REGULAR ARTICLE

HIDING IN PLAIN SIGHT: GENETIC CONFIRMATION OF PUTATIVE LOUISIANA FATMUCKET LAMPSILIS HYDIANA (MOLLUSCA: UNIONIDAE) IN ILLINOIS

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ABSTRACT

Understanding the status and distribution of species is fundamental for conservation. However, recent genetic work has challenged the known distributions of some unionid taxa. The recognized range of the Louisiana Fatmucket Lampsilis hydiana spans watersheds from east Texas northward to southern Arkansas and eastward to western Mississippi. Specimens with morphological similarities to L. hydiana have been observed in Illinois and were presumed to be Fatmucket Lampsilis siliquoidea based on known distributions of Lampsilis species in Illinois. We examined specimens from Illinois and completed comparative genetic analyses using the mitochondrial genes cox1 and nad1 for species resembling L. siliquoidea. Our results show two morphologically similar, yet genetically distinct, species in Illinois. One of these species was genetically similar to L. siliquoidea, and one of these species showed little-to-no genetic difference from topotypic L. hydiana. The confirmation of L. hydiana populations within Illinois is significant for documenting the faunal diversity of the state. The varying degree of phenotypic separation confirms the need for further morphological research within Lampsilis, as well as genetic research throughout the updated known range of L. hydiana.

KEY WORDS: Fatmucket, Louisiana Fatmucket, Illinois, Lampsilis hydiana, Lampsilis siliquoidea

INTRODUCTION

Accurate knowledge of the status and distribution of biota is fundamental for proper conservation of natural resources. Diversity is significant within unionid mollusks in the Mississippi basin (van der Schalie and van der Schalie 1950; Johnson 1980; Turner et al. 2000), yet an incomplete understanding of the genetic structure of many taxa (e.g., Campbell et al. 2005, Graf and Cummings 2007) leads to uncertainty regarding species distributions. Illinois has a diverse, well-documented freshwater mussel fauna that historically consisted of more than 80 species of Unionidae and one species of Margaritiferidae (Baker 1906, 1912; Parmalee 1967; Cummings and Mayer 1997; Tiemann et al. 2007). Range updates, such as discovering Bankclimber Plectomerus dombeyanus (Valenciennes, 1827) in Illinois in 2012, have been documented through sporadic or systematic surveys (Tiemann et al. 2007, 2013). Publication of such findings is valuable to regional conservation efforts, because federal and state agency conservation plans can apply only to species that are known to be present.

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More than 20 species of *Lampsilis* are currently recognized in North America (Williams et al. 2017; FMCS 2019), and seven of those have been documented in Illinois by live material or shell (Tiemann et al. 2007). This diverse genus ranges across eastern and central North America and has shell morphology that varies from ovate—like Pink Mucket *Lampsilis abrupta* (Say, 1831)—to elongate and terete—like the newly described Canary Kingshell *Lampsilis sietmani* Keogh and Simons 2019 (Keogh and Simons 2019; Fig. 1). Fatmucket *Lampsilis siliquoidea* (Barnes, 1823) is one of the most widespread unionids in the world and has stable populations across most of its range. It occurs widely in the Mississippi and Great Lakes basins and is commonly encountered in Illinois rivers (Tiemann et al. 2007; Watters et al. 2009). Louisiana Fatmucket *Lampsilis hydiana* (Lea, 1838) (Fig. 1c)—a species previously reported from eastern Texas, Oklahoma, and Arkansas and east to Alabama (Burch 1975; Howells et al. 1996)—has a similar morphology to *L. siliquoidea* (Fig. 1f), but *L. hydiana* has never been genetically confirmed to exist in Illinois. Neither *L. hydiana* nor *L. siliquoidea* is of conservation concern in Illinois or at the federal level.

*Lampsilis hydiana* is described as having an elliptical, rayed, somewhat inflated shell and is distinguished from *L. siliquoidea* by a pearlier nacre, an umbo that is anterior, and an overall smaller average total length (Lea 1838). However, these two species have been considered indistinguishable at times (Vaughn et al. 1996) or as synonyms (Call 1895), which has led to uncertainty regarding their distributions. Based on literature reports and museum shell records, these species presumably co-occur in several drainages, such as the Big Black and Yazoo rivers in Mississippi (Jones et al. 2005). Additionally, specimens from Arkansas initially identified as *L. hydiana* included three genetically distinct groups that represented *L. hydiana* and two additional undescribed species (Harris et al. 2009). These divisions were supported by a shape
analysis, though there was some overlap in morphology (Harris et al. 2004; Harris et al. 2009). Thus, the range extent of *L. hydiana* remains unknown, and morphological characteristics to distinguish among *L. siliquoidea*, *L. hydiana*, and other similarly shaped *Lampsilis* species are lacking.

Certain specimens in several southern Illinois watersheds morphologically resemble *L. hydiana* (Fig. 2), though collection localities are well outside the published range of this species (Fig. 3). These specimens were typically identified as *L. siliquoidea*, despite morphologic resemblance to *L. hydiana*. The objective of our study was to determine taxonomic placement of the specimens that morphologically resemble *L. hydiana* to gain a better understanding of the distribution of *L. siliquoidea* and related species in Illinois.

**METHODS**

Mantle tissues of putative *L. hydiana* and *L. siliquoidea* from Illinois (*n = 83* specimens from 25 sites) were collected from fresh, frozen, or ethanol-preserved individuals, used for DNA extraction, and catalogued in the Illinois Natural History Survey (INHS) Mollusk Collection, Champaign, Illinois (Appendix 1). Specimens came from the Big Muddy, Cache, Embarras, Kaskaskia, Little Wabash, Little Vermilion, Mackinaw, Sangamon, and Skillet Fork drainages and direct
tributaries to the Ohio River (Big Grande Pierre, Lusk, and Rose creeks); images of external and internal valves of each specimen were made available via the Illinois Data Bank (https://doi.org/10.13012/B2IDB-5609050_V1). Initial species identifications were made from external shell morphology of each specimen prior to genetic analysis. Those that were more inflated, had a pearlier nacre, and had a shorter average total length in mature individuals were identified as putative *L. hydiana* (*n* = 46; Fig. 2a), while specimens that were more compressed, had a duller nacre, and had a longer average total

Figure 3. Approximate locations of reference materials used for this study. The previously published range for *Lampsilis hydiana* was adapted from Burch (1975) and Howells et al. (1996).
length in mature individuals were identified as *L. siliquoidea* \((n = 37; \text{Fig. 2b})\). Most of the putative *L. hydiana* were from specimens collected from the southern half of Illinois. Specimens used in this study were collected as part of other research projects, primarily during a statewide mussel survey for Illinois from 2009 to 2012. Funding constraints or curated tissue quality prevented us from using all available tissue samples from putative *L. hydiana* or *L. siliquoidea* in Illinois. Four *L. hydiana* specimens were collected from the Boeuf River, Louisiana, to provide comparative material (INHS 87783). In addition, comparative sequences were obtained from GenBank (Appendix 2).

DNA was extracted from approximately 2 mm \(\times\) 2 mm mantle and muscle biopsies using the MagMAX–96 DNA Multi-Sample Kit (ThermoFisher Scientific, Waltham, MA, USA) according to the manufacturer’s instructions, except samples were eluted in 40 \(\mu\)l of elution buffer 1 and 2 instead of 100 \(\mu\)l. Polymerase chain reactions (PCR) and primers for *cox1* and *nad1* DNA amplification followed Campbell and Lydeard (2012). PCR products were sequenced on a Life Technologies 3730xl DNA Analyzer (Applied Biosystems, University of Illinois Chicago Genome Research Core). The *cox1* region was 660 base pairs long, and the *nad1* region was 834 bases long (including 30 bases of tRNA-Leu). Not all reads clearly resolved all bases, however, and unreadable bases were entered as unknowns. Sequences were aligned using BioEdit (Hall 1999). The sequence alignments are available at the Illinois Data Bank (https://doi.org/10.13012/B21DB-5609050_V1).

The relationships between species currently assigned to *Lampsilis* are not well resolved (Keogh and Simons 2019). To determine appropriate comparison taxa for our specimens, we performed preliminary phylogenetic analyses (details below) of all available *cox1* and *nad1* sequences for species currently assigned to *Lampsilis* (based on Williams et al. 2017), along with representatives of other genera in the tribe Lampsilini. These supported a clade of morphologically similar taxa that included *L. siliquoidea* and *L. hydiana*, along with Guadalupe Fatmucket *Lampsilis bergmanni* Inoue & Randklev, 2020, Arkansas Fatmucket *Lampsilis powelli* (Lea, 1852), Eastern Lampmussel *Lampsilis radiata* (Gmelin, 1791), *L. sietmani*, Rough Fatmucket *Lampsilis straminea* (Conrad, 1834), and Alabama Lampmussel *Lampsilis virens* (Lea, 1858). In turn, this *siliquoidea* clade was most closely related to a clade that included Mucket *Ortmanniana ligamentina* (Lamarck, 1819), *L. abrupta*, and Higgins Eye *Lampsilis higginsii* (Lea, 1857), consistent with previous findings (Porto-Hannes et al. 2019; Inoue et al. 2020). Nomenclature follows Williams et al. (2017), with updates from recent works for *O. ligamentina* (Pfeiffer et al. 2019; Graf and Cummings 2021). As noted by Keogh and Simons (2019), confident assessment of the phylogenetic relationships of *Lampsilis* species within Lampsilini will require extensive sampling. Our goal was to find appropriate taxa for comparison with our *L. siliquoidea*–like and *L. hydiana*–like populations from Illinois, and we did not pursue the general phylogeny further. Based on these preliminary results, we included all available *cox1* and *nad1* sequences from the *siliquoidea* clade in our detailed analyses and used the *ligamentina* clade as the outgroup. The sequence identified as *L. powelli* in GenBank was treated as *L. hydiana* in our analyses (MF326971). Walters et al. (2021) also found this sequence to be *L. hydiana*, whereas true *L. powelli* was nearest to *L. siliquoidea*. A few sequences currently listed as *L. radiata* in GenBank (*cox1*: HQ153601, HQ153602, HQ153605; *nad1*: HQ153683, HQ153684, HQ153687, and HQ153691) were found to represent the “Cryptic *Lampsilis* sp.” of McCartney et al. (2016). Those sequences did not place in the *siliquoidea* clade based on McCartney et al. (2016) and our preliminary analyses, thus we excluded them from the present analyses. Percent differences and number of base-pair differences were calculated for all sequences from the *siliquoidea* clade using PAUP*4.0a167 (Swofford 2002).

Because many individuals had only one gene or the other sequenced, *cox1* and *nad1* were compared separately in these analyses. These calculations omit bases with uncertainty (e.g., A versus N is not counted as a difference, nor is that position counted in the total number of bases for calculating percentage). We used the program ABGD (Puillandre et al. 2012) to test the differentiation between species in the *siliquoidea* clade. To test the cutoff for different divisions, the number of steps was increased to 20 and relative gap width decreased to one; other settings used the default values.

For phylogenetic analyses, we used both parsimony and Bayesian approaches and included all individuals with data for both *nad1* and *cox1*. We concatenated the two genes, omitting the tRNA-Leu region. *Lampsilis sietmani* and *L. abrupta* had no *nad1* data available, but we included representative *cox1* sequences. In the ABGD analysis, one published *cox1* sequence identified as *L. hydiana* (EF033270, from the Cossatot River in Arkansas), the Escambia River *L. straminea* (four sequences), and the Neches River sequence of *L. sietmani* (two individuals with identical sequences) were somewhat divergent from the other sampled individuals, so they were also included despite having only *cox1* data available. If two individuals had the same haplotype for both *cox1* and *nad1*, that combined haplotype was included only once in the phylogenetic analyses. Maximum parsimony and “Group present/Contradicted” (GC) bootstrap analysis (Goloboff et al. 2003) in the computer program TNT 1.5 (Goloboff and Catalano 2016) used all the “new technology” search options. Parsimony analysis used 500 random addition replicates, and the bootstrap analysis used 500 bootstrap replicates, each with 10 random addition replicates. Bayesian analyses used 10,000,000 generations with 10 runs, each with eight chains. We used PAUP* to test data partitions, setting the codon positions as data blocks. Using likelihood criteria and the “greedy” heuristic, the AICc criterion supported a GTR+1 model for *cox1* positions 1 and 3 and *nad1* position 2, GTR for *cox1* position 2 and *nad1* position 3, and GTR+G for *nad1* position 1. MrBayes 3.2.7 was used for Bayesian analyses (Ronquist et al. 2012). Each codon position was treated as a separate partition. The parameters revmat, shape,
pinvar, and statefreq were all unlinked. Convergence was determined by examining the standard deviation of split frequencies and confirming that they were under 0.01 (Ronquist et al. 2011), as well as by examination of the ESS values and trace plot in Tracer 1.7.1 (Rambaut et al. 2018). Tracer showed all ESS values well over 200, and the trace plot did not show any anomalies, so the standard 25% burn-in was used. We used PAUP* to calculate a majority-rule consensus of the Bayesian trees to obtain posterior probabilities, which facilitated outputting the tree as a graphic. Additionally, haplotype networks were constructed for *L. siliquoidea* and *L. hydiana* using median joining in PopART (Leigh and Bryant 2015).

