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Radular morphology of extinct pleurocerids (Gastropoda: Cerithioidea: Pleuroceridae)

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Abstract: The freshwater gastropod family Pleuroceridae suffers from a disproportionately high number of imperiled species and recent extinctions. As pleurocerid diversity has been lost, so too has our ability to study the biology of these animals. However, many extinct species were deposited in natural history collections before their demise. I extracted radulae from dried tissue left in shells of seven extinct species in three genera (*Leptoxis* Rafinesque, 1819, *Gyrotoma* Shuttleworth, 1845, *Lithasia* Haldeman, 1840) to gain insights into the morphological differences separating species and provide data for future researchers. There were notable intergeneric and interspecific differences in radular morphology such as shape of cusps (*e.g.*, dagger-like vs. blunt) and number of denticles on the rachidian, lateral, and marginal teeth. Interestingly, the degree of radular differences among *Leptoxis* spp. likely corroborates previous hypotheses that the genus is not a natural group. These data are a resource for future studies and should aid in determining the feeding habits and relationships of extinct pleurocerids.

Key words: radula, microscopy, freshwater gastropod, *Gyrotoma*, *Leptoxis*, *Lithasia*

Pleuroceridae (Gastropoda: Cerithioidea) is one of the most imperiled families of freshwater gastropods in the world with 79% of 162 species considered at risk of extinction or already extinct (Johnson *et al.* 2013). Pleurocerids are most speciose in the Mobile and Tennessee River basins (Lydeard and Mayden 1995, Johnson *et al.* 2013), but they are found in North America from east of the Rocky Mountains to the Atlantic Coast (Strong and Köhler 2009). Although historically abundant (Tryon 1873), many species have gone extinct as a result of river pollution and impoundment (Johnson *et al.* 2013). In fact, the damming of the Coosa River in Alabama and Georgia caused what is considered the single largest mass extinction event in human history (Williams *et al.* 2008); of the approximately 34 Coosa River snails that went extinct, 28 were pleurocerids (Johnson *et al.* 2013). These Coosa River endemics most likely went extinct between 1914 when the first Coosa River hydropower dam, Lay dam, was built and 1967 when Bouldin dam was finished (Johnson *et al.* 2013). Pinpointing when other pleurocerid extinctions happened is more difficult, but likely occurred in the mid-20th century as a result of anthropogenic habitat modifications (Neves *et al.* 1997).

Little is known about extinct pleurocerids aside from their shell morphology and historical distributions. A poignant example of this is the extinct genus *Gyrotoma* Shuttleworth, 1845—a genus of six species from the Coosa River (Goodrich 1924). *Gyrotoma* is distinguished by a fissure in the shell above the body whorl, but little else is known of their biology (*e.g.*, anatomy, diet, life history traits). Pleurocerid shells are known to exhibit high levels of intraspecific variation (Minton *et al.* 2008, Whelan *et al.* 2012a) and are of limited use for determining relationships among genera (Minton 2002). Shells also provide little information concerning the general biology of a species (*e.g.*, diet, life history). Radulae, on the other hand, are likely better for characterizing at least some aspects of pleurocerid biology. For example, if extinct gastropods have similar radular morphologies to extant species then grazing habit and diet could be inferred under the assumption that radular morphology has been optimized for specific diets.

The goal of this study was to use material deposited in natural history collections to document the radular morphology of some recently extinct pleurocerids. This material was deposited before these species went extinct and is a valuable, but limited, resource for providing anatomical data on extinct species.

MATERIALS AND METHODS

Dried tissues left in shells of extinct species were removed with forceps from lots at the Michigan Museum of Natural History (MMNH), North Carolina Museum of Natural Sciences (NCSM), and Florida Museum of Natural History (FLMNH) (Table 1). Snails were identified based on identifications of the original collector and on descriptions by Goodrich (1922, 1924). Taxonomy follows Johnson *et al.* (2013). Radulae were isolated by digesting surrounding tissue with the method of Holznagel (1997). Radulae were mounted on aluminum studs, coated in gold, and visualized under a Hitachi TM3000 scanning electron microscope at the National Museum of Natural History in Washington, D.C.

