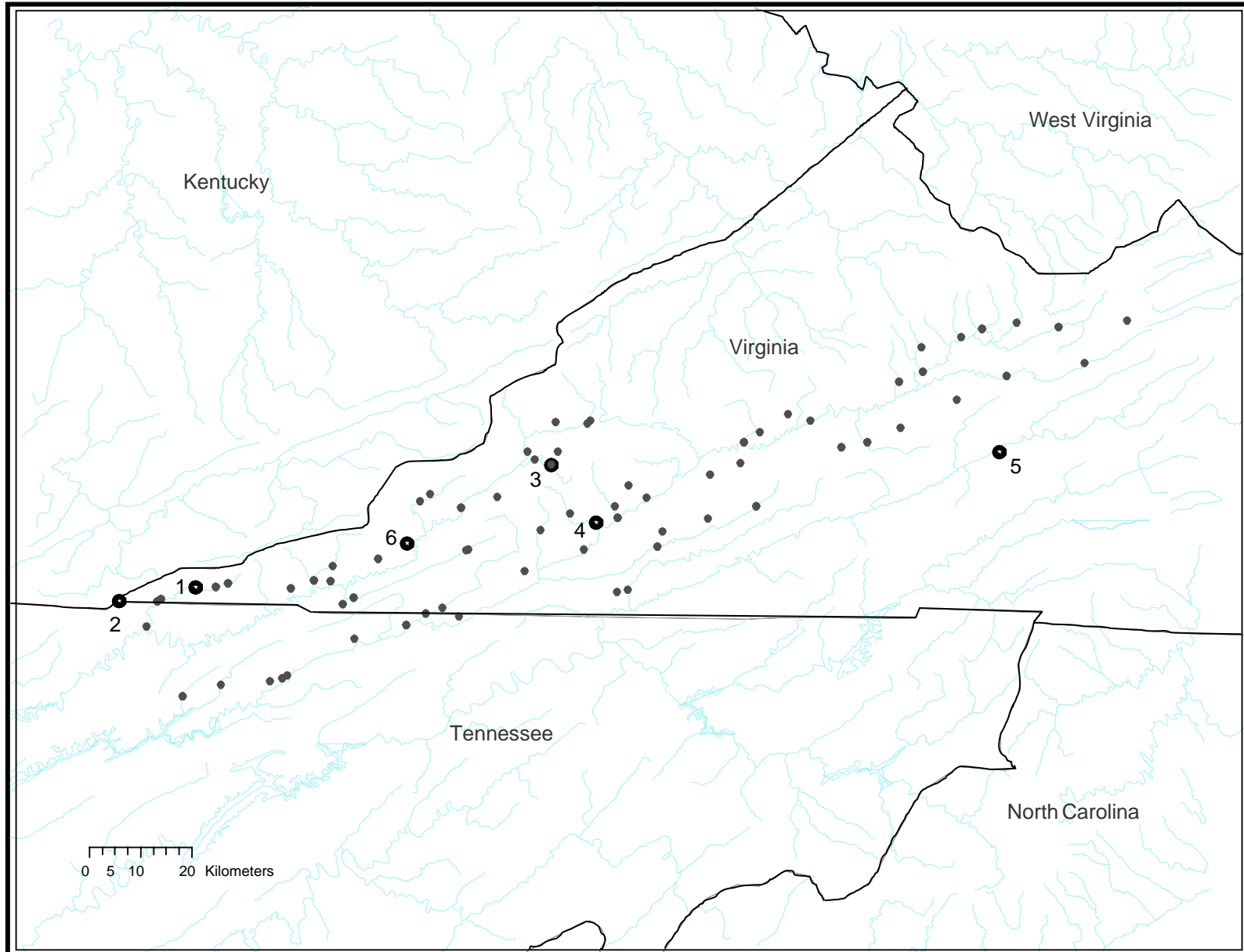
