## **Aquatic Surveys of Delaware River of Texas**

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Revised on June 21, 2018 to accommodate genetic analysis of the Headwater Catfish.

## By:

Timothy H. Bonner, Ph.D.
Texas State University
Department of Biology/Aquatic Station
601 University Drive
San Marcos, Texas 78666
TBonner@txstate.edu

In collaboration with:

Bradley M. Littrell BIO-WEST, Inc. 1812 Central Commerce Court Round Rock, Texas 78664 blittrell@bio-west.com

## **Study Purpose**

Purpose of this study was to document occurrence of Texas Hornshell *Popenaias popeii*, Rio Grande Cooter *Pseudemys gorzugi*, Gray Redhorse *Moxostoma congestum*, Blue Sucker *Cycleptus elongatus*, and Pecos Springsnail *Pyrgulopsis pecosensis* in the Delaware River (Culberson County), Texas.

Objectives were to compile historical records (i.e., biota, habitat descriptions) for the basin, assess spatial trends in groundwater (and surface water) contributions, quantify water quality and characteristics of habitats and sites along a longitudinal gradient, and quantify occurrences or abundances of gastropods, mussel, fish, and turtle communities.

#### Methods

Historical records on the Delaware River of Texas were searched and compiled for gastropods, mussels, fishes, turtles, hydrogeology, geology, hydrology, water quality, and riparian vegetation.

Field collections were made to quantify aquatic biota, water quality, and stream habitat characteristics in May, June, and August 2016. Sites were selected to represent a longitudinal gradient along the perennially flowing Delaware River (Culberson County, Texas). Three

reaches were delineated within the basin based on stream geomorphology and gradient. Upper Reach was located upstream from Ranch to Market (RM) 652 and consisted of sites with gently sloping gradients, well defined riffle, run, and pool geomorphic units, and substrate sizes ranging from silt to bedrock. Middle Reach was located downstream from RM 652 and also consisted of sites with gently sloping gradients, diversity of geomorphic units, and substrate sizes ranging from clay to cobble. Middle reach generally terminated at a point where the river became a lower gradient stream. Lower reach was within the low gradient section of the river. Riffle habitats were well defined, but runs and pools habitats were less distinct, consisting of slower current velocities, greater water depths, deeper silt substrates, and more cobble substrates.

Multiple sites were selected within each reach. At each site, all available habitats were sampled. In addition, water quality, stream habitat characteristics, freshwater snails, mussels, fishes, and turtles were quantified.

Water quality parameters were measured with a YSI water quality multi-probe. Measured parameters were water temperature ( $^{\circ}$ C), dissolved oxygen (mg/l), specific conductance ( $\mu$ S/cm), pH, turbidity (NTU).

In May and August 2016, fishes were quantified by geomorphic units (i.e., backwater, pool, run, and riffle) and sampled with a common-sense seine (3 m x 1.8 m, mesh size: 3.2 mm) with single to multiple passes or used as a block seine for downstream substrate kick. Current velocity and water depth were quantified for each geomorphic unit with multiple point estimates using a Marsh-McBirney Flow-Mate Model 2000 flow meter and incremental wading rod. Percent substrate, percent large woody debris, and percent vegetation coverage were visually estimated. Length and width of geomorphic unit or area sampled with seines were recorded. Fishes were identified to species. Vouchers were taken at each site, anesthetized with MS-222, and fixed in 10% formalin.

In May and June 2016, freshwater mussels and snails were surveyed using timed searches with both visual and tactile search methods. Shallow habitats were surveyed with the aid of a mask and snorkel. Surface-supplied-air from a Brownie's Third Lung Hookah System was used to allow divers to survey deeper areas. Although surveys were focused on identifying live mussels and snails, any shell material found was also noted. A dip net was also used to collect snails from submerged vegetation.

In May 2016, all turtles encountered during fish and mussel surveys were captured by hand and identified. In June 2016, turtles were surveyed using both passive and active survey techniques. Passive surveys consisted of hoop traps placed in likely habitat and checked periodically. Traps were baited with a variety of baits including canned sardines, fresh cut fish, and melons. Active surveys consisted of visually searching likely habitats using snorkel or dive gear, and catching turtles by hand. All turtles encountered were identified to species and released. For all Rio Grande Cooters captured, standard morphometric measurements (carapace length [mm], carapace width [mm], weight [g]) were taken, the shell was notched, and a blood sample was collected for later use. We only report occurrence and abundance data here, as blood samples were not processed as part of this study.