Table 1. Species examined.

Species	Catalog number	Number of radulae imaged	Collection Locality
Leptoxis lirata	NCSM 59379	3	Coosa River, below Logan Martin Dam, Alabama
Leptoxis showalterii	HAD 6575[a]	2	Coosa River, 0.8 km below Rockport, Alabama
Leptoxis occultata	NCSM 59480	2	Choccolocco Creek, Jackson Shoals, Alabama
Leptoxis trilineata	UMMZ 134076	2	Ohio River, Cincinnati, Ohio
Gyrotoma pyramidata	NCSM 57380	2	Coosa River, Ten Island Shoals
Gyrotoma excisa	FLMNH 80358	1	Coosa River, Alabama
Lithasia hubrichti	NCSM 59904	1	Big Black River, 4.8 km northwest of Edwards, Mississippi

[a]Part of NCSM.

RESULTS

Intact radulae were retrieved from extinct species in three genera (*Leptoxis* Rafinesque, 1819, *Lithasia* Haldeman, 1840, *Gyrotoma*) and three drainages (Coosa River, Ohio River, Big Black River; Table 1). Despite the thousands of extinct pleurocerid shells in natural history collections examined, few had tissue left in them and even fewer had enough tissue for an intact radula to be present. *Elimia* Adams and Adams, 1854 species were not considered here, but there are extinct *Elimia* species lots at the North Carolina Museum of Natural Sciences that have dried tissue left in shells (N. Whelan *pers. observ.*).

Leptoxis lirata (Goodrich, 1922)

Rachidian hexagonal with a flat edged lower margin and basal projections extending from the base of the lateral sides (Figs. 1A, 2A). Cutting edge with spade-like central cusp arriving at a prominent point. Two prominent, triangulate denticles on either side of the central cusp with a weakly developed third outer denticle on either side extending from the lateral side of rachidian. Lateral teeth with a broad, rhomboidal cusp and two inner denticles, the innermost of which often weakly developed and half the height of the first denticle (Figs. 1A, 2A). Outer denticle on lateral teeth long and flimsy (Figs. 1A, 2A: arrows). Inner marginal teeth with four to six wide, long, rectangular denticles (Figs. 1A, 3A). Marginal teeth with 12 to 15 narrow, short denticles ending in a sharply rounded point (Figs. 1A, 3A). Both inner and outer marginal teeth with a flange covering over 2/3 of tooth shaft width, more prominent on inner teeth.

Leptoxis occultata (Goodrich, 1922)

Rachidian hexagonal with a flat lower margin and basal projections extending from the base of the lateral sides (Figs. 1B, 2B). Rachidian cusp spade-like with a sudden, sharp point. Two to four denticles on either side of rachidian cusp; outer most denticle on each side often flimsy in appearance and curves under cutting edge (Fig. 2B: asterisk). Lateral teeth with a broad, rhombiodal cusp (Figs. 1B, 2B). One, or rarely two, inner denticles on each lateral tooth; when present, second inner denticle approximately 1/16 the height of first inner denticle. A single, weakly developed, and flimsy outer denticle on most lateral teeth that appears more like a tissue flap than a denticle (Fig. 2B: arrows). Inner marginal teeth with four to five wide, long, flat tipped denticles (Figs. 1B, 3B). Outer marginal teeth with nine to eleven narrow denticles. Both inner and outer denticles with a flange on shaft of tooth, but wider on the outer denticle than on inner denticle.