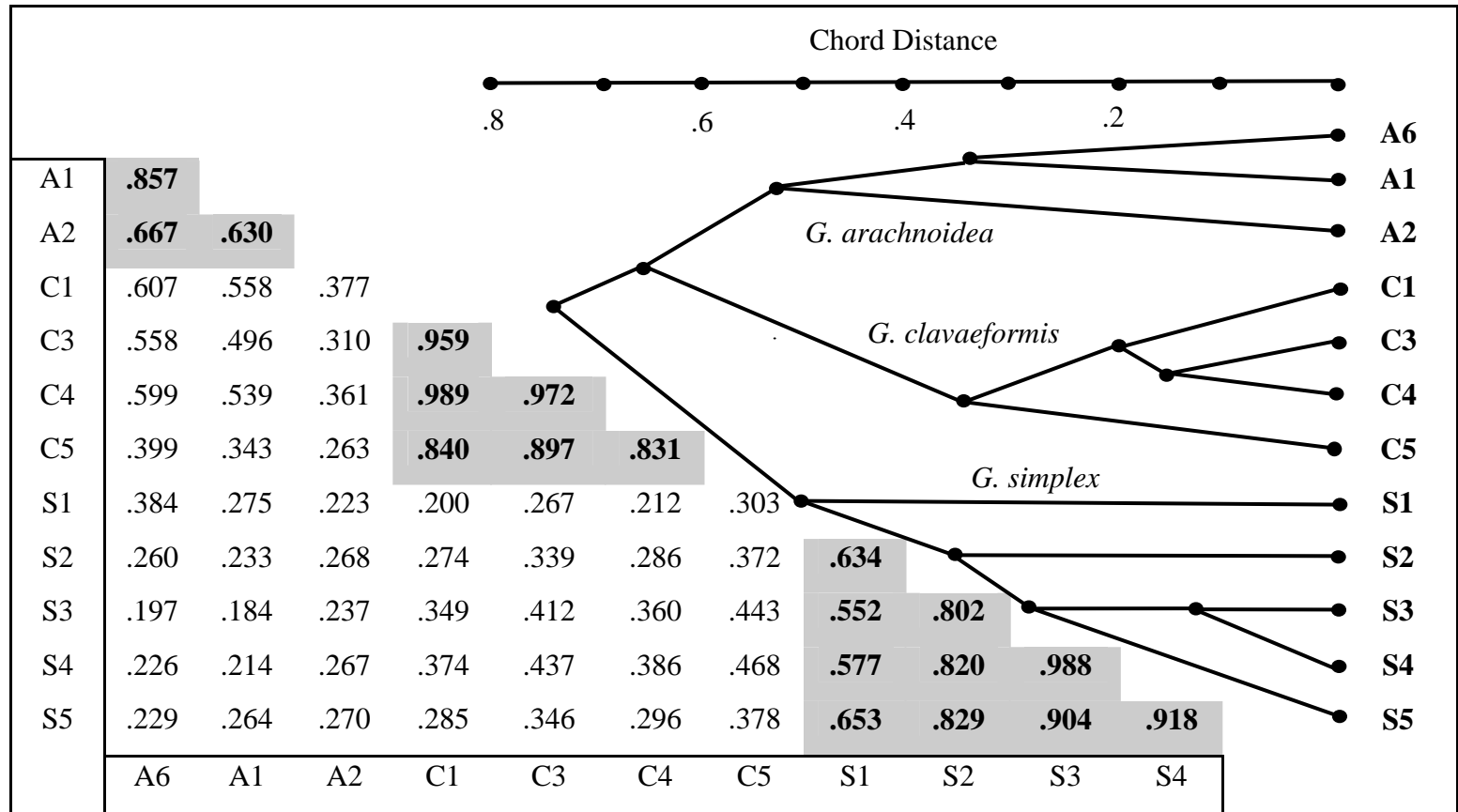


