Final Report

ENDANGERED SPECIES RESEARCH PROJECTS ON THE DISTRIBUTION AND ABUNDANCE OF THE TEXAS HEELSPLITTER (*POTAMILUS AMPHICAENUS*) AND LOUISIANA PIGTOE (*PLEUROBEMA RIDDELLII*)

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Introduction

North American unionid freshwater mussels: Background

Freshwater mollusks (Order Bivalvia, Class Unionidae) have been integral members of global aquatic environments for the last 400 million years (Howells et al. 1996), and they are a subject of keen environmental, economic, and biological interest (Graf and Cummings 2007). Historically, they have been poorly studied and only recently has their status as an imperiled taxon been brought to light and their ecological significance been realized (Graf and Cummings 2007). Mollusks are one of the most endangered taxa in the world, and in North America, freshwater unionids are considered to be one of the most endangered animal groups (Burlakova et al. 2012). Thus far, of all Texas unionid species, studies have revealed 65% of them to be rare; 15 of these were recently added to the threatened species list while 11 are currently considered to be federally listed by the U. S. Fish and Wildlife service (Burlakova et al. 2012). The Neartic region, extending from North America to central Mexico (Escalante et al. 2010), has the highest global diversity of Unionidae, with 5 families, 59 genera, and 302 species (Bogan 2008). This can be partially attributed to the particularly robust diversity found within southeastern United States: 42 genera and 271 species (Bogan 2008). Texas is among the top states in species diversity; accordingly, Texas ranks fourth in the number of species extinctions (Burlakova et al. 2012). East Texas has been noted as a hotspot for unionid diversity with nearly every river hosting 17 to 28 species (Burlakova et al. 2012).

Mollusks can be characterized by a calcium-rich, proteinaceous shell with two halves, or valves, protecting the inner soft tissues (Howells et al. 1996). Shell morphology demonstrates a high degree of variability, ranging along a spectrum of extreme bumps and ridges to complete smoothness (Howells et al. 1996). Shells have growth rings that may be apparent or hard to discern, and while correlated to the age of the individual, are not necessarily annular marks (Howells et al. 1996). Shell shape can be round, rhomboidal, oval, elliptical, triangular, or quadrate (Howells et al. 1996). Connecting the two halves of the shell are soft tissue shell muscles: the anterior and posterior adductors, which regulate whether the shell is open or closed (Howells et al. 1996). The mantle, a thin tissue that encloses the entirety of the soft tissue body, secretes the three shell layers, contains sensory organs, and functions in respiration and feeding (Howells et al. 1996). A third component of the mussel soft tissue is the foot, which serves primarily in orienting, substrate anchorage, and general movement (Howells et al. 1996). Gills are located within the mantle cavity and are the site of gas exchange and assist in reproduction. Mollusks are filter-feeders: as water flows through the mussel, gills trap particulate matter with secreted mucosa (Howells et al. 1996). Food materials include diatoms, filamentous algae, desmids, and other algal species (Howells et al. 1996).

Freshwater mussels have five life cycle stages; developing eggs are retained in the female brood pouch and are released into the water column as microscopic glochidia, which if successful, attach to a fish host (Howells et al. 1996). They are carried for some time (sometimes for several months) until reaching the juvenile stage, when they release from their host and remain mostly sedentary through adulthood (Howells et al. 1996). As feeding and reproductive strategies are highly dependent on freely-moving water, freshwater mussels are typically found within rivers and streams, although there are some that can thrive in lakes, ponds, canals and

reservoirs (Howells et al. 1996). Different species of unionids have variable habitat suitability requirements, such as bottom types (sand, silt, clay, etc.), flow velocity, water depth, water chemistry, climate, and vegetation (Howells et al. 1996).

Study subjects

Over 60% of freshwater mussels are imperiled in Texas, and of those, 15 species are state-listed as threatened, with 11 of them currently being federally considered; 2 of those are the subject of this study: *Potamilus amphicaenus* (Texas Heelsplitter) and *Pleurobema riddelli* (Louisiana Pigtoe) (Burlakova et al. 2012).