### **Results**

# Objective 1: Compile historical records for the basin

Delaware River drainage originates on the eastern slope of Guadalupe Mountains (Culberson County, Texas) and terminates about 100 km downstream into the Pecos River (Eddy County, New Mexico). Surface flows are intermittent from its origin for about 40 km and range from intermittent to perennial within the Upper Reach (40 to 55 km from origin), Middle Reach (55 to 70 km), Lower Reach (70 to 85 km), and New Mexico Reach (85 to 100 km). The current Delaware River channel bisects a mix of Holocene deposits and Permian sandstones, siltstones, and limestones, most notably Bell Canyon formation within the Upper Reach, Castile Formation in the Middle Reach, Rustler Formation in the Lower Reach, and Rustler Formation and Holocene deposits in the New Mexico Reach (US Geological Survey 2015). Fresh to brackish groundwater emerges as seeps and springs within the Bell Canyon Formation (Uliana 2001, Brune 2002), Castile Formation (Brune 2002, Stafford 2013), and Rustler Formation (Boghici and Broekhoven 2001).

Median average daily flow is 2 CFS between 1938 through 2016 (USGS Station 08408500; located in the New Mexico Reach; Figure 1). Among daily flow records, 13% report 0 CFS. Zero flow days are common during summer months and extend up to 4 months. Maximum daily flow was 22,000 CFS in October 1955 with a maximum 15-min observation of 81,400 CFS. Discharges >10,000 CFS (15-min observations) occur about once a decade, up to four times in the 1950s, and between the months of May and October.

Removal of invasive riparian vegetation (e.g., Salt cedar *Tamarix*) began within the New Mexico Reach in the 1990s (Brandenburg et al. 2011).

The Texas Hornshell is known to occur in the Black River, New Mexico (a tributary to the Pecos River upstream from the Delaware River confluence) as well as several locations within the Rio Grande drainage of Texas and south into Mexico. Although the Delaware River is within the historical range of the Texas Hornshell (Lang 2001), survey information from the Texas portion of the Delaware is lacking. In an attempt to establish additional populations of Texas Hornshell within New Mexico, the New Mexico Department of Game and Fish (NMDGF) restocked the New Mexico portion of the Delaware River with Texas Hornshell collected from the Black River in 2013 and again in 2015 (Dan Trujillo, NMDGF, personal communication). Many of the adult mussels released have been subsequently located and some were determined to be gravid. However, long-term success of this reintroduction is yet to be determined, and data from the Texas portion of the basin are still lacking.

Among museum records, six species of fishes are reported in the Delaware River of Texas (Hendrickson and Cohen 2015). Four species of fishes (i.e., Red Shiner *Cyprinella lutrensis*, Fathead Minnow *Pimephales promelas*, Plains Killifish *Fundulus zebrinus*, and Green Sunfish *Lepomis cyanellus*) were taken from the Upper Reach in 1968. Four species

of fishes (i.e., Red Shiner, Fathead Minnow, Mexican Tetra *Astyanax mexicanus*, Headwater Catfish *Ictalurus lupus*) were taken from the Middle Reach in 1982.

Among collections taken from New Mexico, 21 fish species are reported in the Delaware River within the New Mexico Reach between 1947 and 2008 (Brandenburg et al. 2011). Among the 12 species reported within the Delaware River in Texas herein (see below), 11 were previously reported in the Delaware River within the New Mexico Reach. The one exception is Gray Redhorse, which was recently stocked in the New Mexico Reach along with Texas Hornshell (Dan Trujillo, NMDGF, personal communication). Among the additional 10 species reported by Brandenburg et al. (2011) from the New Mexico portion of the Delaware, all are reported in the Pecos River (Hoagstrom 2003) and likely represent an established or transitory use of the Delaware River-New Mexico Reach by species more common in the Pecos River. Two additional notable observations from the Brandenburg et al. (2011) report: collections of Headwater Catfish and collections of Roundnose Minnow Dionda episcopa only in the 1940s. Headwater Catfish population within the New Mexico Reach represents one of several introgressed populations within the greater Rio Grande Drainage (McClure-Baker 2011). Roundnose Minnow and *Dionda* in general are more common in tributaries of the main stem Pecos River of New Mexico and Texas (Sublette et al. 1990, Hubbs et al. 2008).

