

**Rediscovery, systematic position, and re-description of
“*Leptoxis*” *melanoides* (Conrad, 1834)
(Mollusca: Gastropoda: Cerithioidea: Pleuroceridae)
from the Black Warrior River, Alabama, U.S.A.**

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Abstract.—The rediscovery of *Leptoxis melanoides*, a pleurocerid snail from the Black Warrior River drainage of Alabama, previously considered to be extinct, is presented. The radula of *L. melanoides* more closely resembles that of *Elimia*, and an analysis of 16S rDNA sequences positions the species within a monophyletic clade of *Elimia*. Therefore, we re-describe the species as *E. melanoides*, designate a neotype, precise type locality, and illustrate the specimen and its radula.

The Mobile River basin, which drains much of Alabama and parts of Georgia, Mississippi, and Tennessee, is not only one of the largest drainage systems east of the Mississippi River (70,252 km²; Lydeard et al. 1997), but was also home to more freshwater gastropod taxa than anywhere else in the world, accounting for 118 species in nine families (Bogan et al. 1995). Unfortunately, based on recent surveys and literature records, 38 (32%) of the gastropod species in the basin are now presumed extinct and many others have exhibited a marked decline in their distribution. One family that makes up a large component of the aquatic gastropod fauna in the basin is the Pleuroceridae.

Pleurocerids are freshwater, gill-breathing, operculate snails that reach their highest diversity in the southeastern United States (Burch & Tottenham 1980, Lydeard & Mayden 1995, Neves et al. 1997). Unfortunately, 25 pleurocerid species and one genus (*Gyrotoma*) are now presumed extinct due to the inundation of shoal areas by impoundment and habitat degradation from

poor land use practices (Stein 1976, Bogan et al. 1995, Lydeard & Mayden 1995, Neves et al. 1997). Besides *Gyrotoma*, the genus *Leptoxis* has fared equally poorly in recent times, as 11 of 15 *Leptoxis* species in the Mobile River system are presumed extinct. Eight of the presumed 11 species were restricted to the main channel of the Coosa River of Alabama (Bogan et al. 1995), which is now a highly modified and regulated system (Hershler et al. 1990). Of the three other species, *L. compacta* was restricted to the middle portion of the Cahaba River, which, although lacking major dams, was and remains subject to high environmental stress from urban development and activity near the city of Birmingham. Another presumed extinct species, *L. melanoides*, was restricted to the Black Warrior River of Alabama. Recent surveys of the Coosa (Bogan & Pierson 1993a, J. Godwin, pers. comm.) and Cahaba (Bogan & Pierson 1993b) Rivers failed to find any of the presumed extinct *Leptoxis* species.