Potamilis amphicaenus is a mussel endemic to northeastern Texas. It is elongated, has a thin shell up 177 mm in length, and is brown on the beak, which darkens as it extends radially (Burch 1973). Internally, the shell is bluish-white to bluish-purple (Burch 1973). Distribution ranges from the Sabine to the Neches and the Trinity rivers and is also found within larger bodies of water within these river systems (Burch 1973). The Texas Parks and Wildlife department currently lists *P. amphicaenus* as legally threatened; in addition, it is on the verge of being federally listed with the U.S. Fish and Wildlife Service (Walters and Ford 2013). From 1898 to 2010, just over 300 specimens had been found (Walters and Ford 2013), which is a relatively low number.

Pleurobema riddellii is a mussel found primarily in western Louisiana and eastern Texas, where it is primarily observed within the Trinity, Neches, and Sabine river systems (Simpson 1914; Burch 1973). It only occurs in lotic (stream and river) habitats with low to moderate flow, and prefers a combination of silty-sand, gravel, and loose clay substrate (Williams et al. 1993). The shell can grow to 36 mm in length and 34 mm in height, and is short, rounded to triangular,

and substantially solid (Simpson 1914; Walters and Ford 2013). It is of a greenish-brown coloration externally and is an iridescent bluish-white internally (Simpson 1914; Walters and Ford 2013). Like *P. amphicaenus*, *P. riddellii* is extremely rare; as of 2013, there were only 38 records for the species (Ford et al. 2013), and few additional records have been added since then (Ford et al. 2017). Its habitat in Texas appears to be confined to undisturbed areas of the Neches River and its main tributaries, the Angelina and Attouyac Rivers (Ford et al. 2017; Walters et al. 2017), suggesting that it is particularly sensitive to pollution (Ford et al. 2017). It is currently under review for federal listing by the U.S. Fish and Wildlife Service (Williams et al. 1993).

Study objectives

Because both *P. amphicaenus* and *P. riddellii* are extremely rare and are actively under consideration for federal listing as threatened or endangered (Williams et al. 1993; Walters and Ford 2013), the purpose of this study was to better delineate specific locales of the two species within their ranges, using field and genetic verification, in order to inform the listing process and the delineation of critical habitat. We visited reservoirs in East Texas where *P. amphicaenus* has been historically recorded, to verify whether the species can still be detected there, and to provide our qualitative assessment of whether those sites are still good habitat. We also surveyed for new records of *P. riddellii* by visiting sites that were first scouted by the Lower Neches Valley Authority (https://lnva.dst.tx.us/), primarily on the lower Neches River south of the B. A. Steinhagen Reservoir.

Materials and Methods

Study Area

This study took place from July 15th – October 29th 2020. It included sites on the Trinity, Neches, Angelina, and Sabine Rivers, Big Cypress Creek, and some of their tributaries (Figure 1). The following reservoirs were re-surveyed for the presence of *Potamilus amphichaenus*, based on historical records (Neck 1986; Howells et al. 2000; US Fish & Wildlife Service 2017; Pandolfi and Orsak 2019): Lewisville, Grapevine, Livingston, B. A. Steinhagen, and Tawakoni. 19 – 24 sites per lake were surveyed, covering as much of the area as possible (Figures 2 – 6). The following areas were surveyed for the presence of *P. riddellii*: the Sabine River in Gregg County (Figure 1), the Neches River in between Anderson and Cherokee Counties (Figure 1), the Angelina River in between Cherokee and Nacogdoches Counties (Figure 1), the Angelina River directly downstream of the Sam Rayburn Reservoir (Figure 1),
Big Cypress Creek and Little Cypress Bayou downstream of Lake O' the Pines (Figure 7), and Big Cypress Bayou upstream of the Lake O' the Pines (Figure 7).

Field sampling and measurements

Field sampling protocols differed for the lakes versus the river segments. For the lakes, we followed Carlson et al. (2008). Sampling was done in one of two ways, with 3 – 5 person-hours per site:

- For sites less than an arm's length in depth, a timed handpicking search was performed while wading (Tiemann et al. 2009).
- For sites greater than an arm's length in depth, Surface Air (Hooka) or SCUBA diving equipment was used following all applicable safety rules.

- When logistically feasible, other promising sites in the vicinity of existing sites were opportunistically sampled. These sites were chosen by visual inspection along the banks of the reservoirs, especially the cove areas, as well as by handpicking the shallows in the direction away from the bank of the lake.