**RESULTS**

The genetic results indicate that the *L. siliquoidea* and putative *L. hydiana* specimens from Illinois represent two distinct but closely related *Lampsilis* species. Sequences obtained for this study are available in GenBank (accession numbers MH560712-MH560762, MH560764-MH560777, MH588322-MH588394, MT537705-MT537725; Appendices 1, 2). Parsimony and Bayesian methods produced nearly identical results, with no differences in the affinities of the Illinois specimens. Parsimony analyses produced 319 trees of length 545 (as counted by TNT, which collapses polytomies, making a much smaller number of trees than PAUP*). In the Bayesian analysis, the standard deviation of split frequencies reached 0.01 after 1,735,000 generations. *Lampsilis hydiana* and *L. siliquoidea* were not sister taxa, but instead placed on different branches within the larger *siliquoidea* clade (Fig. 4). *Lampsilis siliquoidea* and *L. radiata* are sister taxa with relatively low genetic divergence, while *L. hydiana* is sister to *L. bergmanni*.

Sequences for *L. hydiana* versus *L. siliquoidea* had an average of 5.67% difference between them in *cox1* and 7.43% in *nad1*, similar to most other interspecies differences within the clade (Table 1). In contrast, the average differences within *L. hydiana* and within *siliquoidea* for both genes were under 0.5%, with some Illinois specimens sharing haplotypes with specimens from elsewhere. In particular, identical haplotypes were found in some Illinois specimens and some of the toptotypic *L. hydiana* specimens sampled in this study (Appendix 2, Figs. 5, 6). Likewise, the haplotype networks show much larger differences between *L. hydiana* and *L. siliquoidea* than within them. In ABGD for *cox1*, all partitions with gap priors between 0.0183 and 0.00162 separated the Illinois specimens (along with many from other states) into two groups, corresponding to *L. hydiana* and *L. siliquoidea*. No partitions supported any further division of *L. hydiana* or *L. siliquoidea*, except for recognizing the Cossatot River, Arkansas “*L. hydiana*” as distinct for priors of 0.00886 or less in the initial partition and 0.0144 or less in the recursive partition. The species most difficult to distinguish from *L. hydiana* were *L. bergmanni* and Mobile Basin *L. straminea*, which separated only at gap priors of 0.00428 or less, whereas *L. siliquoidea* and *L. radiata* were separated at gap priors of 0.0546 or less. Gap priors of 0.00127 or less split up individual variation, which produced 116 groups. For *nad1*, partitions with gap priors between 0.0183 and 0.00264 separated *L. hydiana* and *L. siliquoidea* without dividing either one. Again, separation between *L. hydiana* and *L. bergmanni* or *L. straminea* was less clear, requiring gap priors of 0.00207 or less, which also began to split off divergent sequences within *L. hydiana*. Separation of *L. siliquoidea* from *L. radiata* was supported with the recursive partition at a gap prior of 0.0183 or less and the initial partition at a gap prior of 0.0144 or less. Intermediate gap priors generally agreed with currently recognized species, though some currently recognized species were divided into more than one group, especially if there was a geographic gap in the sampling (such as *L. sietmani* from Texas versus the upper Mississippi drainage).

Our results support the presence of *L. hydiana* in the Big Muddy, Cache, Embarras, Kaskaskia, Sangamon, Ohio, and Little Wabash drainages of Illinois (Fig. 7). Most drainages that we examined contained only *L. siliquoidea* or *L. hydiana*; however, both *L. siliquoidea* and *L. hydiana* were confirmed in the Sangamon River basin and in Horse, Big Grande Pierre, and Lusk creeks. Our morphological identifications matched the genetic confirmation in most cases (72 of 83 individuals were identified correctly; Appendix 1). Ten specimens that were determined morphologically to be *L. siliquoidea* were genetically confirmed as *L. hydiana*, and one specimen that was determined morphologically to be *L. hydiana* was genetically confirmed as *L. siliquoidea*. Three of four sites where these mismatches occurred had both *L. hydiana* and *L. siliquoidea* genotypes present (Big Grande Pierre Creek, Lusk Creek, and Horse Creek; Fig. 2c, 2d). The only individual sequenced from Salt Creek (of two total specimens from the Sangamon River drainage) was genetically confirmed as *L. hydiana* but was determined morphologically to be *L. siliquoidea*.

**DISCUSSION**

We used genetic analyses to confirm the presence of *L. hydiana* in Illinois. This genetic confirmation supports the species determinations by Anson A. Hinkley and Frank C. Baker more than a century ago (Illinois Natural History Survey, Prairie Research Institute 2021 [INHS Collections Data], referenced via previous identification field), that were made prior to the availability of genetic tools. It is unclear why *L. hydiana* was never included on Illinois species lists even though shells were deposited in the INHS Mollusk Collection bearing this identification. Regardless, we now have genetic support that the range of *L. hydiana* extends to latitude 40.1° N in the Sangamon River drainage, which is well north of the previously published range limit of latitude 34.6° N (Burch 1975; Howells et al. 1996; Inoue et al. 2020). While historical literature proclaimed the morphological differences between *L. hydiana* and *L. siliquoidea* to be “very clear cut” (Isley 1924,
Figure 4. Phylogram of the Bayesian majority-rule consensus tree. Numbers on branches are Bayesian posterior probability/bootstrap GC percentage. * indicates under 50% bootstrap support; * denotes branches that did not have room for labeling the probabilities directly on the branch. Numbers after a name indicate \textit{cox1} haplotype, followed by \textit{nad1} haplotype (see Appendix 2). Letters after a name indicate a new sequence from Illinois, topotype \textit{Lampsilis hydiana} (t), the sequence identified as \textit{Lampsilis powellii} (p), or haplotypes found in other published sequences and in new ones from Illinois (o). \textit{L. hydiana} indicates the divergent Cossatt River sequence, and \textit{L. "straminea"} indicates the Escambia River population.
we obviously did not find that to be the case for all the individuals analyzed. At sites where both *L. hydiana* and *L. siliquoidea* genotypes were present, we were unable to separate these individuals using only shell morphology (Fig. 2c, 2d). A more detailed morphological analysis may reveal additional characters that we did not consider, such as quantifying height to length ratio or measuring shell thickness (Keogh and Simons 2019). We recognize that our study’s

|        | Intraspecific         | To *hydiana*          | To *siliquoidea*       |
|--------|-----------------------|-----------------------|------------------------|
| **cox1** |                       |                       |                        |
| *L. bergmanni* | 0.08% (0.00–0.33)  | 1.74% (1.18–2.74)   | 5.21% (4.19–6.12)     |
|          | 0.55 bp (0–2)       | 11.18 bp (7–18)     | 32.86 bp (22–37)      |
| *L. hydiana* | 0.32% (0.00–1.98)  | 0.32% (0.00–1.98)   | 5.67% (4.38–7.29)     |
|          | 2.02 bp (0–13)      | 2.02 bp (0–13)      | 35.63 bp (20–43)      |
| “*L. hydiana*” | n/a                 | 3.48% (3.09–4.45)   | 5.35% (4.80–6.00)     |
|          | n/a                  | 20.19 bp (14–26)    | 31.00 bp (25–34)      |
| *L. radiata* | 0.63% (0.00–1.53)  | 5.37% (4.04–7.07)   | 2.23% (1.39–3.03)     |
|          | 3.42 bp (0–8)       | 31.64 bp (16–42)    | 13.11 bp (8–19)       |
| *L. sietmani* | 0.66% (0.00–2.60)  | 6.46% (5.88–8.16)   | 7.17% (6.46–8.45)     |
|          | 4.23 bp (0–16)      | 41.20 bp (26–53)    | 45.43 bp (34–52)      |
| *L. siliquoidea* | 0.46% (0.00–1.74) | 5.67% (4.38–7.29)   | 0.46% (0.00–1.74)     |
|          | 2.89 bp (0–11)      | 35.63 bp (20–43)    | 2.89 bp (0–11)        |
| *L. straminea* | 0.46% (0.16–0.69) | 2.08% (1.55–3.53)   | 5.60% (4.44–6.24)     |
|          | 2.67 bp (1–4)       | 12.64 bp (8–23)     | 33.74 bp (23–39)      |
| “*L. straminea*” | 0.39% (0.16–0.63) | 3.08% (2.50–4.10)   | 5.72% (4.83–6.50)     |
|          | 2.50 bp (1–4)       | 19.56 bp (16–27)    | 35.64 bp (25–40)      |
| *L. virescens* | 0.87% (0.00–2.01)  | 4.66% (3.35–6.89)   | 7.42% (5.91–9.17)     |
|          | 3.52 bp (0–8)       | 22.64 bp (16–31)    | 36.05 bp (29–44)      |
| **nad1** |                       |                       |                        |
| *L. bergmanni* | 0.21% (0.00–0.70)  | 1.70% (0.67–3.10)   | 7.50% (6.19–8.39)     |
|          | 1.22 bp (0–4)       | 9.92 bp (2–17)      | 43.16 bp (23–49)      |
| *L. hydiana* | 0.63% (0.00–2.36)  | 0.63% (0.00–2.36)   | 7.43% (5.34–8.86)     |
|          | 3.73 bp (0–15)      | 3.73 bp (0–15)      | 47.68 bp (16–61)      |
| *L. radiata* | 0.60% (0.00–1.66)  | 6.76% (5.37–8.66)   | 4.91% (3.43–5.71)     |
|          | 4.30 bp (0–12)      | 41.49 bp (21–61)    | 32.52 bp (13–39)      |
| *L. siliquoidea* | 0.31% (0.00–0.96) | 7.43% (5.34–8.86)   | 0.31% (0.00–0.96)     |
|          | 2.06 bp (0–7)       | 47.68 bp (16–61)    | 2.06 bp (0–7)         |
| *L. straminea* | 11.70% (11.70–11.70)| 6.95% (1.26–12.30) | 10.12% (7.13–12.39)   |
|          | 57 bp (57 bp)       | 38.17 bp (3–66)     | 56.12 bp (25–72 bp)   |
| *L. virescens* | n/a                 | 5.62% (4.17–6.76)   | 5.55% (5.10–6.24)     |
|          | n/a                 | 37.42 bp (16–42)    | 40.43 bp (22–44)      |

| Illinois intraspecific | Topotype *hydiana* | Other conspecific specimens |
|------------------------|-------------------|-----------------------------|
| *L. hydiana* Illinois  | 0.16% (0.00–1.52) | 0.20% (0.00–1.23)          |
|                       | 1.06 bp (0–10)    | 1.30 bp (0–8)              |
| *L. siliquoidea* Illinois | 0.01% (0.00–0.15) | n/a                        |
|                       | 0.42 bp (0–4)     | n/a                        |

| **nad1** |                       |                       |                        |
| *L. bergmanni* | 0.21% (0.00–0.70)  | 1.70% (0.67–3.10)   | 7.50% (6.19–8.39)     |
|          | 1.22 bp (0–4)       | 9.92 bp (2–17)      | 43.16 bp (23–49)      |
| *L. hydiana* | 0.63% (0.00–2.36)  | 0.63% (0.00–2.36)   | 7.43% (5.34–8.86)     |
|          | 3.73 bp (0–15)      | 3.73 bp (0–15)      | 47.68 bp (16–61)      |
| *L. radiata* | 0.60% (0.00–1.66)  | 6.76% (5.37–8.66)   | 4.91% (3.43–5.71)     |
|          | 4.30 bp (0–12)      | 41.49 bp (21–61)    | 32.52 bp (13–39)      |
| *L. siliquoidea* | 0.31% (0.00–0.96) | 7.43% (5.34–8.86)   | 0.31% (0.00–0.96)     |
|          | 2.06 bp (0–7)       | 47.68 bp (16–61)    | 2.06 bp (0–7)         |
| *L. straminea* | 11.70% (11.70–11.70)| 6.95% (1.26–12.30) | 10.12% (7.13–12.39)   |
|          | 57 bp (57 bp)       | 38.17 bp (3–66)     | 56.12 bp (25–72 bp)   |
| *L. virescens* | n/a                 | 5.62% (4.17–6.76)   | 5.55% (5.10–6.24)     |
|          | n/a                 | 37.42 bp (16–42)    | 40.43 bp (22–44)      |