In 1996, the authors conducted a gastropod survey in one of the few remaining

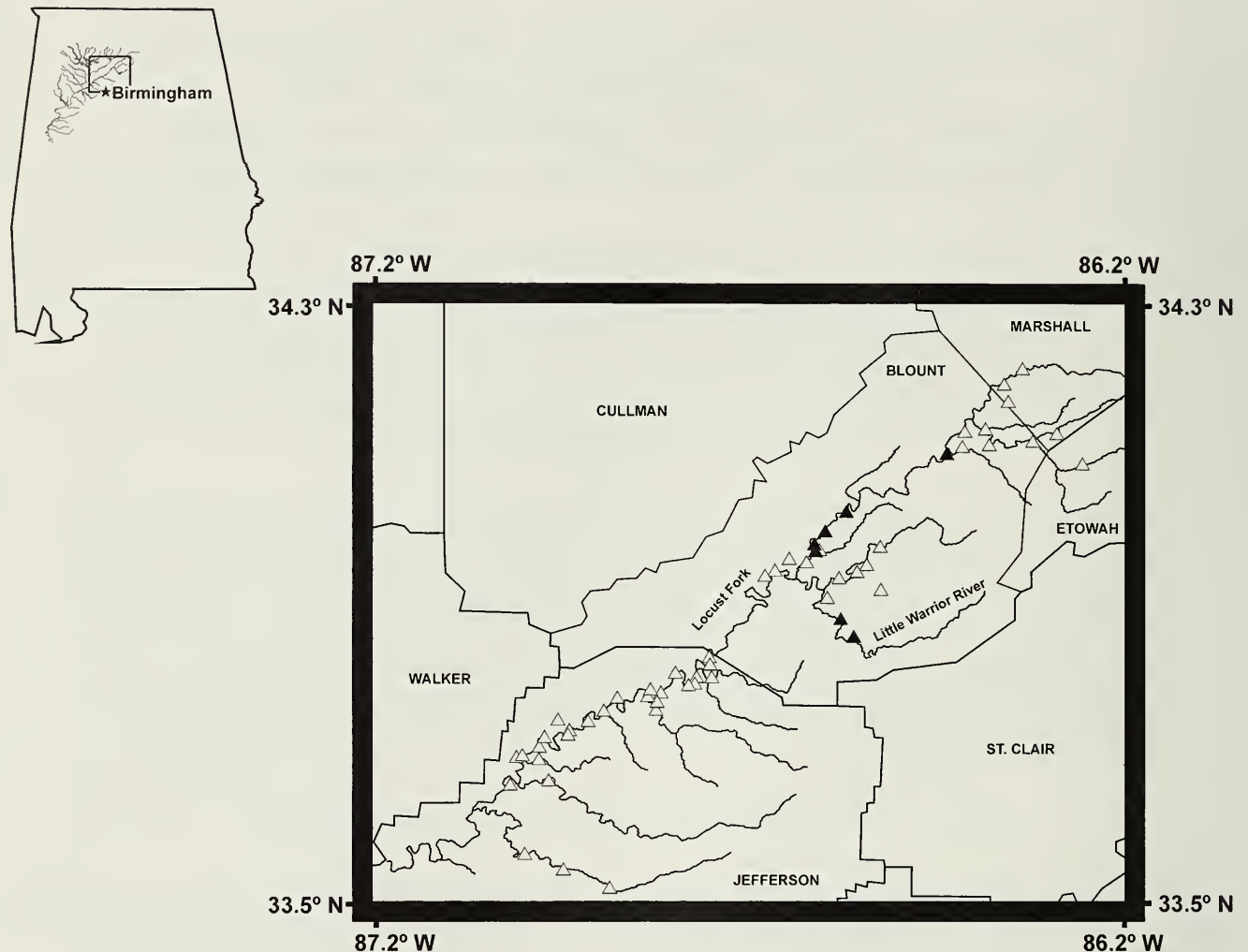


Fig. 1. Map of sites visited in this study. Upper map shows location of Black Warrior River drainage, lower map shows individual sites in Locust Fork portion. Closed triangles indicate those sites where *Leptoxis melanoides* was present.

free-flowing rivers in Alabama, the Locust Fork of the Black Warrior River. The main objective of the study was to determine the distribution of the third species, *L. plicata*, now restricted to the Locust Fork. During this survey, we rediscovered putative *L. melanoides*. This paper reviews the taxonomic and conservation history of *L. melanoides*, examines the historical and recent distribution of the species, and evaluates its phylogenetic position based on an analysis of mitochondrial 16S rDNA sequences. In addition, causes for the evident contraction of the distribution of the species are provided, along with a complete re-description of the species.

Methods

During the survey, 57 sites were visited in the Locust Fork drainage of the Black

Warrior River in Blount, Etowah, Jefferson, and Marshall Counties (Fig. 1 & Appendix I). *Leptoxis melanoides* was found living in a total of seven localities in Blount County, five in the Locust Fork proper, and two in the Little Warrior River. Five individuals of *L. melanoides* from the Little Warrior River in Blount County, Alabama were collected in 2002 and used in this study. Radulae were prepared and visualized using the methods of Holznagel (1998) and compared to other pleurocerid radulae as figured in Holznagel (2000).

Genomic DNA was isolated from head tissues using standard phenol/chloroform methods. Sequences were obtained for an amplified segment of the mitochondrial large ribosomal subunit (16S) gene using the SR14231/SNL002 and H3080/L2510

primer pairs of Holznagel & Lydeard (2000) and Palumbi et al. (1991) respectively. The primers amplify an approximately 1 kb fragment of the gene and were used for amplification and sequencing. Double-stranded amplifications via PCR were generated using 50–500 ng of template genomic DNA in 25 μ l volumes (10 mM Tris, 50 mM KCl, 2.5 mM MgCl₂, 1 μ M each primer, 0.1 mM each dNTP, 1.5 units Taq DNA polymerase). The amplification regime consisted of 35 cycles of denaturation (92°C for 40 sec), annealing (52°C for 60 sec), and extension (72°C for 90 sec). Double-stranded products were purified using the Qiagen PCR Cleanup kit and provided the template for cycle sequencing using the ABI BigDye 2.0 kit following manufacturer's instructions. Reactions were purified using Qiagen DyeEx spin columns and sequenced on an ABI 3100 genetic analyzer.

