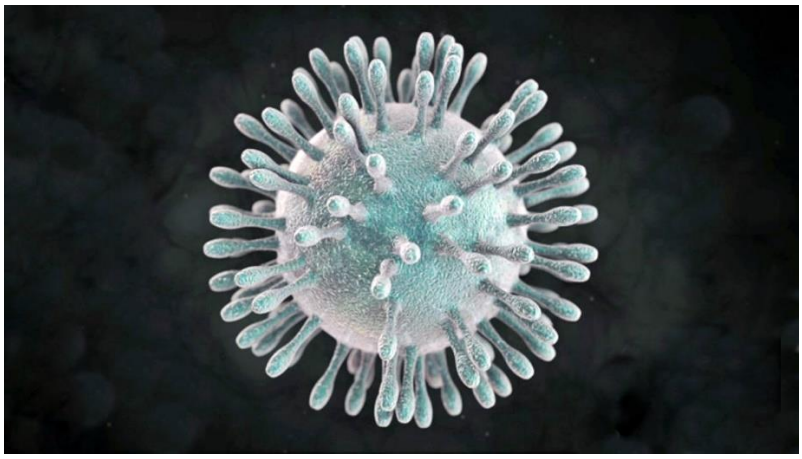
Newsletter of the Freshwater Mollusk Conservation Society
Vol. 22 – No. 3 ISSN 2689-2936 September 2020



FMCS will NOT Hold an In-Person Symposium in 2021

As a result of discussions among members of the 2021 Local Planning Committee and the Executive Committee, the decision has been made that FMCS will **not** hold an in-person Symposium during 2021, either in Portland, Oregon, or elsewhere. Given the present state of COVID-19-related issues, and the uncertainties surrounding possible travel restrictions, group gathering prohibitions, and agency budget constraints, we recognized that it will not be possible to gather together any time soon in the way we have at past Symposia.

But all may not be lost for 2021! With the decision made not to assemble for a regular Symposium, we are exploring a variety of options for holding some sort of

Contributed Articles

The following articles have been contributed by FMCS members and others interested in freshwater mollusks. These contributions are incorporated into Ellipsaria *without peer review and with minimal editing. The opinions expressed are those of the authors.*

Reproductive Isolation Between *Lithasia* Populations of the *geniculata* and *duttoniana* Forms in the Duck River, Tennessee

Robert T. Dillon, Jr., Freshwater Gastropods of North America Project.
P.O. Box 31532, Charleston, South Carolina, USA 29417 dillonr@fwgna.org

Goodrich (1940) recognized two species of *Lithasia* in the Duck River – the famous populations of *Lithasia geniculata* (with three subspecies) which he featured in his landmark (1934) study on shell morphology in the Pleuroceridae, and the only-slightly-less famous *Lithasia duttoniana*. Populations of *Lithasia geniculata* inhabit midstream high-current areas down almost the entire length of the river, bearing smooth shells in the headwaters at DRM 275, developing bumpy shoulders in the lower reaches (Dillon 2020). Populations that Goodrich identified as *Lithasia duttoniana* only inhabit lower reaches of the Duck River, from about DRM 186 to the mouth, generally reaching higher densities at the stream margins. Their shells are not bumpy so much as carinate or slightly spiny, bearing more acute protuberances around a periphery lower on the shell whorl.

Minton and Lydeard (2003) conducted an extensive survey of mtDNA sequence variation across a broad sample of North American *Lithasia* populations, ultimately reporting CO1 sequence data from 27 populations representing 11 nominal species and subspecies. The four unique sequences they obtained from 19 Duck River snails (1 *jayana*, 4 *duttoniana*, 1 *geniculata geniculata*, 7 *geniculata fuliginosa*, and 6 *geniculata pinguis*, in 9 Genbank submissions) did not resolve into any consistent clades. Thus Minton and Lydeard synonymized all Duck River populations under a single specific nomen, *Lithasia fuliginosa*. Minton and colleagues (2008) went on to lump Duck River *Lithasia* populations of all historic nomina into a single study of shell morphological variation down the entire length of the river.

