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Final Report

A SYSTEMATIC STUDY OF THE MORPHOLOGICAL FORMS OF ELLIPSTOMID SNAILS (MOLLUSCA: GASTROPODA: PLEUROCERIDAE) IN THE DUCK RIVER, TENNESSEE, USING ELECTROPHORETIC ANALYSIS

Carol B. Stein and David H. Stansbery
Museum of Zoology

For the Period
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DEPARTMENT OF THE INTERIOR
Fish and Wildlife Service
Office of Endangered Species
Washington, D.C.

Contract No. 14-16-0004-79-092

July 1984

The Ohio State University Research Foundation
1314 Kinnear Road
Columbus, Ohio 43212

OSU

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30 July 1984

Ms. Ruth Slette
Contracting Officer
Division of Contracting and General Services
U.S. Department of the Interior
Fish and Wildlife Service
75 Spring Street SW
Atlanta, GA 30303

Dear Ms. Slette:

Enclosed herewith is the Final Technical Report fulfilling the requirements of Contract No. 14-16-0004-79-092.

The original report contains color photographs. As you requested, we have submitted not only the original, but also five additional copies. These additional copies are in black and white.

Sincerely,

Carol B. Stein

Carol B. Stein
Curator, Division of
Gastropod Mollusks

College of Biological Sciences
Department of Zoology

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by

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INTRODUCTION

River snails of the family Pleuroceridae are abundant members of the benthic animal communities of most relatively unpolluted swift-flowing rivers and streams of North America. Members of the family also occur in Central and South America, Africa, and Asia (Morrison, 1954).

Some 700 different scientific names have been applied to members of this family in North America. The only comprehensive monograph of Pleuroceridae on this continent is that of Tryon, published in 1873. In that work the author recognized some 500 species as valid, noting that fully two thirds of these inhabited the upper Tennessee River and its tributaries or the Coosa River system of the Mobile Basin.

During the past century the streams of both the Tennessee and the Coosa River basins have been greatly altered from their original state by the construction of many impoundments, which convert long stretches of shallow, free-flowing rivers into still-water lakes. In addition, these waterways have carried many kinds of effluents from human activities, from mine wastes and sewage to agricultural pesticides and silt from denuded hillsides. These alterations of the natural riverine environments have greatly changed the conditions of life for the aquatic plants and animals of the southern

Appalachian and Cumberland Plateaus.

Until the past decade, the Duck River in Tennessee had remained relatively free of impoundments. It has continued to support large populations of several forms of pleurocerid snails, many of which are apparently endemic to this river and its tributaries, or which are known from only a few isolated populations outside the Duck River basin.

In the late 1970's the construction of a large Tennessee Valley Authority dam at Normandy eliminated several miles of riverine habitat in the upper reaches of the Duck River. Another major dam at Columbia, now virtually complete, is expected to eliminate an even larger stretch of the middle portion of the Duck River.

On January 12, 1977, the U.S. Department of Interior listed 15 taxa of Pleuroceridae which it proposed for listing as Threatened or Endangered Species pursuant to the Endangered Species Act of 1973 (Greenwalt, 1977). The following five taxa were listed as occurring in the Duck River of Tennessee:

COMMON NAME	SCIENTIFIC NAME	PROPOSED STATUS
Dutton's River Snail	Io armigera duttoniana	Threatened
Jay's River Snail	Io armigera jayana	Threatened
Geniculate River Snail	Io geniculata geniculata	Threatened
Small Geniculate River Snail	Io geniculata penguis (sic)	Threatened
Rugged River Snail	Io salebrosa	Endangered

This proposal aroused considerable discussion among malacologists familiar with these forms. It soon became apparent that both the taxonomic relationships and the nomenclature of these forms were the subject of much controversy and were not at all stable. These taxa are still under review, and

have been listed in the May 22, 1984, Federal Register (Arnett, 1984: 21673) as follows:

COMMON NAME	SCIENTIFIC NAME
Dutton's River Snail	Lithasia duttoniana (Lea, 1841)
Geniculate River Snail	Lithasia geniculata Haldeman, 1840
Jay's River Snail	Lithasia jayana (Lea, 1841)
Small Geniculate River Snail	Lithasia pinguis (Lea, 1852)
Rugged River Snail	Lithasia salebrosa (Conrad, 1834)

This project was undertaken in order to analyze the taxonomic relationships of these five forms, together with all other congeneric and presumably closely related forms which could be found in the Duck River, a tributary of the Tennesse River entirely within the state of Tennessee. This included the smooth form described as fuliginosa by Lea in 1841, which was originally described from Big Bigby Creek, a Duck River tributary, in Maury County, Tennessee.

Virtually all that is known of the systematics and distribution of these river snails is based on previous authors' descriptions and interpretations of their various shell forms and sculpture patterns. We believed it would be advantageous to compare large series of specimens of this complex taken from a variety of localities throughout the length of the river with the original descriptions, figures, and (where reasonably possible) holotypes of the species which had been described in this complex.

Several relatively new taxonomic tools have been proposed as methods of obtaining additional information of value in the study of the systematics of various biological groups. One of the most promising of these is the

application of electrophoretic techniques and histochemical staining methods to the study of the variation of enzymatic proteins. The systematic value of data obtained in this manner has been reviewed by Avise (1975).

Ayala and Powell (1972) studied enzyme differences controlled by alleles at a single locus (allozymes) in natural populations of sibling species of fruit flies of the Drosophila willistoni group. They concluded that allozymic differences could be used as species-diagnostic characters, and reported that between 15% and 32% of the loci studied were diagnostic for any pair of the sibling species they studied. A locus was considered diagnostic if an individual could be correctly assigned to one of two species with a probability of 99% or higher.

Chambers (1978) used the technique of starch gel electrophoresis to analyze specimens of pleurocerid snails of the genus Goniobasis [Elimia] in the Ichetucknee River of Florida. He discovered that there were two distinct groups of snails which lacked common electromorphs at 8 of the 18 loci studied. Instead of the single species previously believed to inhabit the river, he found that two electrophoretically distinct sibling species were living there sympatrically. After comparing the electrophoretic patterns with the shell shape and sculpture, Chambers was able to recognize previously overlooked morphological characteristics which could be used to identify most (but not all) of the shells of these two very similar species.

In a later paper, Chambers (1980) compared genetic diversity as determined by starch gel electrophoresis with the diversity of shell sculptural characteristics of 18 Florida populations of Goniobasis. He discovered that divergence in shell sculptural characters between populations of the G. floridensis complex was often accompanied by little or no genetic divergence, as indicated by the electrophoretic data.

Dillon and Davis (1980) used electrophoretic techniques and shell morphology to study Goniobasis [Elimia] populations in the upper New River and surrounding drainages in the Appalachian Mountains. They reported that three species, identified as G. proxima, G. semicarinata, and G. simplex, "were found to be very distinct using starch gel electrophoresis," and concluded that "compared to electrophoresis, species identification using shell morphology alone was found to be unreliable."

In this study we have applied the techniques of starch gel electrophoresis, as used by Chambers and by Dillon and Davis, to a complex group of river snails, variously known as Pleurocera, Ellipstoma, Io, Lithasia, and Angitrema, in the Duck River. We have also initiated a study of variation in shell morphology among these forms. By comparing the two types of data, we hope to arrive at a better understanding of the actual systematic relationships of these forms, and to determine whether they represented several distinct species, two polymorphic species with clinal variation along the length of the river, or a single interbreeding species with a great amount of polymorphism in shell shape and sculpture.

SPECIMENS EXAMINED

Extensive series of Pleuroceridae collected in the Duck River and other localities by the authors and other staff members and associates of the Ohio State University Museum of Zoology are deposited in the OSUM Gastropod Research Collection. These specimens were available for morphological study, but additional living material was required for electrophoresis.

Most of the specimens used in electrophoresis were collected during an expedition in October, 1982, which included stops at 24 sites in the Duck River

and a few of its major tributaries, from the head of the Kentucky Lake impoundment at the mouth of the Duck (Duck River Mile 11.6) upstream to Fredonia (DRM 275.7).

All specimens were collected by hand picking from cobbles, boulders, bedrock ledges, and occasionally from mud substrates in water less than three feet deep, generally in moderate to swift water. Animals which appeared to belong to the species complex under study were placed in plastic bags with field labels at the site, and were immediately frozen on dry ice in portable insulated containers. At the end of the collecting trip the specimens were placed in a freezer, where they were kept until they were electrophoresed.

At least 100 large, presumably adult specimens of this complex were collected at each of 8 sites. Localities from which specimens were electrophoresed are shown in Figure 1. Representative series of shells from each locality are also figured. The collecting sites are:

1. SR13. Duck River at DRM 24.6, at the State Route 13 bridge (= the Walter H. and C.B. Jones Bridge) 2.5 miles SSE of Hurricane Mills, 10.3 miles S of Waverly, Humphreys Co., Tennessee.

2. BHR. Duck River at DRM 32.2, at Barren Hollow Road bridge 1 mile WNW of Only, 7.4 mi. NW of Coble, Hickman Co., Tennessee.

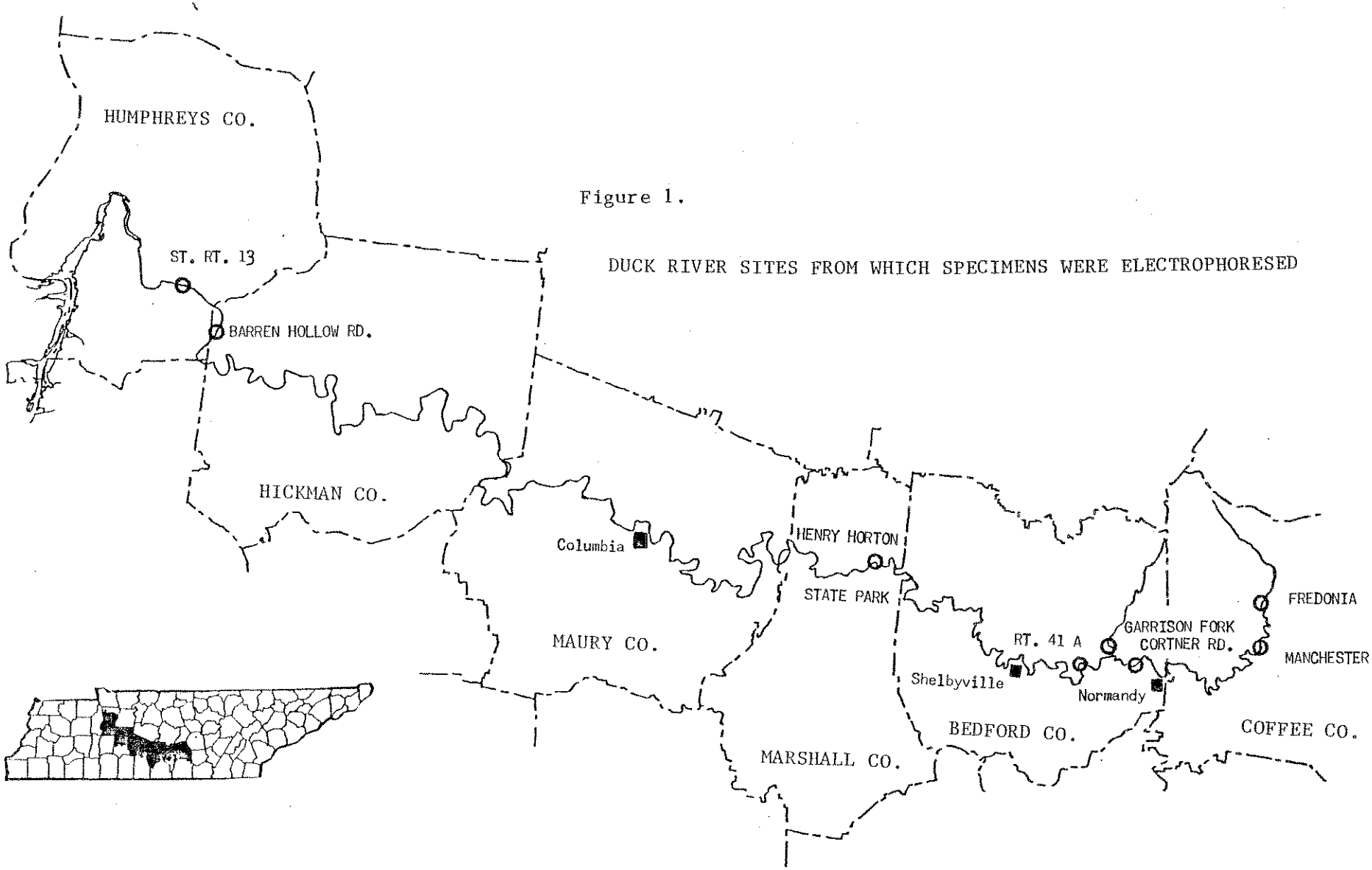
3. HHSP. Duck River at DRM 186.5, at Henry Horton State Park, 2.4 miles S of Chapel Hill, 11 miles NNE of Lewisburg, Marshall Co., Tennessee.

4. R41A. Duck River at DRM 235.3, at Mullins Mill Access Area above the U.S. Route 41A bridge 4.8 miles E of Shelbyville, 4.8 miles SSW of Wartrace, Bedford Co., Tennessee.

5. GFK. Garrison Fork 0.5 mile above its confluence with Duck River, at Haley Road bridge (=Cannon Bridge) 2.9 miles S of Wartrace, 1.5 miles SW of Bugscuffle, 6.0 miles E of Shelbyville, Bedford Co., Tennessee.

Figure 1.

DUCK RIVER SITES FROM WHICH SPECIMENS WERE ELECTROPHORESED



6. CORT. Duck River at DRM 242.5, at Cortner Road bridge (=Dement Bridge) 1.0 mile W of Cortner, 2.2 miles WNW of Normandy, Bedford Co., Tennessee.

7. MAN. Duck River at DRM 269.4 at the foot of low dam at Old Stone Fort State Park Visitors' Center, above the mouth of Little Duck River, in NW Manchester, Coffee Co., Tennessee.

8. FRE. Duck River at DRM 275.7, at bridge just E of Fredonia, 4.7 miles N of Manchester, Coffee Co., Tennessee.

Specimens of the form described as geniculata from the Barren Hollow Road (BHR) population were chosen as a reference population or standard against which to compare other populations and morphological forms for three reasons: 1. A very large population of animals of this form occurred at BHR. 2. This form seemed to be more widely divergent from the other described forms in the lower reaches of the Duck River than in the headwaters. 3. The site, which was located just off I-40, was easy to reach, and the river was relatively easily accessible at this point.

A few specimens of the pleurocerid snail Mudalia (Elimia ?) livescens (Menke, 1830) from Rocky Fork Creek, at the U.S. Rt. 62 bridge 3.7 miles NE of Gahanna, Franklin Co., Ohio, were also electrophoresed for comparison with the Duck River forms.

ELECTROPHORESIS

Snails to be used in electrophoresis were stored in plastic bags at -20°C until the day they were to be electrophoresed. The specimens to be used that day were removed from the freezer and placed in a dish of ice and water, where they were allowed to warm to the freezing point. Only large specimens, presumed to be adult, were used. The snail's columellar muscle was severed with a concavely curved scalpel blade, and the body was then "unscrewed" from the shell and placed in a small iced glass dish. The operculum was removed and placed with the shell in a numbered box. The snail

body was then examined with a dissecting microscope to determine its sex. Specimens having an egg-laying groove in the right side of the body, as described and figured by Morrison (1954), were considered to be females; specimens lacking such a groove were assumed to be males.

The entire animal body was then minced, using a scalpel and forceps. A small amount of distilled water, approximately equal to the volume of the minced animal body, was added to a test tube along with the minced snail tissue. The test tube was then placed in a beaker of crushed ice and water where it was held as the tissue was homogenized. A Brinkman Polytron homogenizer equipped with a 12 mm diameter sawtooth probe generator combined mechanical shearing action with ultrasonic vibration to produce a liquid snail homogenate. The test tube of homogenate was then placed in a numbered slot in a test tube rack which was kept chilled in a pan of ice and water.

The tissue samples were applied to the gels as Whatman filter paper tabs, approximately 3 x 8 mm, which were dipped into the snail homogenate samples, blotted, and inserted into the slit of the starch gel in numerical sequence from left to right. Results obtained with centrifuged homogenates were indistiguishable from those obtained without centrifugation, so we did not centrifuge them.

Each tab number corresponded with the number of the individual shell, its operculum, and the vial of homogenate, which was frozen for future reference. In this way, any electrophoretic variation could be compared directly with the shell morphology of the particular specimen.

All shells of specimens used in the electrophoresis were cleaned and deposited as voucher specimens in the Ohio State University Museum of Zoology Gastropod Research Collection.

On each gel, the samples were applied in sequential groups of 2 or more specimens from one locality or described form alternating with several

specimens from another locality or form. In this way, similarities and differences of electromorphs between the sample populations could be recognized easily.

Horizontal starch gel electrophoresis was carried out using the methods described and figured by Schaal and Anderson (1974). These are essentially the same as the methods used by Dillon and Davis (1980) and by Chambers (1977) in their electrophoretic studies of Pleuroceridae.

Electrophoresis was carried out in a large refrigerator kept at 4°C, with pans of ice and water placed on top of the gels to prevent them from overheating as they were running. Current to the gels was regulated with Heathkit high voltage power supply units, Model IP-2717. Usually five gels were electrophoresed simultaneously, each bearing the same sequence of samples from the same specimens.

Following electrophoresis each gel was removed from its tray and the upper left-hand corner of the main gel and the lower left-hand corner of the backslice were trimmed diagonally so the starting point of the numerical sequence of samples could be recognized. Each gel was then sliced horizontally into three slices, each of which was placed in a staining dish with a particular histochemical stain. The gel slices and staining solutions were incubated as necessary, and when the zymograms reached full development they were plced on a light box and photographed with their label, using color slide film. They were then fixed overnight in a solution of 5 parts methanol, 5 parts distilled water, and 1 part glacial acetic acid (Bush & Huettel, 1972: 16), after which they were wrapped in saran wrap, labeled, and placed in a refrigerator for storage. Gels so fixed were photographed as much as two years later to produce the gel illustrations accompanying this report. Gels

developed using agar overlays could not be fixed, so we used agar as rarely as possible---mainly for MPI and some OCT gels.

Stains used in this study were screened on several of the following buffer systems, and the buffer giving the best results was selected for each stain.

Stains which produced scorable results (see Table 1) were:

ACPH/D+D	Acid Phosphatase	Dillon & Davis, 1980: 88
ALPH/B+H	Alkaline Phosphatase	Bush & Huettel, 1972: 21
GOT/D+D	Glutamate-Oxaloacetate Transaminase	Dillon & Davis, 1980: 88
MPI/D+D	Mannose-6-Phosphate Isomerase	Dillon & Davis, 1980: 88
OCT/D+D	Octopine Dehydrogenase	Dillon & Davis, 1980: 88
PGI/B+H	Phosphoglucose Isomerase	Bush & Huettel, 1972: 28

Stains which were generally not scorable, but which were sufficiently well resolved to provide some evidence bearing on the problem were:

ACPH/B&H	Acid Phosphatase	Bush & Huettel, 1972: 20
ALDO/S+P	Aldolase	Shaw & Prasad, 1970: 302
EN/B+H	Enolase	Bush & Huettel, 1972: 21
EST/B+H	Esterase	Bush & Huettel, 1972: 21
EST/D+D	Esterase	Dillon & Davis (pers. com.)
EST/S+A	Esterase	Schaal & Anderson, 1974: 9
GP/SEL	General Proteins	Selander et al., 1971: 88
HEXDH/D+D	Hexanol Dehydrogenase and Superoxide Dismutase	Dillon & Davis, 1980: 88
ISDH/B+H	Isocitrate Dehydrogenase	Bush & Huettel, 1972: 25
LAP/D+D	Leucine Aminopeptidase	Dillon & Davis, 1980: 88
MDH/B+H	Malate Dehydrogenase	Bush & Huettel, 1972: 25

PEPSIN/B+H	Pepsinogen	Bush & Huettel, 1972: 22
PGM/AYALA	Phosphoglucomutase	Ayala notes (via Chambers)
PROT/AYALA	Protein	Ayala notes (via Chambers)
SDH/AYALA	Sorbitol Dehydrogenase	Ayala notes (via Chambers)

Stains which gave some indication of enzyme activity, but which could not be clearly or consistently resolved to be useful in providing taxonomic evidence were:

ADH/B+H	Alcohol (Isopropyl) Dehyudrogenase	Bush & Huettel, 1972: 28
AO/B+H	Aldehyde Oxidase	Bush & Huettel, 1972: 22
CAT/S+A	Catalase	Schaal & Anderson, 1974: 8
CK-TO/S+A	Creatine Kinase & Tetrazolium Oxidase	Schaal & Anderson, 1974: 8
FUM/ B&H	Fumarase	Bush & Huettel, 1972: 27
G3PD/AYALA	Glyceraldehyde-3-Phosphate Dehydrogenase	Ayala notes (via Chambers)
G6PD/AYALA	Glucose-6-Phosphate Dehydrogenase	Ayala notes (via Chambers)
MDH-T/B+H	Malate Dehydrogenase	Bush & Huettel, 1972: 25
6PGD/B+H	6-Phosphogluconate Dehydrogenase	Bush & Huettel, 1972: 26
SDH/D+D	Sorbitol Dehydrogenase	Dillon & Davis, 1980: 88
XDH/AYALA	Xanthine Dehydrogenase	Ayala notes (via Chambers)

Stains which were screened with no positive results were:

EADH/S+A	Ethyl Alcohol Dehydrogenase	Schaal & Anderson, 1974: 17
ALDO/DILLN	Aldolase	Dillon, 1982: 142
GAL-DH/B+H	Galactose Dehydrogenase	Bush & Huettel, 1972: 23
GLU-DH/B+H	Glutamate Dehydrogenase	Bush & Huettel, 1972: 24

HEXOK/ B+H	Hexokinase	Bush & Huettel, 1972: 28
ME/AYALA	Malic Enzyme	Ayala notes (via Chambers)
MO/B+H	Monoamine Oxidase	Bush & Huettel, 1972: 22
ODH/B+H	Octanol Dehydrogenase	Bush & Huettel, 1972: 26
TYRO/B+H	Tyrosinase	Bush & Huettel, 1972: 23
XDH/D+D	Xanthine Dehydrogenase	Dillon & Davis, 1980: 88

RESULTS OF ELECTROPHORESIS

Allele (electromorph) frequencies for 6 scored loci are shown in Table 1. In general, the specimens sampled from the seven downstream populations show very little electrophoretic variation. The GOT locus proved monomorphic for all Duck River specimens examined, even the animals representing four different genera and species occurring sympatrically at Henry Horton State Park (see Fig. 6). Only one individual, a Cortner Road specimen, was scored as having a faster allele than the normal one. "Shadow bands" were typical of GOT zymograms on LiOH gels. As shown in Figure 7, specimens from the Fredonia population showed much less intensely stained shadow bands, but this does not appear to represent a genetic or taxonomic difference. The PGI locus also showed little electrophoretic variation, with all Duck River populations sampled showing at least 92.9% "normal" alleles. The Fredonia specimens all were homozygous for this allele. A similar pattern was found at the MPI locus for the populations which could be scored for this enzyme, although the population at the U.S. Route 41A bridge seemed more polymorphic, with a third of its alleles faster than the common electromorph.

The ACPH and ALPH loci also show highly homogeneous results for the seven downstream populations, with only a scattering of variant alleles at these sites, as shown in Table 1 and Figs. 2 and 4. The Manchester population

Table 1. Allele (electromorph) frequencies in 8 populations of pleurocerid snails of the Ellipstoma gibbosa complex in the Duck River basin, Tennessee.

		POPULATION LOCALITY							
LOCUS	ALLELE	R13	BHR	HHSP	R41A	GFK	CORT	MAN	FRE
ACPH	115	---	---	---	---	---	---	---	.333
	111	---	.012	---	---	---	---	---	---
	106	---	---	---	---	---	---	---	.333
	105	---	---	---	---	---	---	---	.333
	102	---	.081	.029	.048	.061	.062	---	---
	100	1.00	.895	.971	.952	.939	.885	1.00	---
	98	---	---	---	---	---	.042	---	---
	97	---	---	---	---	---	.010	---	---
	88	---	.012	---	---	---	---	---	---
Number of Animals Scored		4	166	70	21	66	96	30	6
ALPH	120-125		---	---	---	---	---	---	.867
	105		---	---	.015	---	---	---	---
	104		.032	---	---	---	---	---	.133
	102		.040	.018	.060	.089	.023	---	---
	100		.911	.973	.924	.911	.943	1.00	---
	98		.012	---	---	---	.034	---	---
	97		.006	---	---	---	---	---	---
	95		---	.009	---	---	---	---	---
Number of Animals Scored		0	174	57	33	45	87	31	15
GOT	104	---	---	---	---	---	.016	---	---
	100	1.00	1.00	1.00	1.00	1.00	.983	1.00	1.00
Number of Animals Scored		14	119	30	15	42	60	52	14
PGI	Fast		.024	.042	.049	.071	.014	.071	---
	100		.943	.958	.951	.929	.979	.929	1.00
	Slow		.033	---	---	---	.007	---	---
Number of Animals Scored		0	122	24	41	70	71	7	6
OCT	Fast + 5	.143	---	---	---	---	---	---	---
	Fast	.143	.429	.448	.308	.333	.435	.012	.937
	Slow	.714	.571	.552	.692	.667	.565	.988	.063
Number of Animals Scored		14	111	29	13	6	46	43	16
MPI	Fast	.150	.015	---	.342		.021		
	Normal	.800	.961	.976	.658		.979		
	Slow	.050	.024	.024	---		---		
Number of Animals Scored		10	102	41	38	0	47	0	0

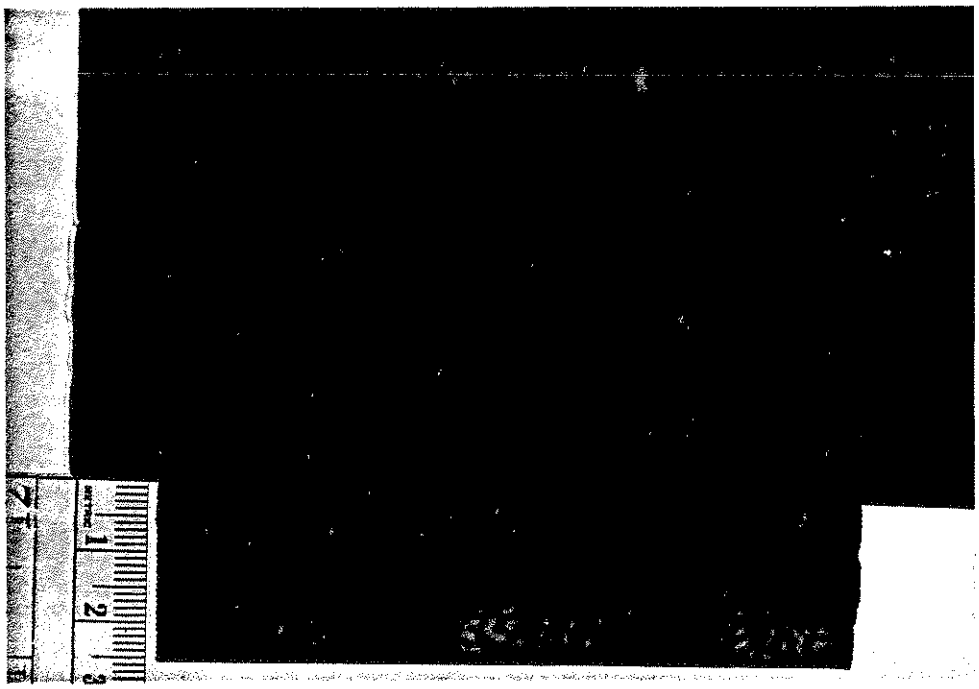


Figure 2. This typical ACPH gel shows samples of E. gibbosa from the widely-separated localities BHR (from left, # 1-5 and 11-15) and CORT (# 6-10, 16-20), all monomorphic for the normal allele.