Sequences of *L. melanoides* were entered in the software program BioEdit (version 5.0.9; Hall 1999), along with the 35 pleurocerid taxa used in Holznagel & Lydeard (2000), who provided the first phylogenetic survey of the Pleuroceridae (Appendix II). Sequences were aligned by eye to the secondary structure models suggested by Holznagel & Lydeard (2000) and Lydeard et al. (2002). Phylogenetic hypotheses were generated in PAUP* 4.0b10 (Swofford 2002) under maximum parsimony using 50 replicates of heuristic search with random addition. The following options were employed: uninformative characters were ignored, branches with a minimum zero length collapsed, and minimal length trees kept. To test the internal stability of the data, a jackknife analysis (Farris et al. 1996; 1000 replicates, 10 random additions per replicate) was performed using PAUP* and Bremer decay indices (Bremer 1994) were determined in SEPAL (Salisbury 2001). Holznagel & Lydeard (2000) showed significant phylogenetic signal and no evidence of base composition bias in their analysis. They also reported no TS/TV sat-

uration up to near 20% genetic distance, so no weighting schemes were employed.

Specimens from the following museums were examined in this study: FMNH—Field Museum of Natural History, Chicago; FLMNH—Florida Museum of Natural History, Gainesville; NCSM—North Carolina State Museum of Natural Sciences, Raleigh; UMMZ—University of Michigan Museum of Zoology, Ann Arbor.

Results

Radulae taken from putative *Leptoxis melanoides* most resemble *Elimia* species, and not *Leptoxis* species (Fig. 2; see below and Fig. 4 for complete description). *Leptoxis melanoides* radulae have more rectangular laterals and more lateral cusps than other *Leptoxis* species, though the main lateral cusps are larger than those normally seen in *Elimia*. Also in *L. melanoides*, denticles are narrower and more numerous on the inner marginals.

Aligned sequences resulted in a matrix of 900 characters, including indels, of which 318 were parsimony informative. Maximum parsimony analysis yielded a single tree (1338 steps, CI = 0.52; Fig. 3). All five specimens of *L. melanoides* shared a single sequence and were resolved in a terminal clade of *Elimia* taxa and not in the polyphyletic groups of *Leptoxis* at the base of the tree. Jackknife values and Bremer indices strongly supported the resolution.

Systematics

Family Pleuroceridae Fischer, 1885

Genus *Elimia* Adams & Adams, 1854 (see Burch 2001)

Elimia melanoides (Conrad, 1834)

Fig. 4

Anculosa melanoides Conrad 1834:64, fig. 19.

Leptoxis melanoides Haldeman 1843–1853: 5, figs. 145–146.

Nitocris melanoides Adams & Adams 1858:308.