For the river segments, sites were selected by prior scouting for mussels and suitable habitat. All mesohabitats present in an area were surveyed: banks, backwater, front of point bar, woody debris. The boundaries of the search areas were measured to obtain their total sizes. Survey effort was evenly spread around all available mesohabitat types at a site using tactile searches. Sites were searched for a minimum of 4 person-hours (p-h) in one p-h intervals. If additional species are found during the last p-h, then an additional p-h of searching was conducted, and so on.

During each search interval, surveyors were evenly distributed in the search area, and each searched one or two mesohabitat types (depending on the number of surveyors and habitat types). At the end of each search interval all live mussels were identified to species and placed in a mesh bag, which were kept submerged in water until the survey was completed, and then returned to the river into the appropriate habitat. If mussels were found in the first p-h then a second survey was performed with surveyors interchanging locations. Snorkel and mask were used in shallow water and surface air (hooka) was used at deeper sites where water depth exceeded 1.5 m.

For each *P. amphicaenus* or *P. riddellii* specimen encountered, information was recorded in the format of the Mussels of Texas database (i.e., in this format: http://musselsdev.nri.tamu.edu). Furthermore, additional information was included beyond the scope of the Mussels of Texas database:

- A unique ID number for each site
- The approximate water depth where the specimens were found
- The surveying method(s) used (tactile searching versus hookah versus SCUBA)
- Whether the genetic barcodes verified the field identifications:
    - For *COX1*, there were three columns corresponding to the top three BLAST matches to the field sequences, and the corresponding percentages of sequence similarities between the field sequences and the BLAST matches.
    - For *ND1*, there were three columns corresponding to the top three BLAST matches to the field sequences, and the corresponding percentages of sequence similarities between the field sequences and the BLAST matches.
    - For *ITS-1*, there were three columns corresponding to the top three BLAST matches to the field sequences, and the corresponding percentages of sequence similarities between the field sequences and the BLAST matches.

Specimens of other species that were encountered were also recorded in this database, but only live specimens (not shells or recent dead) and only the following information:

- Species [genus]
- Species [epithet]
- Common name
- State-listed (Y or N)
- Federally listed (Y or N)
- Mesohabitat (i.e., riffle, run, pool, bank, backwater, boulder/bedrock)
- Drainage (major river drainage)
- Waterbody (river, stream or lake sampled)

- County
- State
- Date
- Year
- Latitude
- Longitude
- Collectors
- Identified by
- experience (years)
- Source (Institution or company)

Finally, information from sites where no mussels were found was also recorded, but only the following information:

- Mesohabitat (i.e., riffle, run, pool, bank, backwater, boulder/bedrock)
- Drainage (major river drainage)
- Waterbody (river, stream or lake sampled)
- County
- State
- Date
- Year
- Latitude
- Longitude

Genetic barcoding verification

For each live or fresh dead *P. amphicaenus* or *P. riddellii* specimen encountered, non-destructive cell samples were obtained by taking viscera swabs and storing them at room temperature in lysis buffer (Karlsson et al. 2013). Specifically, as described in Karlsson et al. (2013), cotton swabs were used to collect material from the outer surface of the visceral mass of each specimen. Following Karlsson et al. (2013), DNA was extracted from the visceral swab samples with the E.Z.N.A. MicroElute Genomic DNA kit (Omega biotek, Norcross, GA) following the protocol for isolation of genomic DNA from cotton swabs. DNA from all viscera swab samples was then eluted in 60 mL of elution buffer.

For each specimen, the barcoding genes *ND1*, *COX1*, and *ITS-1* were amplified. For *ND1*, the primers specified in Serb et al. (2003) were used; for *COX1*, the primers specified in Campbell et al. (2005) were used; and for *ITS-1*, the primers specified in King et al. (1999) were used. A 25µL polymerase chain reaction (PCR) was performed consisting of 2.5µL of 10X PCR buffer (1X), 2µL of 25 mM $MgCl_2$ (2 mM), 0.5µL of dNTPs (10 mM each; 0.2 mM), 1.25 µL of each 10 µM forward and reverse primer (0.5 µM), 0.125µL (0.625 U) of Taq Polymerase (Applied Biosystems), and typically 2 - 3µL of genomic DNA template (~200 ng). Sterile water was used to make up the remaining volume. Reactions were amplified with an Eppendorf Mastercycler gradient thermal cycler with a temperature-controlled lid. Reaction conditions for double-stranded amplification were as follows:

- For *ND1*:
    - An initial denaturation at 94 degrees C for 5 min, followed by 30 cycles of 94 degrees C for 45 s, 54 degrees C for 60 s, and 72 degrees C for 60 s, and a final extension of 72 degrees C for 5 min.
- For *COX1*:

    - An initial denaturation at 94 degrees C for 5 min, followed by 35 cycles of 94 degrees C for 45 s, 55 degrees C for 60 s, and 72 degrees C for 60 s, and a final extension of 72 degrees C for 10 min.

- For *ITS-1*:
    - For *Pleurobema riddellii*:
        - An initial denaturation at 94 degrees C for 2 min, followed by 35 cycles of 94 degrees C for 30 s, 55 degrees C for 30 s, and 72 degrees C for 90 s, and a final extension of 72 degrees C for 10 min.
    - For *Potamilus amphicaenus*:
        - An initial denaturation at 94 degrees C for 2 min, followed by 35 cycles of 94 degrees C for 30 s, 59 degrees C for 30 s, and 72 degrees C for 90 s, and a final extension of 72 degrees C for 10 min.

PCR products were visualized on 1% agarose gels with SYBR Safe gel stain. Products will then be purified and sequenced at the University of Texas at Austin's DNA Sequencing Facility on an Applied Biosystems 3730 DNA Analyzer. Consensus contig sequences were assembled and edited using Geneious version 10.2.3 (http://geneious.com; Kearse et al. 2012). In order to genetically confirm each specimen's species identity, the sequences were uploaded to the Basic Local Alignment Search Tool (BLAST) website at the National Center for Biotechnology (NCBI) (Boratyn et al. 2013) and compared against the sequences in their database, according to the percent sequence similarity.

Results

Field results

A total of 151 unique sites were visited (Figure 1). Out of these, 106 sites contained freshwater mussels of some sort, and 30 sites contained either *Potamilus amphichaenus* or *Pleurobema riddellii*, or both, as confirmed by genetic barcode (full database: https://docs.google.com/spreadsheets/d/18NxL7eXzjJTUPxhni4ZTeZMEeMtT8EDeSFEvzQ7kYts/edit?usp=sharing). There were a total of 69 specimens of *P. amphicaenus*, spread across 25 different sites (Figure 8), that were confirmed by genetic barcode; and there were a total of 54 specimens of *P. riddellii*, spread across 10 different sites (Figure 9), that were confirmed by genetic barcode (Table 1). There was one specimen that was misidentified as *P. amphicaenus* that was actually *Leptodea fragilis* (on the lower Neches River below B. A. Steinhagen reservoir), and there were two specimens that were misidentified as *P. riddellii* that were actually *Fusconaia flava* and *Cyclonaias pustulosa*, respectively (both from Little Cypress Creek) (Table 1).

Some specimens that were morphologically identified as either *Potamilus amphicenus* or *Pleurobema riddellii* could not be genetically verified, because the tissue samples and the DNA was of poor quality. These specimens were therefore not counted as being either *Potamilus amphicenus* or *Pleurobema riddellii*. Only genetically verified *Potamilus amphicenus* or *Pleurobema riddellii* specimens are included in the tallies reported here. At Grapevine Lake, there were mussels present at eight of the 19 sites that were visited, but none of them were the target species (Figure 2). At Lake Lewisville, there were mussels present at seven out of 24 sites that were visited, but none of them were the target species (Figure 3). At Lake Tawakoni, there were mussels present at 15 of the 22 sites that were visited, but none of them were the target species (Figure 4). At Lake Livingston, there were mussels present at 23 out of 24 sites that