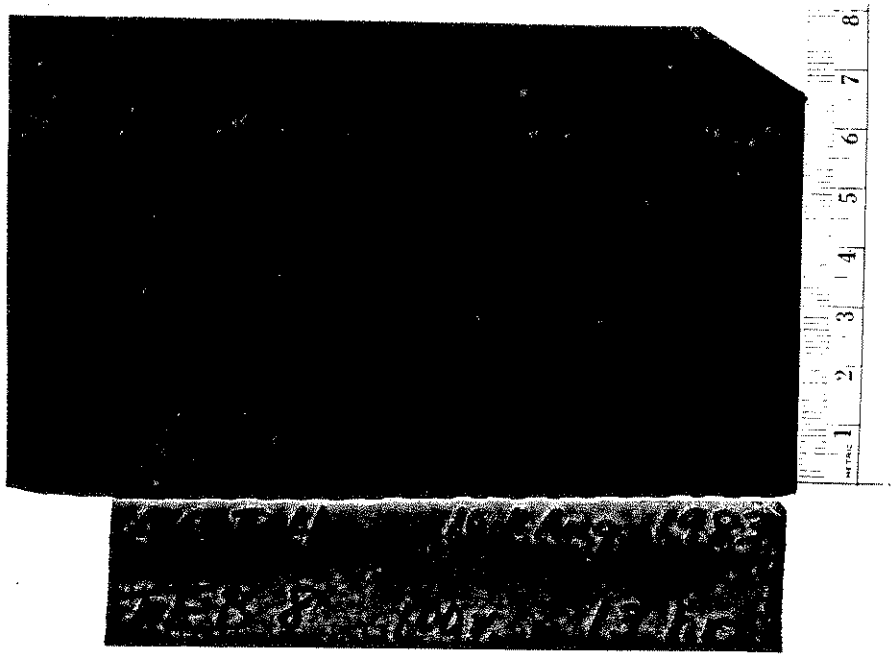


Figure 3. This poorly-resolved and unscorable ACPH gel nevertheless indicates distinct differences between the geographically close but taxonomically distant populations formerly believed to represent pinguis at Manchester (from right, #1-3, 9-11, and 18-20) compared to Fredonia (#4-8, 12-17).

Figure 4. The lack of variation between E. gibbosa populations at BHR (from the right, # 1-5 and 11-15) and at CORT (#6-10 and 16-20) is evident in this typical ALPH zymogram.

Figure 5. A much faster-migrating ALPH electromorph characterizes the so-called "pinguis" population at Fredonia (from left, # 4-8 and 12-17) compared to the Manchester population of E. gibbosa (# 1-3, 9-11, and 18-20).

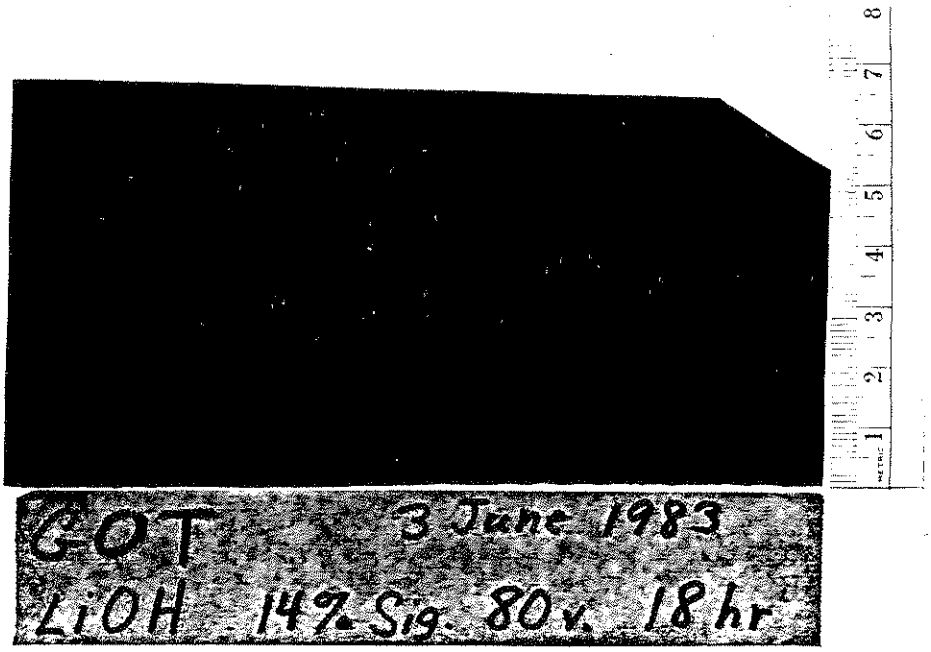


Figure 6. Samples representing four different genera of sympatric Pleuroceridae (from right: #1-5, Elimia laqueata (Say, 1829); #6-10, Leptoxis praerosa (Say, 1821); #11-15, Pleurocera canaliculata (Say, 1821); and #16-20, Ellipstoma gibbosa Rafinesque, 1818, all from HHSP) show very little variation in GOT mobility or staining intensity, except for two of the laqueata which lack the "shadow bands."

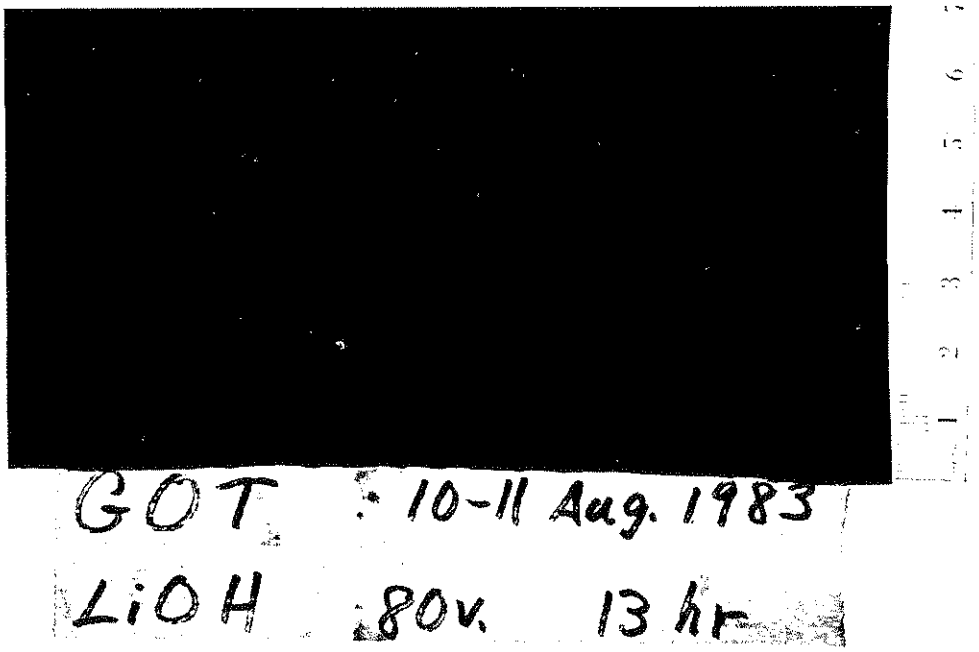


Figure 7. E. gibbosa specimens from MAN (#1-3, 9-11, and 18-20) exhibit much darker staining "shadow bands" than the FRE specimens (#4-8 and 12-17), although all are scored monomorphic for the GOT locus represented by the lower row of bands. Although striking in appearance, this difference is probably not of taxonomic significance.

Figure 8. Four sympatric genera of Pleuroceridae from HHSP exhibit distinctly different electromorph patterns in this LAP zymogram. From the left, the species represented are: #1-5, Elimia laqueata (Say, 1829); 6-10, Leptoxis praerosa (Say, 1821); 11-15, Pleurocera canaliculata (Say, 1821); and 16-20, Ellipstoma gibbosa Rafinesque, 1818.

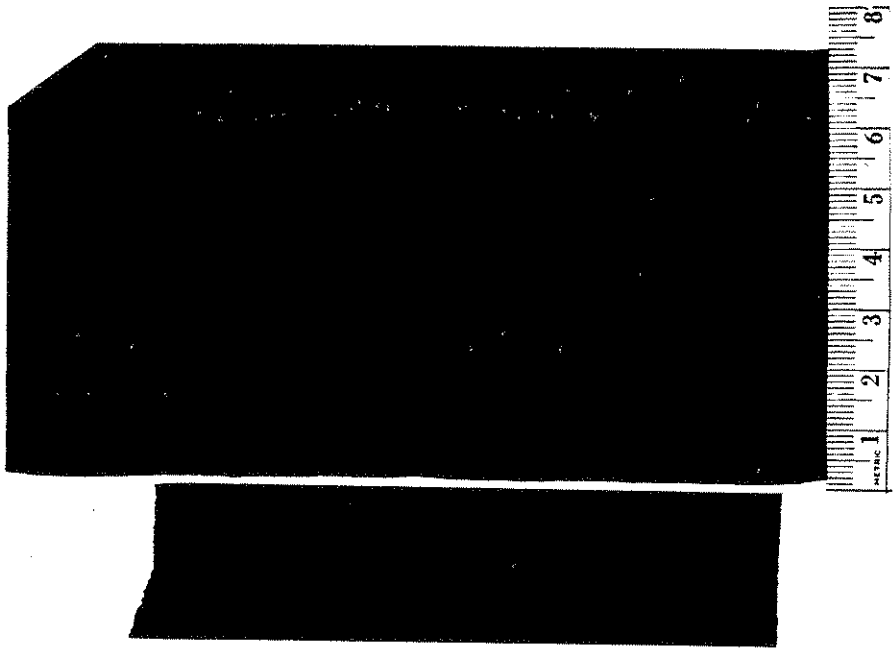


Figure 9. There appear to be two alleles present but not clearly resolved in the row of bands shown between 40 and 50 mm from the origin in this LAP zymogram. However, no sharp differences at this locus can be distinguished between the two E. gibbosa populations sampled: from left, 1-2, 10-11, and 19-20 are from BHR; 3-9 and 12-18 are from R41A. BHR specimens usually, but not always, exhibit additional bands on LAP gels, such as the one shown here. These migrate only about half as far as the normal electromorph, and probably represent a different locus. These slow bands were not seen on gels from other Duck River populations sampled.

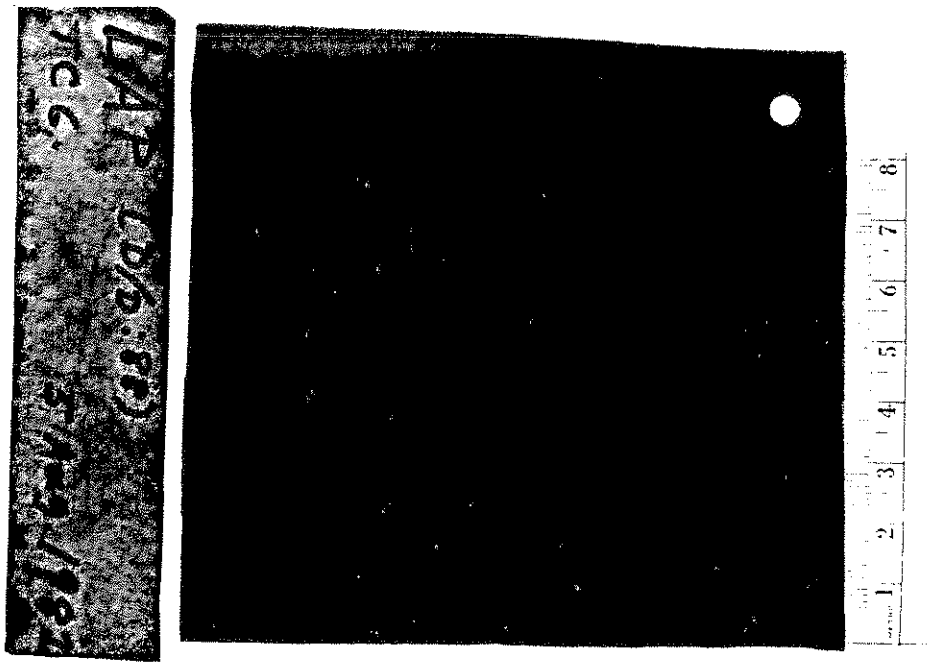


Figure 10. Gel heterogeneity apparently caused the LAP enzymes at the right to migrate more slowly than those at the left in this zymogram, creating a slanted row of bands. Nevertheless, a distinct difference can be seen between the electromorph pattern shown by the five Ohio specimens of Mudalia livescens in the center (#6-10) and that of the BHR specimens of E. gibbosa form geniculata (#1-5, 11-15).

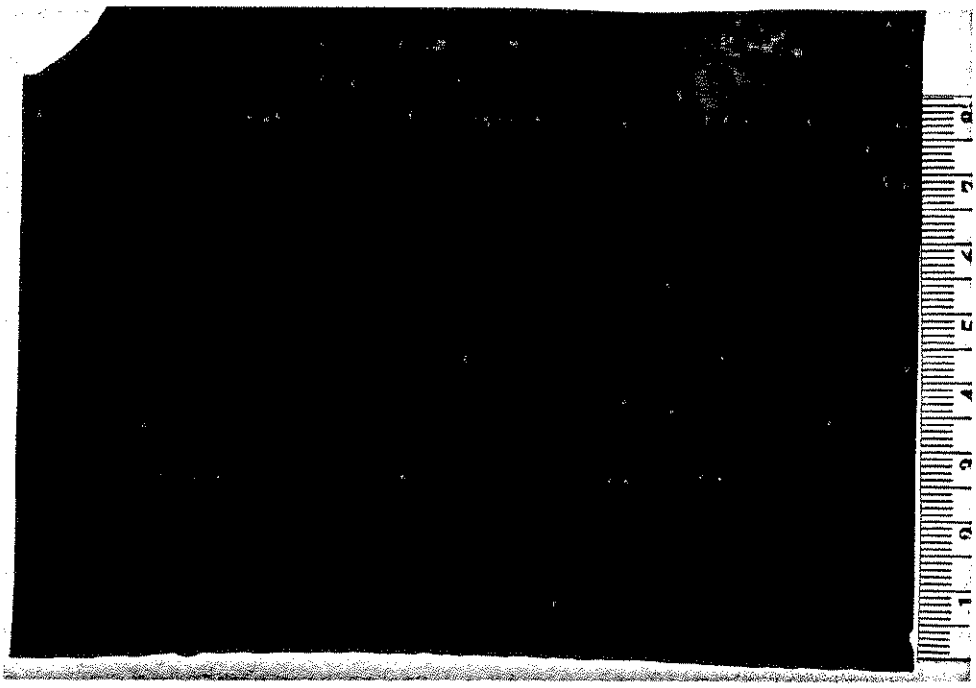


Figure 11. The sharp differences in LAP mobility between the MAN population (from left, # 1-3, 9-12, and 18-20) and the FRE population (#4-8, 12-17) were not seen in any of the Duck River populations sampled from Manchester to the river's mouth. The Fredonia specimens consistently show a slower electromorph than do the specimens found at or below Manchester.

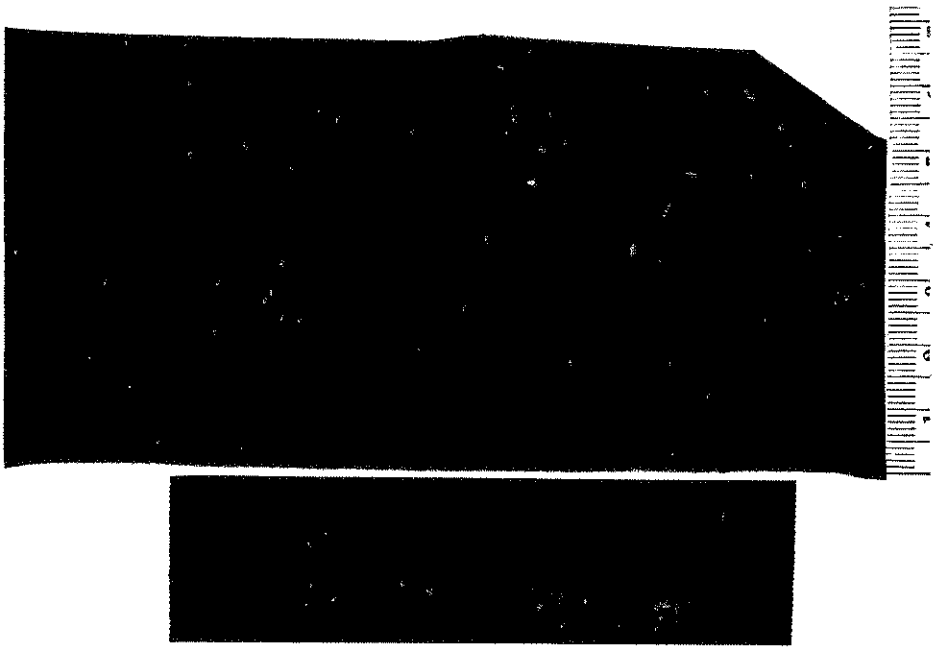


Figure 12. Both fast and slow alleles of OCT are common in populations of E. gibbosa below Manchester, and heterozygotes are common, as shown in this comparison of specimens from BHR (from right, # 1-6 and 13-16) and from CORT (# 7-12 and 17-20).

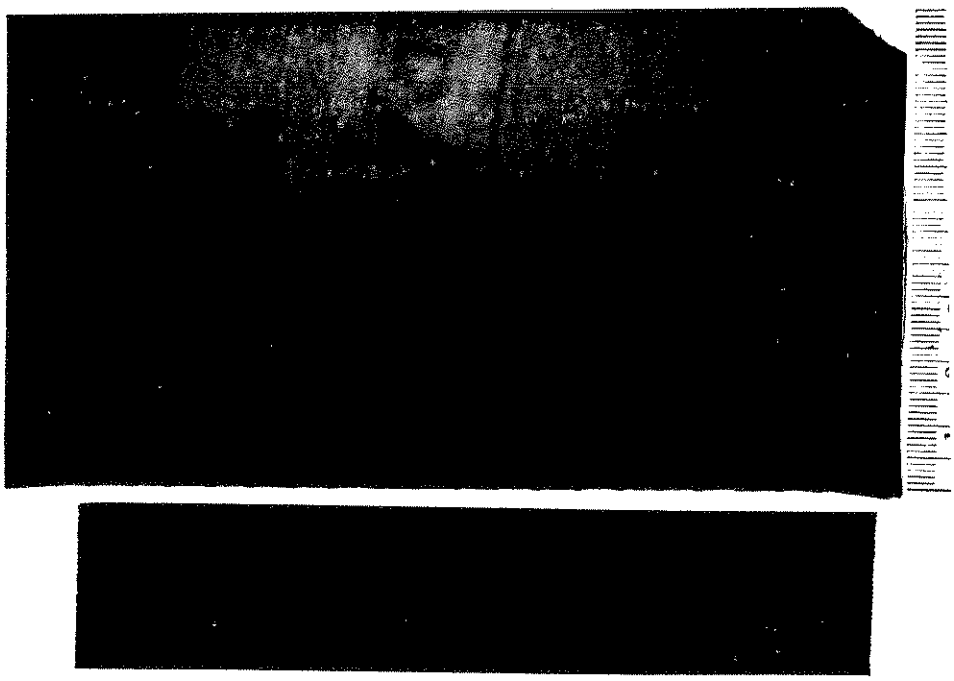


Figure 13. At Manchester, the slow allele of OCT has a frequency of .988, while the fast allele was very rare, with a frequency of only .012. This reduction in variability parallels the lessened variability in shell sculpture which is also found at Manchester. Specimens #3-8 and 12-18 in this photograph are from MAN; the others are from the highly variable BHR population.

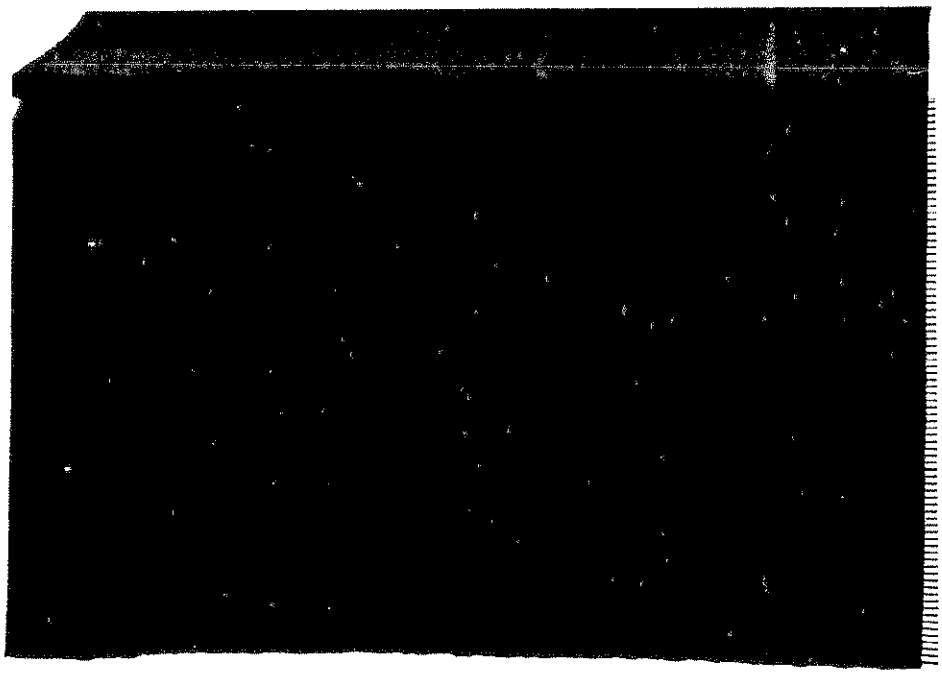


Figure 14. Sharp differences in gene frequencies at the OCT locus occur between the populations sampled at Manchester (#1-3, 9-11, and 18-20), which is virtually fixed for the slow allele, and the Fredonia population (#4-8 and 12-17) which is nearly fixed for the fast allele. One heterozygous FRE specimen, # 17, is visible in this figure. While the two electromorphs of the FRE population appear to be identical to the two alleles found in the E. gibbosa complex, they may not be genetically homologous.

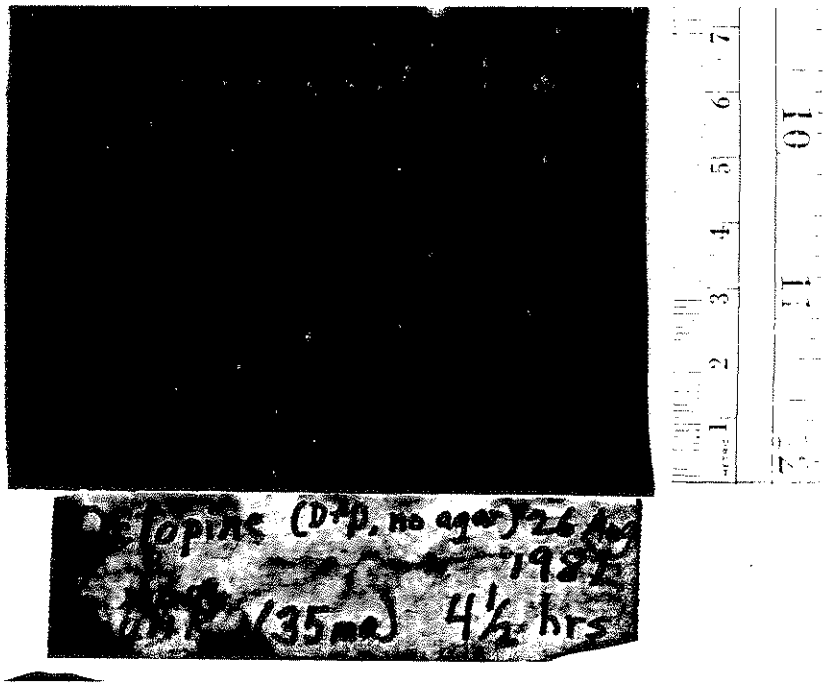


Figure 15. The five M. livescens (#6-10) from Rocky Fork Creek near Gahanna, Ohio, exhibit a monomorphic, fast-migrating OCT electromorph which differs sharply from both alleles of the BHR E. gibbosa (#1-5, 11-15).

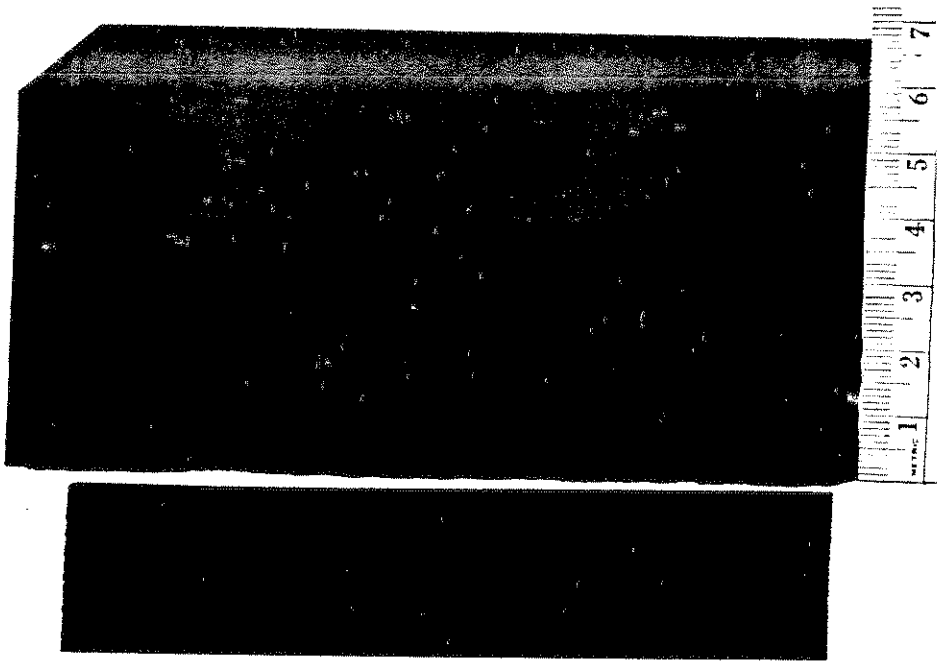


Figure 16. The PGI locus was essentially monomorphic in all of the Duck River populations sampled, as shown in this zymogram. Occasional clearly defined bands found well above the normal electromorph, as shown in #16 and #18 here, were interpreted as alternate alleles in the frequency table, but it is possible that they actually represent another locus. Specimens #1-2, 10-11, and 19-20 are from BHR; #3-9 and 12-18, from R41A.