Figure 2. Typical shells of (left to right) *Goniobasis clavaeformis*, *G. simplex*, and *G. arachnoidea*.



Figure 3. Nei's (1978) unbiased genetic identities are shown below the diagonal, with conspecific comparisons shaded. Above the diagonal is the result of a UPGMA cluster analysis based on Cavalli-Sforza and Edwards (1967) genetic distances.



Appendix 1. Locality Data and gastropod species observed. The six sites sampled for the genetic study are listed first, followed by sites visited in the general distributional survey.

**Site 1** (renumbered from VG054) Lee Co., VA. Indian Ck. at Co. 684, Kesterson Mill. Powell drainage. 36.6283; -83.5019. *Goniobasis arachnoidea*, *G. clavaeformis*, *G. simplex*.

**Site 2** (renumbered from VG051) Claiborne Co., TN. head of Gap Creek in town of Cumberland Gap. Powell drainage. 36.6001; -83.6681. *Goniobasis arachnoidea*, *G. simplex*.

**Site 3** (renumbered from VG035B) Wise Co., VA. Big Spring Run, 2 km SE of East Stone Gap by Co. 612. Powell drainage. 36.8581; -82.7299. *Goniobasis clavaeformis*, *G. simplex*.

**Site 4** (renumbered from VG104) Scott Co., VA. Cove Ck., 1 km NE of Kerns by Co. 649. Clinch drainage. 36.7572; -82.6292. *Goniobasis clavaeformis*, *G. simplex*.

**Site 5** (renumbered from VG111) Smythe Co., VA. North Fork Holston R., 1 km NE of Saltville at Va. 91. Holston drainage. 36.8907; -81.7491. *Goniobasis clavaeformis*, *G. simplex*.

**Site 6** (renumbered from VG101) Lee Co., VA. Shafer Ck., 2 km W of Collier Mill at Co. 641. Powell drainage. 36.7146; -83.0426. *Goniobasis arachnoidea*, *G. simplex*.

VG001 Claiborne Co., TN. Little Sycamore Ck., 5 km E of Tazwell at Little Sycamore Rd. Clinch drainage. 36.4363; -83.5229. *Goniobasis arachnoidea*, *G. clavaeformis*, *G. simplex*.

VG002 Claiborne Co., TN. Big Sycamore Ck, 4 km SW of Howard Quarter at Rowe Rd. Clinch drainage. 36.4572; -83.4415. *Goniobasis clavaeformis*.

VG003 Hancock Co., TN. Big Ck., 4 km S of town of Big Creek at Big Creek Rd. Clinch drainage. 36.4659; -83.3351. no snails.

VG004 Hancock Co., TN. Clinch R. at old mill, 10 km SE of Sneedville at by Caney Valley Rd. Clinch drainage. 36.4723; -83.3083. *Goniobasis clavaeformis*, *L. praerosa*.

VG005 Hancock Co., TN. Swan Ck., 10 km SE of Sneedville at Caney Valley Rd. Clinch drainage. 36.4770; -83.2975. no snails.

VG006 Hancock Co., TN. Panther Ck., 6 km W of Sneedville at Jimmie Brooks Rd. Clinch drainage. 36.5442; -83.1521. *Goniobasis clavaeformis*.

VG007 Hancock Co., TN. Clinch R., Kyles Ford at TN 70. Clinch drainage. 36.5712; -83.0400. *Goniobasis clavaeformis*, *Io fluvialis*, *L. praerosa*.

VG008 Hancock Co., TN. North Fork Clinch R. by Co. 626 at Dona. Clinch drainage. 36.5915; -82.9975. *Goniobasis clavaeformis*, *L. praerosa*.

VG009 Scott Co., VA. North Fork Clinch R., 6 km SW of Fairview at Co 621 ford. Clinch drainage. 36.6030; -82.9626. *Goniobasis clavaeformis*.