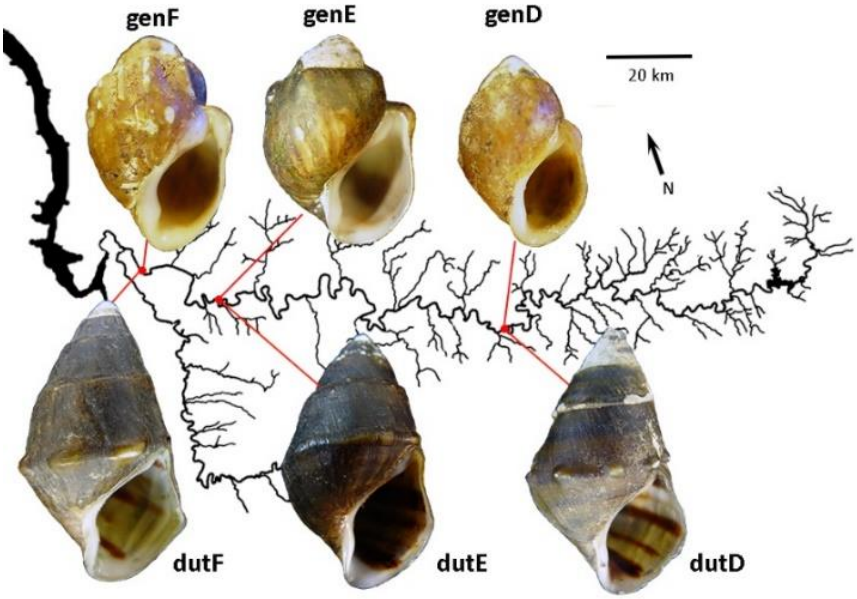


Figure 1. The Duck River of Middle Tennessee, showing sample sites. The shell length of dutF is 24.6 mm; the other shells are to scale.

Dillon (2020) has recently reported the results of a survey of allozyme variation in a set of seven *L. geniculata* populations incidentally sampled during a 2000-02 survey of the Duck River unionid mussel fauna by Ahlstedt and colleagues (2017), transmitted by P. D. Johnson. Samples of *Lithasia* bearing the *duttoniana* shell morphology were also collected with the *geniculata* samples at the three lowest sample sites, site D from the Fountain Creek confluence (TNC94, 35.5695, -86.9682), site E from Wright Bend (TNC110, 35.8267, -87.6657), and site F at the Watered Hollow boat launch (35.9322, -87.7475). See Figure 1.

Here I report a survey of allozyme variation in Duck River *Lithasia* of the *duttoniana* form, conducted alongside the study of the *geniculata* form reported by Dillon (2020). A sample of ten *duttoniana* individuals from site E (dutE) was also analyzed along with the samples from pinA and genE referenced in my 2020 study and screened for polymorphism at 17 enzyme loci using the methods of Dillon (1982, 1985, 1992). And again, as reported in 2020, allozyme variation interpretable as the product of codominant Mendelian inheritance was discovered at just three loci: mannose phosphate isomerase (Mpi) on buffers TrisCit6 and TEB8, octopine dehydrogenase (Odh) on buffers TrisCit6 and Poulik, and hexanol dehydrogenase (Hexdh) on buffers TEB8 and Poulik. Genetic variation was subsequently assessed at these three loci only for the remainder of the populations and individuals.

Gene frequencies in *geniculata*-form and *duttoniana*-form samples were strikingly different at all three sites where they were collected together (Table 1). The frequency of Hexdh99 ranged from 0.214 – 0.426 in *duttoniana* sampled from sites D, E, and F, while rising no higher than 0.015 in co-occurring *geniculata*. The Fisher's exact probability that genF and dutF were drawn from the same population of Hexdh alleles was 0.0003, and much lower comparing *geniculata* and *duttoniana* at sites D and E. The absence of Odh114 and Odh111 (together) in *geniculata* was also extremely significant (Fisher's exact $p < 0.0001$ at sites E and F), as well as the near absence of Mpi91 in *duttoniana* sampled from the same sites. The (Nei 1978) genetic distances among samples genD, genE, and genF ranged from 0.096 - 0.227, and those among samples dutD, dutE, and dutF ranged from 0.107 - 0.245, showing no overlap with the 0.276 - 0.360 range of between-group genetic distances. This constitutes strong evidence of reproductive isolation between *Lithasia* populations of the *geniculata* and *duttoniana* forms in the Duck River.

Table 1. Gene frequencies at three allozyme-encoding loci in three Duck River populations of *Lithasia geniculata* and co-occurring populations bearing the *duttoniana* shell form.