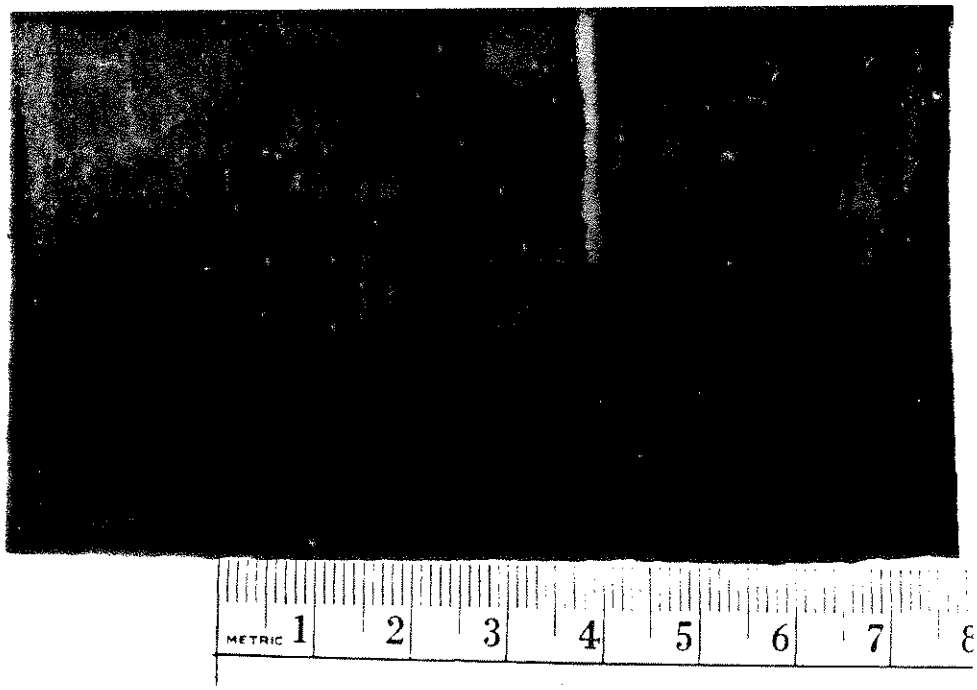


Figure 17. A distinctly slower-migrating PGI electromorph characterized all specimens of the taxonomically and geographically distant M. livescens specimens from Ohio which were electrophoresed in this study as a comparison to the Duck River E. gibbosa. On this gel, from the left, #1-2 and 6-10 are M. livescens; #3-5 and 11-15 are from BHR.

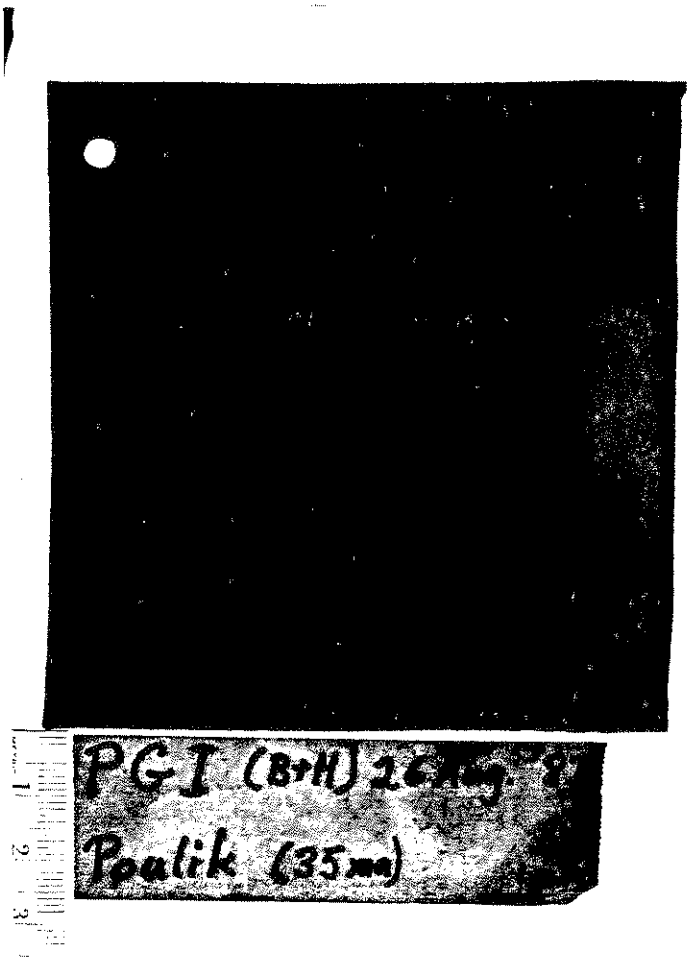


Figure 18. Taxonomically significant variation in electrophoretic mobility at the PGI locus is clearly shown in this zymogram. The five M. livescens (#5-10) appear to have a monomorphic electromorph which is slower than that of the BHR specimens (1-5 and 11-15) on each side of it, though #9 is not clearly resolved and would not be scored.

proved 100% monomorphic for the normal ACPH and ALPH alleles. However, less than five miles north of Manchester, at the Fredonia site, no specimens having any ACPH or ALPH alleles in common with any of the downstream populations were found. All of the Fredonia specimens which could be scored (and even some unscorable ones---see Fig. 3) exhibited faster-migrating electromorphs for these loci than did the specimens collected at or below Manchester.

The OCT locus appeared to be dimorphic in the Duck River, with both fast and a slow alleles common in the populations from Barren Hollow Road upstream to the Cortner Road bridge near Normandy. The slower of these two alleles was consistently more common than the faster one in these populations, however, and at Manchester the slower electromorph was virtually fixed, with a frequency of .988 in the 43 specimens scored. Yet just a few miles upstream at Fredonia, the frequency of this electromorph dropped to .063, and the fast electromorph virtually replaced it, with a frequency of .937. At the farthest-downstream site, State Route 13 bridge, 8 specimens were homozygous for the slow allele, 4 were heterozygous for both fast and slow alleles, and two specimens were homozygous for a third electromorph, which migrated faster than either of the two "normal" ones. This was the only population in which this allele was found. Its presence in this lowermost population suggests that the jayana form found there may have been exchanging genes with a genetically different population in the Tennessee River, at least prior to the habitat interruption created by the Kentucky Lake dam.

The LAP locus was not scored because the resolution of the zymograms was insufficient to determine whether one or two alleles occurred at the zone of enzymatic activity. However, the populations of E. gibbosa sampled in the Duck River from Manchester to State Route 13 were remarkably uniform in their electrophoretic mobility at the LAP locus, as shown in Figure 9. Many specimens from Barren Hollow Road showed not only the normal electromorph, but

also a second band which migrated only half as fast, and which probably represents a second locus. Sharp differences in LAP mobility were found between the Manchester population, which showed the same electromorph found in the downstream populations, and the Fredonia population, which exhibited a consistently slower electromorph at this locus, as shown in Figure 11.

The LAP zymogram of Elimia laqueata (Say, 1829), Leptoxis praerosa (Say, 1821), Pleurocera canaliculata (Say, 1821), and Ellipstoma gibbosa Rafinesque, 1818, all from Henry Horton State Park, shows dramatically different electromorphic patterns for each of these four sympatric pleurocerid species (Fig. 8). A distinct difference in electrophoretic mobility was also seen in specimens of Mudalia (Elimia?) livescens (Menke, 1830) from Rocky Fork Creek in Ohio when compared with E. gibbosa from Duck River at Barren Hollow Road (Fig. 10).

The similarities and differences found in the electrophoretic patterns of the various populations examined have been summarized in Table 2. The sharp differences in allele (electromorph) frequencies found at four of the six loci between the populations sampled at Fredonia and Manchester strongly support the inference that these two populations represent different species, and probably different genera. This agrees with our evidence for their separation on the basis of conchological characteristics.

No such differences were found to occur in any of the populations of this species complex examined from Manchester, Cortner Road, Garrison Fork, U.S. Route 41A, Henry Horton State Park, Barren Hollow Road, or State Route 13. Nor could any electrophoretic variation be found correlated with the variation in shell sculpture which occurs at these localities. We therefore conclude that the electrophoretic evidence available supports the conchological evidence that these forms intergrade with one another and are a single interbreeding species, not sibling species or even subspecies.

Table 2. Summary of inferences drawn from the electrophoretic data concerning the genetic similarities and differences among the pleurocerid snail populations examined during this study.

LOCUS	All *E. gibbosa*: R13, BHR, HHSP, R41A, GFK, Cort, and MAN	Manchester versus Fredonia	4 Genera of Pleuroceridae: H. Horton St.Pk.	Ohio *M. livescens* versus BHR *E. gibbosa*
ACPH	SAME (Fig. 2)	DIFFERENT (Fig. 3)	SAME	SAME
ALPH	SAME (Fig. 4)	DIFFERENT (Fig. 5)	PROBABLY SAME	DIFFERENT
GOT	SAME	PROBABLY SAME (Fig. 7)	SAME (Fig. 6)	NO DATA
LAP	SAME (Fig. 9)	DIFFERENT (Fig. 11)	DIFFERENT (Fig. 8)	DIFFERENT (Fig. 10)
OCT	SAME (Figs. 12, 13)	DIFFERENT (Fig. 14)	DIFFERENT	DIFFERENT (Fig. 15)
PGI	SAME (Fig. 16)	SAME	PROBABLY SAME	DIFFERENT (Figs. 17, 18)
PROBABLE TAXONOMIC STATUS	SAME	DIFFERENT	INCONCLUSIVE	DIFFERENT

The electrophoretic differences seen in the fixation of alternate electromorphs between the four pleurocerid genera at Henry Horton State Park and also between the Ohio specimens of M. livescens and the E. gibbosa from Barren Hollow Road indicates that these electrophoretic methods are of value in pleurocerid systematics.

METHODS OF MORPHOLOGICAL ASSESSMENT

The examination of the morphological variability of Duck River Ellipstoma shells was undertaken largely independently of the electrophoretic work in an effort to discover what this approach could provide in the way of data to be used in working out the systematic relationships of the various described taxa. We examined all of the OSUM lots of this complex, representing nearly every point of access on the main stem and from a number of tributaries. These were mainly large lots, several including more than 500 individuals. We also examined the shells of the specimens which were used in electrophoresis.

Morphological characteristics of some of the headwaters lots were recorded in tabular form, but this approach was so time-consuming that doing it for all specimens examined was beyond the scope of this project. Our primary objective was to examine the variability exhibited by the specimens and to relate this to the various described taxa of this complex from each site throughout the length of the river. Specimens were laid out in various patterns according to their morphological characteristics and degree of intergradation between taxon-groups, as well as the site-to site variation, until inferences as to their probable relationships could be drawn.

Original descriptions, figures, previous literature, and, where possible, type specimens were studied in order to produce the best possible taxonomic and nomenclatorial treatment of this complex.

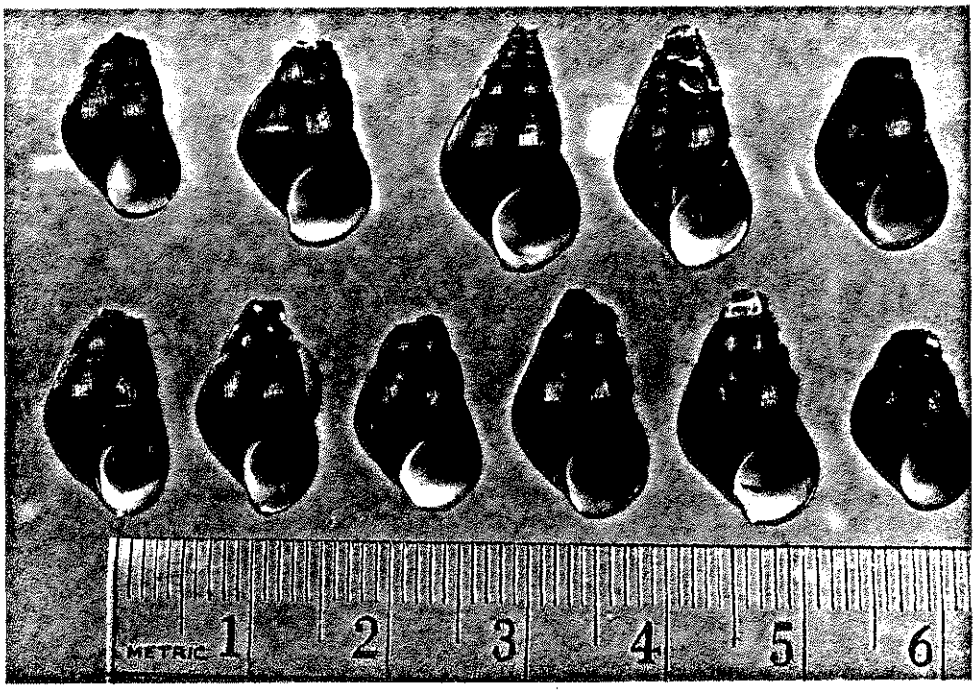


Figure 19. Electrophoresed Pleuroceridae from the Duck River at Fredonia, seen from the apertural side. These specimens are distinct from all other Duck River populations examined in this study, both electrophoretically and morphologically. They are also taxonomically distinct from pinguis of the Cumberland River basin on the basis of shell morphology.

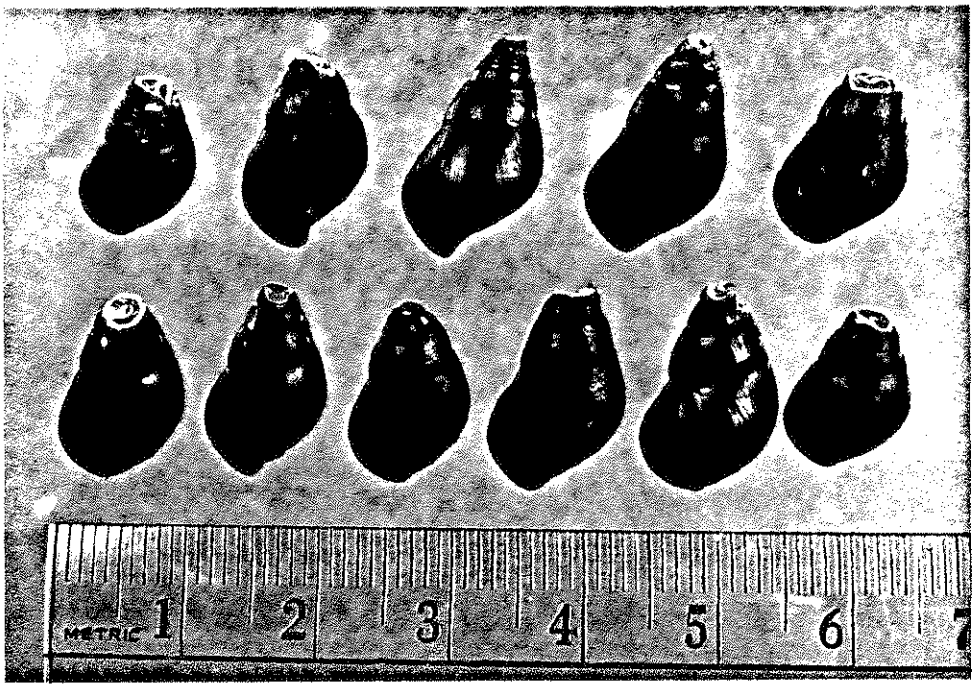


Figure 20. Dorsal view of the same specimens shown in Figure 19. Note the spiral sculpture on the early whorls, a character not found in either E. gibbosa or pinguis.

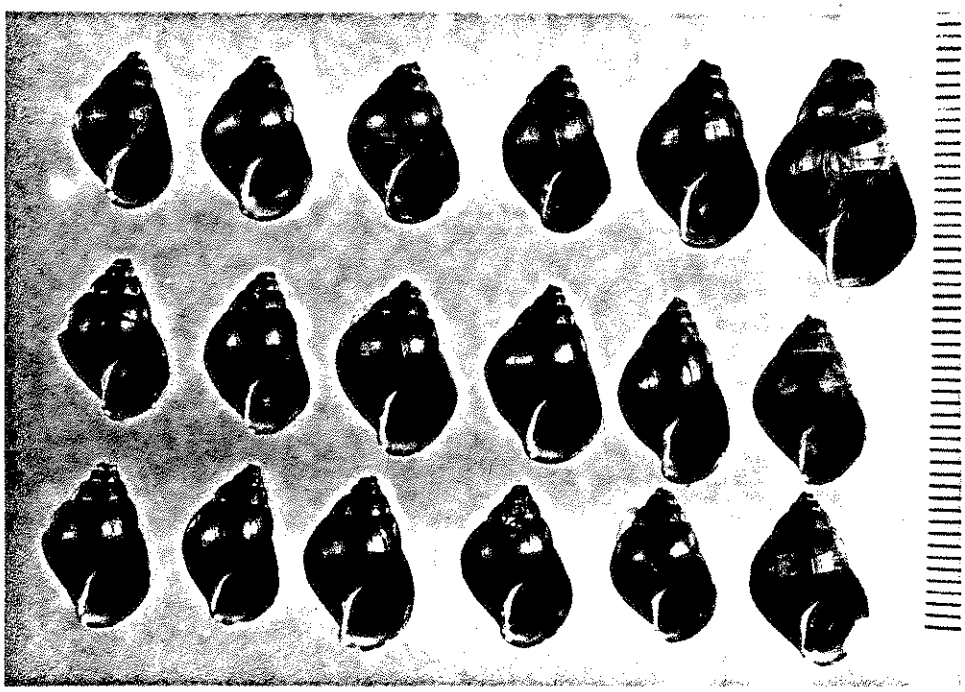


Figure 21. Young specimens of E. gibbosa form fuliginosa collected at Manchester, from the same lot as the electrophoresed specimens illustrated in Figures 23 and 24.

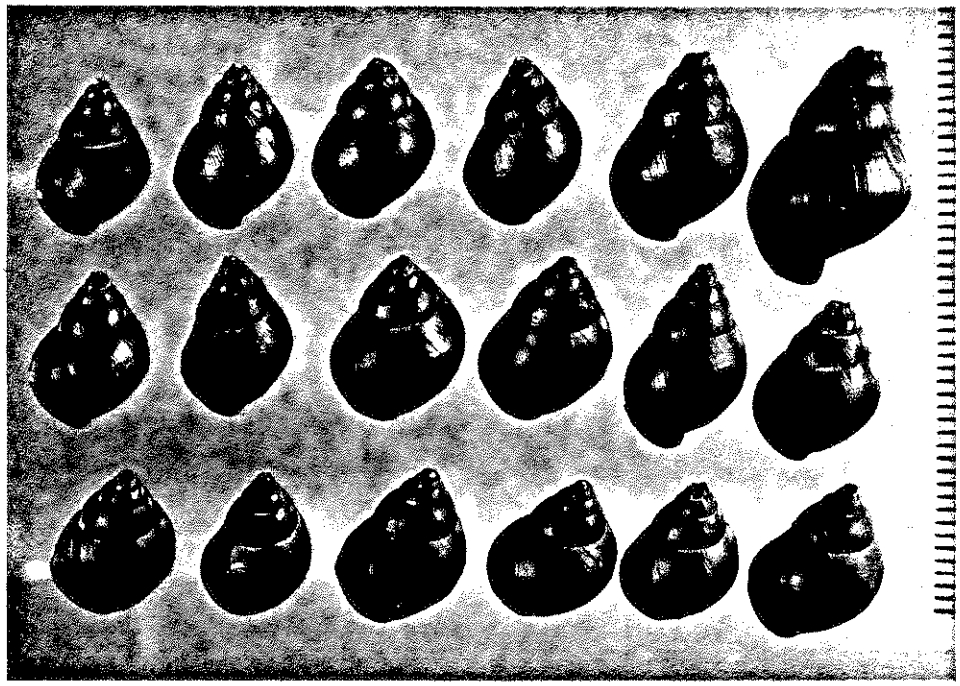


Figure 22. Dorsal view of the same specimens illustrated in Figure 21. Note the absence of spiral sculpture on the early whorls, in contrast to the Fredonia specimens shown in Figs. 19 and 20. The white band immediately below the suture is also consistently present in this population, but absent in the shells taken at Fredonia.

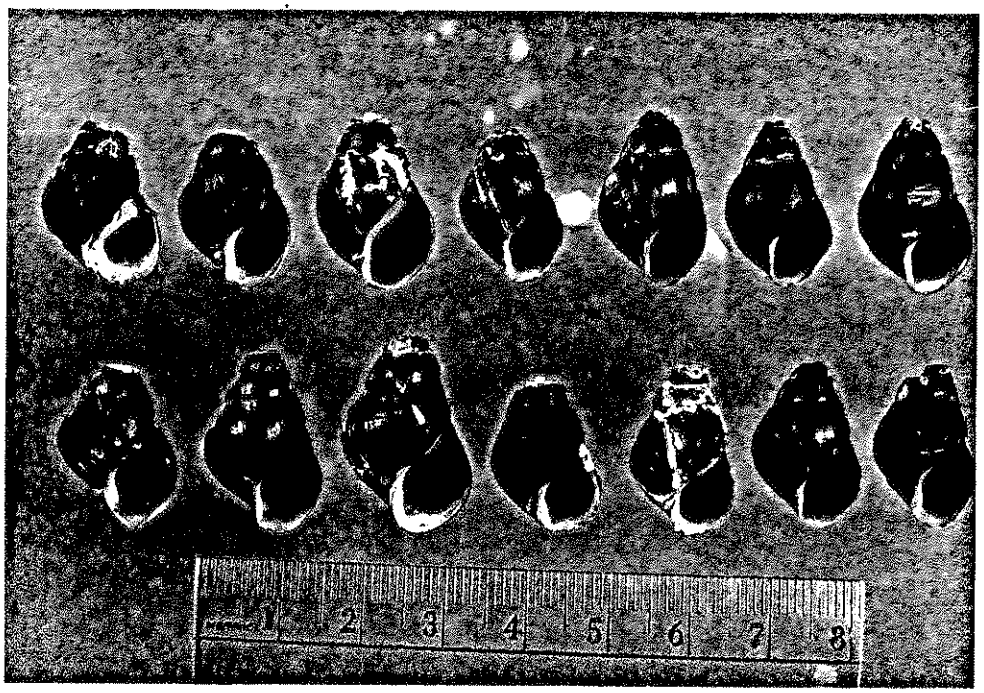


Figure 23. E. gibbosa form fuliginosa from Manchester, electrophoresed 28 April 1983.

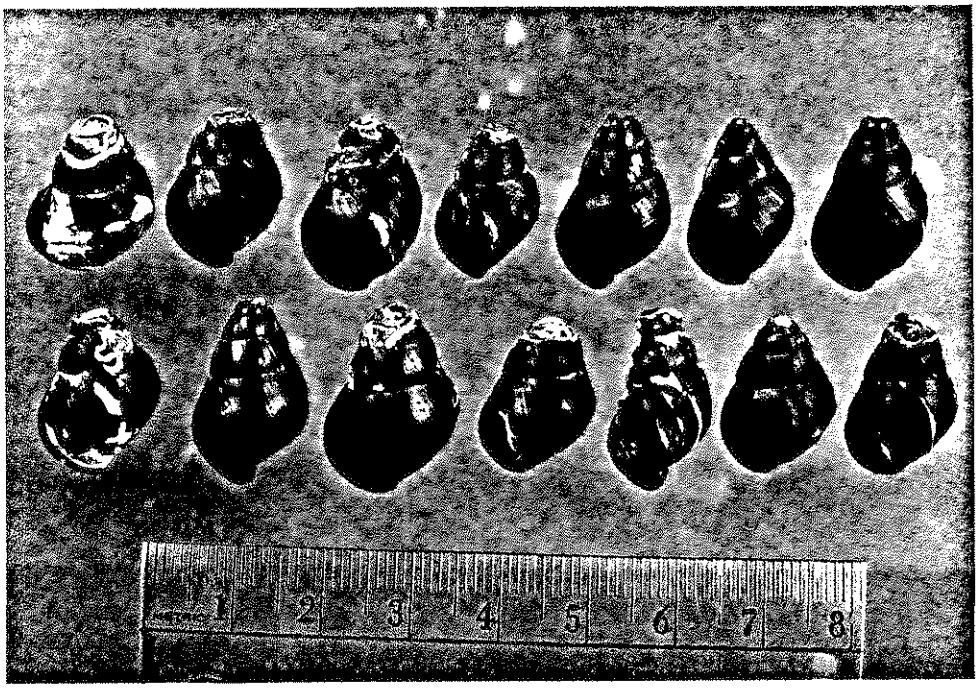


Figure 24. Dorsal view of the same specimens figured above in Fig. 23.