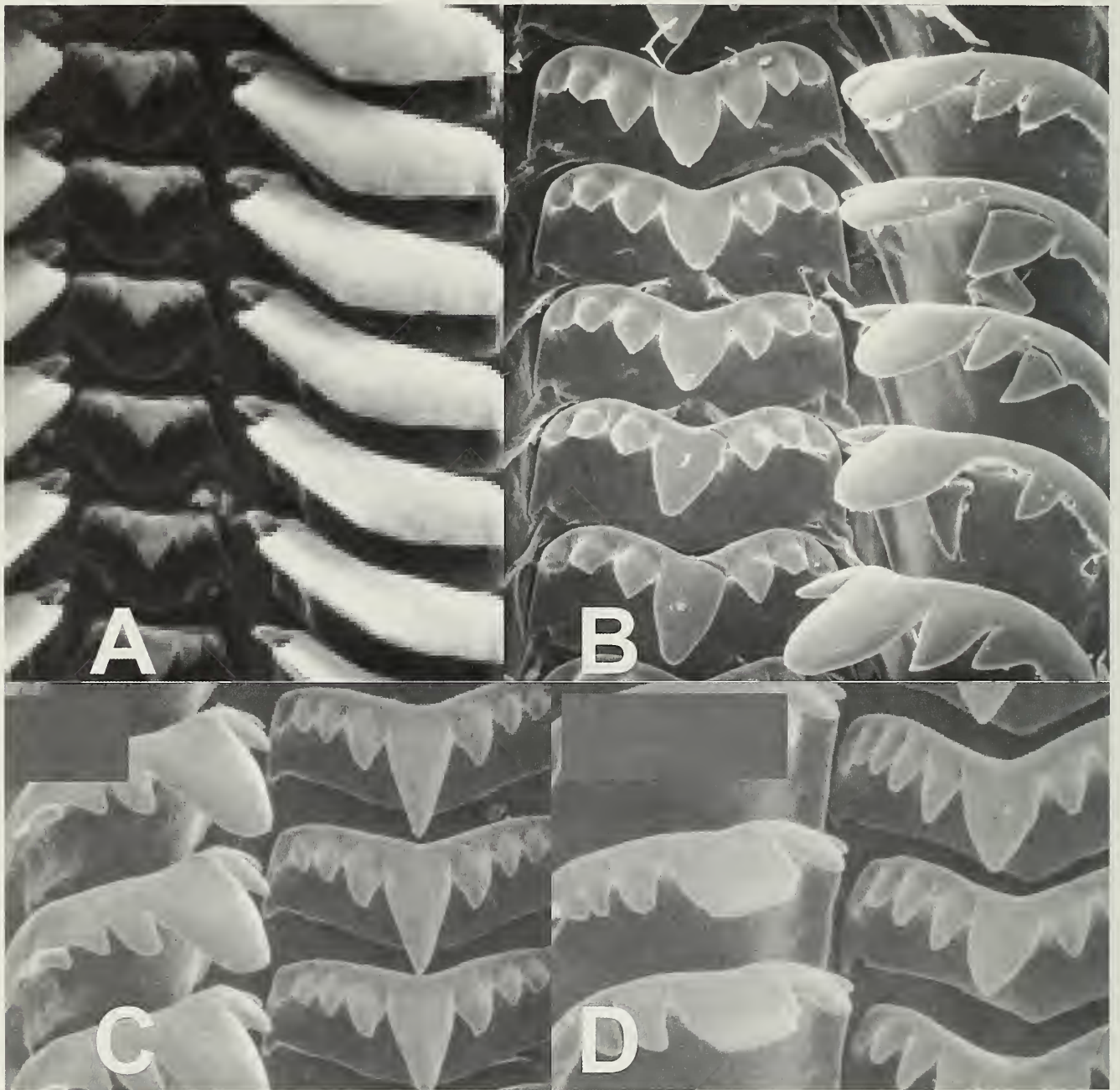


Fig. 2. Representative radulae from pleurocerids. A. *Leptoxis ampla*. B. *Elimia* sp. C. *Elimia alabamensis*. D. *Elimia showalteri*.

Anculosa turgida Haldeman 1840:2.

Leptoxis turgida Haldeman 1843–1853:5, fig. 151.

Type designation and locality.—Neotype FMNH 301993, Little Warrior River, dirt road bridge crossing off County Highway 15, R1E, T13S, sec. 30, Blount County, Alabama, U.S.A., here selected, in accordance with article 75.3.4 of the International Code of Zoological Nomenclature (I.C.Z.N. 1999). Neoparatypes FMNH 301889 (4 specimens).

Material examined (all in Alabama).—FMNH 33749, Black Warrior River; FMNH 57344, Black Warrior River; FMNH 76034, Black Warrior River; FMNH 80200, Warrior River, Jefferson County; FMNH 80243, Warrior River, Jefferson County; FLMNH 18219, Black Warrior River; FLMNH 81398, Locust Fork of Warrior River, Trafford, Jefferson County; FLMNH 81399, Black Warrior River, the forks; FLMNH 81400, forks of Warrior River, Walker County; FLMNH 81401;