	Fountain Cr. conf.		**Wright Bend**		**Watered Hollow**	
	genD	**dutD**	**genE**	**dutE**	**genF**	**dutF**
Odh	32	34	49	44	33	35
114	0.0	0.0	0.0	0.011	0.0	0.129
112	0.547	0.456	0.582	0.239	0.561	0.057
111	0.0	0.059	0.0	0.136	0.0	0.186
109	0.453	0.485	0.418	0.614	0.439	0.629
Mpi	32	34	49	44	33	35
97	0.0	0.0	0.0	0.0	0.015	0.0
94	1.00	0.985	0.867	1.00	0.667	0.986
91	0.0	0.0	0.133	0.0	0.318	0.014
90	0.0	0.015	0.0	0.0	0.0	0.0
Hexdh	25	34	41	37	33	35
99	0.0	0.426	0.012	0.432	0.015	0.214
93	1.00	0.574	0.988	0.568	0.985	0.786

These results have important implications. In recent years, at least four separate studies have returned evidence of double-digit mtDNA sequence divergence within pleurocerid populations: Dillon & Frankis (2004) Dillon and Robinson (2009, 2016), and Whelan and Strong (2016). Such results, termed "mitochondrial superheterogeneity" by Dillon (2019), have been interpreted both as evidence that pleurocerid populations are highly fragmented, and that they are extraordinarily old.

In the Duck River, however, a pair of reproductively isolated *Lithasia* species apparently demonstrate no mtDNA sequence divergence at all. It is certainly possible that *Lithasia* populations of the *geniculata* and *duttoniana* forms have speciated so recently that no mtDNA sequence divergence has as yet accumulated. The interspecific levels of allozyme divergence reported here are as strikingly low as their COI sequence divergence (e.g., Dillon 1984). It is also possible that the CO1 sequences shared by both species (GenBank AF435744 and variants) are ancestral to the genus *Lithasia*, preserved by populations of both species in parallel for millions of generations after their divergence.

In either case, the results presented here, together with those such as have been reported by Dillon and Robinson (2009) and Whelan and Strong (2016), should strongly caution workers relying on so-called "DNA Barcoding" methods to work out specific relationships among populations of freshwater gastropods.

References:

Ahlstedt, S. A., J. R. Powell, R. S. Butler, M. T. Fagg, D. W. Hubbs, S. F. Novak, S. R. Palmer & P. D. Johnson. 2017. Historical and current examination of freshwater mussels (Bivalvia: Margaritiferidae: Unionidae) in the Duck River basin Tennessee, USA. *Malacological Review* 45:1-163.

Dillon, R. T., Jr. 1982. *The correlates of divergence in isolated populations of the freshwater snail, Goniobasis proxima* (Say). Ph.D. Dissertation, The University of Pennsylvania.

Dillon, R.T., Jr. 1984. Geographic distance, environmental difference, and divergence between isolated populations. *Systematic Zoology* 33:69-82.

Dillon, R. T., Jr. 1985. Correspondence between the buffer systems suitable for electrophoretic resolution of bivalve and gastropod isozymes. *Comparative Biochemistry and Physiology* 82B:643-645.

Dillon, R. T., Jr. 1992. Electrophoresis IV, nuts and bolts. *World Aquaculture* 23(2):48-51.

Dillon, R.T., Jr. 2019. Mitochondrial superheterogeneity: What we know. Pp 145 - 153 in *The Freshwater Gastropods of North America Volume 3, Essays on the Prosobranchs*. FWGNA Press, Charleston.

Dillon, R. T., Jr. 2020. Population genetic survey of *Lithasia geniculata* in the Duck River, Tennessee. *Ellipsaria* 22(2):19 – 21.

Dillon R. T., Jr. and R. C. Frankis. 2004. High levels of DNA sequence divergence in isolated populations of the freshwater snail, Goniobasis. *American Malacological Bulletin* 19:69 – 77.

Dillon, R. T., Jr. and J. D. Robinson. 2009. The snails the dinosaurs saw: Are the pleurocerid populations of the Older Appalachians a relict of the Paleozoic Era? *Journal of the North American Benthological Society* 28:1 – 11.

Dillon, R. T., Jr. and J. D. Robinson. 2016. The hazards of DNA barcoding, as illustrated by the pleurocerid gastropods of East Tennessee. *Ellipsaria* 18(1):22-24.

Goodrich, C. 1934. Studies of the gastropod family Pleuroceridae - I. *Occasional Papers of the Museum of Zoology, University of Michigan* 286: - 17.

Goodrich, C. 1940. The Pleuroceridae of the Ohio River drainage system. *Occasional Papers of the Museum of Zoology, University of Michigan* 417:1 - 21.

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.

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

Nei, M. 1978 Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89:583–590.

Whelan, N.V. and E. E. Strong. 2016. Morphology, molecules and taxonomy: extreme incongruence in pleurocerids (Gastropoda, Cerithiodea, Pleuroceridae). *Zoologica Scripta* 45:62 – 87.