were visited, but none of them were the target species (Figure 5). At B. A. Steinhagen Reservoir, there were mussels present at 14 of the 20 sites that were visited, and 12 of these sites contained *P. amphicaenus* that were verified genetically (Figure 6; Table 1). In the Caddo Lake drainage area, there were mussels present at four out of five of the sites that were visited, but none of them were the target species (Figure 7). On the Neches River south of B. A. Steinhagen Reservoir, there were mussels present at all 40 of 40 sites visited, and 17 of these sites contained *P. amphicaenus* and five of these sites contained *P. riddellii* that were verified genetically (Figures 10 and 11; Table 1). On the Angelina south of the Sam Rayburn Reservoir but north of the B. A. Steinhagen Reservoir, there were mussels present at one of the two sites that were visited, and one of those sites contained *P. amphicaenus* that was verified genetically (Figure 8; Table 1). Along the Sabine River in Gregg County, there were mussels present at all three of the sites that were visited, and one of those sites contained *P. riddellii* that was verified genetically (Figure 9; Table 1). Along the Angelina River in between Cherokee and Nacogdoches Counties, there were mussels present at both sites that were visited, and both of those sites contained *P. riddellii* that was verified genetically (Figure 9; Table 1). Along the Neches River in between Anderson and Cherokee Counties, there were mussels present at the one site that was visited, and that sire contained *P. riddellii* that was verified genetically (Figure 9; Table 1).

Genetic barcoding verification

Most specimens were successfully sequenced at the three barcoding genes, *COX1*, *ND1*, and *ITS-1*. However, as in several other recent unionid mussel studies (Jones et al. 2006; Campbell et al. 2008; Plants-Paris 2016; Pratt 2017; Pieri et al. 2018), we found that *ITS-1* was not useful as a barcoding gene, because it could not reliably distinguish among species.

Therefore, we do not present the *ITS-1* barcoding results here. They can be accessed in the full database at: https://docs.google.com/spreadsheets/d/18NxL7eXzjJTUPxhni4ZTeZMEeMtT8EDeSFEvzQ7kYts/edit?usp=sharing. There were a total of 93 specimens that were successfully sequenced at both *COX1* and *ND1* (Table 1). Of these, 59 were *P. amphicaenus* and 31 were *P. riddellii*. Another 10 *P. amphicaenus*, and 23 *P. riddellii*, specimens were successfully sequenced at one gene but not the other (Table 1). In most instances, the morphological identification in the field matched the genetic barcode identification of the species for one or both genes (Table 1). This provides more support for the genetic verifications of the morphological identifications, and confirms the presence of the species at each of those locations. There were only three instances where specimens were clearly misidentified morphologically: one *Leptodea fragilis* was misidentified as *P. amphicaenus*, and one *Fusconaia flava* and one *Cyclonaias pustulosa* were misidentified as *P. riddellii* (Table 1, rows highlighted in yellow). There were a couple of other minor discrepancies, such as specimen numbers NF2 and NF7, which were identified morphologically as *P. ridellii* but that were barcoded as *F. flava* for *ND1*; however, in both of those instances, *COX1* revealed the correct barcode (*P. riddellii*) (Table 1; see).

Discussion

In this study, we definitively documented 30 sites throughout East Texas as having one or both of the preciously rare target species (*Potamilus amphichaenus* or *Pleurobema riddellii*). Before this study, only a few hundred *P. amphicaenus* and less than 100 *P. riddellii* specimens had ever been observed anywhere in the world. **Our intensive site surveys have now added**

substantially to this record, adding about 20% to this count for *P. amphicaenus* and over 100% to this count for *P. riddellii*. And we have demonstrated with genetic barcoding at two different genes that our surveys are accurate, with minimal levels of morphological misidentification. Among the few discrepancies are two instances where *ND1* reveals a "mismatched" barcode, as compared to the morphological identification, whereas *COX1* reveals a barcode that matched the morphological identification of the specimens. While *ND1* generally works reliably as a barcode for telling unionid mussel species apart (Jones et al. 2006; Campbell et al. 2008; Plants-Paris 2016; Pratt 2017; Marshall et al. 2018; Pieri et al. 2018), *COX1* is considered to be the gold standard in genetic barcoding, both in general for animals (Hebert et al. 2003), and in particular for unionids (Zanatta and Murphy 2006; Campbell et al. 2008). Therefore, if the *COX1* barcode matches the morphological identification, even if there is a discrepancy between *ND1* and *COX1*, we believe it is appropriate to consider the specimen to be genetically verified as matching its morphological identification. The sites that we visited were chosen in collaboration with various stakeholders (US Fish & Wildlife Service, Texas Parks & Wildlife Department, the Comptroller of Public Accounts of the State of Texas, the Lower Neches Valley Authority) so as to fill in gaps in our understanding of the current ranges and of these species. Therefore, this information is directly applicable to the species management plans being considered for these species. The fact that we provide many new records of the species that are genetically verified, usually by two different barcoding genes that have proven reliable for unionids, shows that our conclusions are robust and not contaminated by misidentifications in the field. We also note that our swabbing technique was non-destructive, so as not to damage any precious specimens in the wild.