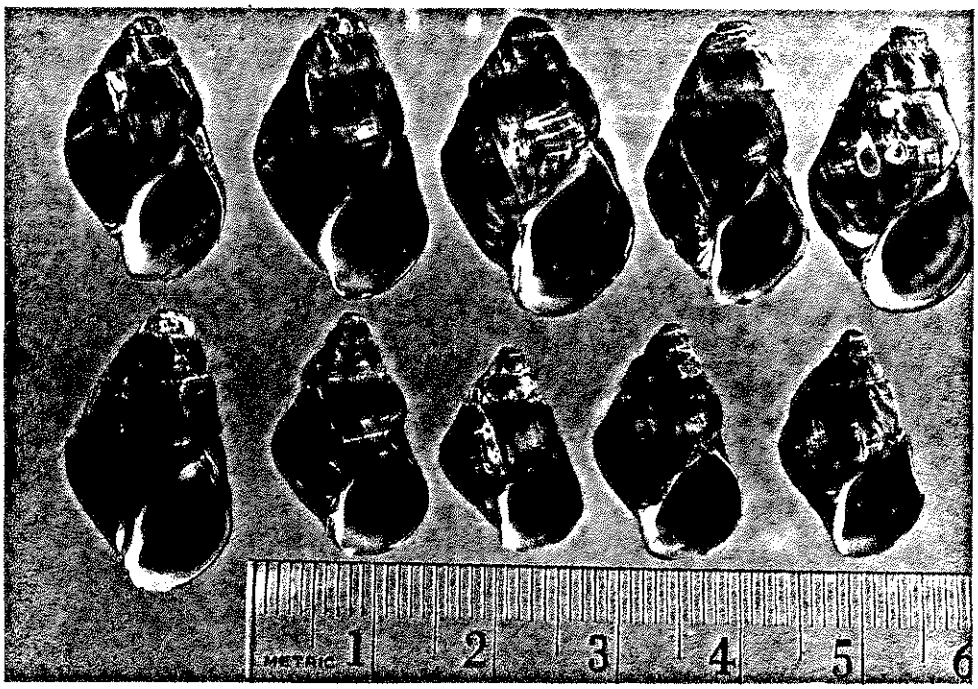


Figure 25. Specimens of E. gibbosa which were collected at the Cortner Road bridge and which were electrophoresed 1 June 1983. Most of these specimens exhibit the characteristic costae on the early whorls (# 3-6). In some shells these persist into the body whorl, where they are developed into various intergrades of the shoulders, keels, and tubercles which are so pronounced in many specimens farther downstream.

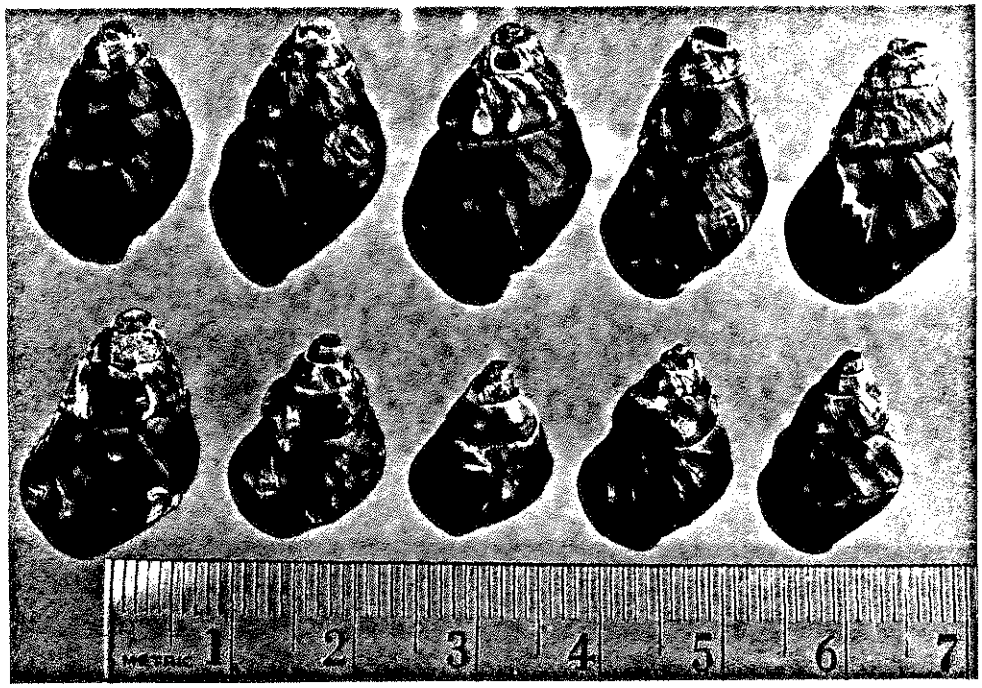


Figure 26. The same specimens as illustrated above (Fig. 25), viewed from the dorsal side. Note that different aspects of the shell often show somewhat different sculpture patterns.

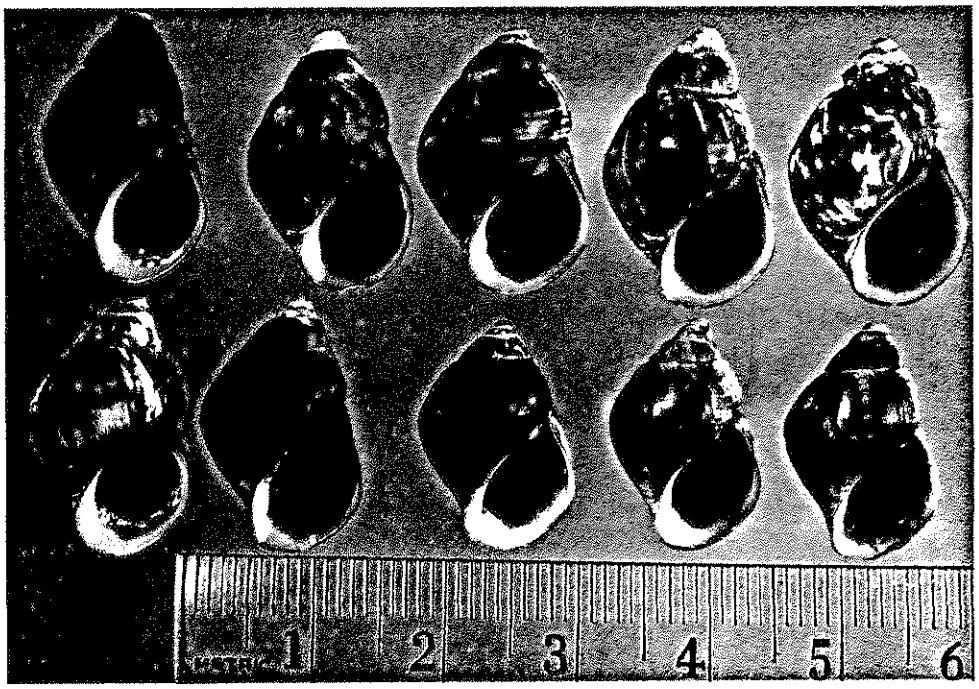


Figure 27. Garrison Fork specimens of E. gibbosa electrophoresed 17-18 May 1983. These specimens represent the form fuliginosa, which is the characteristic form of the E. gibbosa complex in the Duck River tributaries.

Figure 28. The same specimens illustrated in Figure 27, as seen from the dorsal side.

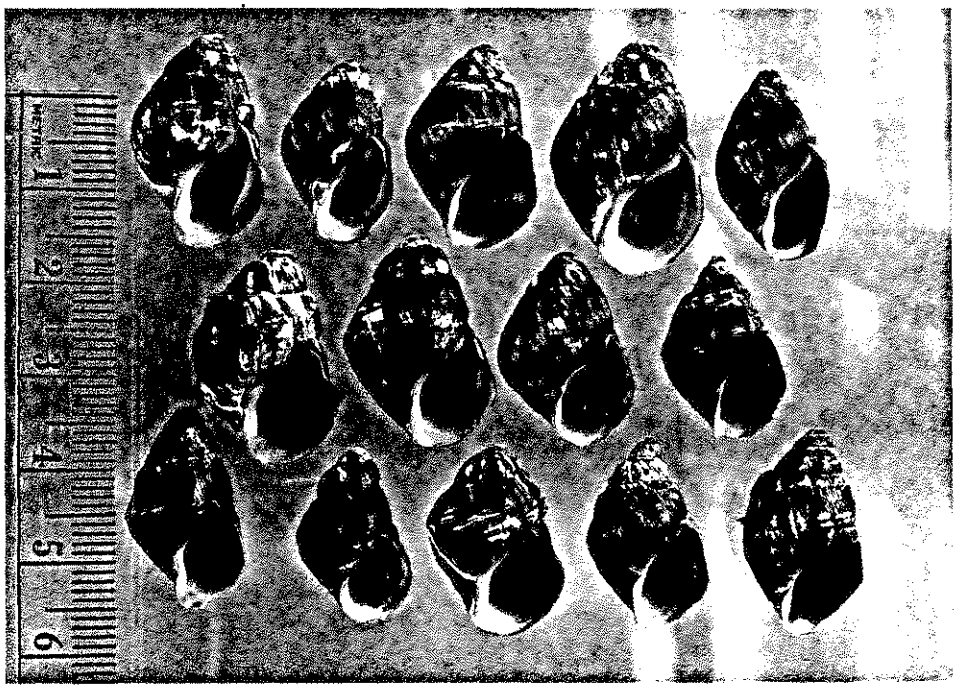


Figure 29. E. gibbosa specimens from U.S. Route 41A bridge site on the Duck River. These specimens were electrophoresed 19 April 1983. The gradual downstream transformation from the simple fuliginosa form into the forms having more pronounced sculpture is evident here. The sculpture patterns characteristic of forms geniculata, gibbosa, jayana, and zonalis are all beginning to be evident in these specimens.

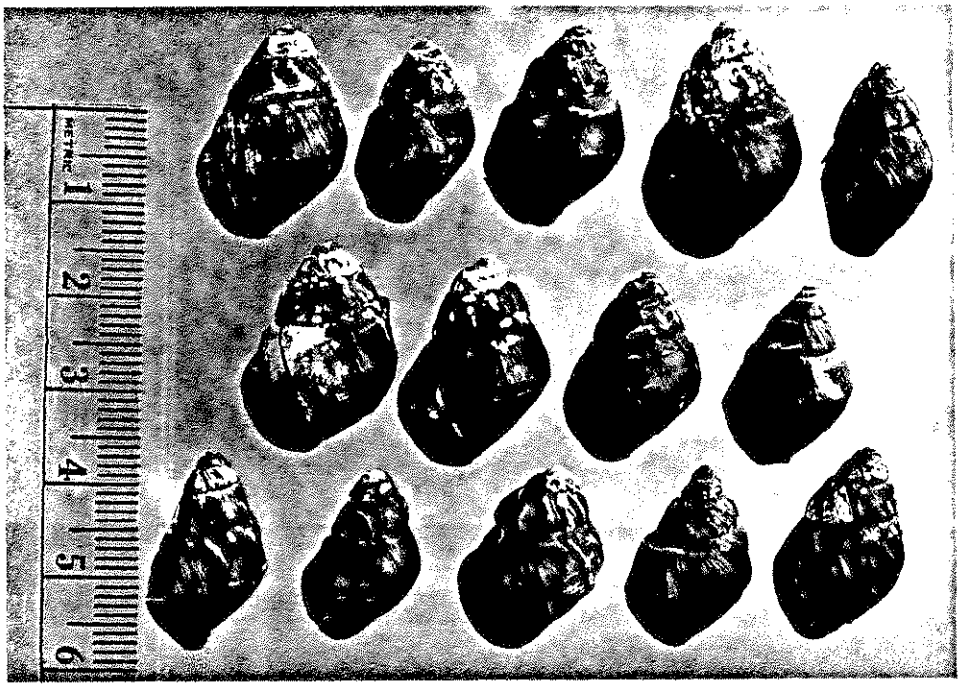


Figure 30. A dorsal view of the specimens pictured in Figure 29, above.

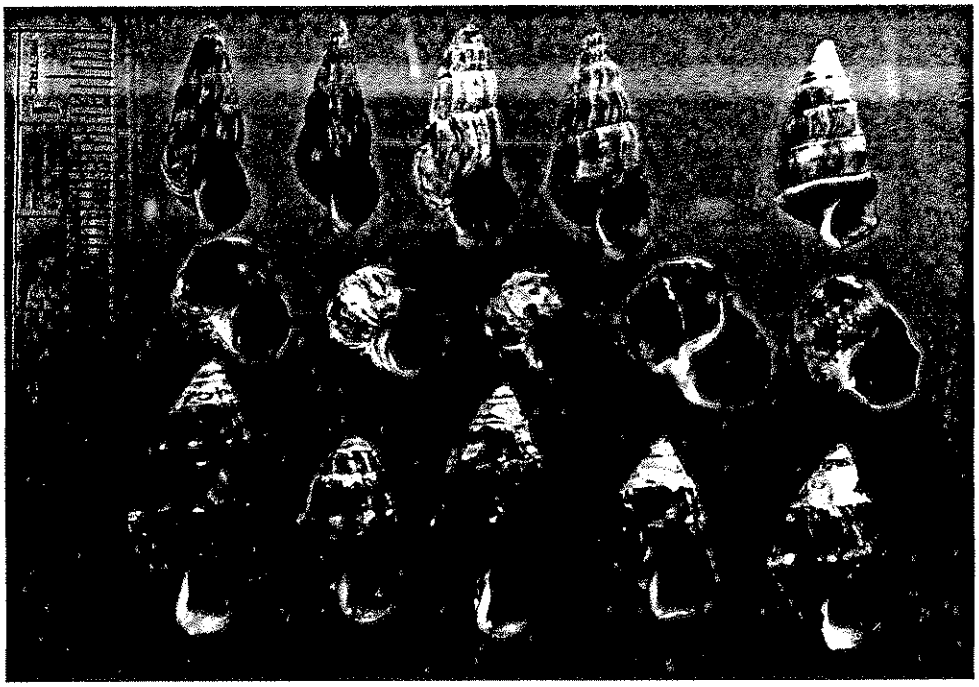


Figure 31. Four genera of Pleuroceridae which occur sympatrically in Duck River at Henry Horton State Park were electrophoresed on the same gels 3 June 1983, and are figured here. In the top row are 4 Elimia laqueata and 1 Pleurocera canaliculata (at right). The middle row shows Leptoxis praerosa, and the specimens in the bottom row are Ellipstoma gibbosa form gibbosa. Although these are the typical small river expression of form gibbosa (also described as duttoniana), there is some sculpture at the shoulder level of these specimens. This sculpture, when fully expressed along with tuberculation on the periphery, produces form jayana.

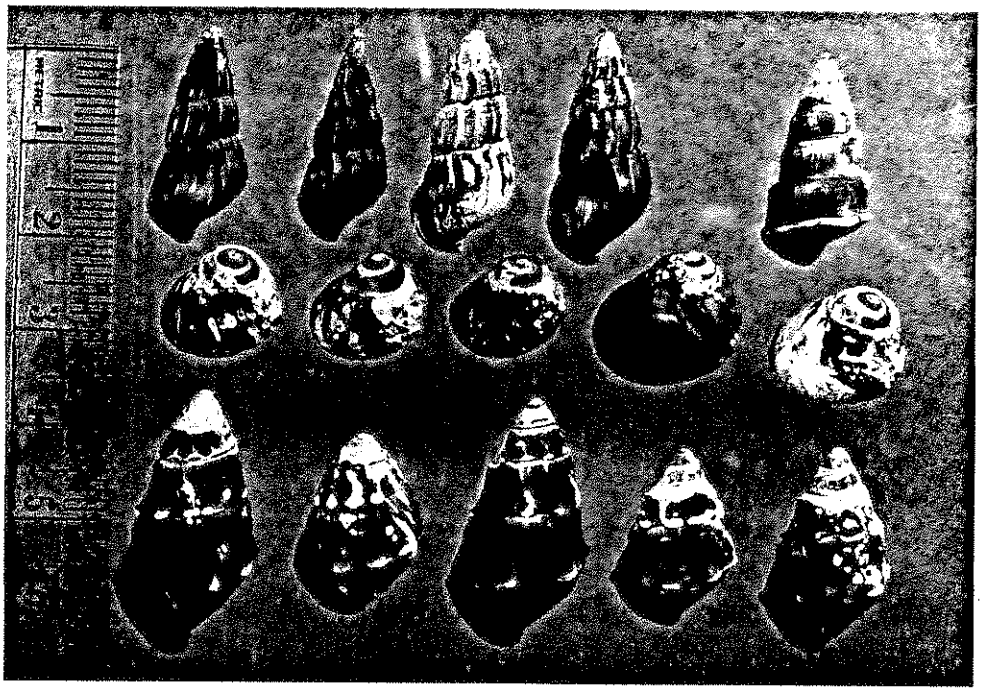


Figure 32. The dorsal view of the specimens figured above in Fig. 31.

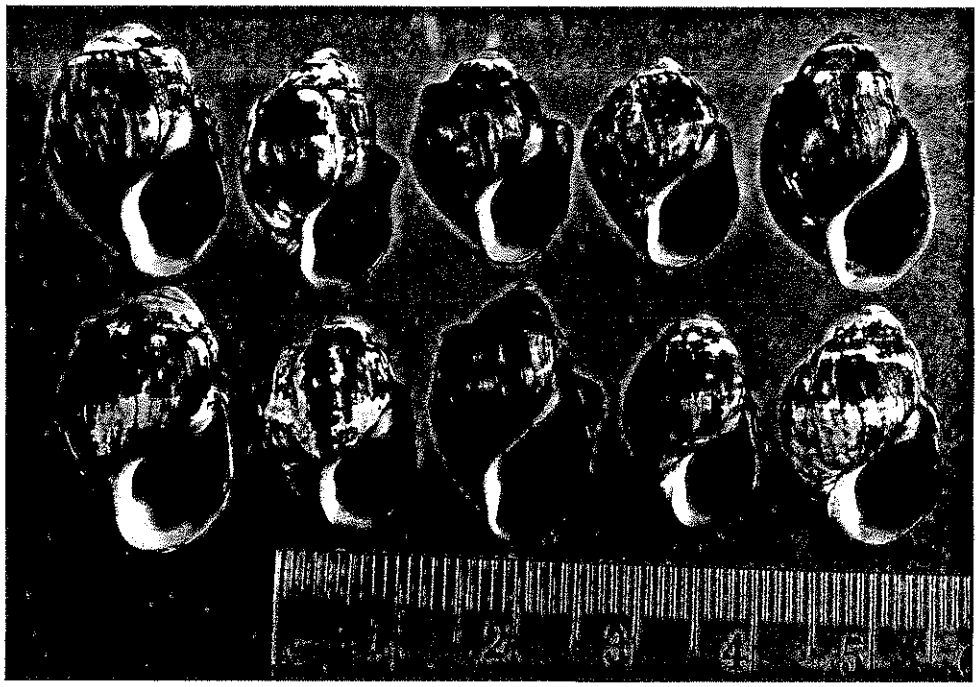


Figure 33. These E. gibbosa specimens from Barren Hollow Road were all electrophoresed 29 March 1983. Many of these shells have reached full sculptural expression. All have the developed shoulders of the zonalis-geniculata-salebrosa forms. Those having strong shoulder tubercles are form geniculata. Those lacking such tubercles are form zonalis. The others are intergrades.

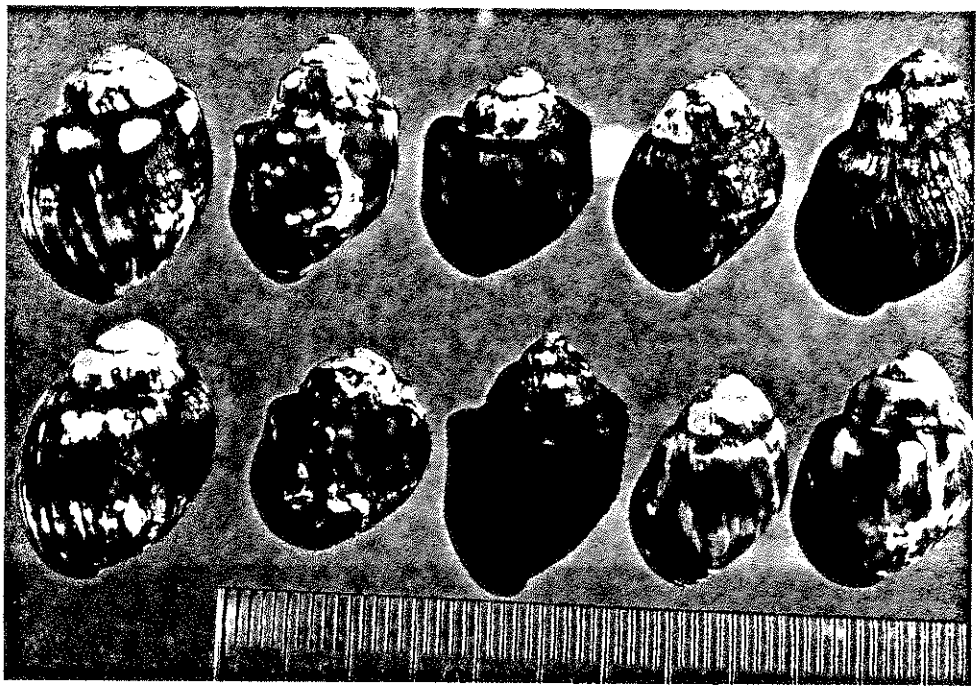


Figure 34. The dorsal view of the specimens shown in Fig. 33.

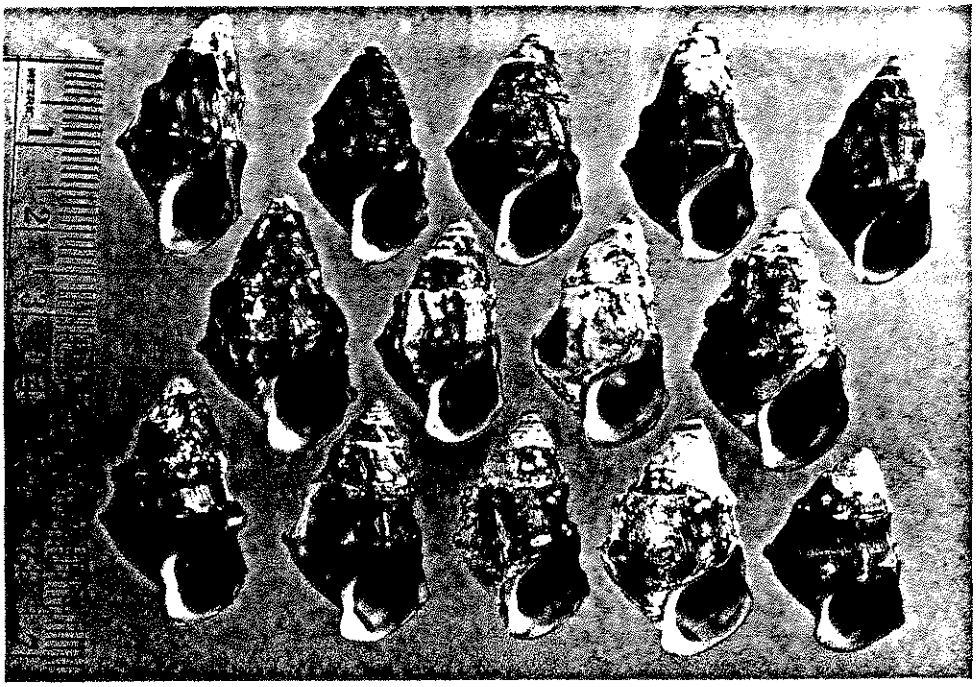


Figure 35. E. gibbosa specimens from Duck River at the State Route 13 bridge, the farthest-downstream site from which specimens were electrophoresed. The shells exhibiting rows of tubercles at both the shoulder and the periphery are form jayana. Those having only peripheral sculpture are form gibbosa. The others are intergrades between these two forms. The specimen at the upper right has shoulder sculpture and a more cuboid form suggestive of the geniculata form.

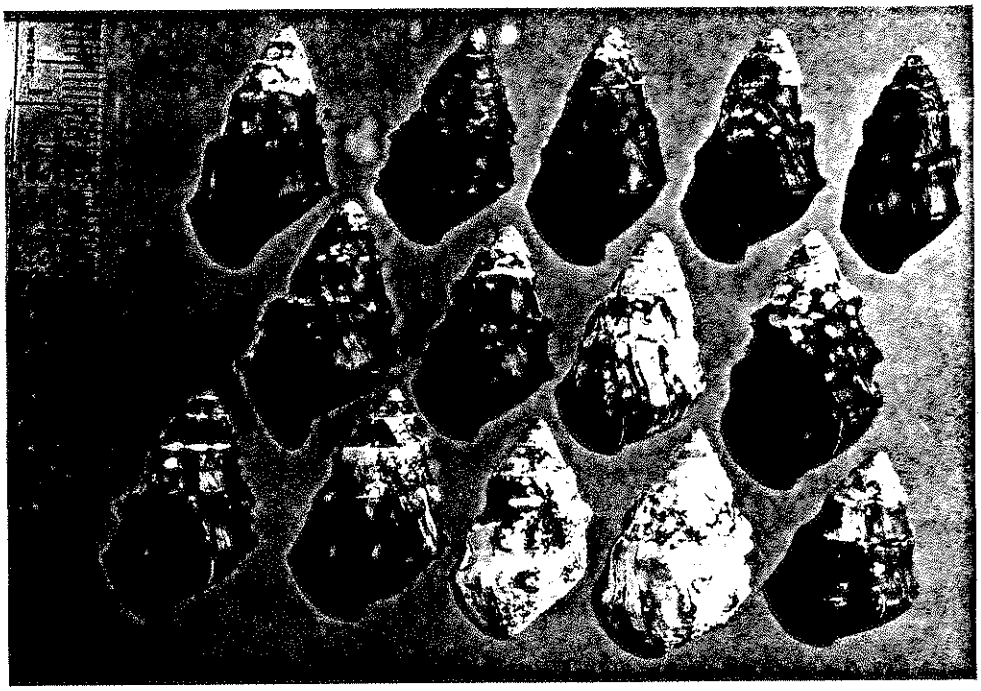


Figure 36. A dorsal view of the same specimens shown in Fig. 35, above.

THE *PINGUIS* LEA, 1852, PROBLEM IN THE DUCK RIVER

Several previous authors have recorded the species described as *Melania pinguis* (Lea, 1852) from the headwaters of the Duck River. Goodrich (1934:8) in his discussion of *Lithasia geniculata* (Haldeman, 1840) in the Duck River, noted that the connection of *pinguis* with this species had "been traced in two streams of Tennessee." He further notes (Goodrich, 1934:9) that the pleurocerids from Manchester, Coffee County, were "All *pinguis*" and that the specimens found "had" the characteristic lithasoid columellae. Specimens collected within Manchester "were nearly round-mouthed." In his review of the Pleuroceridae of the Ohio River system, Goodrich (1940:5) simply lists "Duck River, Coffee County, Tennessee" without specific sites. Davis (1974:31) lists *Io geniculata pinguis* (Lea, 1852) from the Duck River. Although specific sites are not listed in the text, his distribution maps give one site each in the vicinity of Fredonia and of Manchester in Coffee County, Tennessee. Davis points out (1974:31), as did Goodrich (1934:8-9; 1940:5), that "The form *pinguis* is thus *fuliginosa* which had adapted to the small gravel paved headwater streams, ..." and that "The gradation to *fuliginosa* is obvious, however, in collecting successive populations of *fuliginosa* from the headwaters of the Duck River, downstream. There is a continuous gradation to *geniculata*."