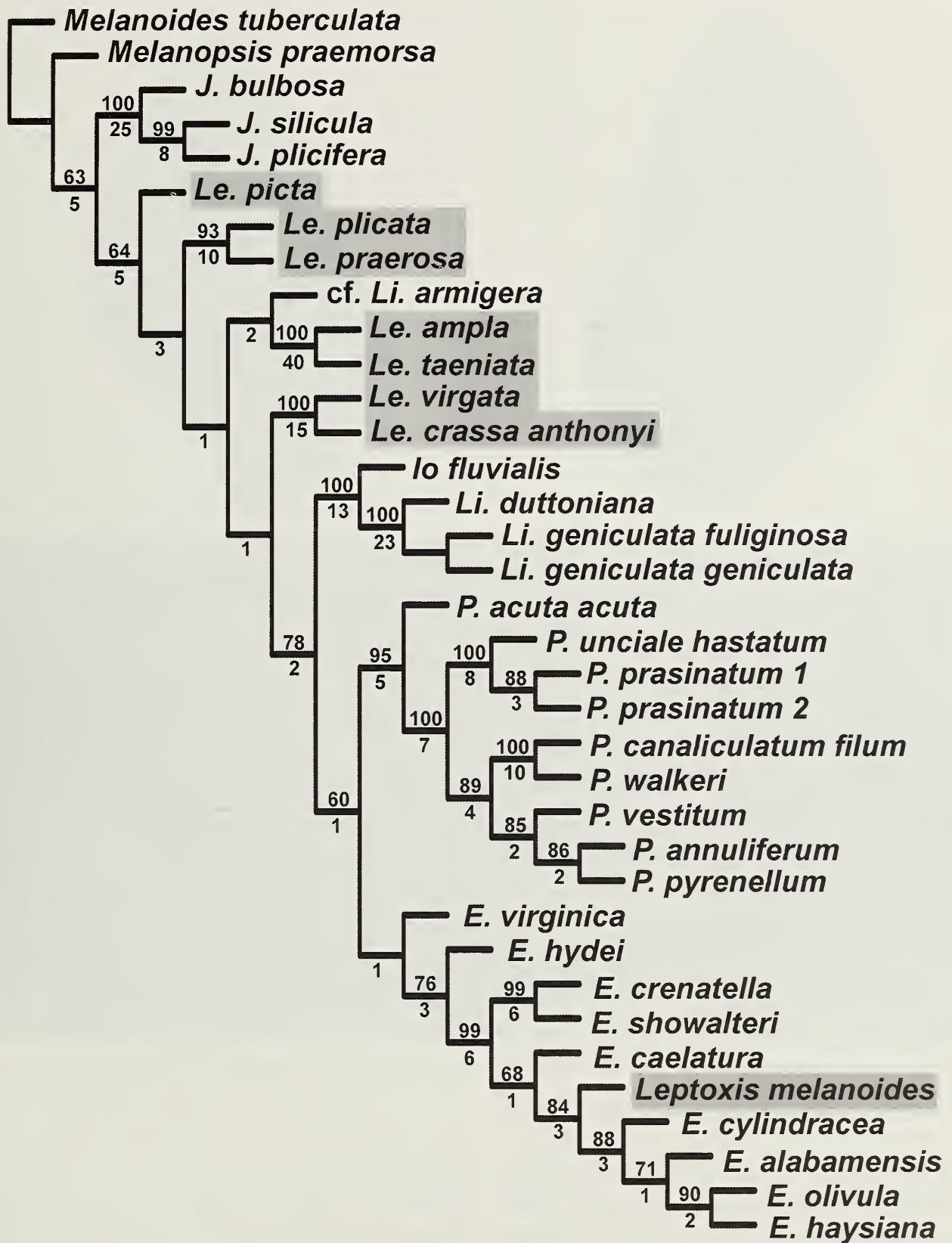


Fig. 3. The single most parsimonious tree generated using 16S rDNA sequences (1338 steps, CI = 0.52). Taxa historically considered *Leptoxis* are shaded. Values above branches are jackknife support, below are Bremer decay indices.