Rio Grande Cooter occurs in the lower Rio Grande and Pecos River drainages of USA and Mexico. They are found in Tamaulipas, Nuevo León, and Coahuila, Mexico, south and west Texas, and southeast New Mexico as far north as the tributaries of Brantley Reservoir, including the Black and Delaware River (Degenhardt et al. 2005, Ernst and Lovich 2009). The Rio Grande Cooter is documented from the Delaware River in New Mexico; however, there is a gap in their distribution along the Delaware River of Texas.

# Objective 2: Assess spatial trends in groundwater (and surface water) contributions

Fresh (829  $\mu$ S/cm) to brackish (mean  $\pm$  1 SD; 4,098  $\pm$  1,410  $\mu$ S/cm) water from seeps and springs of the Bell Formation contribute to surface flows of the Delaware River-Upper Reach (Table 1). The merging of these various sources results in relatively freshwater (1325  $\pm$  143  $\mu$ S/cm) flowing into the Middle Reach, where greater conductivity water (2,525  $\pm$  224  $\mu$ S/cm) from seeps and springs of the Castile Formation elevates conductivity of surface water in the Middle Reach and Lower Reach (range of means: 2,525 – 2,593  $\mu$ S/cm) (Figure 2).

Objective 3: Quantify water quality and characteristics of habitats and sites along a longitudinal gradient

Among reaches, Delaware River was a heterogeneous mix of riffle, run, and pool habitats, ranging in mean depth ( $\pm$  1 SD) from 0.83 m at Site 2 to 1.47 (0.03) m in Sites 8 - 10 and in mean current velocity from 0.02 (0.01) at Site 1 to 0.32 (0.22) at Sites 6 & 7 (Table 1). Dominant substrate was silt among all reaches, although all reaches had sand, gravel, and cobble substrates. Aquatic vegetation ranged from 0% at Site 1 to 100% at Site 2. Mean

water temperature and dissolved oxygen were generally less among tributary sites than main stem sites. Turbidity was greatest at Site 1, but relatively low (<12 NTU) among main stem sites. Riparian vegetation was similar to the New Mexico Reach (Brandenburg et al. 2011) with willow trees, shrubs (e.g., *Baccharis*), grasses, and sedges.

Principal components axes I and II explained 40% of the total variation in habitat and water quality parameters among reaches (Figure 3). Principal component axis I explained 27% of total variation and represented a current velocity and depth gradient. Strongest loadings along PC axis I were riffle (-0.43), current velocity (-0.41), and silt (0.41). Principal component axis II explained 13% of total variation and represented a water quality gradient. Strongest loadings were specific conductance (0.47) and turbidity (0.49). Main stem sites (3 & 4, 6 & 7, and 8 – 10) shifted from shallower to deeper, swifter water to more sluggish water, and lower to higher specific conductance in a longitudinal gradient. Tributary sites (1, 2, and 5) tend to be deeper and Site 1 had higher specific conductance and greater turbidity.

Objective 4: Quantify occurrences or abundances of gastropods, mussel, fish, and turtle communities

# Gastropods

June 2016--Live gastropods were taken from multiple sites. All gastropods collected were identified as members of the Physidae and Planorbidae families. Pecos Springsnail (Hydrobiidae) was not located in the Delaware River.

#### Mussels

May 2016--Despite over 20 person-hours of total search time, live freshwater mussels were not documented. However, multiple long-dead valves of Texas Hornshell were located at two sites within the Middle Reach, and unidentified freshwater mussel shell fragments were identified from one site in the Lower Reach (Table 2).

June 2016-- Approximately 30 person-hours of search time were conducted for freshwater mussels at eight different sites among all reaches. Combined with over 20 person hours conducted in May 2016, this brings the total effort to approximately 50 person-hours of search time within the Texas portion of the Delaware River. Despite this effort, no live freshwater mussels were documented. However, multiple long-dead valves of Texas Hornshell were located at two sites within the Middle reach, and unidentified freshwater mussel shell fragments were identified from one site in the Lower reach.