| Illinois intraspecific | Topotype *hydiana* | Other conspecific specimens |
|------------------------|-------------------|-----------------------------|
| *L. hydiana* Illinois  | 0.12% (0.00–1.45) | 0.20% (0.00–1.45)          |
|                       | 0.98 bp (0–11)    | 1.63 bp (0–11)             |
| *L. siliquoidea* Illinois | 0.20% (0.00–0.84) | n/a                        |
|                       | 1.65 bp (0–7)     | n/a                        |

n/a = not applicable (either only a single sequence was available or irrelevant [*Lampsilis siliquoidea* were not compared with topotypic *Lampsilis hydiana* separately from other *hydiana*]). Number of base-pair differences is affected by including short published sequences.
Figure 5. Haplotype network for *cox1* data. Numbers are the haplotype number (Appendices 1, 2). Bars on connecting lines indicate the number of base-pair differences between specimens; size of circles indicates the number of individuals with that haplotype.
Figure 6. Haplotype network for *nad1* data. Numbers are the haplotype number (Appendices 1, 2). Bars on connecting lines indicate the number of base-pair differences between specimens; size of circles indicates the number of individuals with that haplotype.
Figure 7. Locations of all shell records observed for *Lampsilis hydiana* and *Lampsilis siliquoidea* from Illinois in the INHS Mollusk Collection, Champaign, Illinois (gray closed circles), with genetic confirmation of *L. hydiana* (green triangles) and *L. siliquoidea* (black squares) plotted within each watershed, with pertinent rivers labeled. Watershed shading indicates species assignments based on observed external shell morphology prior to genetic analysis; green shading = shell characters match *L. hydiana* and yellow shading = shell characters match *L. siliquoidea*. 
small sample size limits our understanding of the overall extent of *L. hydiana* in Illinois. Likewise, mitochondrial introgression, selective pressures, or incomplete lineage sorting (Doucet-Beaupré et al. 2012; Chong et al. 2016) could have produced anomalous genetic patterns. Additional nuclear molecular markers and a more detailed morphometric analysis of these populations may provide a clearer picture of relationships of *Lampsilis* populations in Illinois (Graf and Cummings 2006; Bogan and Roe 2008; Chong et al. 2016).

Our results suggest that *L. siliquoidea* and *L. hydiana* are closely related to each other but are not sister taxa. The sister taxon relationship between *L. siliquoidea* and *L. radiata* fits with previous classifications, as *L. siliquoidea* has been treated as a subspecies of *L. radiata* (Watters et al. 2009). Relationships between other members of the *siliquoidea* clade have not been discussed in detail, particularly as *L. siemani* and *L. bergmanni* were described very recently. However, a relationship between *L. straminea*, *L. bergmanni*, and *L. hydiana* would not be surprising on biogeographic grounds, as their ranges adjoin each other.

Our discovery of both *L. hydiana* and *L. siliquoidea* in Illinois highlights the possibility of overlooked diversity elsewhere. Previous studies found some specimens identified as *L. hydiana* from the Arkansas and Red River systems in Arkansas were genetically distinct from topotypic *L. hydiana* (Turner et al. 2000; Lewter et al. 2003; Harris et al. 2004). A *cox1* sequence from one of those populations (Chapman et al. 2008; GenBank accession number EF033270) was divergent from true *L. hydiana* (Keogh and Simons 2019 and present analyses). Similarly, sequences in GenBank identified as *L. powelli* (from Breton et al. 2011 and Robicheau et al. 2018; GenBank accession numbers HM849075 and HM849218) matched topotypic *L. hydiana* (Walters et al. 2021 and present analyses). However, Harris et al. (2004) and Walters et al. (2021) found their sequences for *L. powelli* were closest to *L. siliquoidea*. *Lampsilis straminea* is reported to range from eastern Louisiana to central Florida, but data for *cox1* separated specimens from the Escambia drainage versus those from the Mobile basin; no other populations have been analyzed genetically. Thus, further analyses of the *siliquoidea* clade are likely to reveal additional new records. The recent descriptions of *L. siemani* and *L. bergmanni* highlight the possibility of additional undescribed or incorrectly synonymized species in this group (Inoue et al. 2020; Keogh and Simons 2020).

Our analysis provides additional support showing that the *siliquoidea* clade is one of several distinct groups currently assigned to the genus *Lampsilis*, even though species in this clade are morphologically and genetically distinct from the type of the genus, Pocketbook *Lampsilis ovata* (Say, 1817). Other species seem to be genetically divergent from both the *siliquoidea* clade and from type *Lampsilis*, including Texas Fatmucket *Lampsilis bracteata* (Gould, 1855) (Harris et al. 2004; Porto-Hannes et al. 2019; Inoue et al. 2020), the cryptic *Lampsilis* sp. of McCartney et al. (2016), and the clade of Northern Brokenray *Lampsilis brittsi* Simpson, 1900, Arkan-

sas Brokenray *Lampsilis reeveiana* (Lea, 1852), and Speckled Pocketbook *Lampsilis streckerii* Frierson, 1927 (Harris et al. 2004). One other species recognized in *Lampsilis*, Neosho Mucket *Lampsilis rafinesqueana* Frierson, 1927, has not yet been analyzed genetically but has an unusual combination of anatomical and shell features (Harris et al. 2004). As Keogh and Simons (2019) pointed out, a thorough analysis of *Lampsilini* will be necessary to determine the correct placement of these taxa.

Accurate species delineation is critical to developing sound conservation strategies for freshwater mussels, particularly because many species of conservation concern are managed or closely monitored at the state level. At press time, three *Lampsilis* species are endangered in Illinois: *L. abrupta* and *L. higginsii* are federally protected, while Wavyrayed Lamp-mussel *Lampsilis fasciola* Rafinesque, 1820 is listed only at the state level. Other common, widespread *Lampsilis* species, such as Plain Pocketbook *Lampsilis cardium* (Rafinesque, 1820) and *L. siliquoidea*, are often used by local and state authorities for propagation and augmentation following habitat restoration efforts. Our analysis emphasizes the need for managers to follow best practices during augmentation and reintroduction activities to avoid cross-basin contamination, as hidden diversity may be present even in common, presumably well-understood species (McMurray and Roe 2017; Inoue et al. 2020).

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Appendix 1. Illinois specimens and sequences used in analysis, haplotype number for reference to Figures 4–6, GenBank accession number, INHS catalog number, approximate waterbody location, and our preliminary putative identification based on external shell characteristics.