Leptoxis showalterii (Lea, 1860)

Rachidian hexagonal with a flat lower margin and basal projections extending from base of the lateral sides (Figs. 1C, 2C). Rachidian cusp spade-like with a dull point, or triangulate with a sudden, sharp point. Two to four denticles present on both sides of rachidian cusp, with outer most denticle weakly developed, but robust and on the cutting edge of the rachidian. Lateral teeth with wide, flat-edge cusp; outer denticle flimsy, either underneath or on top of the lateral tooth cutting edge (Figs. 1C, 2C: arrows). Two to three inner denticles on lateral teeth that decrease in length towards the center of the radula and a single, flimsy outer denticle on most lateral teeth. Inner marginal teeth with five to six broad, long denticles that end in a dull point (Figs. 1C, 3C). Outer marginal teeth with twelve to sixteen skinny denticles that come to a dull or round point. Flange on marginal teeth shafts, wider on outer marginal teeth than on inner marginal teeth.

Leptoxis trilineata (Say, 1829)

Rachidian roughly chevron shaped with short or absent basal projections extending from base of the lateral sides (Figs. 1D, 2D). Rachidian spade-like, but long and comes to a sudden point with two to four spade-like outer denticles on each side. Lateral teeth with a broad, convex cusp with one to two sharply pointed outer denticles plus one flimsy outermost denticle that