#### Fishes

A total of 9,251 individuals among 12 species of fishes was taken from the Delaware River of Texas in May and August 2016. *Astyanax mexicanus* was the most abundant species (54% in relative abundance) followed by *Cyprinella lutrensis* (29%) and *Pimephales promelas* (6.5%) among reaches. By reach, abundant species were *Astyanax mexicanus* (70%) and *Fundulus zebrinus* (17%) at Upper Reach, *Astyanax mexicanus* (66%) and *Cyprinella lutrensis* (27%) at Middle Reach, and *Cyprinella lutrensis* (51%) and *Astyanax mexicanus* (32%) at the Lower Reach. See Appendix 1 for results of genetic analysis on *I*.

*lupus*. Genetic analysis was done by D. Lutz-Carrillo, AE Wood Laboratory, Texas Parks and Wildlife Department.

Canonical correspondence model explained 52% (P < 0.01) of the spatial variation in fish community structure based on habitat parameters and reach (Figure 4). Reach and habitat parameters strongly corresponding with CC axis I were Upper Reach (0.90), turbidity (0.34), bedrock (0.31), cobble (-0.28), conductivity (-0.31), and Lower Reach (-0.63). Reach and physical parameters strongly associated with CC axis II were clay (0.46), silt (0.44), Middle Reach (0.37), pool (0.36), Lower Reach (-0.38), cobble (-0.47), and riffle (-0.49). Fundulus zebrinus and Astyanax mexicanus associated with riffle and pool habitats within the Upper Reach, although A. mexicanus was common in all reaches. Moxostoma congestum, Micropterus salmoides, Micropterus punctulatus, and Lepomis macrochirus associated with run and riffle habitats with cobble and boulder substrates in the Lower Reach. Lepomis cyanellus and Cyprinus carpio associated with pool habitats, greater depths, clay to silt substrates, and the Middle Reach. Cyprinella lutrensis and Pimephales promelas were common within run and riffle habitats among all reaches.

### **Turtles**

May 2016--Rio Grande Cooter was documented from two sites within the Middle Reach. Other turtle species documented included Redear Slider *Trachemys scripta* and Texas Spiny Softshell *Apalone spinifera*.

June 2016-- Seven Rio Grande Cooter were captured by hand using active searching. This included two males ranging in carapace length from 125-187 mm, and 5 females ranging in size from 231-256 mm carapace length. All seven of these were captured from the Middle Reach. One Rio Grande Cooter was observed but not captured from the Lower Reach. Other turtle species captured via active searching included Redear Slider, Texas Spiny Softshell, Yellow Mud Turtle *Kinosternon flavescens*, and Common Snapping Turtle *Chelydra serpentina*.

Despite approximately 30 trap-days, no Rio Grande Cooter were captured in the hoop traps. Other turtle species captured in the traps included Redear Slider *Trachemys scripta*, Texas Spiny Softshell *Apalone spinifera*, and Yellow Mud Turtle *Kinosternon flavescens*.

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Table 1. Summary of stream characteristics and water quality taken from multiple sites and reaches of the Delaware River of Texas (Culberson County) May through August 2016.

