Blurred lines in conservation: Freshwater mussels gene flow and species boundaries

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CONTEXT

- Correct identification of species, assessing the potential hybridization (i.e., gene flow between species) and description of hybrid zones is fundamental in developing and implementing measures to conserve and restore threaten taxa
- Unionids (native freshwater mussels) dispersed into the lower Great Lakes from the Ohio and Mississippi River Basins (Interior Basin) and the Atlantic slope after the last glaciation[1,2]. Species range expansions led to secondary contact between closely related species such as *Lampsilis siliquoidea* (Interior Basin) and *L. radiata* (Atlantic slope). It has been suggested that the two species can hybridize[3,4,5], but the prevalence, direction and geographic extent oh hybrids is not well known
- The evidence for potential hybridization comes from the presence of morphological and genetic intermediate forms[5] where these species' range overlaps, which has led to a long history of name confusion and debate on the phylogenetic relationship of the two species.
- *L. siliquoidea* is critically imperiled in the states of OK, imperiled in LA and TN, vulnerable in AR, MS and WV. *L. radiata* is critically imperiled in NC and RI, imperiled in SC, VA, NJ and PA. Both are considered secured in the remaining states where they occur

GOAL

- Determine the phylogenetic relationship and levels of intermixing between *L. siliquoidea* and *L. radiata*.

1. *LAMPSILIS SILIQUOIDEA* AND *L. RADIATA* ARE DIFFERENT SPECIES

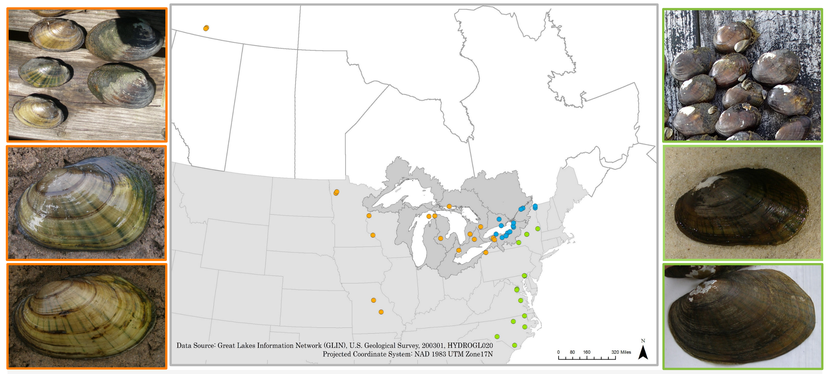


Fig. 1. *L. siliquoidea* and *L. radiata* samples (mantle clips or whole male individuals) were obtained from several locations outside the secondary contact zone and from within the secondary zone (blue).

Species identification outside the secondary zone (depicted in orange and green dots, Fig. 1) was initially based on morphological characters. Unionids have a distinct form of mitochondrial DNA (mtDNA) inheritance, termed doubly uniparental inheritance (DUI) where maternally (m) and paternally (p) inherited mtDNA can be observed and they are considered as independent genetic markers. Identifications were then corroborated using m-and p- inherited mitochondrial DNA (Cytochrome oxidase I, COI)[6].

Haplotype networks (Fig. 2) were constructed in Network version 4.6 using the median-joining algorithm to determine the relationship between the two species outside the secondary contact zone. *L. siliquoidea* and *L. radiata* formed two discrete groups with unique haplotypes, which corroborates that these are two different species.

Some individuals from the secondary contact zone have morphological intermediate forms making classification difficult. Maternally inherited mtDNA haplotypes were used for identification by assigning individuals to either species.(Blue, Fig 2).