In 1977 the U.S. Department of the Interior recorded *Io geniculata penguis* (sic) from the Duck River and Arnett (1984:21673) listed the species as *Lithasia pinguis* (Lea, 1852) in the Federal Register.

A particular effort was made by Clarke (1983:27) to determine the

distribution and relative abundance of pinguis for the U.S. Fish and Wildlife Service. He reported success in locating specimens identified as pinguis at only a single locale among 16 visited in the Duck River and its tributaries near and above Manchester. This population "occurs in a short stretch of the Duck River at Manchester below Big Falls and above the mouth of the polluted Little Duck River." He does note that "Streams above Manchester ... contain a species of Mudalia (?) which resemble L. pinguis." This confirms our observation of some years ago and supports our inferences below. Clarke (1983:27) found pinguis to be "very abundant" in the main stem of the Collins River and several of its free-flowing tributaries. This also agrees with our collecting records over the years.

A comparative study was made of the pleurocerid snails from the upper Duck River system, the main stem and several of the larger tributaries above Normandy. Other than the very common snail of the Elimia laqueata group, only one pleurocerid was found living in the Duck River at Fredonia, our uppermost collecting site. These specimens are represented at OSUM by four collections as follows:

Duck River just NE of Fredonia	1 October 1976
Eaton Branch of Duck River at Fredonia	1 October 1976
Duck River at E edge of Fredonia	2 August 1981
Duck River at E edge of Fredonia	17 October 1982

The electrophoretic analysis revealed clear distinct differences between this form and all members of the E. gibbosa complex found

farther downstream. The Fredonia shells are a uniform olivaceous brown in color, moderately high spired, and smooth except for fine spiral striae on the uppermost several whorls. The aperture is ovoid, angular above, and rounded below, with only the suggestion of a ventral channel in a few. The interior is white with bluish or orange tints in some individuals. Several hundred specimens were examined and found to be quite uniform in their characteristics. A comparison of the Fredonia specimens with material from the Duck River downstream at Manchester revealed two distinctly different forms without intergrades. Two collections from the Little Duck River at Manchester yielded the Fredonia species, while none were found there in the Duck River proper.

A comparison of the Fredonia species with the holotype of Melania pinguis Lea, 1852 (USNM 121219, Figs. 37, 38), demonstrates that it is not that species either. The type of pinguis is a low-spired, globose form which lacks spiral striae. These differences are not surprising since the type locality of pinguis is "Lebanon, Wilson County, Tenn." on Barton Creek, a tributary of the Cumberland River; whereas the Duck River is part of the Tennessee River system. An examination of a number of lots from the Collins River drainage and other sites in the Caney Fork system, which correspond to the general form of pinguis, reveals that the Fredonia species is apparently not shared with the Caney Fork system and that pinguis is far more variable than has been imagined. From this information we conclude that: 1) pinguis is not found in the Duck River system but may be relatively widespread, variable and locally common in the Caney Fork system and its sister streams of the central

Figure 37 (left) and 38 (right) are two views of the holotype of Anculosa pinguis Lea, 1852, USNM-121219, from "Lebanon, Wilson county, Tenn., Mr. J.M. Stafford." (Lea, 1852: 301). This species is not part of the Ellipstoma gibbosa complex, despite its superficial resemblance to some E. gibbosa form fuliginosa specimens from the headwaters of Duck River, especially those at Manchester (see Figures 21 and 22). The population from the Duck River at Fredonia (see Figures 19 and 20) also bears a superficial resemblance to pinguis and to the Manchester E. gibbosa form fuliginosa, but is not conspecific with either of them.

Cumberland River drainage. 2) A species living in the Duck River at Fredonia and in the Little Duck River at Manchester apparently has a very limited distribution in the upper Duck River system and has never, so far as is known, been found elsewhere. The further investigation and elucidation of this species is another project high on our list of priorities. 3) The E. gibbosa complex exists upstream in the Duck River at least as far as Manchester, but it does not extend up Little Duck River; nor does its range extend above Manchester as far as Fredonia.

THE DESCRIBED FORMS OF THE ELLIPSTOMA GIBBOSA RAFINESQUE, 1818, COMPLEX IN THE DUCK RIVER IN CENTRAL TENNESSEE

The E. gibbosa complex first appears in the Duck River at Manchester, Coffee County, Tennessee. It is relatively uniform at this site and can best be described as fusoid in shape, blackish-green to deep olive in color, purplish within, smooth and lacking any sculpture with the exception of a few scarcely discernible interrupted lines typically found near the periphery or on the underside of the body whorl. This species can easily be separated from the Fredonia species, which occurs only sparingly at Manchester, by the presence in E. gibbosa of a narrow white zone subtending the suture. Viewed from above this line appears as a white spiral with its center at or near the apex of the spire. The eroded tip of the spire is purple rather than white due to the dark colored nacre beneath the periostracum. The columella is light colored, axial, and approximates a right angle with the ventral lip of the aperture (Fig. 21). The individuals of this polymorphic species are more uniform in appearance here than at any other site on the river.

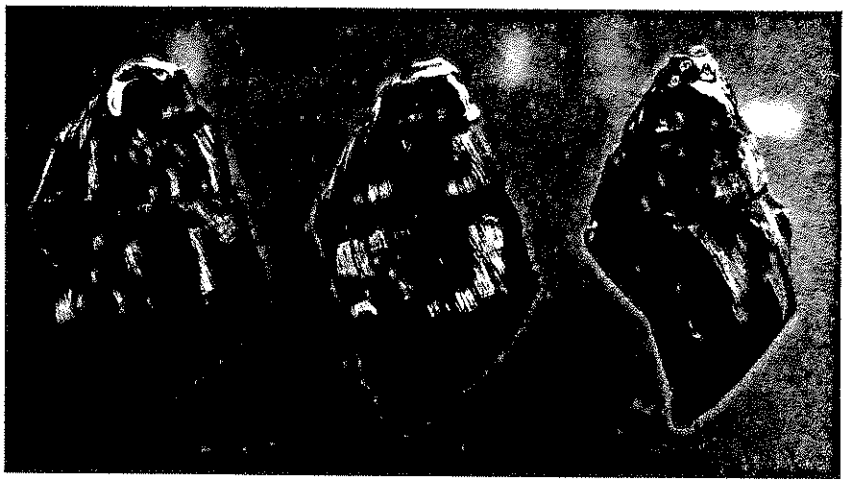
Moving downstream to Normandy, a distance of only 23 miles, one observes a number of morphological changes. The familiar fusoid shell at Manchester with the dark periostracum and light spiral line is represented by only a few individuals. Most specimens are horn color with broad bands, lines or blotches of purple seen best through the aperture when held up to the light. The eroded apices of the horn colored specimens are white and the white spiral appears only on the dark individ-

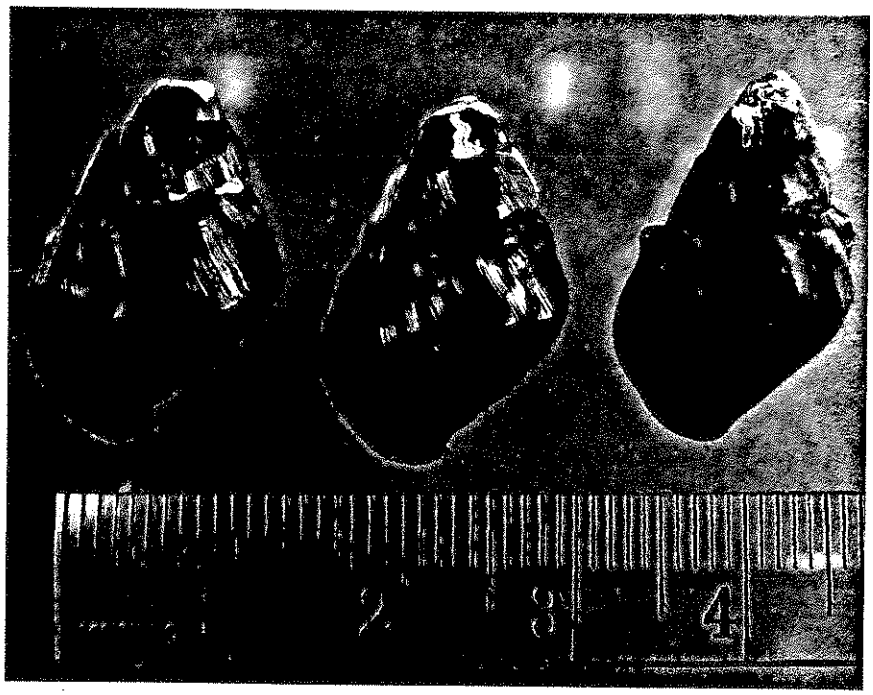
uals.

Perhaps most striking is the presence of costations or plications on the early whorls of most specimens. These vary from scarcely discernible to prominent, from straight to curved, from axial to diagonal, and from complete to "remnant" ends or tubercles. Since most of this sculpture occurs on the upper half of the whorl, the remnants become rows of tubercles either at the shoulder subtending the suture, at the periphery, or both. In some individuals the shoulder tubercles apparently coalesce, forming a prominent shoulder that may be only undulate or quite smooth. On others the costae are few and extend from the upper half of the whorl down over the periphery to flatten out and "disappear" beneath. An examination of over 500 specimens from the Normandy site produced 20 different character combinations. Some specimens exhibited two or three sculpture patterns on as many different sides of the shell. In such individuals one can "change the species" simply by rotating the specimen! Three such specimens showing sculptural changes are illustrated in Figure s 39 - 41.

It should be emphasized that, except for the simple costate forms, none of the sculpture observed in the Normandy specimens is as fully developed as it is at most downstream sites. However, all of the character combinations of this complex recorded from Duck River which have been given scientific names in times past are present here in some degree. Present also are intermediate forms standing between and connecting these described entities by insensible gradations. These intermediate individuals, along with the described forms, constitute something

Figures 39 (top), 40 (middle), and 41 (bottom). The three Normandy E. gibbosa in these figures demonstrate the variability of shell sculpture which often occurs within the same individual as it grows. This phenomenon is common in upper Duck River populations.





of an enigma at the present time. Our electrophoretic analysis indicates a high degree of genetic homogeneity. Since all appear to have come into being and have developed in essentially the same habitat, this genetic homogeneity should produce individuals which are identical or nearly so. Our studies of this complex at a number of sites farther downstream reveal that these differences become generally greater or more exaggerated, while the genetic facies revealed by electrophoresis remains essentially constant and uniform. If the electrophoretic technique gives us a genetic expression sensitive at species level differences, what is the source of the observed variations in shell morphology? Subtle differences in microhabitat have been suggested as responsible for these differences, but we have seen little correlation between shell form and habitat distribution. Where well-developed, well-defined individuals make up nearly all of the population the gibbosa group is generally found in quieter waters while the geniculata group is characteristic of fast waters of the riffles.

Whatever the causative factors responsible for such phenomena, we believe it is best to act conservatively in cases where populations of unique genetic diversity may be in danger of destruction. It is for this reason that we have adopted the following "form" nomenclature. Each form represents a group(s) of morphologically similar individuals which are, in some populations, connected by intergrades with other such groups of the complex and, in yet other populations, seem to be nearly if not quite distinct. The overall picture is one of a number of interrelated forms which have a high degree of interbreeding at some sites

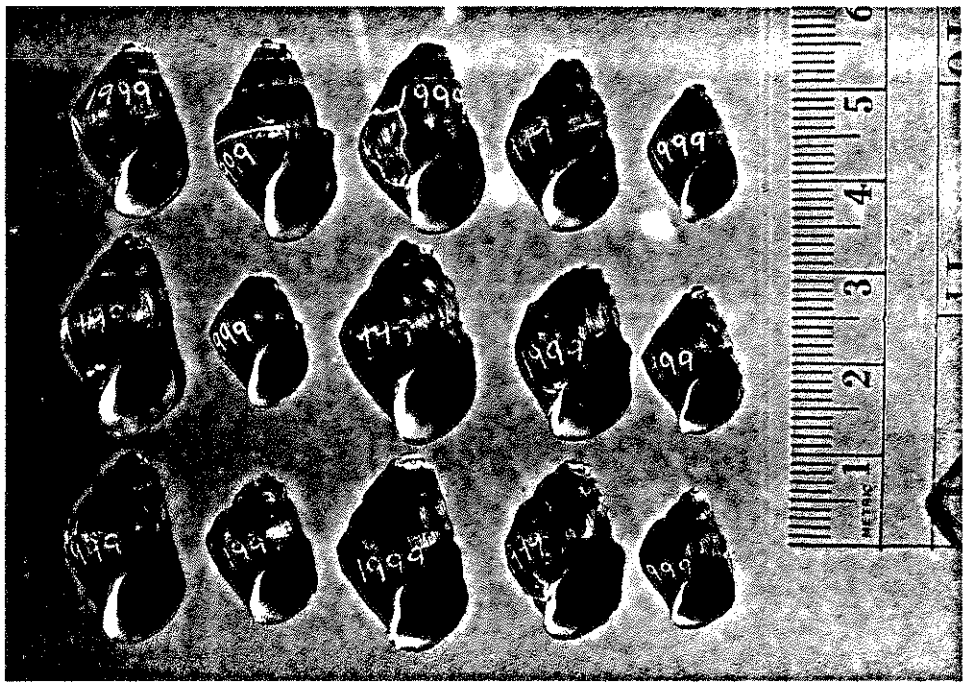


Figure 42. This series of 15 specimens from our 1972 collection at Normandy includes examples of forms fuliginosa, jayana, geniculata, and gibbosa. Even though the sculpture is subdued on these upstream shells, it is still clearly evident. Since the sculpture of an individual often changes as it grows, the identification of form based on a ventral view frequently is different than the identification of the same specimen when it is rotated to show the dorsal view, as shown in the figure below. Sometimes the same individual exhibits the sculpture of two or even three forms on different parts of its shell. This is strong evidence of intergradation of the forms of E. gibbosa.

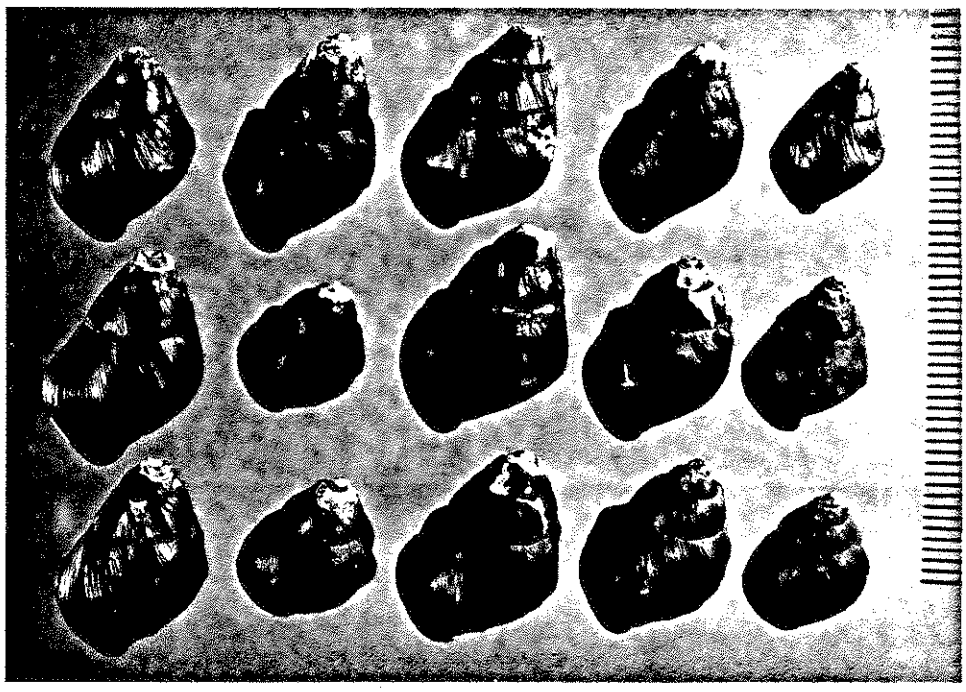


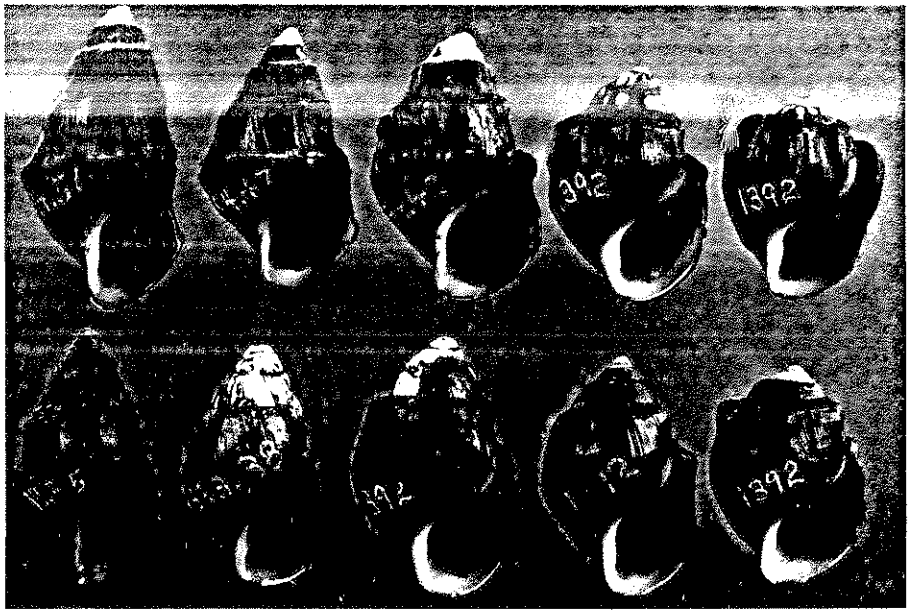
Figure 43. The same specimens illustrated above have been rotated and rephotographed in the same sequence. Note the variation in color, shape, and sculpture.

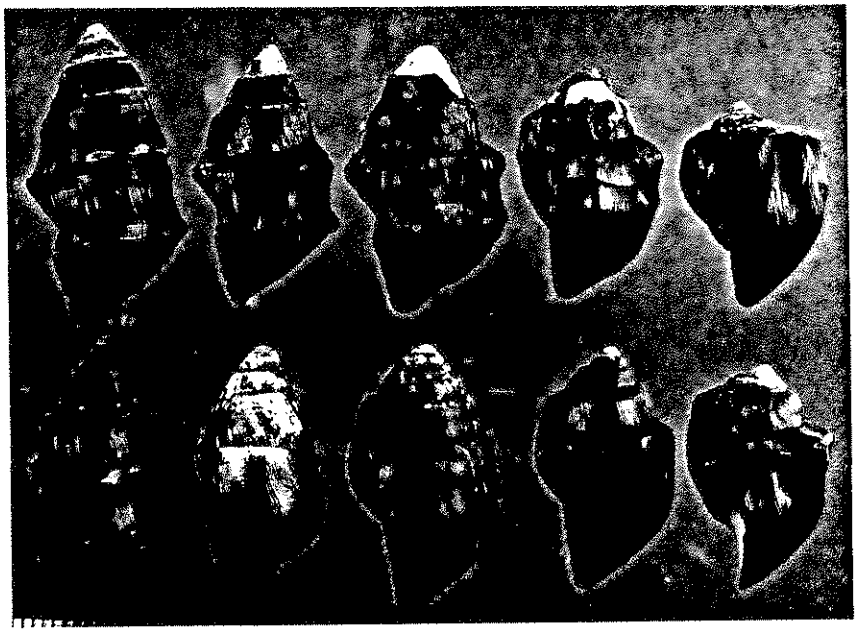
and little if any interbreeding at others. The nomenclature below attempts to express this relatedness within our present system of classification so that these entities can be understood (as best we can at present) and dealt with realistically.

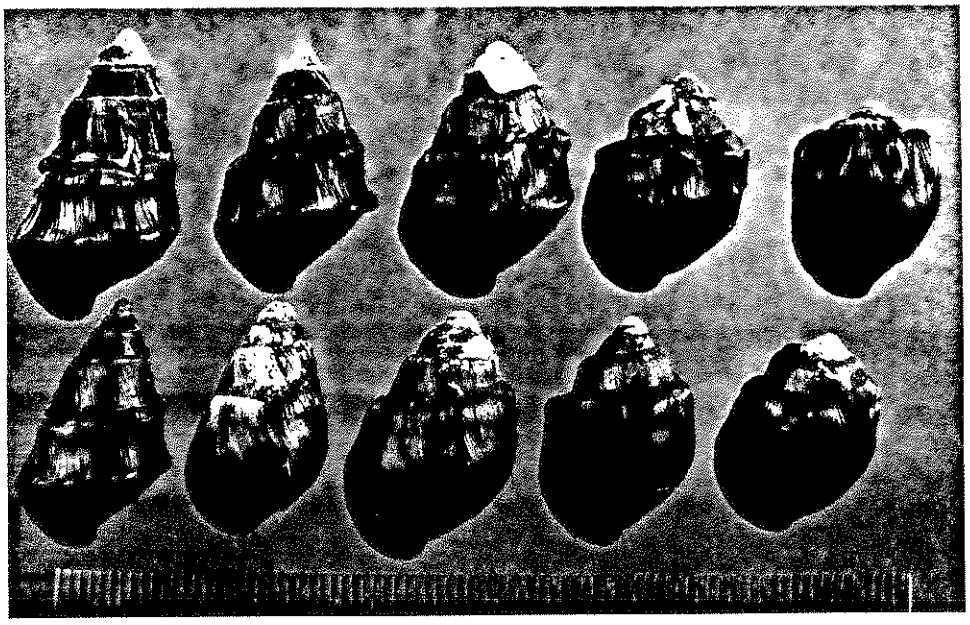
Figures 45 (top), 46 (center), and 47 (bottom).

These three views of the same 10 shells from the Barren Hollow Road site show the strong sculpture which occurs on many specimens in the river's lower reaches.

In this part of the river the forms are more distinct, with less intergradation between them, than in the upper reaches.







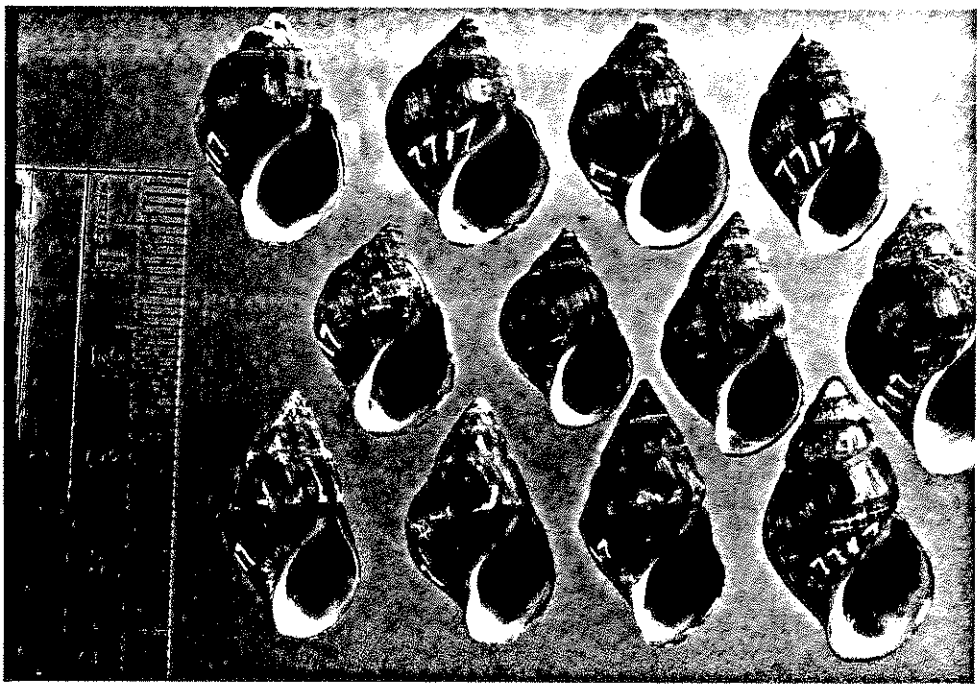


Figure 47. This series of E. gibbosa from Garrison Fork demonstrates that sculptural variation and intergradation is not restricted to main stream populations. The specimens in the top row show transitional stages between form zonalis (left) and form fuliginosa (right). The left specimens in the bottom row are midway between form gibbosa and form fuliginosa.

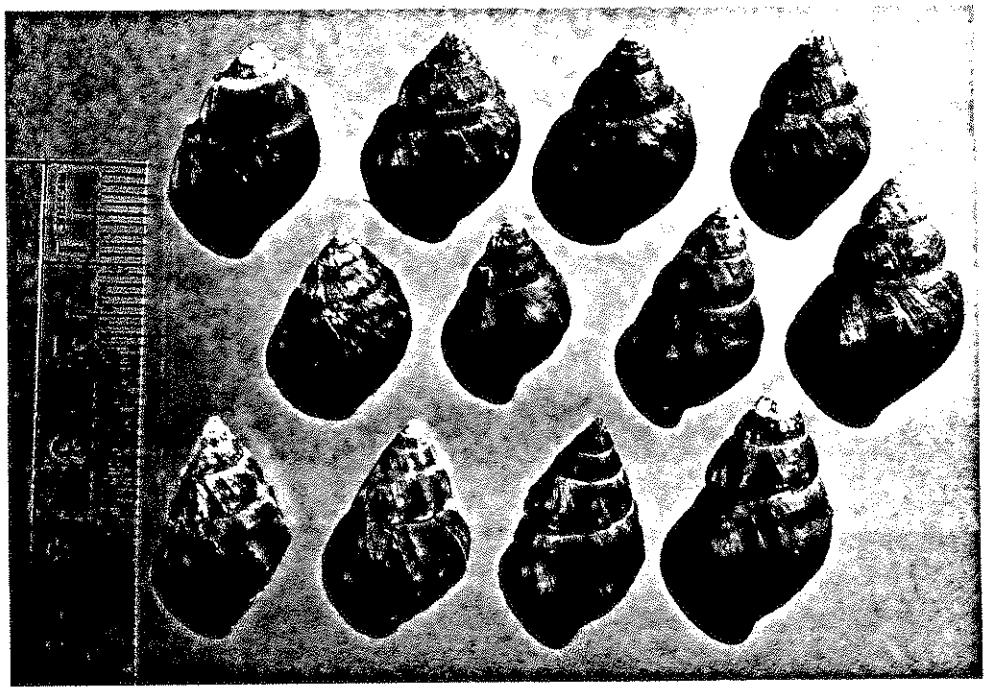


Figure 48. Dorsal view of the same Garrison Fork specimens figured above.