| Species      | Gene | Haplotype | GenBank accession no. | INHS catalog no. | Waterbody                  | Putative species |
|--------------|------|-----------|-----------------------|------------------|----------------------------|-----------------|
| *L. hydiana* | nad1 | 22        | *MT537714             | INHS 35065-1     | Cache River                | *L. hydiana*    |
| *L. hydiana* | cox1 | 5         | *MT537719             | INHS 35065-3     | Cache River                | *L. hydiana*    |
| *L. hydiana* | nad1 | 23        | *MT537715             | INHS 35065-3     | Cache River                | *L. hydiana*    |
| *L. hydiana* | nad1 | 24        | *MT537716             | INHS 35065-4     | Cache River                | *L. hydiana*    |
| *L. hydiana* | cox1 | 5         | *MT537720             | INHS 35065-4     | Cache River                | *L. hydiana*    |
| *L. hydiana* | cox1 | 21        | *MT537721             | INHS 39742-1     | East Fork Kaskaskia River  | *L. hydiana*    |
| *L. hydiana* | cox1 | 3         | *MT537717             | INHS 39742-2     | East Fork Kaskaskia River  | *L. hydiana*    |
| *L. hydiana* | nad1 | 20        | *MT537705             | INHS 39742-4     | East Fork Kaskaskia River  | *L. hydiana*    |
| *L. hydiana* | cox1 | 3         | *MT537718             | INHS 39742-5     | East Fork Kaskaskia River  | *L. hydiana*    |
| *L. hydiana* | nad1 | 21        | *MT537706             | INHS 39742-2     | East Fork Kaskaskia River  | *L. hydiana*    |
| *L. hydiana* | cox1 | 5         | *MH560721             | INHS 86789-1     | Big Grande Pierre Creek    | *L. siliquoidea* |
| *L. hydiana* | nad1 | 25        | *MH560728             | INHS 86789-1     | Big Grande Pierre Creek    | *L. siliquoidea* |
| *L. hydiana* | cox1 | 5         | *MH560723             | INHS 86789-2     | Big Grande Pierre Creek    | *L. siliquoidea* |
| *L. hydiana* | nad1 | 20        | *MH588329             | INHS 45495-1     | Big Muddy River            | *L. hydiana*    |
| *L. hydiana* | cox1 | 5         | *MH560732             | INHS 45495-2     | Big Muddy River            | *L. hydiana*    |
| *L. hydiana* | nad1 | 6         | *MH588342             | INHS 45495-2     | Big Muddy River            | *L. hydiana*    |
| *L. hydiana* | cox1 | 5         | *MH560716             | INHS 45495-3     | Big Muddy River            | *L. hydiana*    |
| *L. hydiana* | nad1 | 20        | *MH588325             | INHS 45495-3     | Big Muddy River            | *L. hydiana*    |
| *L. hydiana* | nad1 | 20        | *MH588326             | INHS 45495-4     | Big Muddy River            | *L. hydiana*    |
| *L. hydiana* | cox1 | 5         | *MH560717             | INHS 45495-5     | Big Muddy River            | *L. hydiana*    |
| *L. hydiana* | nad1 | 6         | *MH588327             | INHS 45495-5     | Big Muddy River            | *L. hydiana*    |
| *L. hydiana* | cox1 | 4         | *MH560739             | INHS 45455-1     | Bradshaw Creek             | *L. hydiana*    |
| *L. hydiana* | nad1 | 8         | *MH588344             | INHS 45455-1     | Bradshaw Creek             | *L. hydiana*    |
| *L. hydiana* | cox1 | 5         | *MH560715             | INHS 45455-2     | Bradshaw Creek             | *L. hydiana*    |
| *L. hydiana* | nad1 | 6         | *MH588345             | INHS 45455-2     | Bradshaw Creek             | *L. hydiana*    |
| *L. hydiana* | cox1 | 5         | *MH588346             | INHS 45455-3     | Bradshaw Creek             | *L. hydiana*    |
| *L. hydiana* | cox1 | 5         | *MH560740             | INHS 45460-1     | Brush Creek                | *L. hydiana*    |
| *L. hydiana* | nad1 | 20        | *MH588347             | INHS 45460-1     | Brush Creek                | *L. hydiana*    |
| *L. hydiana* | cox1 | 5         | *MH560741             | INHS 45460-2     | Brush Creek                | *L. hydiana*    |
| *L. hydiana* | nad1 | 20        | *MH588348             | INHS 45460-3     | Brush Creek                | *L. hydiana*    |
| *L. hydiana* | cox1 | 5         | *MH560742             | INHS 45460-3     | Brush Creek                | *L. hydiana*    |
| *L. hydiana* | nad1 | 20        | *MH588349             | INHS 45460-3     | Brush Creek                | *L. hydiana*    |
| *L. hydiana* | cox1 | 5         | *MH560743             | INHS 45460-4     | Brush Creek                | *L. hydiana*    |
| *L. hydiana* | nad1 | 20        | *MH588350             | INHS 45460-4     | Brush Creek                | *L. hydiana*    |
| *L. hydiana* | cox1 | 5         | *MH560744             | INHS 45460-5     | Brush Creek                | *L. hydiana*    |
| *L. hydiana* | nad1 | 20        | *MH588351             | INHS 45460-5     | Brush Creek                | *L. hydiana*    |
| *L. hydiana* | cox1 | 5         | *MH560751             | INHS 45482-1     | Cypress Creek              | *L. hydiana*    |
| *L. hydiana* | nad1 | 7         | *MH588361             | INHS 45482-1     | Cypress Creek              | *L. hydiana*    |
| *L. hydiana* | cox1 | 25        | *MH560752             | INHS 45482-2     | Cypress Creek              | *L. hydiana*    |
| *L. hydiana* | nad1 | 29        | *MH588362             | INHS 45482-2     | Cypress Creek              | *L. hydiana*    |
| *L. hydiana* | cox1 | 5         | *MH560753             | INHS 45490-1     | East Fork Kaskaskia River  | *L. hydiana*    |
| *L. hydiana* | nad1 | 20        | *MH588363             | INHS 45490-1     | East Fork Kaskaskia River  | *L. hydiana*    |
| *L. hydiana* | cox1 | 3         | *MH560754             | INHS 45490-2     | East Fork Kaskaskia River  | *L. hydiana*    |
| *L. hydiana* | nad1 | 20        | *MH588364             | INHS 45490-2     | East Fork Kaskaskia River  | *L. hydiana*    |
| *L. hydiana* | cox1 | 5         | *MH560755             | INHS 45490-3     | East Fork Kaskaskia River  | *L. hydiana*    |
| *L. hydiana* | nad1 | 20        | *MH588365             | INHS 45490-3     | East Fork Kaskaskia River  | *L. hydiana*    |
| *L. hydiana* | nad1 | 20        | *MH588366             | INHS 45490-4     | East Fork Kaskaskia River  | *L. hydiana*    |
| *L. hydiana* | nad1 | 20        | *MH588367             | INHS 45490-5     | East Fork Kaskaskia River  | *L. hydiana*    |
| *L. hydiana* | cox1 | 5         | *MH560713             | INHS 45491-1     | Elm River                  | *L. hydiana*    |
| Species         | Gene   | Haplotype | GenBank accession no. | INHS catalog no. | Waterbody                  | Putative species       |
|-----------------|--------|-----------|-----------------------|------------------|-----------------------------|------------------------|
| *L. hydiana*    | nad1   | 20        | *MH588322             | INHS 45491-1     | Elm River                  | *L. hydiana*           |
| *L. hydiana*    | cox1   | 1         | *MH560714             | INHS 45491-2     | Elm River                  | *L. hydiana*           |
| *L. hydiana*    | nad1   | 20        | *MH588323             | INHS 45491-2     | Elm River                  | *L. hydiana*           |
| *L. hydiana*    | cox1   | 5         | *MH560745             | INHS 45462-2     | Horse Creek                | *L. hydiana*           |
| *L. hydiana*    | nad1   | 20        | *MH588353             | INHS 45462-2     | Horse Creek                | *L. hydiana*           |
| *L. hydiana*    | cox1   | 5         | *MH560746             | INHS 45462-3     | Horse Creek                | *L. hydiana*           |
| *L. hydiana*    | nad1   | 20        | *MH588354             | INHS 45462-3     | Horse Creek                | *L. hydiana*           |
| *L. hydiana*    | cox1   | 24        | *MH560747             | INHS 45462-4     | Horse Creek                | *L. hydiana*           |
| *L. hydiana*    | nad1   | 20        | *MH588355             | INHS 45462-4     | Horse Creek                | *L. hydiana*           |
| *L. hydiana*    | cox1   | 5         | *MH560735             | INHS 45449-1     | Lake Fork Kaskaskia River  | *L. hydiana*           |
| *L. hydiana*    | nad1   | 20        | *MH588339             | INHS 45449-1     | Lake Fork Kaskaskia River  | *L. hydiana*           |
| *L. hydiana*    | cox1   | 1         | *MH560736             | INHS 45449-3     | Lake Fork Kaskaskia River  | *L. hydiana*           |
| *L. hydiana*    | nad1   | 8         | *MH588340             | INHS 45449-3     | Lake Fork Kaskaskia River  | *L. hydiana*           |
| *L. hydiana*    | nad1   | 20        | *MH588341             | INHS 45449-4     | Lake Fork Kaskaskia River  | *L. hydiana*           |
| *L. hydiana*    | cox1   | 5         | *MH560724             | INHS 86787-1     | Lusk Creek                 | *L. siliquoidea*       |
| *L. hydiana*    | nad1   | 30        | *MH588376             | INHS 86787-1     | Lusk Creek                 | *L. siliquoidea*       |
| *L. hydiana*    | cox1   | 5         | *MH560725             | INHS 86787-2     | Lusk Creek                 | *L. siliquoidea*       |
| *L. hydiana*    | nad1   | 20        | *MH588330             | INHS 86787-2     | Lusk Creek                 | *L. siliquoidea*       |
| *L. hydiana*    | cox1   | 5         | *MH560727             | INHS 86787-3     | Lusk Creek                 | *L. siliquoidea*       |
| *L. hydiana*    | nad1   | 7         | *MH588331             | INHS 86787-3     | Lusk Creek                 | *L. siliquoidea*       |
| *L. hydiana*    | cox1   | 5         | *MH560728             | INHS 86787-4     | Lusk Creek                 | *L. siliquoidea*       |
| *L. hydiana*    | nad1   | 26        | *MH588332             | INHS 86787-4     | Lusk Creek                 | *L. siliquoidea*       |
| *L. hydiana*    | cox1   | 5         | *MH560729             | INHS 86787-5     | Lusk Creek                 | *L. siliquoidea*       |
| *L. hydiana*    | nad1   | 7         | *MH588333             | INHS 86787-5     | Lusk Creek                 | *L. siliquoidea*       |
| *L. hydiana*    | cox1   | 5         | *MH560730             | INHS 86787-6     | Lusk Creek                 | *L. siliquoidea*       |
| *L. hydiana*    | nad1   | 20        | *MH588334             | INHS 86787-6     | Lusk Creek                 | *L. siliquoidea*       |
| *L. hydiana*    | cox1   | 5         | *MH560731             | INHS 86787-7     | Lusk Creek                 | *L. siliquoidea*       |
| *L. hydiana*    | nad1   | 20        | *MH588335             | INHS 86787-7     | Lusk Creek                 | *L. siliquoidea*       |
| *L. hydiana*    | cox1   | 5         | *MH560718             | INHS 22361       | Muddy Creek                | *L. hydiana*           |
| *L. hydiana*    | cox1   | 5         | *MH560719             | INHS 35459       | Salt Creek                 | *L. siliquoidea*       |
| *L. hydiana*    | cox1   | 5         | *MH560748             | INHS 45463-1     | Skillet Fork               | *L. hydiana*           |
| *L. hydiana*    | nad1   | 20        | *MH588356             | INHS 45463-1     | Skillet Fork               | *L. hydiana*           |
| *L. hydiana*    | nad1   | 20        | *MH588357             | INHS 45463-2     | Skillet Fork               | *L. hydiana*           |
| *L. hydiana*    | cox1   | 5         | *MH560749             | INHS 45463-3     | Skillet Fork               | *L. hydiana*           |
| *L. hydiana*    | nad1   | 20        | *MH588358             | INHS 45463-3     | Skillet Fork               | *L. hydiana*           |
| *L. hydiana*    | cox1   | 2         | *MH560750             | INHS 45463-5     | Skillet Fork               | *L. hydiana*           |
| *L. hydiana*    | nad1   | 28        | *MH588360             | INHS 45463-5     | Skillet Fork               | *L. hydiana*           |
| *L. hydiana*    | cox1   | 5         | *MH560737             | INHS 45453-3     | Twomile Slough             | *L. hydiana*           |
| *L. hydiana*    | nad1   | 20        | *MH588342             | INHS 45453-3     | Twomile Slough             | *L. hydiana*           |
| *L. hydiana*    | cox1   | 5         | *MH560738             | INHS 45453-4     | Twomile Slough             | *L. hydiana*           |
| *L. hydiana*    | nad1   | 20        | *MH588343             | INHS 45453-4     | Twomile Slough             | *L. hydiana*           |
| *L. hydiana*    | cox1   | 23        | *MH560734             | INHS 45443       | West Okaw River            | *L. hydiana*           |
| *L. hydiana*    | cox1   | 27        | *MH588338             | INHS 45443       | West Okaw River            | *L. hydiana*           |
| *L. hydiana*    | cox1   | 3         | *MH560733             | INHS 45447       | West Okaw River            | *L. hydiana*           |
| *L. hydiana*    | nad1   | 20        | *MH588337             | INHS 45447       | West Okaw River            | *L. hydiana*           |
| *L. siliquoidea*| nad1   | 6         | *MT537712             | INHS 35786-1     | Little Vermilion River     | *L. siliquoidea*       |
| *L. siliquoidea*| nad1   | 6         | *MT537713             | INHS 35786-4     | Little Vermilion River     | *L. siliquoidea*       |
| *L. siliquoidea*| cox1   | 5         | *MT537707             | INHS 41996-1     | Mackinaw River             | *L. siliquoidea*       |
| *L. siliquoidea*| cox1   | 5         | *MT537722             | INHS 41996-2     | Mackinaw River             | *L. siliquoidea*       |
| Species       | Gene   | Haplotype | GenBank accession no. | INHS catalog no. | Waterbody             | Putative species |
|---------------|--------|-----------|-----------------------|------------------|-----------------------|-----------------|
| *L. siliquoidea* | nad1   | 5         | *MT537708             | INHS 41996-2     | Mackinaw River        | *L. siliquoidea* |
| *L. siliquoidea* | cox1   | 5         | *MT537723             | INHS 41996-3     | Mackinaw River        | *L. siliquoidea* |
| *L. siliquoidea* | nad1   | 5         | *MT537709             | INHS 41996-3     | Mackinaw River        | *L. siliquoidea* |
| *L. siliquoidea* | cox1   | 5         | *MT537724             | INHS 41996-4     | Mackinaw River        | *L. siliquoidea* |
| *L. siliquoidea* | nad1   | 7         | *MT537710             | INHS 41996-4     | Mackinaw River        | *L. siliquoidea* |
| *L. siliquoidea* | cox1   | 7         | *MT537725             | INHS 41996-5     | Mackinaw River        | *L. siliquoidea* |
| *L. siliquoidea* | nad1   | 5         | *MT537711             | INHS 41996-5     | Mackinaw River        | *L. siliquoidea* |
| *L. siliquoidea* | cox1   | 5         | *MH588388             | INHS 35558       | Big Ditch             | *L. siliquoidea* |
| *L. siliquoidea* | nad1   | 2         | *MH588368             | INHS 45613-1     | Big Grande Pierre Creek | *L. siliquoidea* |
| *L. siliquoidea* | cox1   | 5         | *MH588375             | INHS 45613-10    | Big Grande Pierre Creek | *L. siliquoidea* |
| *L. siliquoidea* | nad1   | 2         | *MH588369             | INHS 45613-3     | Big Grande Pierre Creek | *L. siliquoidea* |
| *L. siliquoidea* | cox1   | 5         | *MH586075             | INHS 45613-4     | Big Grande Pierre Creek | *L. siliquoidea* |
| *L. siliquoidea* | nad1   | 2         | *MH588370             | INHS 45613-4     | Big Grande Pierre Creek | *L. siliquoidea* |
| *L. siliquoidea* | cox1   | 5         | *MH586079             | INHS 45613-5     | Big Grande Pierre Creek | *L. siliquoidea* |
| *L. siliquoidea* | nad1   | 2         | *MH588371             | INHS 45613-5     | Big Grande Pierre Creek | *L. siliquoidea* |
| *L. siliquoidea* | cox1   | 5         | *MH586076             | INHS 45613-6     | Big Grande Pierre Creek | *L. siliquoidea* |
| *L. siliquoidea* | nad1   | 2         | *MH588372             | INHS 45613-6     | Big Grande Pierre Creek | *L. siliquoidea* |
| *L. siliquoidea* | cox1   | 5         | *MH586071             | INHS 45613-8     | Big Grande Pierre Creek | *L. siliquoidea* |
| *L. siliquoidea* | nad1   | 2         | *MH588373             | INHS 45613-8     | Big Grande Pierre Creek | *L. siliquoidea* |
| *L. siliquoidea* | cox1   | 5         | *MH586072             | INHS 45613-9     | Big Grande Pierre Creek | *L. siliquoidea* |
| *L. siliquoidea* | nad1   | 2         | *MH588374             | INHS 45613-9     | Big Grande Pierre Creek | *L. siliquoidea* |
| *L. siliquoidea* | cox1   | 5         | *MH588376             | INHS 86788       | Horse Creek           | *L. hydiana*    |
| *L. siliquoidea* | nad1   | 2         | *MH586079             | INHS 45615-10    | Lusk Creek            | *L. siliquoidea* |
| *L. siliquoidea* | cox1   | 5         | *MH586070             | INHS 45615-10    | Lusk Creek            | *L. siliquoidea* |
| *L. siliquoidea* | nad1   | 2         | *MH588382             | INHS 45615-10    | Lusk Creek            | *L. siliquoidea* |
| *L. siliquoidea* | cox1   | 5         | *MH586077             | INHS 45615-12    | Lusk Creek            | *L. siliquoidea* |
| *L. siliquoidea* | nad1   | 2         | *MH588383             | INHS 45615-12    | Lusk Creek            | *L. siliquoidea* |
| *L. siliquoidea* | cox1   | 5         | *MH586071             | INHS 45615-13    | Lusk Creek            | *L. siliquoidea* |
| *L. siliquoidea* | nad1   | 2         | *MH588384             | INHS 45615-13    | Lusk Creek            | *L. siliquoidea* |
| *L. siliquoidea* | cox1   | 5         | *MH586072             | INHS 45615-18    | Lusk Creek            | *L. siliquoidea* |
| *L. siliquoidea* | nad1   | 2         | *MH588385             | INHS 45615-18    | Lusk Creek            | *L. siliquoidea* |
| *L. siliquoidea* | cox1   | 5         | *MH586073             | INHS 45615-19    | Lusk Creek            | *L. siliquoidea* |
| *L. siliquoidea* | nad1   | 2         | *MH586074             | INHS 45615-2     | Lusk Creek            | *L. siliquoidea* |
| *L. siliquoidea* | cox1   | 5         | *MH589377             | INHS 45615-2     | Lusk Creek            | *L. siliquoidea* |
| *L. siliquoidea* | nad1   | 2         | *MH586075             | INHS 45615-3     | Lusk Creek            | *L. siliquoidea* |
| *L. siliquoidea* | cox1   | 5         | *MH586076             | INHS 45615-3     | Lusk Creek            | *L. siliquoidea* |
| *L. siliquoidea* | nad1   | 2         | *MH588378             | INHS 45615-4     | Lusk Creek            | *L. siliquoidea* |
| *L. siliquoidea* | cox1   | 5         | *MH586076             | INHS 45615-4     | Lusk Creek            | *L. siliquoidea* |
| *L. siliquoidea* | nad1   | 2         | *MH588380             | INHS 45615-6     | Lusk Creek            | *L. siliquoidea* |
| *L. siliquoidea* | cox1   | 5         | *MH586078             | INHS 45615-9     | Lusk Creek            | *L. siliquoidea* |
| *L. siliquoidea* | nad1   | 2         | *MH588381             | INHS 45615-9     | Lusk Creek            | *L. siliquoidea* |
| *L. siliquoidea* | cox1   | 5         | *MH586074             | INHS 45471-1     | Rose Creek            | *L. siliquoidea* |
| *L. siliquoidea* | nad1   | 2         | *MH588387             | INHS 45471-1     | Rose Creek            | *L. siliquoidea* |