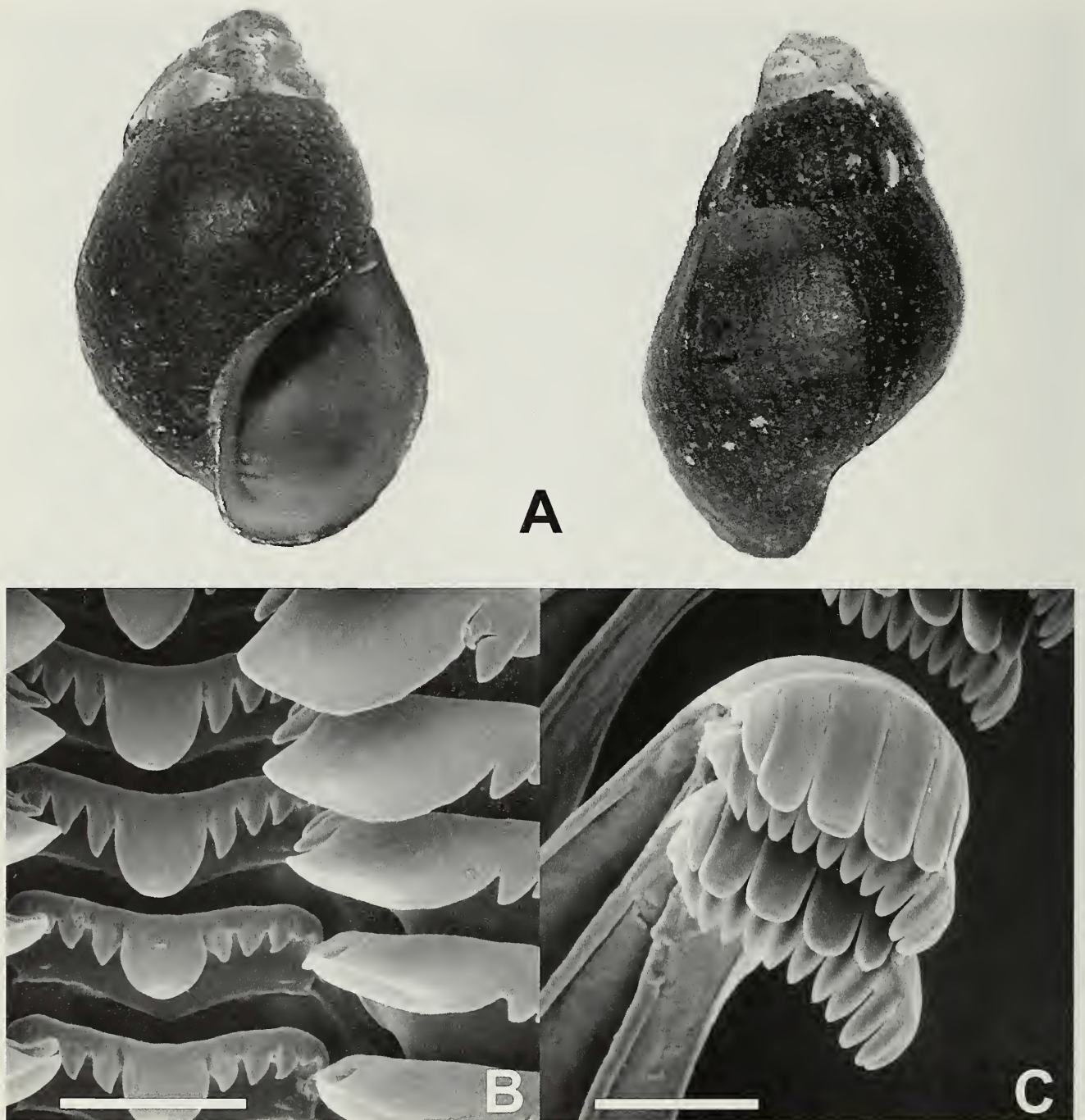


Fig. 4. *Elimia melanoides*. A. Neotype FMNH 301933, 10.8 mm. B. Rachidian and laterals. C. Inner and outer marginals. Scale bars: B, C = 50 μ m.

FLMNH 82143, Locust Branch, Warrior River, Trafford; FLMNH 82144, Black Warrior River, Squaw Shoals, Jefferson County; FLMNH 82145, Warrior River; FLMNH 82146, Black Warrior River; FLMNH 82147, forks of Warrior River, Walker County; FLMNH 82148, Black Warrior River; FLMNH 82149, Warrior River, lock 15, Tuscaloosa County; FLMNH 82150, Black Warrior River, Tuscaloosa, Tuscaloosa County; FLMNH

82151, Valley Creek, Toadvine, Jefferson County; FLMNH 82152, Black Warrior River; FLMNH 82153, Black Warrior River, Squaw Shoals, Jefferson County; FLMNH 230087, Sipsev Fork, Bankhead National Forest N.F. 234, Winston County; FLMNH 230131, Sipsev Fork, Bankhead National Forest N.F. 234, Winston County; FLMNH 230766, Locust Fork, at Black Warrior River, Jefferson County; FLMNH 230803, Sipsev Fork, above Alabama High-

way 33, Winston County; UMMZ 10169; UMMZ 37463, Alabama River; UMMZ 37464, Warrior River; UMMZ 37465, Locust Fork, Black Warrior River, Jefferson County; UMMZ 37466, forks of Black Warrior River, Walker County; UMMZ 49356, Black Warrior River, near mine, Jefferson County; UMMZ 55796 Locust (?) bridge of the Black Warrior River, Blount County; UMMZ 66275, Black Warrior River, Squaw Shoals, Tuscaloosa County; UMMZ 133928, Upper Black Warrior River; UMMZ 185760, Warrior River.