THE NOMENCLATURE OF THE ELLIPSTOMA GIBBOSA RAFINESQUE, 1818, COMPLEX BASED UPON SPECIMENS FROM THE DUCK RIVER IN CENTRAL TENNESSEE

The genetics and shell morphology of the following forms of pleurocerid snails have been investigated in an effort to obtain new evidence on their systematic relationships. All of the material studied, unless noted otherwise, was collected from the Duck River in central Tennessee.

	United States Department of the Interior Published Name (Greenwalt, 1977)	Original Scientific Name of the Form Studied
1)	Io armigera armigera	Ellipstoma gibbosa Rafinesque, 1818.
2)	Io salebrosa	Melania salebrosa Conrad, 1834.
3)	Io armigera duttoniana	Melania duttoniana Lea, 1841.
4)	Io geniculata geniculata	Lithasia geniculata, Haldeman, 1840.
5)	Io armigera jayana	Melania jayana Lea, 1841.
6)	Io geniculata pinguis	Melania pinguis Lea, 1852.

Additional forms have either been previously identified from the Duck River or were found in the course of this study. These additional forms were found to be as closely related to those listed above as those forms are to each other. They are as follows:

8)	Melania fuliginosa Lea, 1841.
9)	Ellipstoma zonalis Rafinesque, 1818.
10)	Angitrema angulata Wetherby, 1876.

All of the above forms, except that assigned above to pinguis, are

connected to each other by insensible gradations of those distinguishing shell characters which were used in their original descriptions. Fredonia "pinguis" differs consistently from all other Duck River forms in several shell characters. The electrophoretic evidence from this study demonstrates sharp differences between the form found at Fredonia and the form found at Manchester. The Manchester form (fuliginosa) could not be separated from any of the other forms of this complex downstream. We therefore conclude that the "pinguis" population at Fredonia is not part of the E. gibbosa complex.

In separating the Duck River specimens into the forms listed above, it was found that a great many of the smaller individuals, as well as some medium-size shells, did not fit any of the described categories. All of these specimens had one thing in common. One or more of the earlier (but not the earliest) whorls had costae. These ribs varied greatly from individual to individual and frequently from one place to another on the same individual and sometimes from one point to another on the same whorl! Costae of the same type were nearly always present on the smaller (but not the smallest) specimens of many other forms found in the Duck that later in life developed a sculpture that placed them into one or another of the described categories. The sculpture of the E. gibbosa complex can be thought of as the enlarged remnants of these costae. This development can be clearly followed as a gradual process on many of the specimens collected. These costae are also reminiscent of those found on the earlier whorls of many Io fluvialis (Say, 1825).

We therefore conclude that many, if not most, of the smaller individuals having costations are the young of larger specimens which have developed different sculpture which falls into one or more described species of the E. gibbosa complex. The larger costate individuals of this fauna represent, we believe, another form which has somehow escaped description -- or perhaps its description has somehow escaped our attention!

The remaining described forms of the E. gibbosa complex, eight of which are dealt with here, are found within the Duck River system. They are interconnected by individuals comprising combinations of intermediate characteristics. A relatively few such intermediate individuals might be explained as the extremes of population variation or perhaps as mutant monsters or environmental variants. While some specimens appear by their rarity or their damaged condition or their correlation with environmental factors to be such variants the vast majority of intermediate specimens give the appearance of normal individuals except that they do not fit the man-made categories prepared to receive them -- the described species of the E. gibbosa complex.

Some sites have relatively few intermediates while other sites have mostly such individuals. Especially impressive are those individuals which could be placed in any one of as many as two or three described form categories depending upon which side of the specimen is examined.

The prime thrust of this study was the use of electrophoretic analysis to detect genetic differences between the different forms, rather than a study of variation of shell morphology within the complex. The

striking electrophoretic similarity between and among these forms led to this brief but revealing examination of shell variation. Time did not permit the in-depth analysis of shell variation indicated. However, it is high on our priority list of future projects.

In view of the striking similarity of electrophoretic patterns among these forms and the great number of individuals having shell characters intermediate between these forms, we conclude that the E. gibbosa complex in the Duck River is a single highly polymorphic species. As such it is very similar to the Io fluvialis (Say, 1825) complex of the upper Tennessee River system (Adams, 1900, 1915), a pleurocerid snail which, in our opinion, is closely related to E. gibbosa. Rosewater (1960:203-4) studied the Pleurocera canaliculata (Say, 1821) complex in the Ohio River drainage system and concluded that 113 described forms were in fact all variants of a single species. It appears that highly polymorphic species are not rare in the Pleuroceridae.

Even with the high degree of intergradation demonstrated among the forms of this species in the Duck River, there remains some morphological evidence for either a fairly high degree of breeding isolation between forms at some sites or a high degree of gene linkage some places and not at others. Whatever the explanation, some forms at some sites are relatively distinct with few intermediate individuals. We interpret this to indicate genetically different breeding populations which may in fact not be interbreeding at some sites. If distinct species could be reproductively isolated at some sites and interbreeding freely at other sites, this would serve to explain the variation in shell morphology ob-

served in Duck River E. gibbosa. We are at a loss to explain why these sometimes striking differences in shell morphology are not reflected in the electrophoretic analysis.

We are persuaded by the evidence cited above to combine these forms into a single species, but to retain their names as forms, at least for the present, since there is at least some evidence that these forms represent genetic diversity. This diversity may be greater than present electrophoretic evidence indicates or perhaps greater than present technology reveals. Where irreplaceable genetic diversity may be at stake, we prefer to be conservative.

MELANIA LAMARCK, 1799.

The oldest generic name used for forms of this complex is the time-honored Melania Lamarck, 1799. This genus has an African species, M. amarula Linne, 1758, as its type (monotypy) and is presently restricted to a group of river snails that do not live in North America. The name is not available, although it was used generally for most North American pleurocerid species for well over the first half of the last century.

PLEUROCERA RAFINESQUE, 1818.

The first generic name described especially for this group of North American river snails is Pleurocera Rafinesque, 1818. This genus had P. verrucosa Rafinesque, 1818, as its type by monotypy. This usage has, however, been suppressed by the plenary powers of the International Commission on Zoological Nomenclature, Opinion 1195 (1981:259). The species acutus Rafinesque in Blainville, 1824, has been made the type species of Pleurocera Rafinesque, 1818, so that P. verrucosa Rafinesque, 1820, not being congeneric with acutus, must then take the next available generic name.

ELLIPSTOMA RAFINESQUE, 1818.

The first generic name, after Pleurocera, described for a group of river snails including forms in the gibbosa complex was Ellipstoma Rafinesque, 1818. The author of this species included the names and descriptions of three of its species along with the generic description.

These species are gibbosa, zonalis and rugosa. This generic name has E. gibbosa as the type (subsequent designation by Hannibal, 1912:168) (See also Morrison, 1954:363). This was a fortunate selection, since the description, although simple, clearly refers to a single variable but familiar form. Rafinesque's E. gibbosa was described as an Ellipstoma from the Ohio and Wabash Rivers which is half an inch in length, has four whorls and "a large knob behind the outward lip." This is clearly the form described three years later by Say (1821:178) as Melania armigera.

Although Pleurocera and Ellipstoma were both described in 1818, they were described in consecutive volumes of the American Monthly Magazine and Critical Review. Pleurocera was described in Volume 3, page 354, and Ellipstoma was described in Volume 4, page 39.

IO LEA, 1831.

If for any reason Ellipstoma should prove to be unavailable for use here, the next name to be considered is Io Lea, 1831. This genus has Fusus fluvialis Say, 1825, as its type by monotypy.

A careful examination of the forms of the E. gibbosa complex and the various forms of Io fluvialis in the OSUM collection leads us to the opinion that these species may be congeneric. Shell characters differentiating Ellipstoma from Io are: 1) the larger size attained by most individuals of Io; 2) the longer siphonal canal produced by all Io we have examined; and 3) the greater degree of spine development exhibited by the spinose forms of Io. It should be noted, however, that all of these

characteristics are a matter of degree. The differences, however, are striking. No intergrading forms have been seen. The process of ontogenetic development from smooth shell through costations or plicae to knobs, nodules or spines is essentially the same in both E. gibbosa and Io fluvialis. Davis (1974) lists the described forms of the gibbosa complex in the Genus Io, indicating the close relationship of these species. We prefer to retain these species in separate genera, as has been customary, until additional evidence of their congeneric status has been obtained. Additional evidence from electrophoretic analysis and/or serum antibody studies might help solve this problem.

LITHASIA HALDEMAN, 1840.

The Genus Lithasia was established by Haldeman in 1840 with L. geniculata Haldeman, 1840, as its type by monotypy. Should all earlier names prove to be unavailable, this name can still be used. Lithasia Haldeman, 1840, at present is a junior synonym of Ellipstoma Rafinesque, 1818.

ANGITREMA HALDEMAN, 1841.

The Genus Angitrema was established by Haldeman in 1841 with Melania armigera Say, 1821, as the type by monotypy. Should all earlier names prove to be unavailable, this name can still be used. Angitrema Haldeman, 1841, at present is a junior synonym of Ellipstoma Rafinesque, 1818.

ELLIPSTOMA GIBBOSA FORM GIBBOSA RAFINESQUE, 1818

Synonymy

Ellipstoma gibbosa Rafinesque, 1818 (Rafinesque, 1818:42)

Original Description: Farther account of discoveries in natural history in the Western States. Amer. Monthly Mag. Crit. Rev., 4:42.

Type Locality: "From the Ohio and Wabash, ..."

Type Material: unknown

Principal Descriptive Characteristics: "4 spires [whorls], a large knob [nodule, tubercle] behind the outward lip. ... length half an inch."

Melania armigera Say, 1821 (Say, 1821:178)

Melania duttoniana Lea, 1841 (Lea, 1841)

* Melania pallidula Anthony, 1854 (Anthony, 1854)

* Meseschiza grosvernori Lea, 1864 (Lea, 1864)

Angitrema armigera (Say, 1821) (Haldeman, 1841:p. 3 of cover)

Io armigera (Say, 1821) (Reeve, 1860:fig. 11)

Pleurocera fluviatilis armigera (Say, 1821) (Hannibal, 1912:171)

Lithasia armigera (Say, 1821) (Walker, 1918:35, Fig. 126)

Melania stygia Say, 1829 (Say, 1829: 261)

* Lithasia armigera stygia (Say, 1829) (Goodrich, 1940: 3)

* Lithasia armigera parva (Wetherby, 1876) (Goodrich, 1940: 3)

* Lithasia downiei Lea, 1862 (Goodrich, 1940: 3)

* Angitrema angulata Wetherby, 1876 (Goodrich, 1940: 3)

Lithasia duttoniana (Lea, 1841) (Goodrich, 1940: 4)

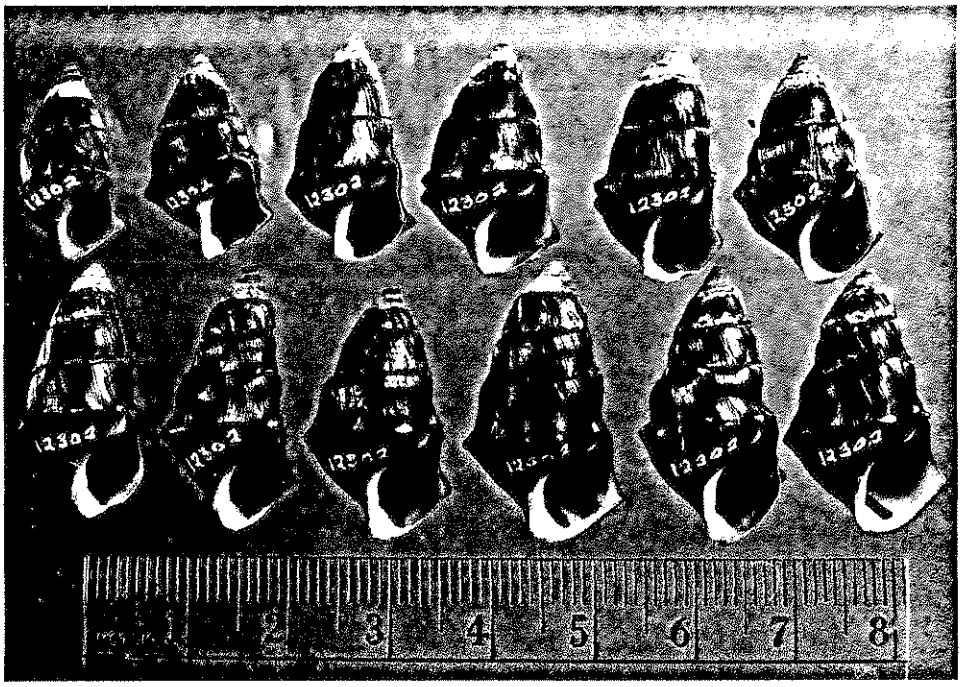


Figure 50. These specimens of E. gibbosa form gibbosa were collected from the type locality of the species, the Ohio River. Some represent the classical expression of armigera, described from the Ohio and Wabash Rivers, while others agree with the characteristic duttoniana from the Duck River. Both armigera and duttoniana are junior synonyms of gibbosa.

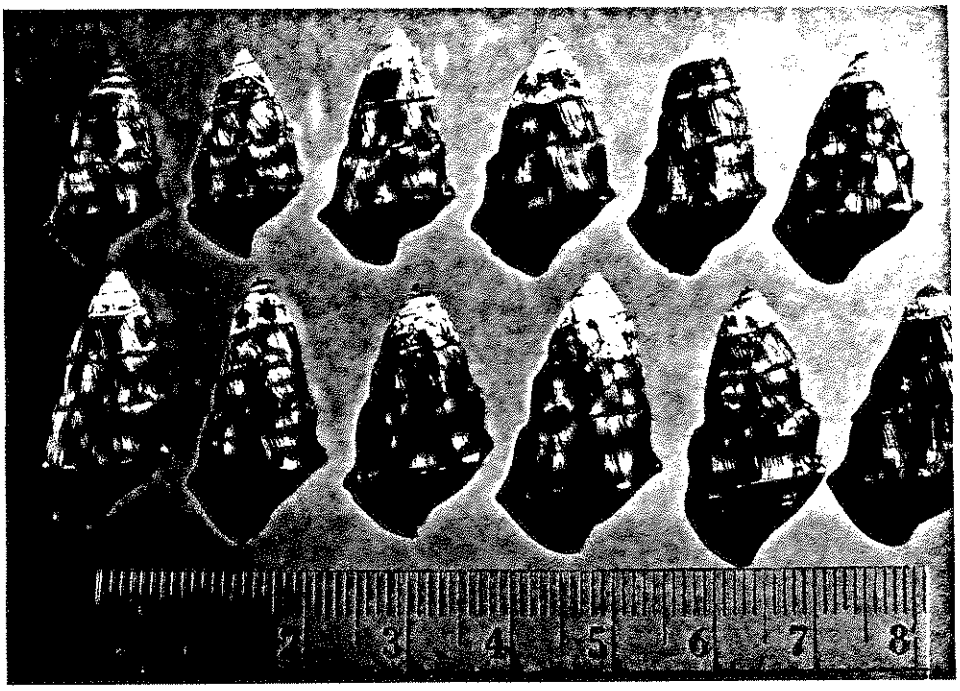


Figure 51. This view of the same specimens figured in the previous illustration shows their dorsal surfaces.

Io fasciolata Reeve, 1860	(Reeve, 1860: Io, #14)
Io armigera (Say, 1821)	(Davis, 1974: 10)
Io armigera form duttoniana (Lea, 1841)	(Davis, 1974: 12)

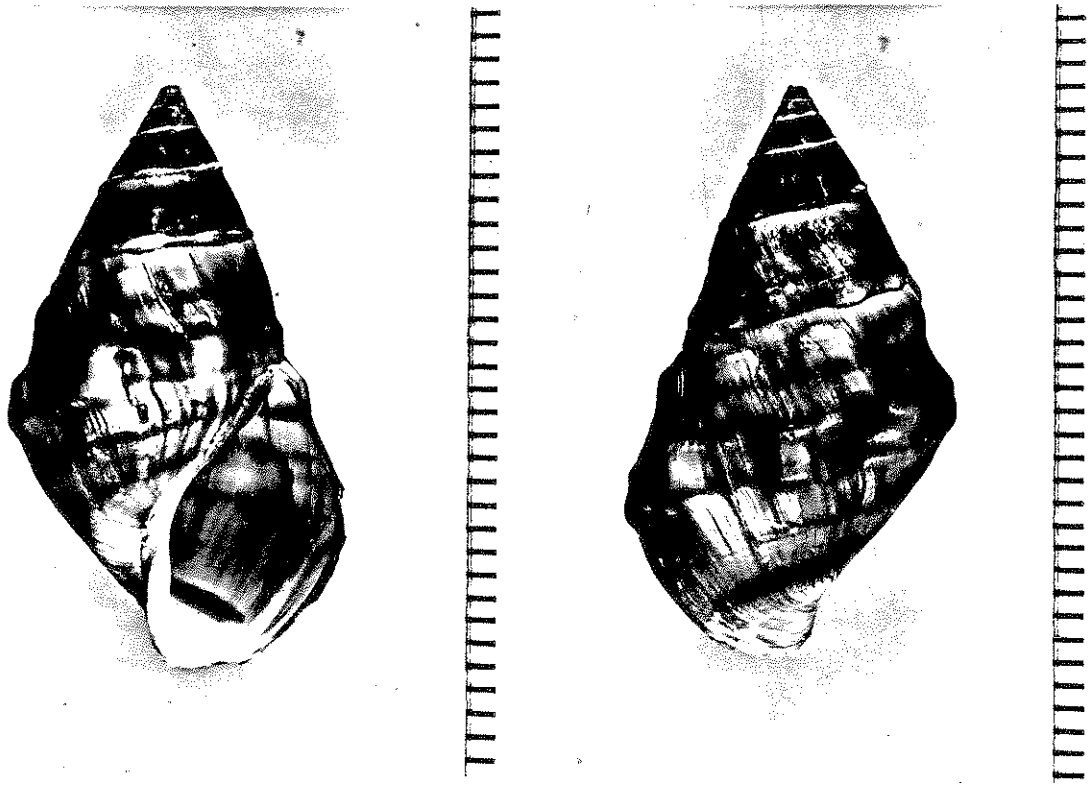
* Listed as a synonym or subspecies of E. gibbosa form gibbosa (as Lithasia armigera (Say, 1821)) by Goodrich, 1940: 3

Morphology and Distribution in Duck River

The form gibbosa is identified by the presence of a row of protuberances (variously termed knobs, nodules, tubercles or spines) on the periphery of a conoid shell. This sculpture has its origin several whorls down from the apex and typically becomes more prominent as the shell becomes larger. It shares this type of sculpture with Io fluvialis, but they are allopatric in range.

In the Duck River the gibbosa form first appears at Normandy as a fusoid-conoid shell having a single series of undulations or low tubercles developed upon a weak to moderately strong ridge. Moving downstream the shell becomes more conoid, the peripheral ridge sharper and the tubercles larger and more distinct. Intergrading forms, largely variations in shape and sculpture, are common in the Normandy collection, but become generally fewer downstream but rarely, if ever, drop out completely.

The duttoniana Lea, 1841, comes close to typifying the form gibbosa in the Duck River. The classical form gibbosa is found in the lower Ohio, lower Wabash, Cumberland and Tennessee rivers. A recent collection from the lower Ohio River (OSUM 12302) shows variation from duttoniana through "classical" gibbosa and even comes close to form jayana (Figs. 50 and 51).



Ellipstoma gibbosa form gibbosa Rafinesque, 1818

Figures 52 & 53. The specimen figured here, USNM 121739, is the designated holotype of Melania duttoniana Lea, 1841. It is a small to medium river expression of the form gibbosa which is also found occasionally with the typical morph in the Ohio River. The specimen above is labeled "Florence, Tenn." and was collected by Mr. Dutton. The specimen matches the figured type. Lea's published type locality designation is: "Hab. Waters of Tennessee.---Dr. Troost. Duck River, Maury Co. Tenn.---Mr. Dutton." We have not been able to locate Florence, Tennessee, and strongly suspect that it is an error for Florence, Alabama, which is on the Tennessee River near Mussel Shoals.

ELLIPSTOMA GIBBOSA FORM JAYANA (LEA, 1841)

Synonymy

Melania jayana Lea, 1841 (Lea, 1841:83)

Original Description: Continuation of Mr. Lea's paper on fresh water and land shells. Proc. Amer. Philos. Soc. 2:83.

Type Locality: "Caney Fork, DeKalb Co., Tenn., Dr. Troost"

Principal Descriptive Characteristics: "Shell tuberculate, subfusiform, thick, pale horn colour; spire exserted; sutures linear and curved; whorls rather convex, impressed in the middle, surrounded by a double series of tubercles; columella incurved, thickened above; aperture trapezoidal, whitish within."

Redescription: (Lea, 1841) Continuation of Mr. Lea's paper on fresh water and land shells. Trans. Amer. Philos. Soc. 9:20, fig.

Melania robulina Anthony, 1850 (Anthony, 1850:263)

Io robulina (Anthony, 1850) (Reeve, 1860:fig. 15)

Io jayana (Lea, 1841) (Brot, 1862:29)

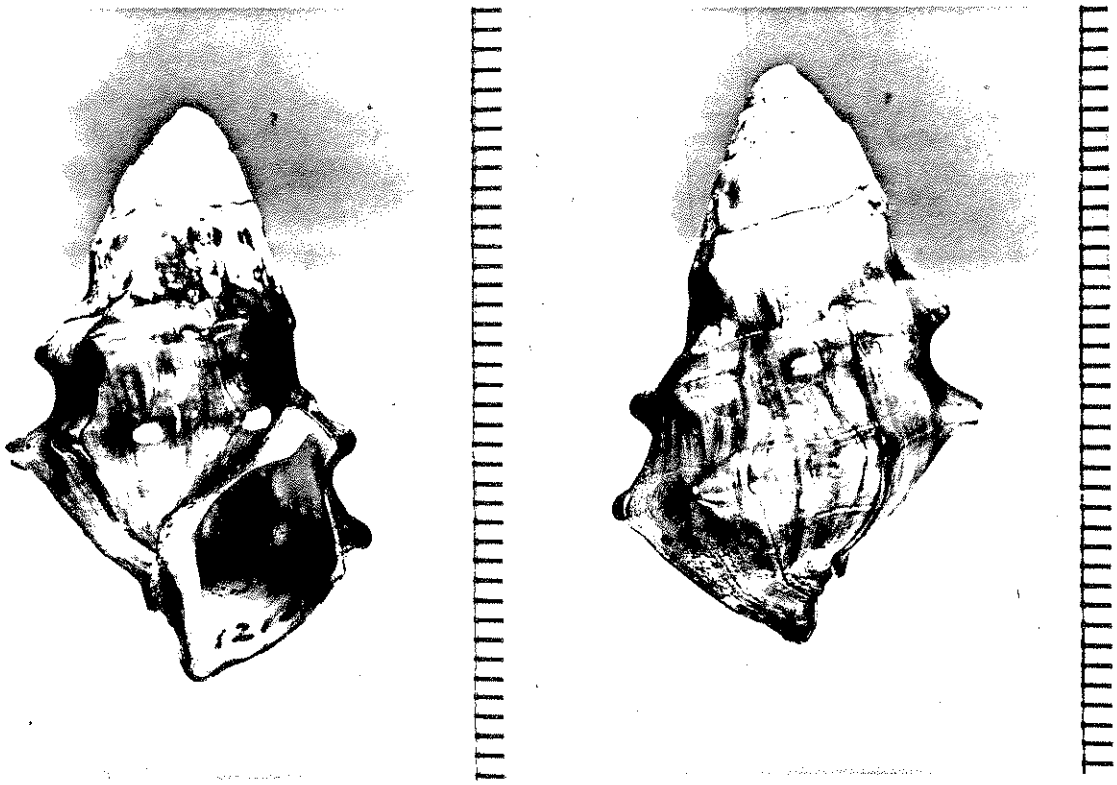
Lithasia jayana (Lea, 1841) (Goodrich, 1940:4)

Io armigera form *jayana* (Lea, 1841) (Davis, 1974:16)

Morphology and Distribution in Duck River

The form *jayana* is produced when both ends of the basic sculptural costations are preserved on the upper half of the whorl of the shell and the centers of the costae become greatly reduced or obliterated.

In the case of individual variants, when the upper or shoulder row



Ellipstoma gibbosa form jayana (Lea, 1841)

Figures 54 & 55. This shell, USNM-121631, is the designated holotype of Melania jayana Lea, 1841. It is from "Cany Fork, De Kalb Co. Tenn.---Dr. Troost." Lea apparently never figured the holotype or any other specimen of his jayana. The photographs above, by A.E. Spreitzer, may be the first illustrations of the holotype, since neither of Tryon's jayana figures (1873: 17, figs. 51 & 52) is of this specimen.

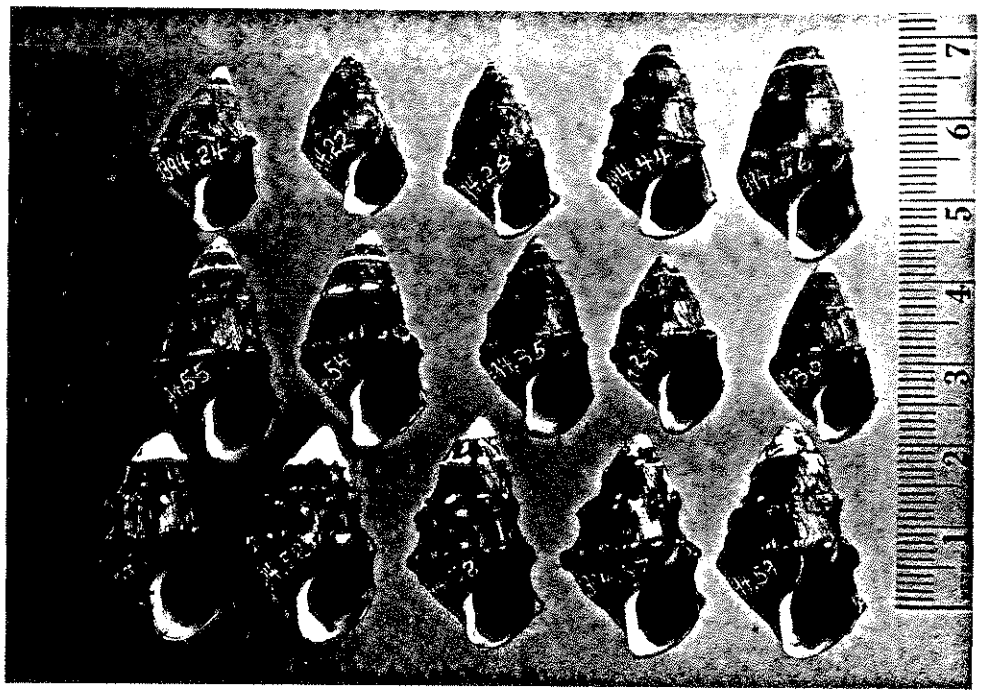


Figure 56. These conoid specimens from the Barren Hollow Road site represent the typical gibbosa form (e.g. the middle specimen in the top row) and typical jayana form (e.g. the middle specimen in the bottom row), along with several intergrades. Most of the Duck River shells of the gibbosa form would be classified as duttoniana under previous taxonomic arrangements.