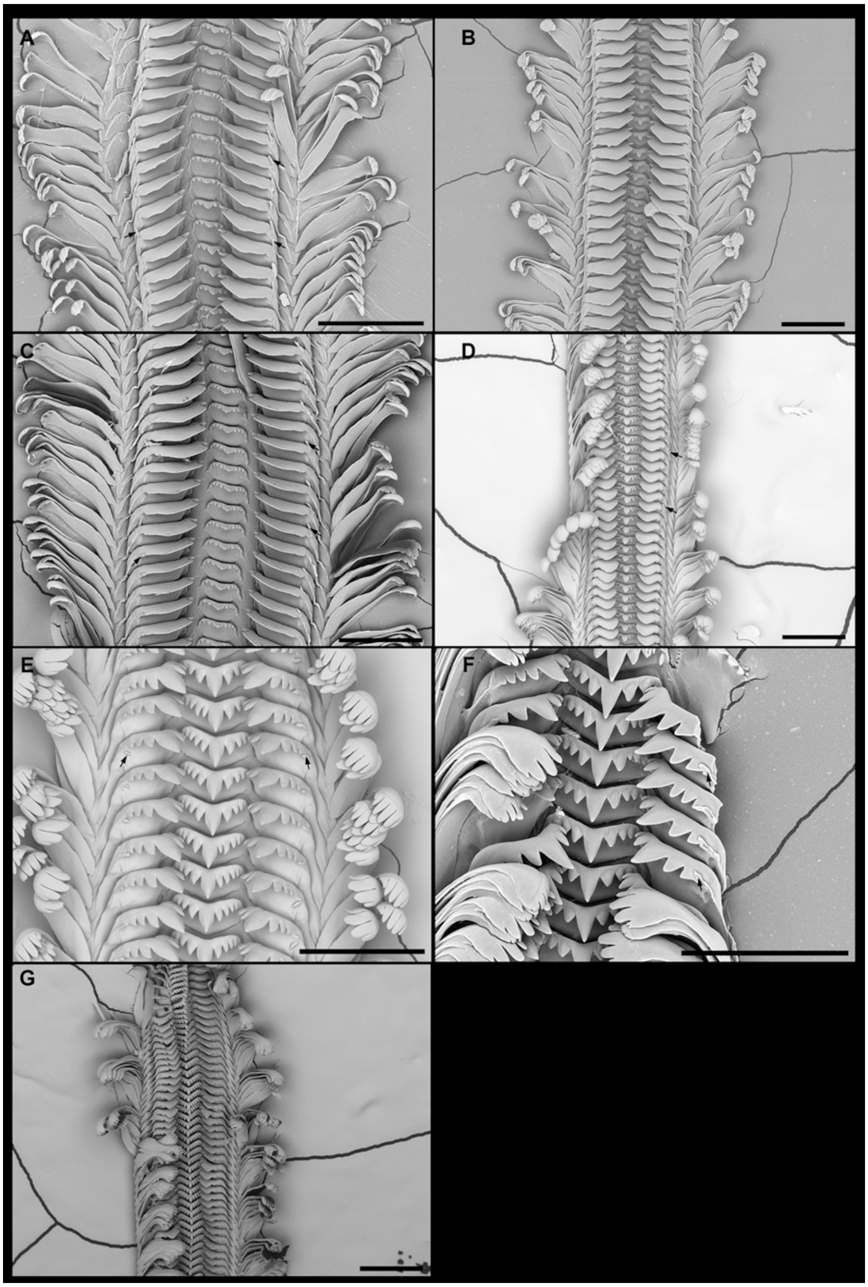

Figure 1. Top-down view of radular ribbon. **A**, *Leptoxis lirata.* **B**, *L. occultata*. **C,** *L. showalterii.* **D**, *L. trilineata.* **E**, *Gyrotoma excisa.* **F**, *G. pyramidata.* **G**, *Lithasia hubrichti*. Scale bar = 200 µm. Arrows point to representative outermost lateral denticles that appeared flimsy in appearance. Asterisk emphasizes flimsy outermost rachidian denticle in *L. occultata*.

appears to stem from the side of the tooth rather than the cutting edge (Figs. 1D, 2D: arrows). One to two inner denticles on lateral teeth. Inner marginal teeth with six wide, long denticles and flat or slightly rounded cutting edges (Figs. 1D, 3D). Outer marginal teeth with ten relatively long denticles and generally round points. Flange on each marginal tooth, approximately half the tooth stem on some, but wider on others.

***Gyrotoma excisa* (Lea, 1843)**

Rachidian wide, roughly hexagonal (Figs. 1E, 2E) with convex lower margin. Short, broad, basal projections on lateral sides of the rachidian. Rachidian cusp dagger-like with prominently pointed tip, over twice the length of rachidian denticles. Three to four denticles on either side of rachidian cusp. Wide, dagger-like cusp on lateral teeth, with four outer denticles, the outer most of which appears flimsy (Figs 1E, 2E: arrow), and three inner denticles (Figs. 1E, 2E). Inner and outer lateral teeth similar in appearance with five to six wide, long, dully pointed denticles (Figs. 1E, 3E). Flanges of at least 2/3 the width of the tooth shaft on marginal teeth.

***Gyrotoma pyramidata* (Shuttleworth, 1845)**

Quality of retrieved radulae were generally poor, but still characterizable. Rachidian wide, roughly hexagonal (Figs. 1F, 2F). Lower rachidian margin broadly convex with short, narrow basal projections extending from lateral sides. Rachidian cusp dagger-like and at least twice as long as rachidian denticles. Four to five denticles on either side of rachidian cusp. Dagger-like cusp on lateral teeth with three to four outer denticles and three inner denticles (Figs. 1F, 2F). Outer-most denticle on lateral teeth, long and flimsy in appearance (Figs 1F, 2F: arrows); inner-most denticle often short and sometimes blunt, but robust, in appearance. Inner marginal teeth with five denticles about 1.5 times as wide as denticles on outer marginal teeth (Figs. 1F, 3F). Outer marginal teeth with eight broad, bluntly pointed denticles. Flange of at least 2/3 the width of the tooth shaft on marginal teeth.

***Lithasia hubrichti* Clench, 1956**

Rachidian wide, but short, with a dagger-like central cusp and five pointed denticles on both sides (Figs. 1G, 2G). Convex rachidian base with short or absent basal projections on the lateral sides of the rachidian. Lateral teeth with a wide, rectangular cusp and a single, pointed outer denticle