Diagnosis.—Shell small relative to sympatric pleurocerids, ovately conic and unsculptured, with even sutures. Shell more conic and aperture more ovate than typical *Leptoxis*. Columella white, frequently with pink to purple tinge. Distribution limited to Black Warrior River drainage, Blount County, Alabama.

Description.—Shell to 13 mm length, thin, ovately conic and smooth. Some early whorls and apex eroded. Fine transverse growth lines visible. Sutures flat to slightly impressed. Aperture oval, about one-half the length of the shell. Columella smooth and white, tinged with pink, red, or violet. Shell color green to brown, occasionally with brownish transverse bands. Operculum thin, dark, and oval, impressed at occasional growth lines (see Goodrich 1922, pl. 3, fig. 28). Radula rachidian tooth with nine triangular cusps, central cusp largest, slightly longer than wide. Lateral tooth broad, top edge straight with five cusps. Central cusp rectangular, broader and longer than others. Inner marginals with six to eight denticles, all equal size except for outer denticle on each side. Outer marginals with 12–14 denticles, all equal size except for outer denticle on each side.

Distribution.—Historically included the upper half of the Black Warrior River drainage, from Tuscaloosa and above. Currently restricted to stretches of the Locust Fork of the Black Warrior River and the Little Warrior River, Blount County, Alabama.

Remarks.—Conrad's (1834) original de-

scription of *Anculosa melanoides* was “Shell conical, with three entire volutions; apex eroded; whorls flattened, rounded only at the sutures; lines of growth prominent; body-whorl abruptly rounded; epidermis blackish, obscurely banded; aperture elliptical, about half the length of the shell” and the type locality, rivers of North Alabama, was ambiguous. Tryon (1873) reproduced Conrad's (1834) original description of *A. melanoides* and provided three figures of the species. Goodrich (1922) included an observation by H. H. Smith, who doubted that *A. melanoides* belonged in *Anculosa*. Goodrich (1941) later suggested that *A. melanoides* more closely resembled northern species in the genus and that its taxonomic position may change when its radula is obtained. A neotype designation was required, as the type specimen, once “in the possession of Mr. Anthony” (Tryon 1873: 400), was not available to Goodrich (1922), could not be located at the Museum of Comparative Zoology (Graf 2001) or the Academy of Natural Sciences in Philadelphia (Baker 1964), and has apparently been lost. The type locality has also been subsequently restricted to the drainage where the species is currently extant, following recommendation 76A of the code, as the original type locality could include the Tennessee River drainage, where the species does not occur.

Discussion

The rediscovery of putative *Leptoxis melanoides* was important and exciting, especially given the overall status of both many North American pleurocerid species and especially those in the Mobile River basin. This study helps to illustrate the need for thorough survey work combined with systematic analysis to rediscover and identify potentially “lost” or extinct taxa. Stein (1976:31) reviewed the conservation status of Alabama gastropods and stated “. . . it is doubtful whether living populations of this species still exist.” In a subsequent report

on rare and endangered invertebrates, Harris (1990) listed *L. melanoides* as endangered without evidence to suggest it still existed. Several formal and informal surveys had been conducted on various portions of the Black Warrior River and Locust Fork by U.S. Fish and Wildlife personnel and other investigators, but none documented the presence of *L. melanoides*. Given that the species had not been documented in over 50 years, Bogan et al. (1995) listed it as presumed extinct, a decision maintained by Turgeon et al. (1998). Comparisons of our material with museum specimens and cited illustrations support our notion of rediscovering *L. melanoides* in the Black Warrior River.

Of the 57 sites visited in the Locust Fork of the Black Warrior River drainage, *L. melanoides* was found at seven sites in two disjunct areas. Although it is possible the species may be more or less continuously distributed between the two areas, it still represents a small portion of the river system. It is evident, however, that based on historical distribution the species has declined drastically since the studies of Goodrich in the 1920's and 1940's. Blount County is home to poultry farms and coal mines which undoubtedly impact the surrounding water quality.

Analysis of mitochondrial 16S rDNA sequences suggests that *L. melanoides* should be placed in the genus *Elimia*, as it is resolved in a well-supported monophyletic clade of *Elimia* from the Mobile River basin. Such proposed generic reassignments are not uncommon in light of phylogenetic studies of freshwater mollusks (e.g., Lydeard et al. 2000, Minton & Lydeard 2003). The observed radulae are consistent with other species of *Elimia* (Holznagel 2000), and combined with shell morphology, make this species diagnosable in the upper reaches of the Locust Fork drainage. A redescription of what we are now considering *Elimia melanoides*, along with redesignation of type material, was needed given the pre-

sumed loss of the holotype and the phylogenetic placement resolved in this study.