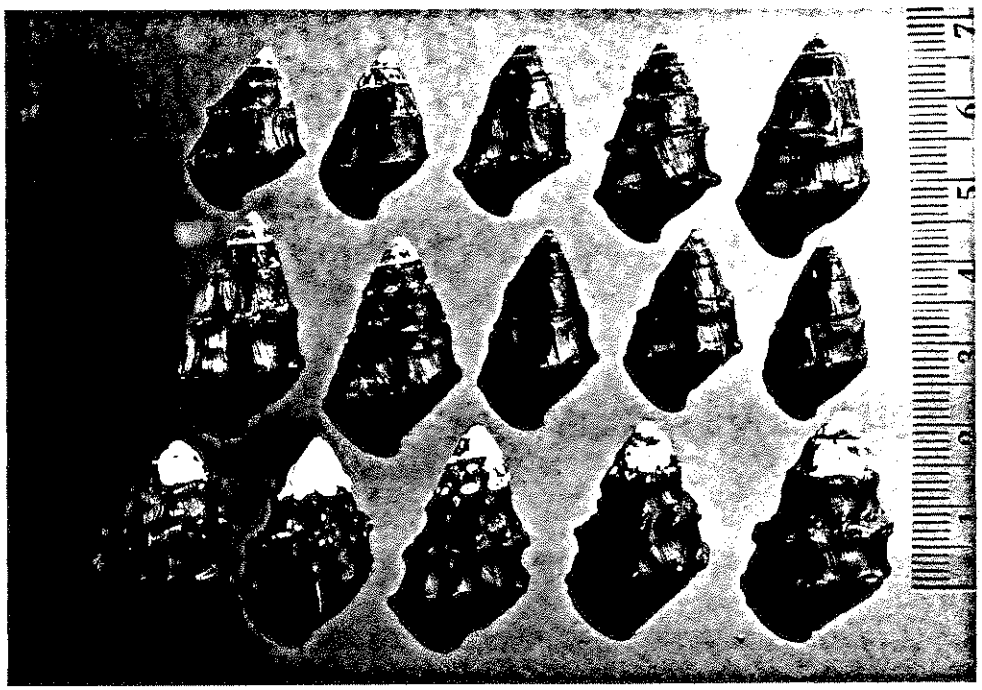


Figure 57. Dorsal view of the same specimens shown in the upper picture.

of tubercles is missing the specimen "becomes" form gibbosa; when the lower or peripheral row of tubercles is missing the specimen "becomes" form geniculata. It is only when both rows are present that the specimen "becomes" form jayana. Intergrades between these named forms are relatively common upstream at Normandy but persist, though in fewer numbers, into the downstream sites. An occasional specimen may exhibit all three forms on different parts of its shell, but such specimens are not common.

ELLIPSTOMA GIBBOSA FORM SALEBROSA (CONRAD, 1834)

Synonymy

Melania salebrosa Conrad, 1834 (Conrad, 1834: 51, pl. 54, fig. 5)

Original Description: New fresh water shells of the United States with coloured illustrations, and a monograph on the genus Anculotus of Say; also a synopsis of the American Naiades. Page 51, pl. 5, fig. 5.

Type Locality: ". . . in the Tennessee river, at Florence, . . ." ". . . in the Holston river, in Tennessee."

Principal Descriptive Characteristics: "Shell short, suboval, thick, ventricose, with a series of very elevated nodes on the shoulder of the body whorl, and generally two series of smaller nodes beneath; spire very short; apex much eroded; aperture about half the length of the shell, contracted; within purplish; columella with a callus above, and another near the base.

"This singular shell approaches the genus Anculotus, in form, but the aperture is that of a Melania."

** Anculotus salebrosus (Conrad, 1834) (Reeve, 1860: Anculotus # 6, pl. 1, fig. 6)

** Leptoxis salebrosa (Conrad, 1834) (Brot, 1862: 25)

** Lithasia salebrosa (Conrad, 1834) (Binney, 1860: No.303)

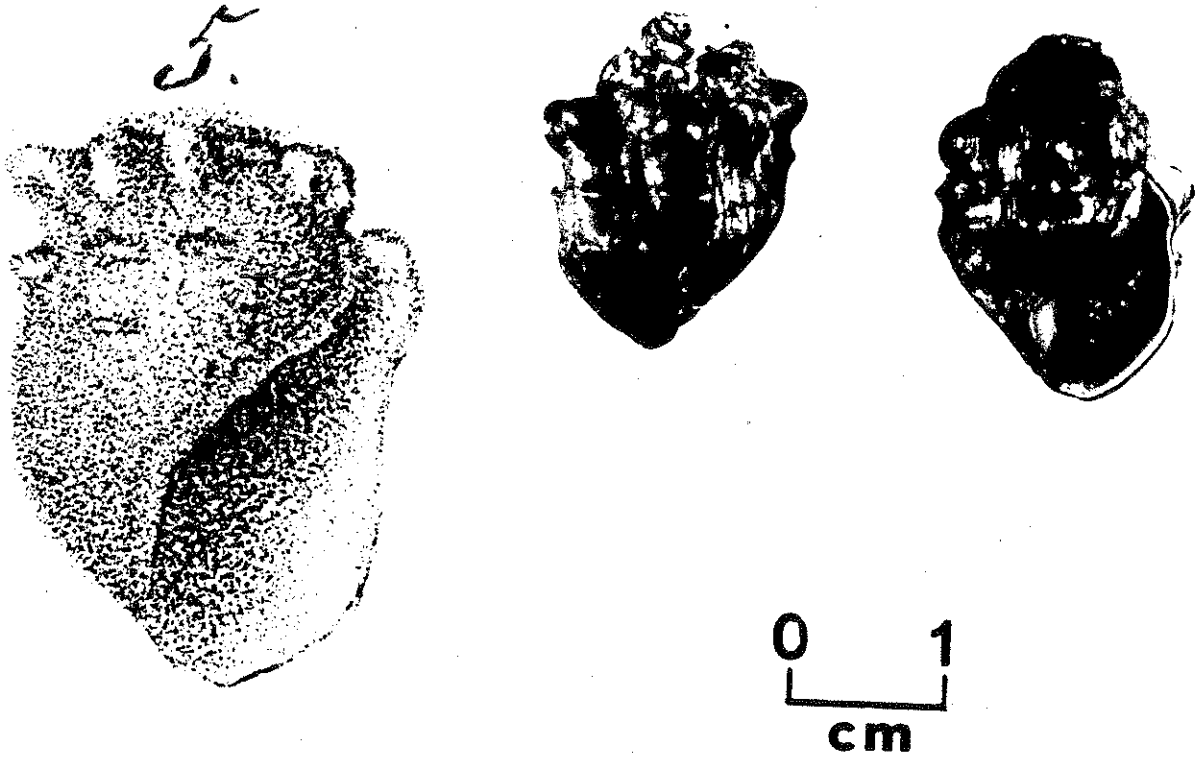
** Lythasia salebrosa (Conrad, 1834) (Adams, 1854; vol. 1: 308)

* Lithasia subglobosa Lea, 1861 (Lea, 1861: 55)

* Melania florentiana Lea, 1841 (Lea, 1841: 15)

** Io florentiana (Lea, 1841) (Adams, 1854: 299)

* Anculosa squalida Lea, 1845 (Lea, 1845: 167)



Ellipstoma gibbosa form salebrosa (Conrad, 1834)

Figure 58, at left above, is an enlargement of the illustration which accompanied Conrad's original description of salebrosa.

Figure 59, above right, shows specimens collected from the Tennessee River, Mile 257.8, prior to August, 1979, by Mr. Billy G. Isom of the Tennessee Valley Authority. This photograph was made available through the courtesy of Dr. Arthur E. Bogan.

* Melania grisea Anthony, 1860	(Anthony, 1860: 61-62)
* Lithasia tuomeyi Lea, 1861	(Lea, 1861: 55)
* Lithasia imperialis Lea, 1861	(Lea, 1861: 55)
Angitrema salebrosa (Conrad, 1834)	(Tryon, 1873: 14, fig. 45, 46)
Angitrema tuomeyi (Lea, 1861)	(Tryon, 1873: 16, fig. 49)
Angitrema subglobosa (Lea, 1861)	(Tryon, 1873: 15, fig. 47, 48)
Io salebrosa (Conrad, 1834)	(Davis, 1974: 35)

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* Placed in the synonymy of, or made a synonym of, salebrosa by Goodrich, (1940: 4).

** Placed in the synonymy of salebrosa by Tryon (1873:14)

Morphology and Distribution in Duck River

The classic concept of form salebrosa is a cuboid shell with a row of prominent, elevated tubercles or knobs developed on a strong shoulder and subtended by one or two rows of smaller tubercles. This concept differs from that of the form geniculata by the presence of one or two rows of smaller tubercles. The few salebrosa collected in Duck River appear to be simply form geniculata with the additional row of tubercles. The answer to the question of whether or not these Duck River specimens are salebrosa depends upon what salebrosa really is. This cannot be properly evaluated without detailed study of topotypical material from the Tennessee River in Alabama. A set of shells labeled as salebrosa from the Tennessee River at Mussel Shoals, the type locality, collected by H.H. Smith, is in the OSUM collection. These specimens, however, do not fit the description of this form, since all but one of them lack a second row of tubercles. Several even lack the series of very elevated nodes on the shoulder of the body whorl.

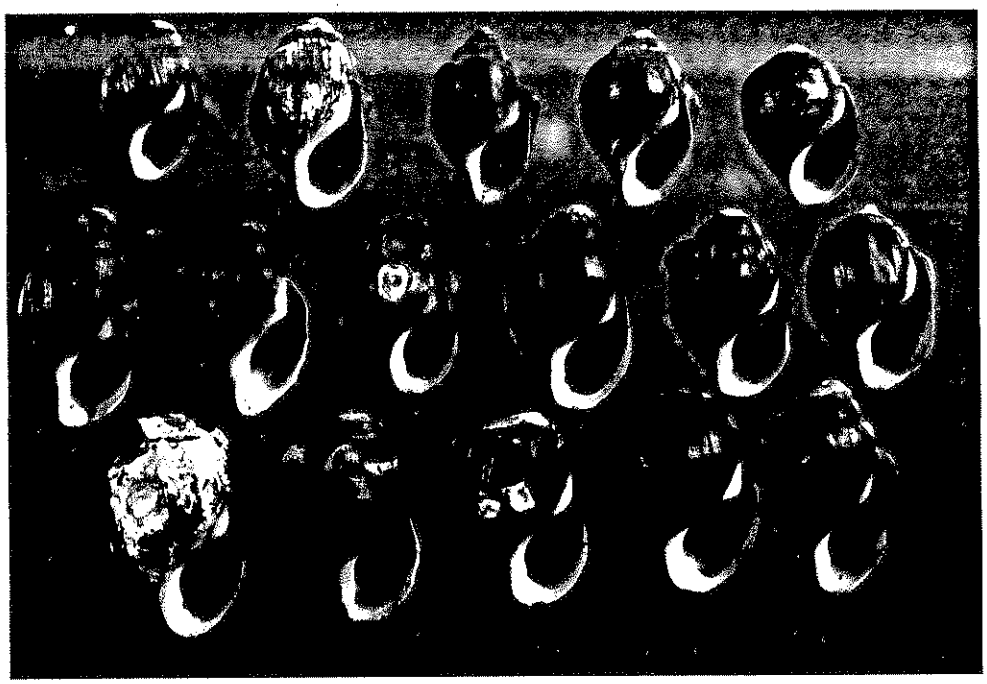


Figure 60. A series of 16 individuals of the E. gibbosa complex from the type locality of form salebrosa, the Mussel Shoals of the Tennessee River, near Florence, Alabama. Some of these specimens resemble form salebrosa; most appear to be form geniculata; and the extreme left specimens in rows 1 and 2 fit the description of form zonalis.

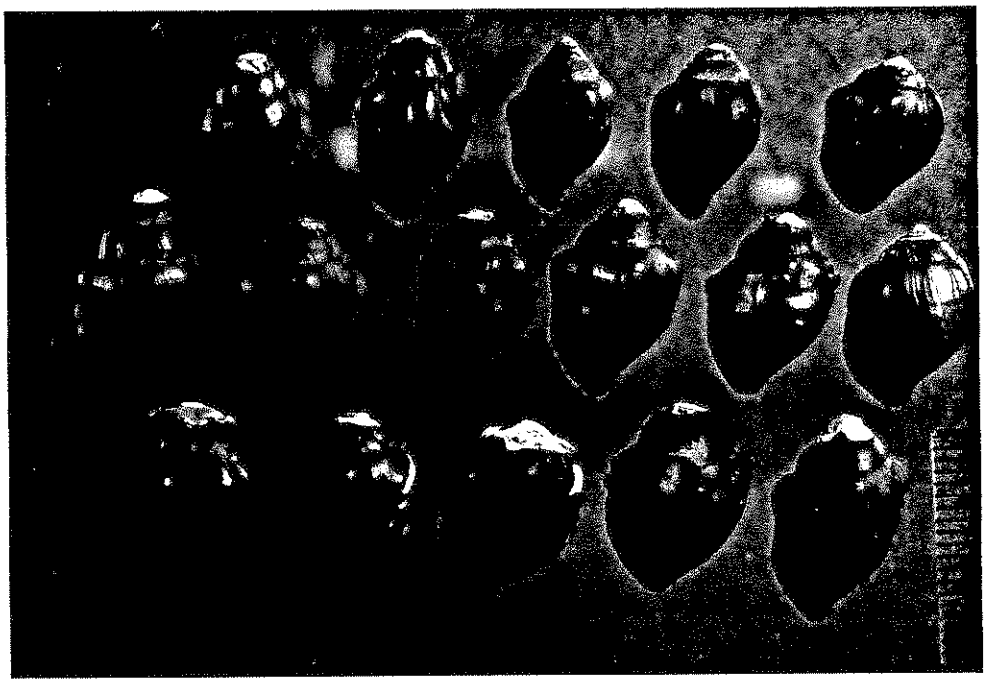


Figure 61. These are the same specimens as shown in the upper figure, rotated to show the other side of the shells.

There are reports (Isom, 1979: 69; McCaleb, personal communication; Bogan, personal communication) that typical specimens of this now rare form have been collected in recent years from one or more sites on the main stem of the Tennessee River. Photographs of some of these specimens forwarded to OSUM leave little doubt as to their identity.

It appears from our scant Duck River material that salebrosa, or at least Duck River specimens identified as this form, may be only a variant of the form geniculata. If they are included in the same species, the older name of salebrosa Conrad, 1834, would have priority over the later name geniculata Haldeman, 1840. However, the name gibbosa Rafinesque, 1818, has priority over both.

ELLIPSTOMA GIBBOSA FORM GENICULATA (HALDEMAN, 1840)

Synonymy

Anculosa (Lithasia) geniculata Haldeman, 1840 (Haldeman, 1840: 1-2)

Original Description: Supplement to number one of "A monograph of the Limniades, or freshwater univalve shells of North America," containing descriptions of apparently new animals in different classes, and the names and characters of the subgenera in Paludina and Anculosa.

Published October, 1840, for gratuitous distribution. 3 pp.

Type Locality: "Hab. East Tennessee."

Principal Descriptive Characteristics: "Shell short and ponderous; body whirl crowned with a row of conical tubercles: labium with a callus above and below: aperture elliptic, with a sinus at each extremity. Length 3/4 in. Hab. East Tennessee. Obs. Differs from Melania salebrosa, Conrad, in having but a single row of tubercles, and a more abrupt shoulder."

Anculotus geniculatus (Haldeman, 1840)	(Jay, 1850: 276)
Lythasia genicula (Haldeman, 1840)	(Wheatley, 1845: 28)
Leptoxis geniculata (Haldeman, 1840)	(Brot, 1862: 24)
* Melania corneola Anthony, 1860	(Anthony, 1860: 61)
* Melania vesicula Lea, 1861	(Lea, 1861: 118)
Goniobasis umbonata Lea, 1864	(Lea, 1864: 3)
* Eurycaelon umbonatum (Lea, 1864)	(Lea, 1866: 150)
Io geniculata (Haldeman, 1840)	(Davis, 1974: 19)

* Listed as synonyms of geniculata by Goodrich, 1940: 5.

Morphology and Distribution in Duck River

The time-honored distinguishing characteristic of the form geniculata is, as expressed by its describer (Haldeman, 1840), the "body whirl crowned with a row of conical tubercles." In this character it contrasts with the form gibbosa, which develops its row of tubercles around the periphery of the shell. Both forms are found at Normandy, but reach their maximal development downstream in the vicinity of the State Route 13 bridge near Hurricane Mills, Humphreys County, Tennessee, not far above the slack water of the impounded Tennessee River. As noted above, the greatest number of intergrades between these and other forms of the E. gibbosa complex occurred at Normandy prior to construction of the impoundment there. In general, the number of intergrades decreases with distance downstream.

The intergradation of this form with the jayana and gibbosa forms has been mentioned above. At least two additional described forms stand somewhat closer to the form geniculata than to the others dealt with in this report.

In some specimens the crowning tubercles are joined laterally, thus forming a shoulder, which varies from gently undulate to essentially smooth. The shell outline is cuboid, as is form geniculata, but there is no tuberculate sculpture. This is the form zonalis Rafinesque, 1818, perhaps better known by its junior synonym obovata Say, 1829.

When a second or third row of tubercles develops just beneath the "crown of tubercles," the specimen fits the essence of the description of form salebrosa Conrad, 1834. Both form zonalis and form salebrosa are discussed below.

ELLIPSTOMA GIBBOSA FORM ZONALIS RAFINESQUE, 1818

Synonymy

Ellipstoma zonalis Rafinesque, 1818 — Rafinesque (1818: 42)

Original Description: Farther account of discoveries in natural history in Western States. Amer. Monthly Mag. Crit. Rev., vol. 4: 42.

Type Locality: "Kentucky river."

Principal Descriptive Characteristics: "3 spires, smooth, 3 transverse zones violet."

Melania obovata Say, 1841 — (Say, 1829: 276)

** Melania hildrethiana Lea, 1841 — (Lea, 1841: 11)

* Melania nucleola Anthony & Gould, 1851 — (Anthony & Gould, 1851: 360)

* Melania gibbosa Lea, 1852 — (Lea, 1852: 252)

* Melania elegantula Anthony, 1854 — (Anthony, 1854: 103, pl. 3, fig. 2)

* Melania coronilla Anthony, 1854 — (Anthony, 1854: 126, pl. 3, fig.27)

* Melania tabulata Anthony, 1854 — (Anthony, 1854: 118, pl. 3, fig. 18)

* Melania latitans Anthony, 1854 — (Anthony, 1854: 88, pl. 2, fig. 6)

* Melania chalybea Anthony in Brot, 1862 — (Brot, 1862: 37) [nomen nudum?]

** Anculotus obovatus (Say, 1829) — (Jay, 1850: 276)

** Leptoxis obovata (Say, 1829) — (Brot, 1862:25)

** Melania undosa Anthony, 1854 — (Anthony, 1854: 124, pl.3, f. 25)

** Melania rarinodosa Anthony in Reeve, 1860 — (Reeve, 1860: Melania # 268)

** Melania consanguinea Anthony, 1854 — (Anthony, 1854: 125, pl. 3, fig.26)

** Anculotus consanguineus (Anthony, 1854) — (Reeve, 1860: Anculotus # 2)

* Anculosa lewisi Lea, 1861 — (Lea, 1861: 54)

	Lithasia obovata (Say, 1841)	(Adams & Adams, 1854: 308)
	Leptoxis hildrethiana (Lea, 1841)	(Adams & Adams, 1854: 307)
*	Goniobasis gabbiana Lea, 1862	(Lea, 1862: 304)
*	Goniobasis infantula Lea, 1863	(Lea, 1863: 155)
*	Goniobasis louisvillensis Lea, 1863	(Lea, 1863: 155)

** Placed in the synonymy of E. zonalis (as L. obovata) by Tryon (1873: 33).

* Placed in the synonymy of E. zonalis (as L. obovata) by Goodrich (1940:6-7).

NOTE: The above list of synonyms of the form zonalis gives some indication of its variability. The ability to survive as isolated populations in small streams across its range is in part responsible, as well as the fact that the range of zonalis is greater than the range of any other form of the E. gibbosa complex. This list of synonyms could be greatly lengthened if its described "subspecies" and their synonyms were added.

Morphology and Distribution in the Duck River

This is the strong-shouldered, non-sculptured, cuboid form of the complex. It is sometimes characterized as "geniculata without tubercles." The OSUM holdings of this form from the Green River system of Kentucky are extensive and highly variable, but none are sculptured. Collections from several sites in Duck River from Normandy downstream to Barren Hollow Road have yielded specimens which fit the concept of zonalis. Some are essentially identical to zonalis specimens from the Green River system and other locales outside the Tennessee River basin. Within the Duck River zonalis apparently intergrades with other sculptured forms of the gibbosa complex. This is especially true near the headwaters and less so downstream, where the various forms appear to develop nearly pure populations.

There is some hesitation in including zonalis here as a form of E. gibbosa because it is (or was) widespread outside the Duck River, occurring with sculptured forms of this complex without any evidence of intergradation, and because it is not a common form in the Duck River. It is included here because shells of this description have been taken in recent years from the Duck River and intermediate specimens between this form and the other forms of the E. gibbosa complex have been found. It would be interesting to do an electrophoretic study on zonalis specimens from outside the Duck River and compare with the data herein.

ELLIPSTOMA GIBBOSA FORM FULIGINOSA (LEA, 1841)

Synonymy

Melania fuliginosa Lea, 1841 (Lea, 1841: 12)

Original Description: New fresh water and land shells. Proc. Amer. Philos. Soc., 2: 12.

Type Locality: "Big Bigby Creek, Maury Co., Tenn."

Principal Descriptive Characteristics: "Testâ fusiformi, subinflatâ, subcrassâ, tenebroso-fuscâ, laevi; spira obtusâ; suturis impressis; anfractibus senis, subconvexis; apertura magnâ; ad basim angulatâ et canaliculatâ."

** Leptoxis fuliginosa (Lea, 1841) (Adams & Adams, 1854: 307)

* Melania densa Anthony & Gould, 1851 (Anthony & Gould, 1851: 360)

* Lithasia dilatata Lea, 1861 (Lea, 1861: 55)

* Melania abbreviata Anthony & Gould, 1851 (Anthony & Gould, 1851: 360)

Lithasia fuliginosa (Lea, 1841) (Tryon, 1873: 27)

Io geniculata form fuliginosa (Lea, 1841) (Davis, 1974: 23)

Angitrema angulata Wetherby, 1876) (Wetherby, 1876: 11, pl. 1, fig. 5)

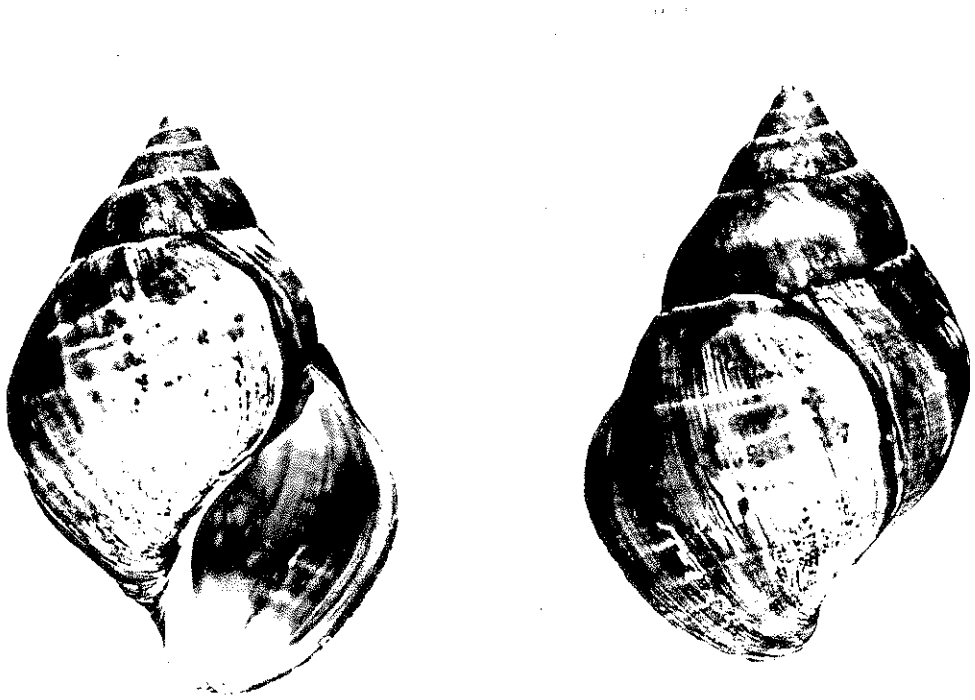
---

* Placed in the synonymy of fuliginosa by Goodrich (1940: 5).

** Placed in the synonymy of fuliginosa by Tryon (1873: 27).

Morphology and Distribution in Duck River

The original description of form fuliginosa leaves no doubt that this is the basic pattern or generalized template upon which, by some changes in form and especially in sculpture, all other forms of this complex can be readily derived. It is, as in Lea's 1842 redesdription (pp. 170-171):



Ellipstoma gibbosa form fuliginosa (Lea, 1841)

Figures 62 and 63. This specimen, USNM-121915, is the designated holotype of Melania fuliginosa Lea, 1841, collected from "Big Bigby Creek, Maury Co. Tenn.---Mr. Dutton." Big Bigby Creek is a tributary of Duck River. This specimen differs in color, number of whorls, and form of spire from the original description.

> . . . Smooth, fusiform, somewhat inflated, rather thick, dark brown; spire obtuse; sutures impresse,; whorls 6, somewhat convex; aperture large, at the base angular and channeled.

The designated holotype, USNM-121915, is unfortunately not the specimen described above and figured by Lea (1842: 170) in his redescription. The designated holotype is not smooth; rather, it has gentle swellings or varices on the body whorl. It is light horn color, rather than dark brown, and it is a relatively thin shell. Lea (1842: 171) also noted that the substance of the shell is disposed to be purple, but there is no purple or dark pigment on the designated holotype either internally or externally. However, this specimen is labeled "Big Bigby Creek, Maury Co., Tenn.", the type locality, and it typifies the form found in the lower reaches of the larger tributaries of the lower Duck River. While it is not the color of the fuliginosa form found in the upper Duck River at Manchester, there is no doubt but that they are the same species.