VG010 Hancock Co., TN. Big Springs, 8 km E of Willis. Clinch drainage. 36.5880; -82.9248. no snails.

VG012 Scott Co., VA. Copper Ck., 4 km E of Carrico Mills at Co. 671. Clinch drainage. 36.7177; -82.4951. *Goniobasis clavaeformis*, *L. praerosa*.

VG013 Scott Co., VA. Valley Ck., 6 km W of Nickelsville at Co. 671. Clinch drainage. 36.7439; -82.4836. *Goniobasis simplex*.

VG014 Scott Co., VA. Jesse Br., 4 km NE of Nickelsville at Va 71. Clinch drainage. 36.7685; -82.3844. *Goniobasis simplex*.

VG015 Russell Co., VA. Copper Ck., 4 km NW of Willow Spring at Co. 678. Clinch drainage. 36.7909; -82.2795. *Goniobasis clavaeformis*, *G. simplex*.

VG016 Russell Co., VA. Little Cedar Ck. at US 19, Lebanon. Clinch drainage. 36.8965; -82.0942. *Goniobasis simplex*, *Physa gyrina*.

VG017 Russell Co., VA. Big Cedar Ck., 4 km E of Lebanon at US 19. Clinch drainage. 36.9068; -82.0388. *Goniobasis clavaeformis*, *G. simplex*.

VG018 Russell Co., VA. Elk Garden Ck., 5 km SE of Rosedale at Va 80. Clinch drainage. 36.9327; -81.9655. *Goniobasis simplex*.

VG019 Russell Co., VA. Hogwallow Br. at Co. 642, Belfast Mills. Clinch drainage. 36.9832; -81.8433. *Goniobasis simplex*.

VG020 Tazwell Co., VA. Little River, 5 km NE of Midway at Co. 610. Clinch drainage. 37.0249; -81.7331. *Goniobasis clavaeformis*, *G. simplex*.

VG021 Tazwell Co., VA. Maiden Spring Ck., 5 km SW of Thompson Valley at end of Co. 1040. Clinch drainage. 37.0488; -81.5623. *Goniobasis simplex*.

VG022 Tazwell Co., VA. South Fork Clinch, 4 km E of Tazwell by Va 61. Clinch drainage. 37.1249; -81.4705. *Goniobasis simplex*.

VG023 Tazwell Co., VA. Clinch R. at Co. 637, Taylors Mill. Clinch drainage. 37.1121; -81.6204. *Goniobasis clavaeformis*, *Physa gyrina*.

VG024 Tazwell Co., VA. Indian Ck., Mouth of Laurel at Co. 627. Clinch drainage. 37.1201; -81.7124. *Goniobasis clavaeformis*.

VG025A Tazwell Co., VA. West Fork Big Ck., 1 km N of Richlands at Va 67. Clinch drainage. 37.1091; -81.7889. *Physa gyrina*.

VG025B Tazwell Co., VA. Big Creek, 1 km N of Richlands by Va 67. Clinch dr. 37.1086; -81.7889. no snails.

VG026 Tazwell Co., VA. Mudlick Ck., at US 460, Doran. Clinch drainage. 37.0929; -81.8343. no snails.

VG027 Russell Co., VA. Sulphur Spring Branch, 1 km N of Dye by Co. 632. Clinch drainage. 37.0746; -81.9219. no snails.

VG028 Russell Co., VA. Clinch R. at Co. 636, town of Swords Creek. Clinch drainage. 37.0324; -81.9174. *Goniobasis clavaeformis*, *L. praerosa*.

VG029 Russell Co., VA. Lewis Ck. at Va 80, Honaker. Clinch drainage. 37.0141; -81.9692. *Goniobasis simplex*, *Physa gyrina*.

VG030 Wise Co., VA. Thacker Branch, 1 km W of Norton at Co. 621. Powell drainage. 36.9318; -82.6527. *Physa gyrina*.