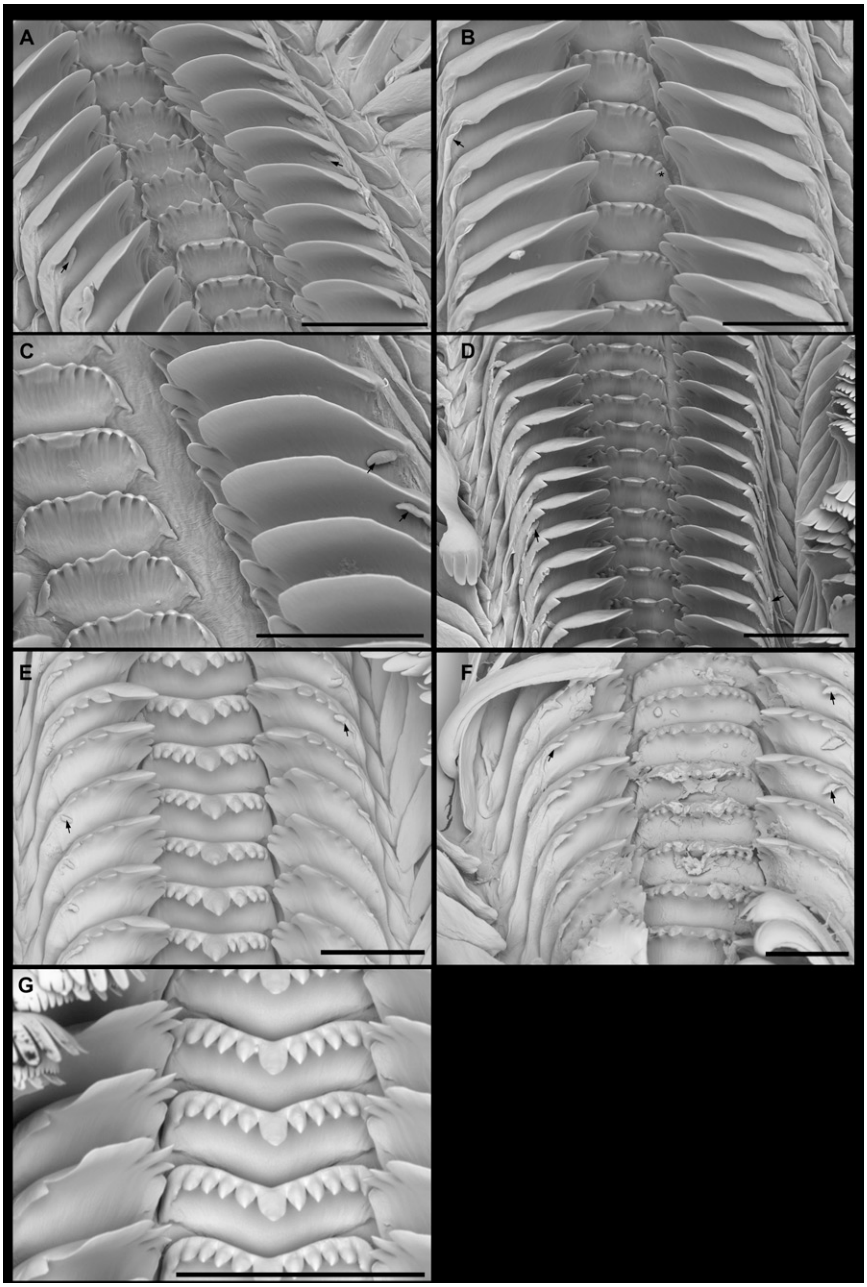

Figure 2. Rachidian and lateral teeth tilted 45°. **A**, *Leptoxis lirata.* **B**, *L. occultata.* **C**, *L. showalterii.* **D**, *L. trilineata.* **E**, *Gyrotoma excisa.* **F**, *G. pyramidata.* **G**, *Lithasia hubrichti.* Scale bar = 100 µm. Arrows point to representative outermost lateral denticles.

(Figs 1G, 2G). First two inner denticles on lateral teeth pointed; innermost denticle blunt and often only as a small protrusion of the tooth rather than a full denticle when compared to the other two inner denticles. Inner marginal teeth with six denticles of medium length that all have a space between neighboring denticle(s) (Figs. 1G, 3G). Outer marginal teeth with 13–15 skinny denticles. Flange on marginal teeth stems that is nearly the whole length of each tooth stem.