*Newly generated sequences.
Appendix 2. Additional sequences used in analysis, with haplotype reference number (see Figs. 4–6), GenBank accession number, specimen identification, and approximate waterbody location.

| Species     | Gene     | Haplotype | GenBank accession no. | Specimen ID | Waterbody                             | Reference         |
|-------------|----------|-----------|-----------------------|-------------|---------------------------------------|-------------------|
| L. abrupta  | coxl     |           | *MH560776             | UAUC3531    | Tennessee River, Diamond Island       | New               |
| L. bergmanni| nadl     | 2         | MK672463              | UGUA01      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 2         | MK672464              | UGUA02      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 2         | MK672465              | UGUA03      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 2         | MK672466              | UGUA04      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 2         | MK672467              | UGUA05      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 2         | MK672468              | UGUA07      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 2         | MK672469              | UGUA08      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 2         | MK672470              | UGUA09      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 2         | MK672471              | UGUA10      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 2         | MK672472              | UGUA11      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 2         | MK672473              | UGUA12      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 2         | MK672474              | UGUA13      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 2         | MK672475              | UGUA14      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 2         | MK672476              | UGUA15      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 1         | MK672477              | UGUA16      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 1         | MK672478              | UGUA17      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 2         | MK672479              | UGUA18      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 1         | MK672480              | UGUA19      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 1         | MK672481              | UGUA20      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 3         | MK672482              | UGUA21      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 2         | MK672483              | UGUA22      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 1         | MK672484              | UGUA23      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 2         | MK672485              | UGUA24      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 1         | MK672486              | UGUA25      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 2         | MK672487              | UGUA26      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 3         | MK672488              | UGUA27      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 2         | MK672489              | UGUA28      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 1         | MK672490              | UGUA29      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 3         | MK672491              | UGUA30      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 1         | MK672492              | UGUA31      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 2         | MK672493              | UGUA32      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 2         | MK672494              | UGUA33      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 3         | MK672495              | UGUA34      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 1         | MK672496              | UGUA35      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 2         | MK672497              | UGUA36      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 1         | MK672498              | UGUA37      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 3         | MK672499              | UGUA39      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| nadl     | 3         | MK672500              | UGUA40      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| coxl     | 1         | MK672718              | UGUA01      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| coxl     | 1         | MK672719              | UGUA02      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| coxl     | 1         | MK672720              | UGUA03      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| coxl     | 1         | MK672721              | UGUA04      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| coxl     | 1         | MK672722              | UGUA05      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| coxl     | 1         | MK672723              | UGUA07      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| coxl     | 1         | MK672724              | UGUA08      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| coxl     | 1         | MK672725              | UGUA09      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| coxl     | 1         | MK672726              | UGUA10      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| coxl     | 1         | MK672727              | UGUA11      | Guadalupe River drainage              | Inoue et al. 2020 |
| L. bergmanni| coxl     | 1         | MK672728              | UGUA12      | Guadalupe River drainage              | Inoue et al. 2020 |
| Species          | Gene | Haplotype | GenBank accession no. | Specimen ID | Waterbody Reference                  |
|------------------|------|-----------|-----------------------|-------------|--------------------------------------|
| L. bergmanni     | cox1 | 1         | MK672729              | UGUA13      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 1         | MK672730              | UGUA14      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 1         | MK672731              | UGUA15      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 1         | MK672732              | UGUA16      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 1         | MK672733              | UGUA17      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 1         | MK672734              | UGUA18      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 1         | MK672735              | UGUA19      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 1         | MK672736              | UGUA20      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 2         | MK672737              | UGUA21      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 1         | MK672738              | UGUA22      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 1         | MK672739              | UGUA23      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 1         | MK672740              | UGUA24      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 1         | MK672741              | UGUA25      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 1         | MK672742              | UGUA26      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 2         | MK672743              | UGUA27      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 1         | MK672744              | UGUA28      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 1         | MK672745              | UGUA29      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 2         | MK672746              | UGUA30      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 1         | MK672747              | UGUA31      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 1         | MK672748              | UGUA32      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 1         | MK672749              | UGUA33      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 2         | MK672750              | UGUA34      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 1         | MK672751              | UGUA35      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 1         | MK672752              | UGUA36      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 1         | MK672753              | UGUA37      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 2         | MK672754              | UGUA39      | Guadalupe River drainage             |
| L. bergmanni     | cox1 | 2         | MK672755              | UGUA40      | Guadalupe River drainage             |
| L. higginsii     | nad1 |           | EF213061              |             | Upper Mississippi drainage           |
| L. higginsii     | cox1 |           | GU085287              | 1           | Upper Mississippi drainage           |
| L. “hydiana”     | cox1 | 18        | EF033270              | H1230       | Coosat River, Red River, Arkansas    |
| L. hydiana       | cox1 | 17        | *MH560720             | INHS 87783-2| Bœuf River                           |
| L. hydiana       | nad1 | 31        | *MH588389             | INHS 87783-1| Bœuf River                           |
| L. hydiana       | nad1 | 8         | *MH588390             | INHS 87783-2| Bœuf River                           |
| L. hydiana       | nad1 | 8         | *MH588391             | INHS 87783-3| Bœuf River                           |
| L. hydiana       | nad1 | 8         | *MH588392             | INHS 87783-4| Bœuf River                           |
| L. hydiana       | nad1 | 12        | MK672437              | BRA01       | Brazos River drainage                |
| L. hydiana       | nad1 | 9         | MK672438              | BRA02       | Brazos River drainage                |
| L. hydiana       | nad1 | 12        | MK672439              | BRA03       | Brazos River drainage                |
| L. hydiana       | nad1 | 9         | MK672440              | BRA04       | Brazos River drainage                |
| L. hydiana       | nad1 | 12        | MK672441              | BRA05       | Brazos River drainage                |
| L. hydiana       | nad1 | 12        | MK672442              | BRA06       | Brazos River drainage                |
| L. hydiana       | nad1 | 12        | MK672443              | BRA07       | Brazos River drainage                |
| L. hydiana       | nad1 | 9         | MK672444              | BRA08       | Brazos River drainage                |
| L. hydiana       | nad1 | 12        | MK672445              | BRA09       | Brazos River drainage                |
| L. hydiana       | nad1 | 46        | MK672446              | BRA10       | Brazos River drainage                |
| L. hydiana       | nad1 | 9         | MK672447              | BRA11       | Brazos River drainage                |
| L. hydiana       | nad1 | 12        | MK672448              | BRA12       | Brazos River drainage                |
| L. hydiana       | nad1 | 12        | MK672449              | BRA13       | Brazos River drainage                |
| L. hydiana       | nad1 | 12        | MK672450              | BRA14       | Brazos River drainage                |