VG031 Wise Co., VA. Powell R. at Co. 610, Norton. Powell drainage. 36.9380; -82.6461. no snails.

VG032 Wise Co., VA. Roaring Fork, 8 km NE of Appalachia by Co. 603. Powell drainage. 36.9342; -82.7227. no snails.

VG033 Wise Co., VA. Powell R., 1 km N of Big Stone Gap by US 23 business. Powell drainage. 36.881; -82.7832. *Goniobasis arachnoidea*.

VG034 Wise Co., VA. Butcher Fork, 3 km E of East Stone Gap at Co. 610. Powell drainage. 36.8825; -82.7166. no snails.

VG035A Wise Co., VA. South Fork Powell R., 2 km SE of East Stone Gap at Co. 612. Powell drainage. 36.8585; -82.7290. *Goniobasis clavaeformis*.

VG036 Wise Co., VA. South Fork Powell R., A. K. Fraley Park by Co. 613, Big Stone Gap. Powell drainage. 36.8669; -82.7664. *Goniobasis clavaeformis*.

VG037 Lee Co., VA. Wade Spring Branch, 2 km S of Olinger by Co. 728. Powell drainage. 36.8002; -82.8474. no snails.

VG038 Lee Co., VA. Powell R., 1 km E of Dryden at US 58A. Powell drainage. 36.7803; -82.9247. *Goniobasis clavaeformis*, *L. praerosa*, *Physa gyrina*.

VG039 Lee Co., VA. North Fork Powell R., 3 km N of Pennington Gap at Co. 606. Powell drainage. 36.7900; -83.0158. no snails.

VG040 Lee Co., VA. Jones Ck., 5 km SW of Robbins Chapel at Co. 628. Powell drainage. 36.8029; -82.9938. no snails.

VG041A Lee Co., VA. Dry Creek at Co. 612, Stickleyville. Powell drainage. 36.7066; -82.9082. no snails.

VG041B Lee Co., VA. Blue Spring, 1 km SW of Stickleyville at Co. 612. Powell drainage. 36.7046; -82.9131. *Goniobasis simplex*.

VG042 Lee Co., VA. Town Branch, 1 km E of Jonesville at US 58. Powell drainage. 36.6868; -83.1040. *Goniobasis arachnoidea*, *G. simplex*.

VG043 Lee Co., VA. Powell R., 10 km SW of Jonesville at Co. 758. Powell drainage. 36.6446; -83.2075. *Goniobasis clavaeformis*, *Io fluvialis*, *L. praerosa*.

VG044 Lee Co., VA. Lone Branch, 4 km SW of Thompson Mill at Co. 612. Powell drainage. 36.6057; -83.1800. *Goniobasis arachnoidea*, *G. simplex*.

VG045 Lee Co., VA. Wallen Ck. at Co. 615, Thompson Mill. Powell drainage. 36.6169; -83.1554. *Goniobasis clavaeformis*, *G. simplex*, *L. praerosa*.

VG046 Lee Co., VA. Dry Creek, 8 km W of Jonesville at Co. 880. Powell dr. 36.6724; -83.2030. no snails.

VG047 Lee Co., VA. Hardy Ck., 3 km E of Wilson Hill at Co. 661. Powell drainage. 36.6463; -83.2430. *Goniobasis arachnoidea*, *G. clavaeformis*, *G. simplex*, *L. praerosa*.

VG048 Lee Co., VA. Yellow Ck., 2 km SW of Wilson Hill by Co. 667. Powell drainage. 36.6311; -83.2929. *Goniobasis arachnoidea*, *G. simplex*.

VG049 Lee Co., VA. Indian Ck. at old US 58, Cowan Mill. Powell drainage. 36.6302; -83.4567. *Goniobasis arachnoidea*, *G. clavaeformis*, *G. simplex*.