A few other important conclusions arise from our project. Firstly, most of the North and East Texas reservoirs (Grapevine, Lewisville, Tawakoni, and Livingston) are not presently good habitat for *P. amphicaenus* or *P. riddellii*. Despite extensive searches, none of the target species were uncovered. In fact, the historical record of *P. amphicaenus* in Lake Tawakoni is dubious to begin with, as the location is not precise, and it could be from an area downstream of the reservoir (Neck 1986; N. Ford, personal observation). While we cannot rule out that *P. amphicaenus* and *P. riddellii* exist in these reservoirs at low density, we believe it is fair to conclude that they do not provide optimal habitat and are not important to the long-term survival of the species. In fact, it makes sense that *P. amphicaenus* and *P. riddellii* would not be adapted to or particularly common in lakes, since lakes are not a natural phenomenon in Texas or nearby states that do not have a history of glacial scouring. We observed that the reservoirs generally appeared too eutrophied and muddy for our region's exclusively riverine-adapted aquatic fauna (L. Williams, personal observation). The one exception to this observation is the B. A. Steinhagen reservoir. We found the highest densities of *P. amphicaenus* in this reservoir as compared to anywhere else in its range. While the reservoir was dried out at the time, as it was experiencing a draw-down for maintenance, we note that the majority of the *P. amphicaenus* specimens were found around the periphery of the lake, near the mouths of creeks and rivers that created sandy substrates more typical of a riverine than lake environments; thus even in B. A. Steinhagen, *P. amphicaenus* appeared to be favoring riverine habitat within the reservoir as opposed to eutrophied or stratified lake habitat. In any event, we certainly believe B. A. Steinhagen is pivotal to the long-term survival of *P. amphicaenus*, due to its readily apparent stronghold there.

Next, we note that, while we have identified several new sites and specimens for both species, *P. amphicaenus* and *P. riddellii* are still extremely rare, even as compared to other rare mussels. We still encountered small numbers of both species (less than 100 of each), despite intensive searches, covering thousands of square kilometers, in the areas where these mussels were supposed to be. We note that we would often find mussels of common species at various sites, but none of the rare ones. That said, we believe it is interesting to point out that *P. riddellii* appears to be even rarer than *P. amphicaenus*. We encountered fewer specimens of P. *riddellii* at fewer sites, as compared to *P riddellii*. This is noteworthy because *P. amphicaenus* is often considered to be the rarest East Texas mussel (Walters and Ford 2013); we believe that *P. riddellii* deserves this distinction instead.

Finally, we caution that our study is based on intensive searches that were meant to fill in gaps in our knowledge of the state of these two species. Therefore, these searches should not be taken as exhaustive in the sense that all possible areas within East Texas were considered equally. The absence of *P. amphicaenus* and *P riddellii* specimens from other areas in our spatial extent does not necessarily imply that the species are not found in those locales; it could simply reflect our concentrated sampling efforts on certain areas. That being said, we believe we surveyed the Grapevine, Lewisville, Tawakoni, and Livingston reservoirs sufficiently to conclude they are not essential habitat for either of these rare mussels, and in fact, there are good biogeographic reasons to believe they would not adapted to them anyway. B. A. Steinhagen is the exception, containing obviously significant habitat for *P. amphicaenus*.

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Literature Cited

Bogan, A. E. 2008. Global diversity of freshwater mussels (Mollusca, Bivalvia) in freshwater. Pp. 139-147 *in* E. V. Balian, C. Lévêque, H. Segers, and K. Martens, eds. Freshwater Animal Diversity Assessment. Springer Netherlands, Dordrecht.