**LAMPSILIS HYDIANA IN ILLINOIS**

Appendix 2, continued.
| Species    | Gene | Haplotype | GenBank accession no. | Specimen ID | Waterbody Reference |
|------------|------|-----------|-----------------------|-------------|---------------------|
| *L. hydiana* | *nad1* | 12 | MK672451 | BRA15 | Brazos River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 11 | MK672685 | BRA01 | Brazos River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 8  | MK672686 | BRA02 | Brazos River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 6  | MK672687 | BRA03 | Brazos River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 11 | MK672688 | BRA04 | Brazos River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 7  | MK672689 | BRA05 | Brazos River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 8  | MK672690 | BRA06 | Brazos River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 11 | MK672691 | BRA07 | Brazos River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 6  | MK672692 | BRA08 | Brazos River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 8  | MK672693 | BRA09 | Brazos River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 11 | MK672694 | BRA10 | Brazos River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 11 | MK672695 | BRA11 | Brazos River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 6  | MK672696 | BRA12 | Brazos River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 8  | MK672697 | BRA13 | Brazos River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 11 | MK672698 | BRA14 | Brazos River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 6  | MK672699 | BRA15 | Brazos River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 11 | MK672700 | BRA16 | Brazos River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 11 | MK672701 | BRA17 | Brazos River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 11 | MK672702 | BRA18 | Brazos River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 11 | MK672703 | BRA19 | Brazos River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 11 | MK672704 | BRA20 | Brazos River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 11 | MK672705 | BRA21 | Brazos River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 11 | MK672706 | BRA22 | Brazos River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 37 | MK672388 | CAL01 | Calcasieu River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 11 | MK672389 | CAL02 | Calcasieu River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 38 | MK672390 | CAL08 | Calcasieu River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 10 | MK672391 | CAL09 | Calcasieu River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 11 | MK672392 | CAL15 | Calcasieu River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 11 | MK672393 | CAL16 | Calcasieu River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 11 | MK672394 | CAL19 | Calcasieu River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 11 | MK672395 | CAL20 | Calcasieu River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 33 | MK672611 | CAL01 | Calcasieu River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 10 | MK672612 | CAL02 | Calcasieu River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 10 | MK672613 | CAL08 | Calcasieu River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 10 | MK672614 | CAL09 | Calcasieu River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 10 | MK672615 | CAL15 | Calcasieu River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 10 | MK672616 | CAL16 | Calcasieu River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 10 | MK672617 | CAL19 | Calcasieu River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 10 | MK672618 | CAL20 | Calcasieu River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 15 | MK226685 | 012TS | Guadalupe River drainage | Porto-Hannes et al. 2019 |
| *L. hydiana* | *cox1* | 16 | MK226686 | 013TS | Guadalupe River drainage | Porto-Hannes et al. 2019 |
| *L. hydiana* | *cox1* | 16 | MK226687 | 016TS | Guadalupe River drainage | Porto-Hannes et al. 2019 |
| *L. hydiana* | *nad1* | 2  | MK226704 | 016TS | Guadalupe River drainage | Porto-Hannes et al. 2019 |
| *L. hydiana* | *nad1* | 1  | MK226709 | 012TS | Guadalupe River drainage | Porto-Hannes et al. 2019 |
| *L. hydiana* | *nad1* | 1  | MK672452 | GUA06 | Guadalupe River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 1  | MK672453 | GUA16 | Guadalupe River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 1  | MK672454 | GUA17 | Guadalupe River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 1  | MK672455 | GUA18 | Guadalupe River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 1  | MK672456 | GUA19 | Guadalupe River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 15 | MK672707 | GUA06 | Guadalupe River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 15 | MK672708 | GUA16 | Guadalupe River drainage | Inoue et al. 2020 |
| Species | Gene | Haplotype | GenBank accession no. | Specimen ID | Waterbody | Reference |
|---------|------|-----------|----------------------|-------------|-----------|-----------|
| L. hydiana | coxl | 15 | MK672709 | GUA17 | Guadalupe River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 15 | MK672710 | GUA18 | Guadalupe River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 15 | MK672711 | GUA19 | Guadalupe River drainage | Inoue et al. 2020 |
| L. hydiana | nadl | 3 | MG030352 | | | Marshall et al. 2018 |
| L. hydiana | coxl | 9 | MK226688 | 138TS | Neches River drainage | Porto-Hannes et al. 2019 |
| L. hydiana | coxl | 10 | MK226689 | 159TS | Neches River drainage | Porto-Hannes et al. 2019 |
| L. hydiana | coxl | 10 | MK226690 | 200TS | Neches River drainage | Porto-Hannes et al. 2019 |
| L. hydiana | coxl | 10 | MK226691 | 214TS | Neches River drainage | Porto-Hannes et al. 2019 |
| L. hydiana | nadl | 5 | MK226705 | 214TS | Neches River drainage | Porto-Hannes et al. 2019 |
| L. hydiana | nadl | 47 | MK226706 | 159TS | Neches River drainage | Porto-Hannes et al. 2019 |
| L. hydiana | nadl | 4 | MK226707 | 200TS | Neches River drainage | Porto-Hannes et al. 2019 |
| L. hydiana | nadl | 3 | MK226708 | 138TS | Neches River drainage | Porto-Hannes et al. 2019 |
| L. hydiana | nadl | 3 | MK672411 | NEC10 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | nadl | 5 | MK672412 | NEC11 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | nadl | 18 | MK672413 | NEC12 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | nadl | 17 | MK672414 | NEC13 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | nadl | 40 | MK672415 | NEC14 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | nadl | 10 | MK672416 | NEC15 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | nadl | 5 | MK672417 | NEC16 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | nadl | 12 | MK672418 | NEC17 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | nadl | 5 | MK672419 | NEC18 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | nadl | 5 | MK672420 | NEC19 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | nadl | 42 | MK672421 | NEC20 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | nadl | 5 | MK672422 | NEC21 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | nadl | 43 | MK672423 | NEC22 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | nadl | 44 | MK672424 | NEC23 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | nadl | 3 | MK672425 | NEC24 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 12 | MK672441 | NEC01 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 10 | MK672424 | NEC03 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 10 | MK672424 | NEC06 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 10 | MK672424 | NEC07 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 10 | MK672424 | NEC09 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 9 | MK672446 | NEC10 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 37 | MK672447 | NEC11 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 38 | MK672448 | NEC12 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 12 | MK672449 | NEC13 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 39 | MK672450 | NEC14 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 40 | MK672451 | NEC15 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 10 | MK672452 | NEC16 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 9 | MK672453 | NEC17 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 10 | MK672454 | NEC18 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 10 | MK672455 | NEC19 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 9 | MK672456 | NEC20 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 10 | MK672457 | NEC21 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 9 | MK672458 | NEC22 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 41 | MK672459 | NEC23 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 9 | MK672460 | NEC24 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 42 | MK672461 | NEC25 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 9 | MK672462 | NEC26 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 10 | MK672463 | NEC27 | Neches River drainage | Inoue et al. 2020 |
| L. hydiana | coxl | 10 | MK672464 | NEC29 | Neches River drainage | Inoue et al. 2020 |
| Species  | Gene | Haplotype | GenBank accession no. | Specimen ID | Waterbody Reference |
|----------|------|-----------|----------------------|-------------|---------------------|
| *L. hydiana* | *cox1* | 43 | MK672665 | NEC30 | Neches River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 10 | MK672666 | NEC31 | Neches River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 10 | MK672667 | NEC32 | Neches River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 9 | MK672668 | NEC33 | Neches River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 5 | MK672669 | NEC34 | Neches River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 10 | MK672670 | NEC35 | Neches River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 10 | MK672671 | NEC37 | Neches River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 10 | MK672672 | NEC38 | Neches River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 9 | MK672673 | NEC40 | Neches River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 5 | MK391871 | JFBM22432 1 | Ohio River drainage | Keogh and Simons 2019 |
| *L. hydiana* | *cox1* | 5 | MK391872 | JFBM22432 2 | Ohio River drainage | Keogh and Simons 2019 |
| *L. hydiana* | *cox1* | 5 | MK391873 | JFBM22432 3 | Ohio River drainage | Keogh and Simons 2019 |
| *L. hydiana* | *cox1* | 5 | MK391874 | JFBM22432 4 | Ohio River drainage | Keogh and Simons 2019 |
| *L. hydiana* | *cox1* | 4 | MK391875 | JFBM22432 5 | Ohio River drainage | Keogh and Simons 2019 |
| *L. hydiana* | *cox1* | 5 | MK391876 | JFBM22432 6 | Ohio River drainage | Keogh and Simons 2019 |
| *L. hydiana* | *cox1* | 5 | MK391877 | JFBM22432 7 | Ohio River drainage | Keogh and Simons 2019 |
| *L. hydiana* | *nad1* | 9 | MK672379 | OUA01 | Ouachita River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 13 | MK672380 | OUA02 | Ouachita River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 13 | MK672381 | OUA03 | Ouachita River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 17 | MK672596 | OUA01 | Ouachita River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 11 | MK672597 | OUA02 | Ouachita River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 27 | MK672598 | OUA03 | Ouachita River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 11 | MK672599 | OUA04 IF01 | Ouachita River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 11 | MK672600 | OUA05 IF02 | Ouachita River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 7 | MK672601 | OUA06 VL01 | Ouachita River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 11 | MK672602 | OUA07 VL02 | Ouachita River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 33 | MK672382 | RED04 | Red River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 34 | MK672383 | RED06 | Red River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 35 | MK672384 | RED07 | Red River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 36 | MK672385 | RED08 | Red River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 14 | MK672386 | RED09 | Red River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 14 | MK672387 | RED10 | Red River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 28 | MK672603 | RED02 | Red River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 11 | MK672604 | RED03 | Red River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 29 | MK672605 | RED04 | Red River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 30 | MK672606 | RED06 | Red River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 31 | MK672607 | RED07 | Red River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 32 | MK672608 | RED08 | Red River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 11 | MK672609 | RED09 | Red River drainage | Inoue et al. 2020 |
| *L. hydiana* | *cox1* | 11 | MK672610 | RED10 | Red River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 17 | MK672396 | SAB01 | Sabine River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 18 | MK672397 | SAB02 | Sabine River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 15 | MK672398 | SAB03 | Sabine River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 18 | MK672399 | SAB04 | Sabine River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 18 | MK672400 | SAB05 | Sabine River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 16 | MK672401 | SAB06 | Sabine River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 16 | MK672402 | SAB07 | Sabine River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 18 | MK672403 | SAB08 | Sabine River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 39 | MK672404 | SAB09 | Sabine River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 18 | MK672405 | SAB10 | Sabine River drainage | Inoue et al. 2020 |
| *L. hydiana* | *nad1* | 17 | MK672406 | SAB11 | Sabine River drainage | Inoue et al. 2020 |
| Species       | Gene   | Haplotype | GenBank accession no. | Specimen ID | Waterbody                  | Reference                |
|--------------|--------|-----------|-----------------------|-------------|----------------------------|--------------------------|