DISCUSSION

Radular morphology varies among pleurocerid species (Holznagel 2000; Minton 2002), and it may be an ideal system for understanding the diversity and possibly relationships among extinct and extant species. However, the level of intra-individual and intraspecific variation is documented here is also of note and has not been documented before in pleurocerids. For example, denticle number on the rachidian, lateral, and marginal teeth can vary widely within individuals and species (see above). Nevertheless, there appears to be species specific radular characteristics for most species, but some are subtle and could be overlooked.

The data here reveal that the degree of variation among any two *Leptoxis* species depends on which species are being compared. *Leptoxis occultata* is distinguished by its outermost denticles on either side of the rachidian that fold under the cutting edge. *Leptoxis lirata* and *L. showalterii*, on the other hand, have strikingly similar radular morphologies. The shells of these species are similar in that they both have many carinae on all shell whorls, but *L. lirata* generally has a higher spire and is less rotund that *L. showalterii*. Given their radular and conchological similarities, these species are likely either recently diverged sister species or, in fact, the same species. Coosa River *Leptoxis* examined here have similar radular morphologies to *L. ampla* (Anthony, 1855) from the Cahaba River and *L. taeniata* (Conrad, 1834) from the Coosa River basin (Minton 2002), but they possess much more compact rachidians than Ohio River basin *Leptoxis* analyzed by Holznagel (2000) and Minton (2002). Coosa River basin *Leptoxis* explored here also have considerably different morphologies from *L. trilineata* (Say, 1829). All *Leptoxis* species analyzed here have different morphologies compared to *L. compacta* (Anthony, 1854) (Whelan *et al.*