Boratyn, G. M., C. Camacho, P. S. Cooper, G. Coulouris, A. Fong, N. Ma, T. L. Madden, W. T. Matten, S. D. McGinnis, Y. Merezhuk, Y. Raytselis, E. W. Sayers, T. Tao, J. Ye, and I. Zaretskaya. 2013. BLAST: a more efficient report with usability improvements. Nucleic acids research 41:W29-W33.

Burch, J. B. 1973. Freshwater inionacean clams (Mollusca: Pelecypoda) of North America, Biota of Freshwater Ecosystems Manual 11. Environmental Protection Agency. https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=20017Q7N.TXT (Accessed 30th January 2020).

Burlakova, L. E., D. Campbell, A. Y. Karatayev, and D. Barclay. 2012. Distribution, genetic analysis and conservation priorities for rare Texas freshwater molluscs in the genera Fusconaia and Pleurobema (Bivalvia: Unionidae). Aquatic biosystems 8:12.

Campbell, D. C., P. D. Johnson, J. D. Williams, A. K. Rindsberg, J. M. Serb, K. K. Small, and C. Lydeard. 2008. Identification of 'extinct' freshwater mussel species using DNA barcoding. Mol Ecol Resour 8:711-724.

Campbell, D. C., J. M. Serb, J. E. Buhay, K. J. Roe, R. L. Minton, and C. Lydeard. 2005. Phylogeny of North American amblemines (Bivalvia, Unionoida): prodigious polyphyly proves pervasive across genera. Invertebrate Biology 124:131-164.

Carlson, S., A. Lawrence, H. Blalock-Herod, K. McCafferty, and S. Abbott. 2008. Freshwater mussel survey protocol for the Southeastern Atlantic Slope and Northeastern Gulf Drainages in Florida and Georgia. U.S. Fish and Wildlife Service: Ecological Services and Fisheries Resources Offices, Georgia Department of Transportation, Office of Environment and Location, Atlanta, GA. https://www.fws.gov/southeast/pdf/methodology/freshwater-mussel-survey-protocol-for-the-southeastern-atlantic-slope-and-northeastern-gulf-drainages-in-florida-and-georgia.pdf. Accessed May 30, 2019.

Escalante, T., G. Rodríguez-Tapia, C. Szumik, J. J. Morrone, and M. Rivas. 2010. Delimitation of the Nearctic region according to mammalian distributional patterns. Journal of Mammalogy 91:1381-1388.

Ford, N., L. Williams, M. Williams, and M. May. 2013. Final Report: Surveys for threatened and endangered mussels and fishes in rivers of Northeastern Texas. Texas Parks and Wildlife Department United States Fish and Wildlife Service Section 6 Grant TX E-130-R.

Ford, N. B., L. R. Williams, M. B. Williams, J. A. Banta, J. S. Placyk Jr., and H. A. Hawley. 2017. Final report: endangered species research projects for freshwater mussels, Region 2, East Texas. Texas Comptroller of Public Accounts. https://comptroller.texas.gov/programs/natural-resources/docs/ComptrollerFinalReport2016reduced.pdf. Accessed 31 January, 2020.

Graf, D. L. and K. S. Cummings. 2007. Review of the systematics and global diversity of freshwater mussel species (Bivalvia: Unionoida). Journal of Molluscan Studies 73:291-314.

Hebert, P. D., A. Cywinska, S. L. Ball, and J. R. deWaard. 2003. Biological identifications through DNA barcodes. Proceedings. Biological sciences 270:313-321.

Howells, R. G., C. M. Mather, and J. A. M. Bergmann. 2000. Impacts of dewatering and cold on freshwater mussels (Unionidae) in B. A. Steinhagen Reservoir, Texas. Tex. J. Sci. 52:93-104.

Howells, R. G., R. W. Neck, and H. D. Murray. 1996. Freshwater mussels of Texas. Texas Parks and Wildlife Department.

Jones, J. W., R. J. Neves, S. A. Ahlstedt, and E. M. Hallerman. 2006. A holistic approach to taxonomic evaluation of two closely related endangered freshwater mussel species, the oyster mussel *Epioblasma capsaeformis* and tan riffleshell *Epioblasma florentina* walkeri (Bivalvia: Unionidae). Journal of Molluscan Studies 72:267-283.