| *L. hydiana* | nad1   | 18        | MK672407              | SAB12       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | nad1   | 18        | MK672408              | SAB13       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | nad1   | 18        | MK672409              | SAB14       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | nad1   | 18        | MK672410              | SAB15       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 12        | MK672619              | SAB01       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 5         | MK672620              | SAB02       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 5         | MK672621              | SAB03       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 5         | MK672622              | SAB04       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 34        | MK672623              | SAB05       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 13        | MK672624              | SAB06       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 13        | MK672625              | SAB07       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 14        | MK672626              | SAB08       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 14        | MK672627              | SAB09       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 14        | MK672628              | SAB10       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 10        | MK672629              | SAB11       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 14        | MK672630              | SAB12       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 14        | MK672631              | SAB13       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 14        | MK672632              | SAB14       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 14        | MK672633              | SAB15       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 2         | MK672634              | SAB16       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 10        | MK672635              | SAB17       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 35        | MK672636              | SAB18       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 36        | MK672637              | SAB19       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 5         | MK672638              | SAB23       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 14        | MK672639              | SAB24       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 5         | MK672640              | SAB25       | Sabine River drainage      | Inoue et al. 2020        |
| *L. hydiana* | nad1   | 2         | MK672457              | SAN01       | San Antonio River drainage | Inoue et al. 2020        |
| *L. hydiana* | nad1   | 1         | MK672458              | SAN02       | San Antonio River drainage | Inoue et al. 2020        |
| *L. hydiana* | nad1   | 2         | MK672459              | SAN03       | San Antonio River drainage | Inoue et al. 2020        |
| *L. hydiana* | nad1   | 2         | MK672460              | SAN04       | San Antonio River drainage | Inoue et al. 2020        |
| *L. hydiana* | nad1   | 1         | MK672461              | SAN05       | San Antonio River drainage | Inoue et al. 2020        |
| *L. hydiana* | nad1   | 2         | MK672462              | SAN06       | San Antonio River drainage | Inoue et al. 2020        |
| *L. hydiana* | nad1   | 15        | MK672712              | SAN01       | San Antonio River drainage | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 15        | MK672713              | SAN02       | San Antonio River drainage | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 15        | MK672714              | SAN03       | San Antonio River drainage | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 15        | MK672715              | SAN04       | San Antonio River drainage | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 15        | MK672716              | SAN05       | San Antonio River drainage | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 15        | MK672717              | SAN06       | San Antonio River drainage | Inoue et al. 2020        |
| *L. hydiana* | nad1   | 2         | MK672428              | SJC01       | San Jacinto River drainage | Inoue et al. 2020        |
| *L. hydiana* | nad1   | 19        | MK672429              | SJC05       | San Jacinto River drainage | Inoue et al. 2020        |
| *L. hydiana* | nad1   | 19        | MK672430              | SJC06       | San Jacinto River drainage | Inoue et al. 2020        |
| *L. hydiana* | nad1   | 2         | MK672431              | SJC07       | San Jacinto River drainage | Inoue et al. 2020        |
| *L. hydiana* | nad1   | 19        | MK672432              | SJC08       | San Jacinto River drainage | Inoue et al. 2020        |
| *L. hydiana* | nad1   | 19        | MK672433              | SJC09       | San Jacinto River drainage | Inoue et al. 2020        |
| *L. hydiana* | nad1   | 19        | MK672434              | SJC10       | San Jacinto River drainage | Inoue et al. 2020        |
| *L. hydiana* | nad1   | 15        | MK672435              | SJC11       | San Jacinto River drainage | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 2         | MK672436              | SJC12       | San Jacinto River drainage | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 16        | MK672676              | SJC01       | San Jacinto River drainage | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 5         | MK672677              | SJC05       | San Jacinto River drainage | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 5         | MK672678              | SJC06       | San Jacinto River drainage | Inoue et al. 2020        |
| *L. hydiana* | cox1   | 16        | MK672679              | SJC07       | San Jacinto River drainage | Inoue et al. 2020        |
| Species        | Gene     | Haplotype | GenBank accession no. | Specimen ID | Waterbody                  | Reference                |
|----------------|----------|-----------|-----------------------|-------------|----------------------------|--------------------------|
| *L. hydiana*   | *cox1*   | 46        | MK672680              | SJC08       | San Jacinto River drainage | Inoue et al. 2020        |
| *L. hydiana*   | *cox1*   | 5         | MK672681              | SJC09       | San Jacinto River drainage | Inoue et al. 2020        |
| *L. hydiana*   | *cox1*   | 5         | MK672682              | SJC10       | San Jacinto River drainage | Inoue et al. 2020        |
| *L. hydiana*   | *cox1*   | 5         | MK672683              | SJC11       | San Jacinto River drainage | Inoue et al. 2020        |
| *L. hydiana*   | *cox1*   | 16        | MK672684              | SJC12       | San Jacinto River drainage | Inoue et al. 2020        |
| *L. hydiana*   | *nad1*   | 45        | MK672426              | TRI05       | Trinity River drainage     | Inoue et al. 2020        |
| *L. hydiana*   | *nad1*   | 2         | MK672427              | TRI06       | Trinity River drainage     | Inoue et al. 2020        |
| *L. hydiana*   | *cox1*   | 44        | MK672674              | TRI05       | Trinity River drainage     | Inoue et al. 2020        |
| *L. hydiana*   | *cox1*   | 45        | MK672675              | TRI06       | Trinity River drainage     | Inoue et al. 2020        |
| *L. hydiana*   | *cox1*   | 17 *MH560712 | INHS 87783-1    | Boeuf River | New                        |                          |
| *L. hydiana*   | *cox1*   | 17 *MH560722 | INHS 87783-3    | Boeuf River | New                        |                          |
| *L. hydiana*   | *cox1*   | 5 *MH560726 | INHS 87783-4    | Boeuf River | New                        |                          |
| *L. hydiana*   | *cox1*   | 14 MH161354 | UAU3C3508           | Naches River | Burlakova et al. 2019     |                          |
| *L. powelli*   | *cox1*   | 17 MF326971 | H2610                | Ouachita River | Robicheau et al. 2018      |                          |
| *L. powelli*   | *cox1*   | 8 MF326971  | H2610                | Ouachita River | Robicheau et al. 2018      |                          |
| *L. powelli*   | *cox1*   | 17 HM849075 | H2610                | Ouachita River | Breton et al. 2011        |                          |
| *L. radiata*   | *cox1*   | 3 MK226692  | 2                     | Hudson River | Porto-Hannes et al. 2019   |                          |
| *L. radiata*   | *cox1*   | 3 MN432619  | mH34                  | Hudson River | Porto-Hannes et al. 2021   |                          |
| *L. radiata*   | *cox1*   | 6 MN432616  | mH31                  | Lake Ontario drainage | Porto-Hannes et al. 2021 |                          |
| *L. radiata*   | *cox1*   | 1 HQ153594  | COX67                 | Lake Waccamaw | McCartney et al. 2016      |                          |
| *L. radiata*   | *cox1*   | 7 HQ153595  | COX68                 | Lake Waccamaw | McCartney et al. 2016      |                          |
| *L. radiata*   | *cox1*   | 1 HQ153596  | COX69                 | Lake Waccamaw | McCartney et al. 2016      |                          |
| *L. radiata*   | *cox1*   | 1 HQ153597  | COX70                 | Lake Waccamaw | McCartney et al. 2016      |                          |
| *L. radiata*   | *cox1*   | 4 HQ153598  | COX71                 | Lake Waccamaw | McCartney et al. 2016      |                          |
| *L. radiata*   | *nad1*   | 2 HQ153676  | NAD55                 | Lake Waccamaw | McCartney et al. 2016      |                          |
| *L. radiata*   | *nad1*   | 3 HQ153677  | NAD56                 | Lake Waccamaw | McCartney et al. 2016      |                          |
| *L. radiata*   | *nad1*   | 3 HQ153678  | NAD57                 | Lake Waccamaw | McCartney et al. 2016      |                          |
| *L. radiata*   | *nad1*   | 3 HQ153679  | NAD58                 | Lake Waccamaw | McCartney et al. 2016      |                          |
| *L. radiata*   | *nad1*   | 3 HQ153680  | NAD59                 | Lake Waccamaw | McCartney et al. 2016      |                          |
| *L. radiata*   | *cox1*   | 19 MN432650 | mH65                  | Potomac drainage | Porto-Hannes et al. 2021 |                          |
| *L. radiata*   | *cox1*   | 20 MN432651 | mH66                  | Potomac drainage | Porto-Hannes et al. 2021 |                          |
| *L. radiata*   | *cox1*   | 7 MN432620  | mH35                  | St. Lawrence drainage | Porto-Hannes et al. 2021 |                          |
| *L. radiata*   | *cox1*   | 8 MN432621  | mH36                  | St. Lawrence drainage | Porto-Hannes et al. 2021 |                          |
| *L. radiata*   | *cox1*   | 9 MN432623  | mH38                  | St. Lawrence drainage | Porto-Hannes et al. 2021 |                          |
| *L. radiata*   | *cox1*   | 10 MN432624 | mH39                  | St. Lawrence drainage | Porto-Hannes et al. 2021 |                          |
| *L. radiata*   | *cox1*   | 11 MN432629 | mH44                  | St. Lawrence drainage | Porto-Hannes et al. 2021 |                          |
| *L. radiata*   | *cox1*   | 12 MN432631 | mH46                  | St. Lawrence drainage | Porto-Hannes et al. 2021 |                          |
| *L. radiata*   | *cox1*   | 13 MN432633 | mH48                  | St. Lawrence drainage | Porto-Hannes et al. 2021 |                          |
| *L. radiata*   | *cox1*   | 14 MN432634 | mH49                  | St. Lawrence drainage | Porto-Hannes et al. 2021 |                          |
| *L. radiata*   | *cox1*   | 15 MN432642 | mH57                  | St. Lawrence drainage | Porto-Hannes et al. 2021 |                          |
| *L. radiata*   | *cox1*   | 16 MN432644 | mH59                  | St. Lawrence drainage | Porto-Hannes et al. 2021 |                          |
| *L. radiata*   | *cox1*   | 17 MN432645 | mH60                  | St. Lawrence drainage | Porto-Hannes et al. 2021 |                          |
| *L. radiata*   | *cox1*   | 18 MN432646 | mH61                  | St. Lawrence drainage | Porto-Hannes et al. 2021 |                          |
| *L. radiata*   | *nad1*   | 4 EF446098  |                       | Lake Erie drainage | Kneeland and Rhymer 2007 |                          |
| *L. radiata*   | *cox1*   | 2 KC408769  | H18                   | Lake Erie drainage | Krebs et al. 2013        |                          |
| *L. radiata*   | *cox1*   | 21 KC408770 | H19                   | Lake Erie drainage | Krebs et al. 2013        |                          |
| *L. radiata*   | *cox1*   | 5 KC408771  | H20                   | Lake Erie drainage | Krebs et al. 2013        |                          |
| *L. radiata*   | *cox1*   | 1 HQ153599  | COX72                 | Waccamaw, Yadkin/Pee Dee, Lumber rivers | McCartney et al. 2016 |                          |
| Species     | Gene | Haplotype | GenBank accession no. | Specimen ID | Waterbody | Reference                      |
|-------------|------|-----------|-----------------------|-------------|-----------|--------------------------------|
| L. radiata  | coxl | 2         | HQ153600              | COX73       | Waccamaw, Yadkin/Pee Dee, Lumber rivers | McCartney et al. 2016 |
| L. radiata  | coxl | 1         | HQ153603              | COX76       | Waccamaw, Yadkin/Pee Dee, Lumber rivers | McCartney et al. 2016 |
| L. radiata  | coxl | 1         | HQ153604              | COX77       | Waccamaw, Yadkin/Pee Dee, Lumber rivers | McCartney et al. 2016 |
| L. radiata  | coxl | 1         | HQ153606              | COX79       | Waccamaw, Yadkin/Pee Dee, Lumber rivers | McCartney et al. 2016 |
| L. radiata  | coxl | 1         | HQ153607              | COX80       | Waccamaw, Yadkin/Pee Dee, Lumber rivers | McCartney et al. 2016 |
| L. radiata  | coxl | 1         | HQ153608              | COX81       | Waccamaw, Yadkin/Pee Dee, Lumber rivers | McCartney et al. 2016 |
| L. radiata  | coxl | 1         | HQ153609              | COX82       | Waccamaw, Yadkin/Pee Dee, Lumber rivers | McCartney et al. 2016 |
| L. radiata  | coxl | 1         | HQ153610              | COX83       | Waccamaw, Yadkin/Pee Dee, Lumber rivers | McCartney et al. 2016 |
| L. radiata  | nadl | 1         | HQ153681              | NAD60       | Waccamaw, Yadkin/Pee Dee, Lumber rivers | McCartney et al. 2016 |
| L. radiata  | nadl | 5         | HQ153682              | NAD61       | Waccamaw, Yadkin/Pee Dee, Lumber rivers | McCartney et al. 2016 |
| L. radiata  | nadl | 3         | HQ153685              | NAD64       | Waccamaw, Yadkin/Pee Dee, Lumber rivers | McCartney et al. 2016 |
| L. radiata  | nadl | 2         | HQ153686              | NAD65       | Waccamaw, Yadkin/Pee Dee, Lumber rivers | McCartney et al. 2016 |
| L. radiata  | nadl | 1         | HQ153688              | NAD67       | Waccamaw, Yadkin/Pee Dee, Lumber rivers | McCartney et al. 2016 |
| L. radiata  | nadl | 1         | HQ153689              | NAD68       | Waccamaw, Yadkin/Pee Dee, Lumber rivers | McCartney et al. 2016 |
| L. radiata  | nadl | 2         | HQ153690              | NAD69       | Waccamaw, Yadkin/Pee Dee, Lumber rivers | McCartney et al. 2016 |
| L. radiata  | nadl | 6         | HQ153692              | NAD71       | Waccamaw, Yadkin/Pee Dee, Lumber rivers | McCartney et al. 2016 |
| L. radiata  | nadl | 3         | HQ153693              | NAD72       | Waccamaw, Yadkin/Pee Dee, Lumber rivers | McCartney et al. 2016 |
| L. sietmani | coxl | 2         | MK391838 TAMUNRI8052 2 | Neches River | Neches River | Keogh and Simons 2019 |
| L. sietmani | coxl | 2         | MK391839 TAMUNRI8052 3 | Neches River | Neches River | Keogh and Simons 2019 |
| L. sietmani | coxl | 1         | MK391843 JFBM22438 1  | Upper Mississippi drainage | Upper Mississippi drainage | Keogh and Simons 2019 |
| L. sietmani | coxl | 1         | MK391844 JFBM22438 2  | Upper Mississippi drainage | Upper Mississippi drainage | Keogh and Simons 2019 |
| L. sietmani | coxl | 1         | MK391845 JFBM22438 3  | Upper Mississippi drainage | Upper Mississippi drainage | Keogh and Simons 2019 |
| L. sietmani | coxl | 1         | MK391846 JFBM22438 4  | Upper Mississippi drainage | Upper Mississippi drainage | Keogh and Simons 2019 |