VG050 Lee Co., VA. Indian Ck., 2 km SW of Wheeler at Co. 692. Powell drainage. 36.6058; -83.5759. *Goniobasis arachnoidea*, *G. clavaeformis*, *G. simplex*.



- VG052 Claiborne Co., TN. mouth of Indian Ck., 2 km SE of Shawanee at Powell River bridge. Powell drainage. 36.5577; -83.6059. *Goniobasis clavaeformis*.
- VG053 Lee Co., VA. Indian Ck., 3 km SW of Wheeler at Co. 693. Powell drainage. 36.6018; -83.5838. *Goniobasis arachnoidea*, *G. clavaeformis*, *G. simplex*, *L. praerosa*.
- VG055 Lee Co., VA. Indian Ck. at Co. 724, Ewing. Powell drainage. 36.6374; -83.4316. *Goniobasis arachnoidea*, *G. clavaeformis*, *G. simplex*.
- VG102 Scott Co., VA. Horton Branch, 2 km W of Clinchport at Co. 600, Fairview Rd. Clinch drainage. 36.6698; -82.7835. *Goniobasis simplex*.
- VG103 Scott Co., VA. Mill Ck., 10 km NE of Clinchport at Va. 65, Clinch River Hwy. Clinch drainage. 36.7104; -82.6554. *Goniobasis simplex*.
- VG105 Scott Co., VA. Big Stony Ck., 2 km N. of Ft. Blackmore by Co. 619. Clinch drainage. 36.7876; -82.589. no snails.
- VG106 Scott Co., VA. Clinch R. at Va. 72, Ft. Blackmore. Clinch drainage. 36.7667; -82.5824. *Goniobasis clavaeformis*, *G. simplex*, *L. praerosa*, *Io fluvialis*.
- VG107 Scott Co., VA. Staunton Ck., 5 km SW of Dungannon at Va. 72. Clinch drainage. 36.8031; -82.5199. *Goniobasis clavaeformis*.
- VG108 Russell Co., VA. Roaring Branch, 4 km WSW of Mew by Co. 684. Clinch drainage. 36.8454; -82.3825. no snails.
- VG109 Russell Co., VA. Castle Run, 1.5 km W of Banners Corner at Va 65. Clinch drainage. 36.8673; -82.3157. no snails.
- VG110 Wise/Russell Co., VA. Clinch R. at US 58A boat landing, St. Paul. Clinch drainage. 36.9035; -82.3080. *Goniobasis clavaeformis*, *G. simplex*, *L. praerosa*.
- VG201 Scott Co., VA. Little Moccasin Ck by RR tracks in Gate City. Holston drainage. 36.6359; -82.5812. *Goniobasis simplex*.
- VG202 Scott Co., VA. Big Moccasin Ck. along SR 619 at Weber City Water Plant. Holston drainage. 36.6402; -82.5580. *Goniobasis clavaeformis*.
- VG203 Scott Co., VA. Stock Ck, 2 km NE of Sunbright at Co. 718. Clinch drainage. 36.7436; -82.7511. no snails.
- VG204 Scott Co., VA. Valley Branch by Co. 653 at Stanleytown. Clinch drainage. 36.7737; -82.6869. *Goniobasis simplex*.
- VG205 Scott Co., VA. Staunton Ck., 6 km N of Ft. Blackmore by Co. 653. Clinch drainage. 36.8246; -82.5596. *Goniobasis simplex*.
- VG206 Russell Co., VA. tiny tributary of Lick Ck at Co. 615, Hamlin. Clinch drainage. 36.9221; -82.2739. *Goniobasis simplex*.
- VG207 Russell Co., VA. Chaney Ck, 2 km W of Clinchfield by Co. 616. Clinch drainage. 36.9545; -82.2123. no snails.
- VG208 Russell Co., VA. tiny tributary of Clinch R., 1 km W of Cleveland by Co. 600. Clinch drainage. 36.9427; -82.1636. *Goniobasis simplex*.