Karlsson, S., B. M. Larsen, L. Eriksen, and M. Hagen. 2013. Four methods of nondestructive DNA sampling from freshwater pearl mussels *Margaritifera margaritifera* L. (Bivalvia:Unionoida). Freshwater Science 32:525-530.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Meintjes, and A. Drummond. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647-1649.

King, T. L., M. S. Eackles, B. Gjetvaj, and W. R. Hoeh. 1999. Intraspecific phylogeography of *Lasmigona subviridis* (Bivalvia: Unionidae): conservation implications of range discontinuity. Molecular Ecology 8:S65-S78.

Marshall, N. T., J. A. Banta, L. R. Williams, M. G. Williams, and J. S. Placyk. 2018. DNA barcoding permits identification of potential fish hosts of unionid freshwater mussels. American Malacological Bulletin 36:42-56.

Neck, R. W. 1986. Freshwater bivalves of Lake Tawakoni, Sabine River, Texas. Tex. J. Sci. 38:241-249.

Pandolfi, G. and E. Orsak. 2019. Texas freshwater mussels of conservation concern. Upper Neches Basin Clean Rivers Program Steering Committee Meeting. https://www.anra.org/divisions/water_quality/crp/pdfs/meetings/2019_Steering_Committee_Meeting/6_ETX_FWM_Pres.pdf. Accessed 31 January, 2020.

Pieri, A. M., K. Inoue, N. A. Johnson, C. H. Smith, J. L. Harris, C. Robertson, and C. R. Randklev. 2018. Molecular and morphometric analyses reveal cryptic diversity within freshwater mussels (Bivalvia: Unionidae) of the western Gulf coastal drainages of the USA. Biological Journal of the Linnean Society 124:261-277.

Plants-Paris, E. 2016. Population dynamics and genetic differentiation of the threatened freshwater mussel species *Fusconaia askewi*, *Fusconaia lananensis*, and *Pleurobema riddellii*. M. S. Thesis, University of Texas at Tyler. https://scholarworks.uttyler.edu/biology_grad/38/. Accessed 31 January, 2020.

Pratt, C. J. 2017. Reevaluating the occurrence and phylogeny of the pigtoe unionid mussels *Fusconaia askewi*, *Fusconaia lananensis*, *Fusconaia flava*, and *Pleurobema riddelli* in Texas. M. S. Thesis, University of Texas at Tyler. https://scholarworks.uttyler.edu/biology_grad/49/. Accessed 31 January, 2020.

Serb, J. M., J. E. Buhay, and C. Lydeard. 2003. Molecular systematics of the North American freshwater bivalve genus *Quadrula* (Unionidae: Ambleminae) based on mitochondrial *ND1* sequences. Mol Phylogenet Evol 28:1-11.

Simpson, C. T. 1914. A descriptive catalogue of the Naiades, or pearly fresh-water mussels. Bryant Walker, Detroit, Mich.

Tiemann, J. S., K. S. Cummings, and C. A. Mayer. 2009. Timed search technique used to evlauate freshwater mussel (Bivalvia: Unionidae) species richness in headwater streams: Is a single one-hour visit enough? Journal of Freshwater Ecology 24:85-92.

US Fish & Wildlife Service. 2017. Texas Heelsplitter fact sheet. https://www.fws.gov/southwest/es/ArlingtonTexas/pdf/Texas%20heelsplitter%20fact%20sheet%2020170301.pdf. Accessed 31 January, 2020.

Walters, A. D., D. Ford, E. T. Chong, M. Williams, N. B. Ford, L. R. WIlliams, and J. A. Banta. 2017. High-resolution ecological niche modeling of threatened freshwater mussels in east Texas, USA. Aquatic Conservation: Marine and Freshwater Ecosystems, in press.

Walters, A. D. and N. B. Ford. 2013. Impact of drought on predation of a state-threatened mussel, *Potamilus amphichaenus*. The Southwestern Naturalist 58:479-481.

Williams, J. D., M. L. Warren Jr., K. S. Cummings, J. L. Harris, and R. J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries 18:6-22.

Zanatta, D. T. and R. W. Murphy. 2006. Evolution of active host-attraction strategies in the freshwater mussel tribe Lampsilini (Bivalvia: Unionidae). Molecular Phylogenetics and Evolution 41:195-208.