| L. sietmani | coxl | 1         | MK391847 JFBM22438 5  | Upper Mississippi drainage | Upper Mississippi drainage | Keogh and Simons 2019 |
| L. sietmani | coxl | 1         | MK391848 JFBM22438 6  | Upper Mississippi drainage | Upper Mississippi drainage | Keogh and Simons 2019 |
| L. sietmani | coxl | 1         | MK391849 JFBM22438 7  | Upper Mississippi drainage | Upper Mississippi drainage | Keogh and Simons 2019 |
| L. sietmani | coxl | 1         | MK391850 JFBM22433    | Upper Mississippi drainage | Upper Mississippi drainage | Keogh and Simons 2019 |
| L. sietmani | coxl | 1         | MK391851 INHS 27760   | Upper Mississippi drainage | Upper Mississippi drainage | Keogh and Simons 2019 |
| L. sietmani | coxl | 1         | MK391853 INHS 32502   | Upper Mississippi drainage | Upper Mississippi drainage | Keogh and Simons 2019 |
| L. sietmani | coxl | 3         | MK391856 JFBM22439    | Upper Mississippi drainage | Upper Mississippi drainage | Keogh and Simons 2019 |
| L. sietmani | coxl | 4         | MK391857 JFBM22439 photo | Upper Mississippi drainage | Upper Mississippi drainage | Keogh and Simons 2019 |
| L. sietmani | coxl | 1         | MK391858 WI River photo | Upper Mississippi drainage | Upper Mississippi drainage | Keogh and Simons 2019 |
| L. siliquoidea | coxl | 49        | MN432647 mH62         | Great Lakes drainage | Great Lakes drainage | Porto-Hannes et al. 2021 |
| L. siliquoidea | coxl | 50        | MN432648 mH63         | Great Lakes drainage | Great Lakes drainage | Porto-Hannes et al. 2021 |
| Species       | Gene | Haplotype | GenBank accession no. | Specimen ID | Waterbody                      | Reference                          |
|---------------|------|-----------|-----------------------|-------------|-------------------------------|-----------------------------------|
| *L. siliquoidea* | *cox1* | 1         | MH012239              | Fatmucket1  | Lake Erie drainage            | Metzger et al. 2018               |
| *L. siliquoidea* | *cox1* | 38        | MN432628              | mH43        | Lake Huron drainage           | Porto-Hannes et al. 2021          |
| *L. siliquoidea* | *cox1* | 6         | MN432617              | mH32        | Lake Michigan drainage        | Porto-Hannes et al. 2021          |
| *L. siliquoidea* | *cox1* | 31        | MN432614              | mH29        | Lake Ontario drainage         | Porto-Hannes et al. 2021          |
| *L. siliquoidea* | *cox1* | 35        | MN432625              | mH40        | Lake Ontario drainage         | Porto-Hannes et al. 2021          |
| *L. siliquoidea* | *cox1* | 42        | MN432636              | mH51        | Lake Ontario drainage         | Porto-Hannes et al. 2021          |
| *L. siliquoidea* | *cox1* | 44        | MN432638              | mH53        | Lake Ontario drainage         | Porto-Hannes et al. 2021          |
| *L. siliquoidea* | *cox1* | 45        | MN432639              | mH54        | Lake St. Clair                | Porto-Hannes et al. 2021          |
| *L. siliquoidea* | *cox1* | 51        | MN432649              | mH64        | Lake St. Clair drainage       | Porto-Hannes et al. 2021          |
| *L. siliquoidea* | *cox1* | 43        | MN432637              | mH52        | Lake Winnipeg drainage        | Porto-Hannes et al. 2021          |
| *L. siliquoidea* | *cox1* | 52        | MN432652              | mH67        | Little Vermilion River, Illinois | Porto-Hannes et al. 2021         |
| *L. siliquoidea* | *cox1* | 53        | MN432653              | mH68        | Little Vermilion River, Illinois | Porto-Hannes et al. 2021         |
| *L. siliquoidea* | *cox1* | 32        | MN432615              | mH30        | Meramec drainage              | Porto-Hannes et al. 2021          |
| *L. siliquoidea* | *nad1* | 5         | MK672508              | MS01        | Mississippi drainage          | Inoue et al. 2020                 |
| *L. siliquoidea* | *nad1* | 5         | MK672509              | MS02        | Mississippi drainage          | Inoue et al. 2020                 |
| *L. siliquoidea* | *nad1* | 5         | MK672510              | MS03        | Mississippi drainage          | Inoue et al. 2020                 |
| *L. siliquoidea* | *nad1* | 5         | MK672511              | MS04        | Mississippi drainage          | Inoue et al. 2020                 |
| *L. siliquoidea* | *nad1* | 3         | MK672512              | MS05        | Mississippi drainage          | Inoue et al. 2020                 |
| *L. siliquoidea* | *nad1* | 5         | MK672513              | MS06        | Mississippi drainage          | Inoue et al. 2020                 |
| *L. siliquoidea* | *nad1* | 3         | MK672514              | MS07        | Mississippi drainage          | Inoue et al. 2020                 |
| *L. siliquoidea* | *nad1* | 5         | MK672515              | MS08        | Mississippi drainage          | Inoue et al. 2020                 |
| *L. siliquoidea* | *cox1* | 5         | MK672774              | MS01        | Mississippi drainage          | Inoue et al. 2020                 |
| *L. siliquoidea* | *cox1* | 5         | MK672775              | MS02        | Mississippi drainage          | Inoue et al. 2020                 |
| *L. siliquoidea* | *cox1* | 5         | MK672776              | MS03        | Mississippi drainage          | Inoue et al. 2020                 |
| *L. siliquoidea* | *cox1* | 5         | MK672777              | MS04        | Mississippi drainage          | Inoue et al. 2020                 |
| *L. siliquoidea* | *cox1* | 5         | MK672778              | MS05        | Mississippi drainage          | Inoue et al. 2020                 |
| *L. siliquoidea* | *cox1* | 5         | MK672779              | MS06        | Mississippi drainage          | Inoue et al. 2020                 |
| *L. siliquoidea* | *cox1* | 5         | MK672780              | MS07        | Mississippi drainage          | Inoue et al. 2020                 |
| *L. siliquoidea* | *cox1* | 5         | MK672781              | MS08        | Mississippi drainage          | Inoue et al. 2020                 |
| *L. siliquoidea* | *cox1* | 36        | MN432626              | mH41        | Mississippi drainage          | Porto-Hannes et al. 2021          |
| *L. siliquoidea* | *cox1* | 37        | MN432627              | mH42        | Mississippi drainage          | Porto-Hannes et al. 2021          |
| *L. siliquoidea* | *cox1* | 34        | MN432622              | mH37        | Nottaway drainage, Hudson Bay, Canada | Porto-Hannes et al. 2021          |
| *L. siliquoidea* | *cox1* | 3         | MF326973              | H2655       | Red River drainage            | Robicheau et al. 2018             |
| *L. siliquoidea* | *nad1* | 1         | MF326973              | H2655       | Red River drainage            | Robicheau et al. 2018             |
| *L. siliquoidea* | *cox1* | 41        | MN432635              | mH50        | Red River drainage            | Robicheau et al. 2018             |
| *L. siliquoidea* | *cox1* | 4         | MK226693              | St. Lawrence drainage | Porto-Hannes et al. 2019  |
| *L. siliquoidea* | *cox1* | 4         | MK226710              | St. Lawrence drainage | Porto-Hannes et al. 2019  |
| *L. siliquoidea* | *cox1* | 33        | MN432618              | mH33        | St. Lawrence drainage         | Porto-Hannes et al. 2021          |
| *L. siliquoidea* | *cox1* | 39        | MN432630              | mH45        | St. Lawrence drainage         | Porto-Hannes et al. 2021          |
| *L. siliquoidea* | *cox1* | 40        | MN432632              | mH47        | St. Lawrence drainage         | Porto-Hannes et al. 2021          |
| *L. siliquoidea* | *cox1* | 46        | MN432640              | mH55        | St. Lawrence drainage         | Porto-Hannes et al. 2021          |
| *L. siliquoidea* | *cox1* | 47        | MN432641              | mH56        | St. Lawrence drainage         | Porto-Hannes et al. 2021          |
| *L. siliquoidea* | *cox1* | 48        | MN432643              | mH58        | St. Lawrence drainage         | Porto-Hannes et al. 2021          |
| *L. siliquoidea* | *cox1* | 2         | MK391878              | JFBM22440 1 | Upper Mississippi drainage    | Keogh and Simons 2019             |
| *L. siliquoidea* | *cox1* | 6         | MK391879              | JFBM22440 2 | Upper Mississippi drainage    | Keogh and Simons 2019             |
| *L. siliquoidea* | *nad1* | 4         | AY094386              | UAUC 882    | Douglas Lake, Cheboygan County, Michigan | Buhay et al. 2002                |
| *L. siliquoidea* | *cox1* | 2         | DQ94752               | UAUC882     | Douglas Lake, Cheboygan County, Michigan | Serb 2006                        |
| Species      | Gene      | Haplotype | GenBank accession no. | Specimen ID | Waterbody Reference          |
|-------------|-----------|-----------|-----------------------|-------------|-----------------------------|
| *L. siliquoidea* | cox1     | 1         | AF156521              | UMMZ 265709a | Huron River, Michigan Graf and Ó Foighil 2000 |
| *L. siliquoidea* | cox1     | 5         | AF156522              | UMMZ 265709b | Huron River, Michigan Graf and Ó Foighil 2000 |
| *L. siliquoidea* | nad1     | 4         | AY158747              | LSILIQ      | Lake Erie drainage Serb et al. 2003 |
| *L. siliquoidea* | cox1     | 8         | KC408744              | H1          | Lake Erie drainage Krebs et al. 2013 |
| *L. siliquoidea* | cox1     | 4         | KC408745              | H2          | Lake Erie drainage Krebs et al. 2013 |
| *L. siliquoidea* | cox1     | 9         | KC408746              | H3          | Lake Erie drainage Krebs et al. 2013 |
| *L. siliquoidea* | cox1     | 10        | KC408747              | H4          | Lake Erie drainage Krebs et al. 2013 |
| *L. siliquoidea* | cox1     | 11        | KC408748              | H5          | Lake Erie drainage Krebs et al. 2013 |
| *L. siliquoidea* | cox1     | 12        | KC408749              | H6          | Lake Erie drainage Krebs et al. 2013 |
| *L. siliquoidea* | cox1     | 13        | KC408750              | H7          | Lake Erie drainage Krebs et al. 2013 |
| *L. siliquoidea* | cox1     | 14        | KC408751              | H8          | Lake Erie drainage Krebs et al. 2013 |
| *L. siliquoidea* | cox1     | 15        | KC408752              | H9          | Lake Erie drainage Krebs et al. 2013 |
| *L. siliquoidea* | cox1     | 16        | KC408753              | H10         | Lake Erie drainage Krebs et al. 2013 |
| *L. siliquoidea* | cox1     | 2         | KC408756              | H13         | Lake Erie drainage Krebs et al. 2013 |
| *L. siliquoidea* | cox1     | 19        | KC408757              | H14         | Lake Erie drainage Krebs et al. 2013 |
| *L. siliquoidea* | cox1     | 20        | KC408758              | H15         | Lake Erie drainage Krebs et al. 2013 |
| *L. siliquoidea* | cox1     | 21        | KC408759              | H16         | Lake Erie drainage Krebs et al. 2013 |
| *L. siliquoidea* | cox1     | 22        | KC408760              | H17         | Lake Erie drainage Krebs et al. 2013 |
| *L. siliquoidea* | cox1     | 23        | KC408761              | H21         | Lake Erie drainage Krebs et al. 2013 |
| *L. siliquoidea* | cox1     | 24        | KC408762              | H22         | Lake Erie drainage Krebs et al. 2013 |
| *L. siliquoidea* | cox1     | 25        | KC408763              | H23         | Lake Erie drainage Krebs et al. 2013 |
| *L. siliquoidea* | cox1     | 26        | KC408764              | H24         | Lake Erie drainage Krebs et al. 2013 |
| *L. siliquoidea* | cox1     | 27        | KC408765              | H25         | Lake Erie drainage Krebs et al. 2013 |
| *L. siliquoidea* | cox1     | 28        | KC408766              | H26         | Lake Erie drainage Krebs et al. 2013 |
| *L. siliquoidea* | cox1     | 29        | KC408767              | H27         | Lake Erie drainage Krebs et al. 2013 |
| *L. siliquoidea* | cox1     | 30        | KC408768              | H28         | Lake Erie drainage Krebs et al. 2013 |
| *L. siliquoidea* | cox1     | 3         | HM849076              | H2655       | Red River drainage Breton et al. 2011 |
| *L. siliquoidea* | nad1     | 5         | HM852926              | BM20297     | Upper Mississippi drainage Boyer et al. 2011 |
| *L. siliquoidea* | nad1     | 5         | HM852927              | BM19948     | Upper Mississippi drainage Boyer et al. 2011 |
| *L. siliquoidea* | cox1     | 17        | KC408754              | H11         | Upper Mississippi drainage Krebs et al. 2013 |
| *L. siliquoidea* | cox1     | 18        | KC408755              | H12         | Upper Mississippi drainage Krebs et al. 2013 |
| *L. straminea*  | cox1     | 4         | MK391881              | JFBM22424   | Escambia River drainage Keogh and Simons 2019 |
| *L. straminea*  | cox1     | 5         | MK672782              | ESC04       | Escambia River drainage Inoue et al. 2020 |
| *L. straminea*  | cox1     | 6         | MK672783              | ESC05       | Escambia River drainage Inoue et al. 2020 |
| *L. straminea*  | cox1     | 7         | MK672784              | ESC06       | Escambia River drainage Inoue et al. 2020 |
| *L. straminea*  | nad1     | 1         | MK391880              | JFBM22423   | Alabama River drainage Keogh and Simons 2019 |
| *L. straminea*  | cox1     | 1         | MK391882              | JFBM:22426  | Tombigbee River drainage Keogh and Simons 2019 |
| *L. straminea*  | nad1     | 2         | MH1588393             | UAM3543     | 32.674–87.765, Black Warrior River drainage New |
| *L. straminea*  | cox1     | 3         | MH161355              | UAUC 3543   | Black Warrior River drainage Burlakova et al. 2019 |
| *L. straminea*  | nad1     | 1         | DQ445163              | UAUC694     | Black Warrior River, near Fosters, Alabama Unpublished |
| *L. straminea*  | cox1     | 2         | EF033271              | H1369       | Not stated Chapman et al. 2008 |
| *L. virescens*  | cox1     | 1         | MK672787              | Lvir TEN01   | Tennessee River drainage Inoue et al. 2020 |
| *L. virescens*  | cox1     | 1         | *MH560775             | AABC        | Paint Rock River Alabama Aquatic Biodiversity Center |
| *L. virescens*  | cox1     | 1         | JF326433              |             | Tennessee River drainage Campbell and Lydeard 2012 |
| *L. virescens*  | nad1     | 1         | JF326443              |             | Tennessee River drainage Campbell and Lydeard 2012 |
| *L. virescens*  | cox1     | 1         | JQ437390              | PR 7106     | Tennessee River drainage Moyer and Díaz-Ferguson 2012 |
| *L. virescens*  | cox1     | 2         | JQ437391              | PR 7108     | Tennessee River drainage Moyer and Díaz-Ferguson 2012 |
| *L. virescens*  | cox1     | 3         | JQ437392              |             | Tennessee River drainage Moyer and Díaz-Ferguson 2012 |
Species | Gene | Haplotype | GenBank accession no. | Specimen ID | Waterbody | Reference |
----------|------|-----------|-----------------------|------------|-----------|-----------|
*L. virescens* | coxl | 4 | JQ437393 | 2 | Tennessee River drainage | Moyer and Díaz-Ferguson 2012 |
*Ortmanniana ligamentina* | coxl | *MH560777* | UAM241 | Kankakee County, Illinois, Kankakee River | New |
*O. ligamentina* | nadl | *MH588394* | UAM241 | Kankakee County, Illinois, Kankakee River | New |

*Newly generated sequences.*