The form fuliginosa was found from Manchester, Coffee County, downstream at least as far as our Barren Hollow Road site at Duck River Mile 32.2. Within this 237-mile distance it has an impressive range of morphological variability. Specimens from downstream sites are relatively narrow, basically horn yellow to tan, and are usually ornamented with purple bands or lines. The development of costae, absent at Manchester and prominent at Normandy, is absent or scarcely perceptible at Barren Hollow Road.

The concept of fuliginosa has been expanded over the years from that of the type, a smooth fusiform shell, to include specimens which have various degrees of sculpture, from subplicate to nodulous. Goodrich (1941: 5) notes, "L. geniculata fuliginosa, belonging to the Duck River of Tennessee and its tributaries, shows a variation in sculpture with reference to position in the stream." He found that the number of sculptured individuals increased with

progression downstream from Bedford to Maury County. The inclusion of costate specimens within the concept of fuliginosa gave these individuals a taxonomic niche. Apparently no costate forms of this complex had ever been described, even though, as noted above, these are connected by insensible gradations to the non-sculptured forms in most populations.

The form described as angulata by Wetherby in 1876 is very similar to the designated holotype of fuliginosa. Both are smooth, light-colored, fusiform shells characterized by the presence of rather prominent wavy varices on the body whorl. Although the type locality of angulata is the Stones River of the Cumberland River basin, we cannot separate the two forms. We therefore place angulata Wetherby, 1876, into the synonymy of E. gibbosa form fuliginosa (Lea, 1841).

SUMMARY

The objective of this study was a biosystematic analysis of the species of the Duck River genus Pleurocera Rafinesque, 1818. The use of the name Pleurocera in its original sense has, in the interim, been suppressed by the International Commission on Zoological Nomenclature (1981). Thus, the next available name, Ellipstoma Rafinesque, 1818, must now be used for this genus.

Several previous efforts to resolve the taxonomic status of these variable snails through the use of morphological characteristics had been made (e.g., Goodrich, 1940; Davis, 1974). Since the results of these studies had not yielded completely satisfactory results, we attempted to obtain additional evidence concerning the taxonomic relationships of these forms by combining an electrophoretic analysis with a review of the shell morphology and the original descriptions, figures, and, where possible, type specimens.

The results of our electrophoretic analysis of 8 populations of Duck River Pleuroceridae which had been referred to this complex indicated that: 1) the population which had previously been referred to this complex in the Duck River at Fredonia is distinct from all other Duck River populations studied; and 2) All of the other described taxa occurring in this river and recognized as part of this generic complex are a single interbreeding species.

That such diverse morphological forms were really a single polymorphic species was difficult to accept, since a number of nominal subspecies, species, and even genera were involved. Because there were so many nominal taxa involved, and because the downstream collections had fewer intermediate specimens, it was believed that some consideration of patterns of variation of shell morphology, beyond that necessary to code electrophoretic samples, would be helpful.

form ZONALIS Rafinesque, 1818: Specimens having a cuboid or conoid-cuboid shell, lacking sculpture, but having a well-developed shoulder.

form FULIGINOSA (Lea, 1841): Specimens having a smooth fusoid or fusoid-conoid shell, lacking sculpture except for costae on whorls 3-6 in some individuals.

Reeve, Lovell A.
1860. Conchologia Iconica: or, illustrations of the shells of molluscous animals. Vol. XII.
Lovell Reeve, London.

Rosewater, Joseph
1960. Geographic variation of Pleurocera canaliculata (Mollusca: Prosobranchia) in the Ohio River Basin.
Unpublished Ph.D. Dissertation, Harvard Univ., Cambridge, MA. 224 pp.

Say, Thomas
1821. Descriptions of univalve shells of the United States.
Jour. Acad. Nat. Sci. Philadelphia, 2 (Pt. 1): 149-179.

1825. Descriptions of some new species of fresh water and land shells of the United States.
Jour. Acad. Nat. Sci. Philadelphia, 5 (Pt. 1): 119-131.

1829. Descriptions of some new terrestrial and fluviatile shells of North America.
Disseminator of Useful Knowledge, New Harmony, Ind., 2 (18): 275-277.

Schaal, Barbara A., and Wyatt W. Anderson
1974. An outline of techniques for starch gel electrophoresis of enzymes from the American oyster Crassostrea virginica Gmelin.
Georgia Marine Science Center, Technical Report Series (unpublished manuscript), (74-3): 1-17, 6 figs.

Selander, R.K., M.H. Smith, S.Y. Yang, W.E. Johnson, and J.B. Gentry
1971. Biochemical polymorphism and systematics in the genus Peromyscus.
Univ. Texas Publ., Studies in Genetics, VI. (7103): 49-90.

Shaw, Charles R., and Rupi Prasad
1970. Starch gel electrophoresis of enzymes --- a compilation of recipes.
Biochemical Genetics, 4: 297-320.

Tryon, George W., Jr.
1873. Land and fresh-water shells of North America. Part IV. Strepomatidae (American Melanians).
Smithsonian Misc. Coll., (253): i-liii, 1-435.

Walker, Bryant
1918. A synopsis of the classification of the freshwater Mollusca of North America, North of Mexico, and a catalogue of the more recently described species, with notes.
Univ. Michigan Museum of Zoology Misc. Pub. (6): 1-213.

Wetherby, A. G.
1876. Remarks on the variation in form of the Family Strepomatidae, with descriptions of new species.
Proc. Cincinnati Soc. Nat. Hist., 1 (1): 1-12, 1 pl.

Appendix 1
ELECTROPHORETIC STAINS USED ON SPECIMENS OF PLEUROCERID SNAILS ELECTROPHORESED FOR THE DUCK RIVER PROJECT

ELECTROPHORESIS DATE (YY/MM/DD)	SOURCE LOCALITIES OF SPECIMENS	STAINS USED						
82/04/15	TN, Duck R., Barren Hollow Rd. AR, Saline R., Tull/Traskwood	CAT/S&A	ODH/S&A					
82/04/21	TN, Duck R., Barren Hollow Rd. AR, Saline R., Tull/Traskwood	MDH/B&H	CAT/S&A	XDH/D&D	OCT/D&D			
82/06/07	TN, Duck R., Barren Hollow Rd.	EST/D&D	G6PD/AYALA	ACPH/B&H	ALDO/S&P			
82/06/08	TN, Duck R., Barren Hollow Rd.	MDH/B&H XDH/AYALA	GOT/D&D GPI/D&D	PGI/B&H	HEXDH/D&D	LAP/D&D	PGM/AYALA	ISDH/B&H
82/06/09	TN, Duck R., Barren Hollow Rd.	ME/AYALA	CAT/S&A	G3PD/AYALA	G6PD/AYALA	SDH/AYALA		
82/06/10	TN, Duck R., Barren Hollow Rd.	LAP/D&D	GP/SEL	OCTOP/D&D	PGM/AYALA			
82/06/11	TN, Duck R., Barren Hollow Rd.	ISDH/B&H	PGI/B&H	GOT/D&D	OCTOP/D&D	XDH/S&A		
82/06/17	TN, Duck R., Barren Hollow Rd.	6PGD/B&H	ODH/B&H	GALDH/B&H	ADH/B&H	EST/S&A	ALPH/B&H	
82/06/18	TN, Duck R., Barren Hollow Rd.	EST/B&H EST/D&D	EST/S&A	ME/AYALA	MDH-T/B&H	AO/B&H	ADH/B&H	EN/B&H
82/06/30	TN, Duck R., Barren Hollow Rd.	GLUDH/B&H PEPSIN/B&H	FUM/B&H MO/B&H	TYRO/B&H ME/AYALA	ACPH/D&D 6PGD/B&H	PGI/B&H MPI/D&D	HEXOK/B&H AO/B&H	CK-TO/S&A
82/07/08	TN, Duck R., Barren Hollow Rd.	ACPH/D&D	ADH/B&H	GOT/B&H				
82/07/09	TN, Duck R., Barren Hollow Rd. OH, Rocky Fork Cr., Columbus	FUM/B&H	MDH/B&H	ALPH/B&H				
82/08/05	TN, Duck R., Barren Hollow Rd. OH, Rocky Fork Cr., Columbus	MDH-T/B&H	SDH/AYALA	LAP/D&D				
82/08/10	TN, Duck R., Barren Hollow Rd. OH, Rocky Fork Cr., Columbus	XDH/AYALA	PGI/B&H	HEXDH/D&D				
82/08/12	TN, Duck R., Barren Hollow Rd. OH, Rocky Fork Cr., Columbus	EST/B&H	EST/D&D	EST/S&A				

Appendix 1, cont.

ELECTROPHORETIC STAINS USED ON SPECIMENS OF PLEUROCERID SNAILS ELECTROPHORESED FOR THE DUCK RIVER PROJECT

ELECTROPHORESIS DATE (YY/MM/DD)	SOURCE LOCALITIES OF SPECIMENS	STAINS USED							
82/08/17	TN, Duck R., Barren Hollow Rd. OH, Rocky Fork Cr., Columbus	CAT/S&A	GP/SEL	G6PD/AYALA					
82/08/19	TN, Duck R., Barren Hollow Rd.	TYRO/B&H	ME/AYALA	ODH/B&H	GLUDH/B&H	GALDH/B&H	EADH/S&A		
82/08/24	TN, Duck R., Barren Hollow Rd.	XDH/SEL	XDH/S&A	PGM/AYALA	HEXOK/B&H	OCTOP/D&D	PROT/AYALA		
82/08/26	TN, Duck R., Barren Hollow Rd.	EST/D&D	SDH/AYALA	LAP/D&D	HEXDH/D&D	ADH/B&H	ALPH/B&H	MDH/B&H	
	OH, Rocky Fork Cr., Columbus	MDH-T/B&H	PROT/AYALA	PGI/B&H	FUM/B&H	OCTOP/D&D			
82/08/31	TN, Duck R., Barren Hollow Rd.	MDH/B&H	LAP/D&D	6PGD/B&H	ACPH/D&D	MDH-T/B&H	SDH/AYALA	G6PD/AY	
	OH, Rocky Fork Cr., Columbus	MDH-T/B&H	G3PD/AYALA	GOT/D&D	CAT/S&A				
82/11/05	TN, Duck R., Barren Hollow Rd.	ACPH/D&D	GOT/D&D	CAT/S&A	PGI/B&H	FUM/B&H	PROT/AYALA	MDH/B&H	
	TN, Duck R., Barren Hollow Rd.	LAP/D&D	EST/D&D	HEXDH/D&D	SDH/AYALA	ALPH/ B&H	MDH-T/B&H	OCTOP/D&D	
82/12/16	TN, Duck R., Barren Hollow Rd.								
82/12/28	TN, Duck R., Barren Hollow Rd.	LAP/D&D	MDH/B&H	PGI/B&H	ACPH/D&D	GOT/D&D	GP/SEL	ALPH/B&H	SDH/Ayala
	TN, Duck R., Cortner Rd.bridge	HEXDH/D&D	EST/D&D	FUM/B&H	AO/D&D	CAT/S&A	OCTOP/D&D	MDH-T/B&H	
82/12/30	TN, Duck R., Barren Hollow Rd.	PGI/B&H	AO/B&H	CAT/S&A	EST/D&D	ACPH/D&D	GP/SEL	HEXDH/D&D	
	TN, Duck R., Cortner Rd.bridge	OCTOP/D&D	LAP/D&D	ISDH/DILLN	ALPH/B&H	FUM/B&H			
83/01/06	TN, Duck R., Barren Hollow Rd.	EST/D&D	EST/S&A	LAP/D&D	EST/B&H	ACPH/D&D	ALPH/B&H	ADH/B&H	
	TN, Duck R., Cortner Rd.bridge	FUM/B&H	HEXDH/D&D	EST/S&A	GP/SEL	PROT/AYALA	AO/DILLON	ALPH/B&H	
83/01/12	TN, Duck R., Barren Hollow Rd.	EST/D&D	EST/S&A	LAP/D&D	EST/B&H	ACPH/D&D	ADH/B&H	FUM/B&H	
	TN, Duck R., Cortner Rd.bridge	HEXDH/D&D	GP/SEL	PROT/AYALA	AO/DILLON	ALPH/B&H	ALDO/S&P		
83/01/14	TN, Duck R., Barren Hollow Rd.	OCTOP/D&D	SDH/D&D	ALDO/S&P					
	TN, Duck R., Cortner Rd.bridge								
83/01/19	TN, Duck R., Barren Hollow Rd.	AO/B&H	ISDH/DILLN	SDH/D&D					
	TN, Duck R., Cortner Rd.bridge								

Appendix 1, cont.

ELECTROPHORETIC STAINS USED ON SPECIMENS OF PLEUROCERID SNAILS ELECTROPHORESED FOR THE DUCK RIVER PROJECT

ELECTROPHORESIS DATE (YY/MM/DD)	SOURCE LOCALITIES OF SPECIMENS	STAINS USED						
83/01/21	TN, Duck R., Barren Hollow Rd.	PEPSIN/B&H	CK-TO/S&A	MPI/D&D				
	TN, Duck R., Cortner Rd.bridge							
83/01/25	TN, Duck R., Barren Hollow Rd.	OCTOP/D&D	G6PD/AYALA	6PGD/B&H	MDH/B&H	GP/SEL	PEPSIN/B&H	
	TN, Duck R., Cortner Rd.bridge							
83/01/27	TN, Duck R., Barren Hollow Rd.	ACPH/D&D	ADH/B&H	ALPH/B&H	FUM/B&H	HEXDH/B&H	ISDH/DILLN	EST/D&D
	TN, Duck R., Cortner Rd.bridge	MDH/B&H	PGI/B&H	GOT/D&D				
83/02/03	TN, Duck R., Barren Hollow Rd.	CK-TO/S&A	MPI/D&D	G6PD/AYALA	FUM/B&H	ALPH/B&H	ALDO/S&P	LAP/D&D
	TN, Duck R., Cortner Rd.bridge	SDH/D&D	PEPSIN/B&H					
83/02/04	TN, Duck R., Barren Hollow Rd.	OCTOP/D&D	XDH/AYALA	HEXDH/D&D	ACPH/D&D	ADH/B&H	ISDH/DILLN	PGI/B&H
	TN, Duck R., Cortner Rd.bridge	GP/SEL	MDH/B&H					
83/02/08	TN, Duck R., Barren Hollow Rd.	XDH/AYALA	ALPH/B&H	AO/DILLON	ACPH/D&D	ISDH/DILLN	FUM/B&H	OCTOP/D&D
	TN, Duck R., Cortner Rd.bridge	PGI/B&H	PGM/AYALA	GP/SEL	PEPSIN/B&H	GOT/D&D	MDH/B&H	EST/D&D
83/02/10	TN, Duck R., Barren Hollow Rd.	HEXDH/D&D	ADH/B&H	ACPH/D&D	AO/DILLON	G6PD/AYALA	OCTOP/D&D	PGM/AYALA
	TN, Duck R., Cortner Rd.bridge	LAP/D&D						
83/02/15	TN, Duck R., Barren Hollow Rd.	ACPH/D&D	ALPH/B&H	FUM/B&H	ISDH/DILLN	ADH/B&H	MDH/B&H	PGI/B&H
	TN, Duck R., Cortner Rd.bridge	ALDO/S&P	GOT/D&D	LAP/D&D				
83/02/17	TN, Duck R., Barren Hollow Rd.	OCTOP/D&D	EST/D&D	EN/B&H	PEPSIN/B&H	GP/SEL	ISDH/DILLN	LAP/D&D
	TN, Duck R., Cortner Rd.bridge	MPI/D&D						
83/02/22	TN, Duck R., Barren Hollow Rd.	ACPH/D&D	ALPH/B&H	FUM/B&H	ISDH/DILLN	MDH/B&H	EST/B&H	OCTOP/D&D
	TN, Duck R., Cortner Rd.bridge	ALDO/S&P	EN/S&P	MPI/D&D	EST/D&D	LAP/D&D	PGI/B&H	GOT/D&D
83/02/25	TN, Duck R., Barren Hollow Rd.	ISDH/DILLN	MDH/B&H	FUM/B&H	6PGD/B&H	GP/SEL	PEPSIN/B&H	GOT/D&D
	TN, Duck R., Cortner Rd.bridge							

Appendix 1, cont.

ELECTROPHORETIC STAINS USED ON SPECIMENS OF PLEUROCERID SNAILS ELECTROPHORESED FOR THE DUCK RIVER PROJECT

ELECTROPHORESIS DATE (YY/MM/DD)	SOURCE LOCALITIES OF SPECIMENS	STAINS USED							
83/03/01	TN, Duck R., Barren Hollow Rd.	ACPH/D&D	ALPH/B&H	FUM/B&H	ISDH/DILLN	ADH/B&H	XDH/AYALA	OCTOP/D&D	PGI/B&H
	TN, Duck R., Cortner Rd.bridge	AO/D&D	PGM/AYALA	LAP/D&D	MDH/D&D	MPI/D&D	GOT/D&D	GP/SEL	
83/03/02	TN, Duck R., Barren Hollow Rd.	EST/D&D	LAP/D&D	MDH/B&H					
	TN, Duck R., Cortner Rd.bridge								
83/03/03	TN, Duck R., Barren Hollow Rd.	ACPH/D&D	ALPH/B&H	FUM/B&H	ISDH/DILLN	OCTOP/D&D	GOT/D&D	GP/SEL	
	TN, Duck R., Cortner Rd.bridge	PGI/B&H	LAP/D&D	MPI/D&D	EST/D&D	MDH/B&H			
83/03/08	TN, Duck R., Barren Hollow Rd.	ACPH/D&D	ALPH/B&H	FUM/B&H	ISDH/DILLN	EST/B&H	MPI/D&D	OCTOP/D&D	
	TN, Duck R., H. Horton St.Park	LAP/D&D	EST/D&D	MDH/B&H	GOT/D&D	PGI/B&H	GP/SEL		
83/03/11	TN, Duck R., Barren Hollow Rd.	PGI/B&H	EST/D&D	MPI/D&D	OCTOP/D&D	MDH/B&H	ISDH/DILLN	ALPH/B&H	
		LAP/D&D	GOT/D&D	FUM/B&H	ACPH/D&D	GP/SEL			
83/03/15	TN, Duck R., Barren Hollow Rd.	ALDO/DILLN	G6PD/AYALA	AO/DILLON	HEXDH/D&D	XDH/AYALA	HEXOK/B&H	ODH/B&H	
		SDH/D&D	ADH/B&H	CK-TO/S&A	6PGD/B&H	PGM/AYALA			
83/03/17	TN, Duck R., Barren Hollow Rd.	OCTOP/D&D	MPI/D&D	PGM/AYALA	6PGD/B&H	MDH/B&H	LAP/D&D	GOT/D&D	
		ISDH/DILLN	ALPH/B&H	ACPH/D&D	PGI/B&H	EST/?			
83/03/29	TN, Duck R., Barren Hollow Rd.	ACPH/D&D	ALPH/B&H	6PGD/B&H	ISDH/DILLN	MPI/D&D	EST/D&D	LAP/D&D	
	TN, Duck R., Cortner Rd.bridge	CAT/S&A	OCTOP/D&D	PGI/B&H	GP/SEL	MDH/B&H	GOT/D&D	PGM/AYALA	
83/03/31	TN, Duck R., Barren Hollow Rd.	ACPH/D&D	ALPH/B&H	6PGD/B&H	ISDH/DILLN	MPI/D&D	EST/D&D	LAP/D&D	
	TN, Duck R., Cortner Rd.bridge	FUM/B&H	OCTOP/D&D	PGI/B&H	GP/SEL	MDH/B&H	GOT/D&D	PGM/AYALA	
83/04/05	TN, Duck R., Barren Hollow Rd.	ACPH/D&D	ALPH/B&H	6PGD/B&H	ISDH/DILLN	MPI/D&D	EST/D&D	LAP/D&D	
	TN, Duck R, H.Horton St. Park	FUM/B&H	OCTOP/D&D	PGI/B&H	GP/SEL	MDH/B&H	GOT/D&D	PGM/AYALA	
83/04/07	TN, Duck R., Barren Hollow Rd.	ACPH/D&D	ALPH/B&H	6PGD/B&H	ISDH/DILLN	MPI/D&D	EST/D&D	LAP/D&D	
	TN, Duck R, H.Horton St. Park	FUM/B&H	OCTOP/D&D	PGI/B&H	GP/SEL	MDH/B&H	GOT/D&D	PGM/AYALA	

Appendix 1, cont.

ELECTROPHORETIC STAINS USED ON SPECIMENS OF PLEUROCERID SNAILS ELECTROPHORESED FOR THE DUCK RIVER PROJECT

ELECTROPHORESIS DATE (YY/MM/DD)	SOURCE LOCALITIES OF SPECIMENS	STAINS USED						
83/04/12	TN, Duck R., Barren Hollow Rd. TN, Duck R, H.Horton St. Park	ACPH/D&D FUM/B&H	ALPH/B&H OCTOP/D&D	PGM/AYALA PGI/B&H	ISDH/DILLN GOT/D&D	MPI/D&D MDH/B&H	EST/D&D	LAP/D&D
83/04/14	TN, Duck R., Barren Hollow Rd. TN, Duck R., US Rt. 41A bridge	ACPH/D&D GP/SEL	ALPH/B&H OCTOP/D&D	PGM/AYALA PGI/B&H	ISDH/DILLN GOT/D&D	MPI/D&D FUM/B&H	EST/D&D	LAP/D&D
83/04/19	TN, Duck R., Barren Hollow Rd. TN, Duck R., US Rt. 41A bridge	ACPH/D&D GP/SEL	ALPH/B&H OCTOP/D&D	PGM/AYALA PGI/B&H	ISDH/DILLN GOT/D&D	MPI/D&D	EST/D&D	LAP/D&D
83/04/21	TN, Duck R., Barren Hollow Rd. TN, Duck R., US Rt. 41A bridge	GOT/D&D PGI/B&H	ALPH/B&H	PGM/AYALA	OCTOP/D&D	MPI/D&D	EST/D&D	LAP/D&D
83/04/26	TN, Duck R., Barren Hollow Rd. TN, Duck R. at Manchester	OCTOP/D&D ACPH/D&D	LAP/D&D GP/SEL	PGM/AYALA GOT/D&D	MPI/D&D PGI/B&H	EST/D&D	ISDH/DILLN	ALPH/B&H
83/04/28	TN, Duck R., Barren Hollow Rd. TN, Duck R. at Manchester	OCTOP/D&D ACPH/D&D	LAP/D&D GP/SEL	PGM/AYALA GOT/D&D	MPI/D&D PGI/B&H	EST/D&D	ISDH/DILLN	ALPH/B&H
83/05/05	TN, Duck R., Barren Hollow Rd. TN, Garrison Fork of Duck R.	OCTOP/D&D ACPH/D&D	LAP/D&D PROT/AYALA	PGM/AYALA GOT/D&D	MPI/D&D PGI/B&H	EST/D&D	ISDH/DILLN	ALPH/B&H
83/05/09	TN, Duck R., Barren Hollow Rd. TN, Garrison Fork of Duck R.	OCTOP/D&D ACPH/D&D	LAP/D&D PROT/AYALA	PGM/AYALA GOT/D&D	MPI/D&D PGI/B&H	EST/D&D	ISDH/DILLN	ALPH/B&H
83/05/10	TN, Duck R., Barren Hollow Rd. TN, Garrison Fork of Duck R.	OCTOP/D&D ACPH/D&D	LAP/D&D PROT/AYALA	PGM/AYALA GOT/D&D	MPI/D&D PGI/B&H	EST/D&D	ISDH/DILLN	ALPH/B&H
83/05/13	TN, Garrison Fork of Duck R.	OCTOP/D&D ACPH/D&D	LAP/D&D XDH/AYALA	PGM/AYALA GOT/D&D	MPI/D&D PGI/B&H	EST/D&D	ISDH/DILLN	ALPH/B&H
83/05/18	TN, Duck R. at Manchester TN, Garrison Fork of Duck R.	OCTOP/D&D ACPH/D&D	LAP/D&D MDH-1/B&H	PGM/AYALA GOT/D&D	MPI/D&D PGI/B&H	EST/D&D	ISDH/DILLN	ALPH/B&H

ELECTROPHORESIS DATE (YY/MM/DD)	SOURCE LOCALITIES OF SPECIMENS	STAINS USED						
83/05/20	TN, Duck R., H.Horton St. Park	OCTOP/D&D	LAP/D&D	PGM/AYALA	MPI/D&D	EST/D&D	ISDH/DILLN	ALPH/B&H
	TN, Garrison Fork of Duck R.	ACPH/D&D	GP/SEL	GOT/D&D	PGI/B&H			
83/05/27	TN, Duck R., Cortner Rd.bridge	OCTOP/D&D	LAP/D&D	PGM/AYALA	MPI/D&D	EST/D&D	ISDH/DILLN	HEXDH
	TN, Garrison Fork of Duck R.	ACPH/D&D	GP/SEL	GOT/D&D	PGI/B&H	MDH/B&H		
83/06/01	TN, Duck R., Cortner Rd.bridge	OCTOP/D&D	LAP/D&D	PGM/AYALA	MPI/D&D	EST/D&D	PGI/B&H	MDH/B&H
	TN, Duck R., H.Horton St. Park	ACPH/D&D	GOT/D&D					
83/06/03	TN, Duck R., H.Horton St. Park	OCTOP/D&D	LAP/D&D	PGM/AYALA	MPI/D&D	EST/D&D	PGI/B&H	GOT/D&D
	TN, Duck R., H.Horton St. Park	ACPH/D&D	ALPH/B&H	ISDH/DILLN	GP/SEL			
83/08/04	TN, Duck R., St. Rt. 13 bridge	OCTOP/D&D	LAP/D&D	PGM/AYALA	MPI/D&D	EST/D&D	PGI/B&H	GOT/D&D
	TN, Duck R., H.Horton St. Park	ACPH/D&D	ALPH/B&H	ISDH/DILLN	GP/SEL	MDH/B&H		
83/08/05	TN, Duck R., St. Rt. 13 bridge	OCTOP/D&D	LAP/D&D	PGM/AYALA	MPI/D&D	EST/D&D	PGI/B&H	GOT/D&D
	TN, Duck R., H.Horton St. Park	ACPH/D&D	ALPH/B&H	ISDH/DILLN	GP/SEL	MDH/B&H		
83/08/11	TN, Duck R. at Fredonia	OCTOP/D&D	LAP/D&D	PGM/AYALA	MPI/D&D	EST/D&D	PGI/B&H	GOT/D&D
	TN, Duck R. at Manchester	ACPH/D&D	ALPH/B&H	ISDH/DILLN	GP/SEL	MDH/B&H		