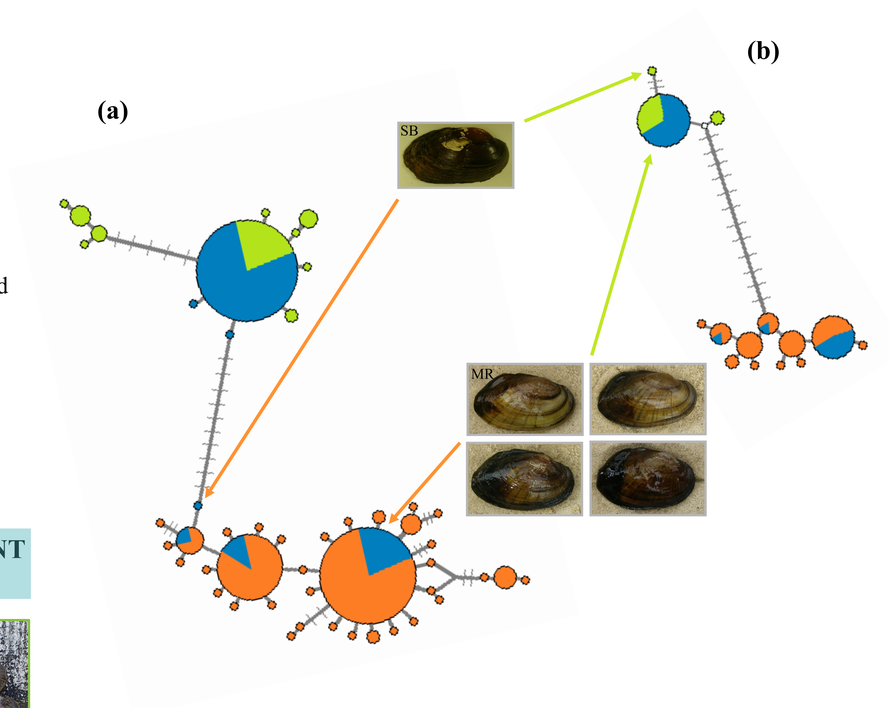


Fig 2. Networks showing the similarity of **(a)** 51 (343 sequences) maternally-inherited and **(b)** 14 (85 sequences) paternally-inherited cytochrome *c* oxidase subunit I (COI) haplotypes. Size of circle is proportional to haplotype frequency; *L. siliquoidea* (orange) and *L. radiata* (green) from outside the secondary contact zone, and from within the secondary contact zone (blue). Small grey lines perpendicular to main axis represent number of mutations between haplotypes, single mutations were not marked. M- and p- inherited haplotypes are not drawn to scale. Arrows identify haplotypes for individuals where there is a mismatch between m- and p-inherited mtDNA suggesting hybridization. Location abbreviations as in Fig 3.

2. INCONGRUENCE BETWEEN m- AND p-INHERITED mtDNA HAPLOTYPES SUGGEST HYBRIDIZATION

Individuals with m-inherited haplotype assigned to one species and p-inherited haplotype assigned to the other species were found in two locations (SB=1 and MR=4, Fig. 2). Incongruence between m- and p-inherited mtDNA haplotypes suggests hybridization. However, incongruence between the two markers can also be caused by incomplete lineage sorting. Further testing is needed.

In GR haplotypes of both species were found, but there was no evidence of hybridization.

3. FURTHER TESTING USING MICROSATELLITES ALSO SUPPORTS HYBRIDIZATION

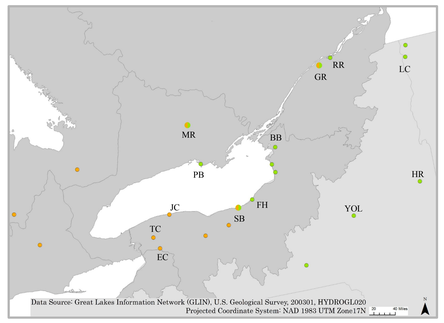


Fig. 3. Classified individuals using m- and p-inherited COI from within the secondary contact zone. Both haplotypes were found at three locations . Ellicott Creek (EC), Tonawanda Creek (TC), Johnson Creek (JC), Moira River (MR), Pleasant Bay (PB), Sodus Bay (SB), Fair Haven State Park (FH), Black River Bay (BB), Grasse River (GR), Raquette River (RR), Lake Champlain (LC), Hudson River (HR) and Young Lake (YL)

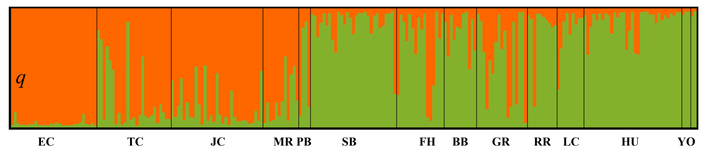


Fig. 4. Bayesian model-based analysis of 5 microsatellite loci with K = 2 distinct genetic clusters. Each vertical line represents one individual. Potential hybrids are represented as admixed individuals that are assigned to *L. siliquoidea* and to *L. radiata* clusters (bicolored lines). Y-axis represent *q,* coefficient of membership (0-1).

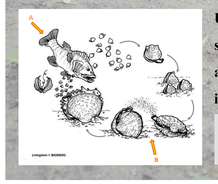
In order to further test the occurrence of hybridization, Bayesian model-based clustering based on 5 microsatellite loci[7] was performed using Structure 2.3.3. No prior population/species information was used and individuals were assigned to one of the clusters. Hybrids were expected to be assigned jointly to clusters of *L. siliquoidea* and *L. radiata* (Fig. 4). This analysis suggests that hybridization may be happening at larger rates and in more locations (e.g. TC, JV, FH) than those suggested by the mtDNA analysis.

CONCLUSIONS AND FUTURE WORK

- *Lampsilis siliquoidea* and *L. radiata* are different species.
- Mitochondrial and microsatellite data supports that hybridization has occurred. However, mtDNA suggests that hybridization is not prevalent, whereas microsatellite data is consistent with more frequent hybridization.
- Further testing is needed, particularly discerning whether or not incongruence between m- and p- inherited mtDNA could be due incomplete lineage sorting.

Get to know Unionids:

Freshwater pearl mussels (Order: Unionacea) are large bivalves that live in the sediments of rivers, streams and lakes. This group has a fascinating life cycle where the larvae are obligate parasites of fishes, and host fish can transport the larvae great distances. North America has approximately 300 of the 847 known species in the world[8]. Although this group is highly diverse, it is the most imperiled taxon (37 extinct species; 165 critically imperiled[8,9]) as a result of human impacts such as overharvesting, pollution, impoundment of rivers, and the introduction of exotic species (e.g. dreissenid mussels)[10].



Unionids are very important in the functioning of aquatic systems (e.g., nutrient cycling, bioturbation, provide habitat and shelter to other aquatic organisms) and their decline has implications in the maintenance of the ecosystem

Unionid life cycle. Arrows show potential means of dispersal. A. Fish, B. Glochidia (larvae) in drift, C. sperm (Image modified from Mulcrone, 2006)

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