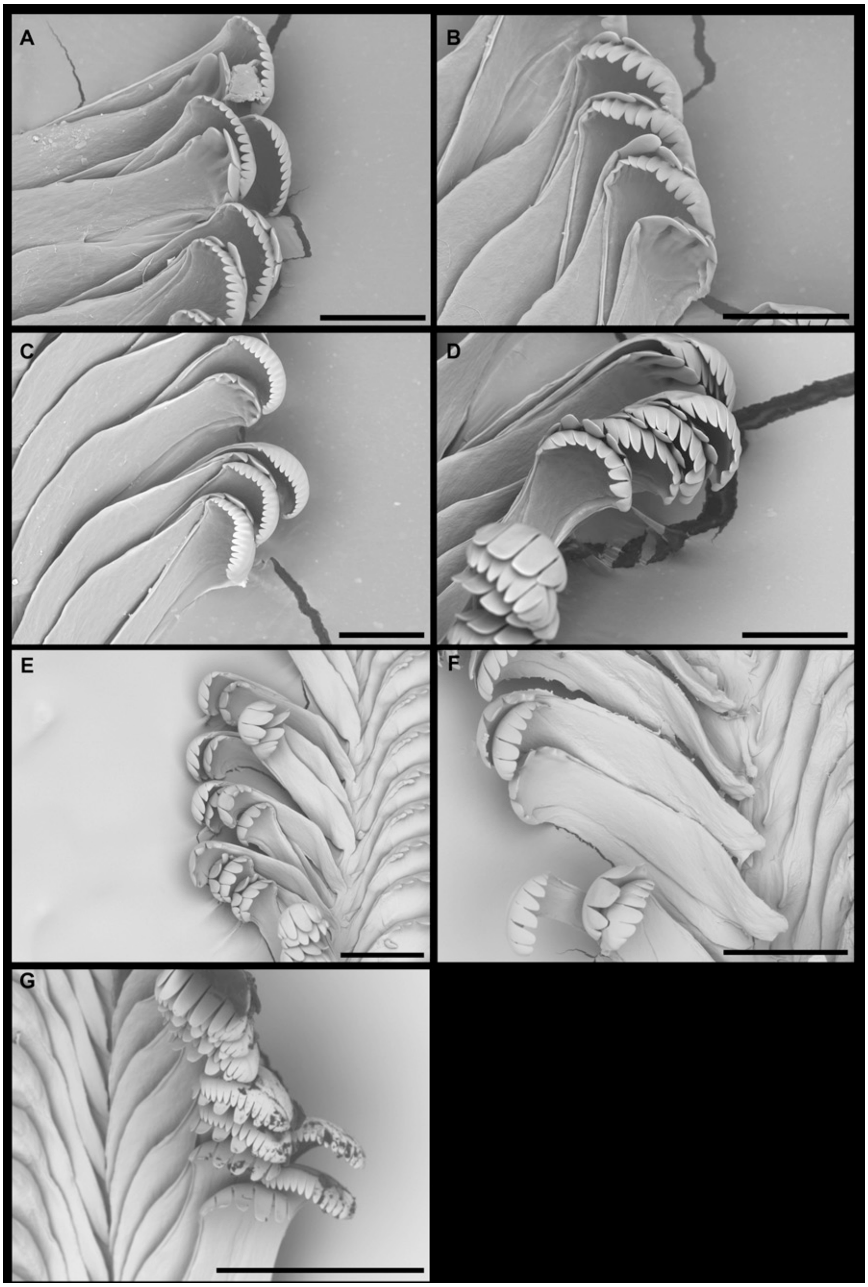

Figure 3. Marginal teeth. **A**, *Leptoxis lirata.* **B**, *L. occultata.* **C**, *L. showalterii.* **D**,. *L. trilineata.* **E**, *Gyrotoma excisa.* **F**, *G. pyramidata.* **G**, *Lithasia hubrichti*. Scale bar = 100 μm.

2012b). These differences lend corroboration to previous hypotheses that *Leptoxis* is not monophyletic (Holznagel and Lydeard 2000, Minton *et al.* 2003, Minton and Lydeard 2003).

Lithasia hubrichti shares many superficial characters with other *Lithasia* species such as a wide, convex rachidian base and at least four dagger like rachidian denticles (Holznagel 2000; Minton, 2002). Furthermore, *Li. hubrichti*, *Li. geniculata* (Haldeman, 1840), and *Li. armigera* (Say, 1821) all have outer marginal teeth with 12 or more denticles and inner marginal teeth with five or six denticles (Fig. 3G; Holznagel 2000; Minton 2002). A phylogenetic analysis, either with radular or other data, would be needed to conclusively place *Li. hubrichti* with other *Lithasia* species, but the data presented here certainly suggests that *Li. hubrichti* is a *Lithasia* and the only species in the genus that resides outside of the Ohio River basin.

In the context of this study, little can be said about what *Gyrotoma* radular morphology implies about the biology of the genus. Nevertheless, feeding behaviors may be the best targets of future analyses to infer the biology of *Gyrotoma*. To data, little is known of pleurocerid feeding habits other than their characterization as grazers. However, if feeding habits (*e.g.*, food ingested) of extant pleurocerids are determined it can be reasonably inferred that extinct species with similar radular morphologies fed in similar manners. Such information would not only shed light onto the life habits of extinct pleurocerids but also reveal historical ecological aspects of anthropogenically modified river systems.

As more species inevitably go extinct, biologists must strive to not only document diversity before it goes extinct, but also utilize available resources to better understand the biology of extinct species so remaining diversity can be put into a historical context. Malacologists should continue to pursue museum resources as a useful source of non-conchological information for extinct species.

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LITERATURE CITED

Goodrich, C. 1922. The *Anculosae* of the Alabama River Drainage. *Miscellaneous Publications of the Museum of Zoology, University of Michigan* **7**: 1–63.

Goodrich, C. 1924. The genus *Gyrotoma*. *Miscellaneous Publications of the Museum of Zoology, University of Michigan* **12**: 1–33.

Holznagel, W. E. 1998. A nondestructive method for cleaning gastropod radulae from frozen, alcohol-fixed or dried material. *American Malacological Bulletin* **14**: 181–184.