Given its relative rarity and restricted distribution, and the overall decline of North American pleurocerids, remaining populations of *E. melanoides* should be monitored. Efforts are underway to list the species as endangered with the U.S. Fish and Wildlife Service. Until formally listed, the species should be treated as endangered and efforts made to preserve its remaining habitat.

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Appendix II

Systematic list of taxa used in this study. Taxon names (Burch & Tottenham 1980) are followed by museum voucher and GenBank accession numbers. FMNH—Field Museum of Natural History, Chicago. NCSM—North Carolina Museum of Natural Sciences, Raleigh.

PLEUROCERIDAE. *Elimia*: *E. alabamensis*, NCSM P4658, U73761; *E. caelatura*, NCSM P4659, AF100988; *E. crenatella*, NCSM P4660, U73762; *E. cylindracea*, NCSM P4661, U73765; *E. haysiana*, NCSM P4662, U73763; *E. hydei*, NCSM P4663, U73764; *E. interrupta*, NCSM 6595, AY010521; *E. melanoides*, FMNH 301889, AF540003; *E. olivula*, NCSM P4664, U73766; *E. showalteri*, NCSM P4665, U73767; *E. virginica*, NCSM P4666, AF100989. *Io*: *Io fluvialis*, NCSM P4667, AF100999. *Juga*: *J. bulbosa*, NCSM P4668, AF101005; *J. plicifera*, NCSM P4669, AF101004; *J. silicula*, NCSM P4670, AF101003. *Leptoxis*: *Le. ampla*, NCSM P4671, U73768; *Le. crassa anthonyi*, NCSM P4672, AF101001; *Le. picta*, NCSM P4673, U73769; *Le. plicata*, NCSM P4674, U73770; *Le. praerosa*, NCSM P4675, AF101002; *Le. taeniata*, NCSM P4676, U73771; *Le. virgata*, NCSM P4677, AF101000. *Lithasia*: cf. *Li. armigera*, NCSM P4678, AF100998; *Li. duttoniana*, NCSM P4679, AF100997; *Li. geniculata*

Appendix I

Localities surveyed in 1996. Data include topographic coordinates; exact locality information is available from the authors. Localities where *E. melanoides* was found are indicated by an asterisk (*).

ALABAMA. Blount County: Berry Creek, T12S, R1E, sec. 33.—Calvert Creek, T13S, R1E, sec. 32.—Calvert Creek, T13S, R1E, sec. 6.—Calvert Prong of Little Warrior River, T12S, R1E, sec. 22.—Dry Creek, T12S, R1W, sec. 23.—*Little Warrior River, T13S, R1E, sec. 30.—*Little Warrior River, T13S, R1E, sec. 32.—Little Warrior River, T13S, R1W, sec. 13.—*Locust Fork, AL 160, T12S, R1W, sec. 23.—Locust Fork, T10S, R2E, sec. 25.—Locust Fork, T10S, R2E, sec. 27.—Locust Fork, T11S, R2E, sec. 1.—*Locust Fork, T11S, R2E, sec. 4.—*Locust Fork, T12S, R1E, sec. 6.—*Locust Fork, T12S, R1W, sec. 13.—*Locust Fork, T12S, R1W, sec. 23.—Locust Fork, T12S, R1W, sec. 27.—Locust Fork, T12S, R1W, sec. 32.—Mill Creek, T13S, R1E, sec. 10.—Sugar Creek, T13S,

fuliginosa, NCSM P4680, AF100995; *Li. geniculata geniculata*, NCSM P4681, AF100996. *Pleurocera*: *P. acuta acuta*, NCSM P4684, AF100994; *P. annuliferum*, NCSM P4685, U73772; *P. canaliculatum filum*, NCSM P4686, AF100991; *P. prasinatum 1*, NCSM P4689, U73774; *P. prasinatum 2*, NCSM P4688, U73773; *P. pyrenellum*, NCSM P4690, AF100990; *P. unciale hastatum*, NCSM P4687, AF100993; *P. vestitum*, NCSM P4691, U73775; *P. walkeri*, NCSM P4692, AF100992. THIARIDAE. *Melanoides tuberculata*, NCSM P4682, AF101006. MELANOPSIDAE. *Melanopsis praemorsa*, NCSM P4683, U73776.