Holznagel, W. E. 2000. *A phylogenetic study of selected North American Pleuroceridae (Gastropoda: Cerithioidea) using molecular and radula data.* Ph.D. Dissertation, Department of Biological Sciences. Tuscaloosa, Alabama, University of Alabama.

Holznagel, W. E. and C. Lydeard 2000. A molecular phylogeny of North American Pleuroceridae (Gastropoda: Cerithioidea) based on mitochondrial 16S rDNA sequences. *Journal of Molluscan Studies* **66**: 233–257.

Johnson, P. D., A. E. Bogan, K. M. Brown, N. M. Burkhead, J. R. Cordeiro, J. T. Garner, P. W. Hartfield, D. A. W. Lepitzki, G. R. Mackie, E. Pip, T. A. Tarpley, J. S. Tiemann, N. V. Whelan, and E. E. Strong. 2013. Conservation status of freshwater gastropods of Canada and the United States. *Fisheries* **38**: 247–282.

Lydeard, C. and R. L. Mayden. 1995. A diverse and endangered aquatic ecosystem of the southeast United States. *Conservation Biology* **9**: 800–805.

Neves R. J., A. E. Bogan, J. D. Williams, S. A. Ahlstedt, and P. W. Hartfield. 1997. Status of aquatic mollusks in the southeastern United States: A downward spiral of diversity. *In*: Benz, G. and D. Colling, eds. *Aquatic Fauna in Peril*. Chattanooga, Tennessee. Pp. 43–86.

Minton, R. L. 2002. A cladistic analysis of *Lithasia* (Gastropoda: Pleuroceridae) using morphological characters. *The Nautilus* **116**: 39–49.

Minton, R. L., J. T. Garner, and C. Lydeard. 2003. Rediscovery, systematic position, and re-description of " *Leptoxis*" *melanoides* (Conrad, 1834) (Mollusca: Gastropoda: Cerithioidea: Pleuroceridae) from the Black Warrior River, Alabama, U.S.A. *Proceedings of the Biological Society of Washington* **116**: 531–541.

Minton, R. L. and C. Lydeard. 2003. Phylogeny, taxonomy, genetics and global heritage ranks of an imperiled, freshwater genus *Lithasia* (Pleuroceridae). *Molecular Ecology* **12**: 75–87.

Minton, R. L., A. P. Norwood, and D. M. Hayes. 2008. Quantifying phenotypic gradients in freshwater snails: A case study in *Lithasia* (Gastropoda: Pleuroceridae). *Hydrobiologia* **605**: 173–182.

Strong, E. E. and F. Köhler. 2009. Morphological and molecular analysis of '*Melania*' *jacqueti* Dautzenberg and Fischer, 1906: From anonymous orphan to critical basal offshoot of the Semisulcospiridae (Gastropoda: Cerithioidea). *Zoologica Scripta* **38**: 483–502.

Tryon, G. W. 1873. *Land and fresh-water shells of North America part IV. Streptomatidae (American melanians)*. Smithsonian Institution, Washington, D.C.

Whelan, N. V., P. D. Johnson, and P. M. Harris. 2012a. Presence or absence of carinae in closely related populations of *Leptoxis ampla* (Anthony, 1855) (Gastropoda: Cerithioidea: Pleuroceridae) is not the result of ecophenotypic plasticty. *Journal of Molluscan Studies* **78**: 231–233.

Whelan, N. V., P. D. Johnson, and P. M. Harris. 2012b. Rediscovery of *Leptoxis compacta* (Anthony, 1854) (Gastropoda: Cerithioidea: Pleuroceridae). *PLoS ONE* **7**: e42499.

Williams, J. D., A. E. Bogan, and J. T. Garner. 2008. *Freshwater Mussels of Alabama and the Mobile Basin in Georgia, Mississippi, and Tennessee*. University of Alabama Press, Tuscaloosa, Alabama.

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