	Upper			Middle		Lower
	Tributary	Tributary	Mainstem	Tributary	Mainstem	Mainstem
	Site 1	Site 2	Sites 3 & 4	Site 5	Sites 6 & 7	Sites 8 - 10
N of habitats sampled	7	1	17	4	19	26
Area (m²)	257	30	1,151	1,943	1,287	2,484
% Riffle			41		37	31
% Run	29	100	29	50	42	46
% Pool	71		29	50	21	19
% Backwater						4
% Clay				25		3
% Silt	77	100	54	75	50	47
% Sand			7		17	2
% Gravel	3		25		17	11
% Cobble	6		6		15	29
% Boulder	1		1		1	7
% Bedrock	13		7			
% Large woody debris			1.5	1.5		3.7
% Total vegetation		100	31	38	45	33
% Chara			19	38	35	24
% Filamentous Algae			7		6	3
% Detrital Algae			4			
% Emergent Vegetation		100	2		3	6
Mean (± 1 SD) Temperature (°C)	19.9 (6.18)	17.5	22.4 (3.12)	19.0 (0.11)	23.1 (5.14)	25.9 (4.42)
Mean (± 1 SD) Dissolved Oxygen (mg/l)	3.3 (1.73)	4.3	9.0 (1.26)	6.4 (1.50)	6.7 (1.45)	6.8 (0.66)
Mean (± 1 SD) Specific Conductance (μS/cm)	4,098 (1,410)	829	1,325 (143)	2,264 (224)	2,525 (209)	2,593 (420)
Median pH	8.0	7.4	8.5	7.3	7.7	8.0
Mean (± 1 SD) Turbidity (NTU)	89 (51.8)	14	8.6 (6.60)	9.7 (3.12)	6.5 (0.41)	11.5 (2.33)
Mean (± 1 SD) Current Velocity (cm/sec)	0.02 (0.012)	0.19	0.24 (0.200)	0.07 (0.083)	0.32 (0.217)	0.19 (0.176)
Mean (± 1 SD) Depth (m)	1.1 (0.55)	0.83	0.85 (0.763)	1.27 (0.83)	1.16 (1.01)	1.41 (1.07)

Table 2. Relative abundances of fishes and occurrences of mussels and turtles taken from multiple sites and reaches of the Delaware River of Texas (Culberson County) May through August 2016.

	Upper			Middle		Lower
	Tributary	Tributary	Mainstem	Tributary	Mainstem	Mainsten
	Site 1	Site 2	Sites 3 & 4	Site 5	Sites 6 & 7	Sites 8 - 10
Fish Relative Abundance (%)						
Common Carp Cyprinus carpio					0.4	0.3
Red Shiner Cyprinella lutrensis			9.2		40	51
Fathead Minnow Pimephales promelas			3.9		0.4	14
Gray Redhorse Moxostoma congestum						0.3
Mexican Tetra Astyanax mexicanus	61		73	86	56	32
Black Bullhead Ameiurus melas			1.9		0.5	< 0.1
Headwater Catfish Ictalurus lupus					1.5	0.9
Plains Killifish Fundulus zebrinus	39		11		0.2	
Green Sunfish Lepomis cyanellus			1.1	14	1.0	1.4
Bluegill Lepomis macrochirus						0.1
Largemouth Bass Micropterus salmoides						0.1
Spotted Bass Micropterus punculatus						0.1
Total N of fishes	616	0	2,552	858	1,656	3,569
Occurrences (0 = absent, 1 = present):						
Mussels:						
Texas Hornshell Popenaias popeii						
Live	0	0	0	0	0	0
Dead (complete valves)	0	0	0	0	1	0
Mussel Shell Fragments (not Corbicula)	0	0	0	0	0	1
Asian Clam Corbicula shells	0	0	1	1	1	1
Furtles:						
Rio Grande Cooter Pseudemys gorzugi	0	0	0	1	1	1
Redear Slider Trachemys scripta	1	0	1	1	1	1
Texas Spiny Softshell Turtle Apalone spinifera	1	0	1	1	1	1
Yellow Mud Turtle Kinosternon flavescens	0	0	0	1	0	0
Common Snapping Turtle Chelydra serpent ina	0	0	0	0	1	0

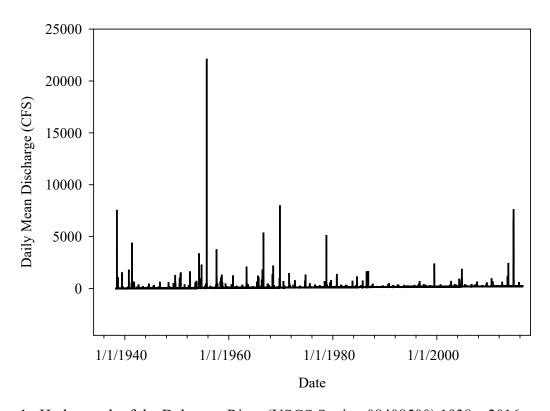


Figure 1. Hydrograph of the Delaware River (USGS Station 08408500) 1938 – 2016.

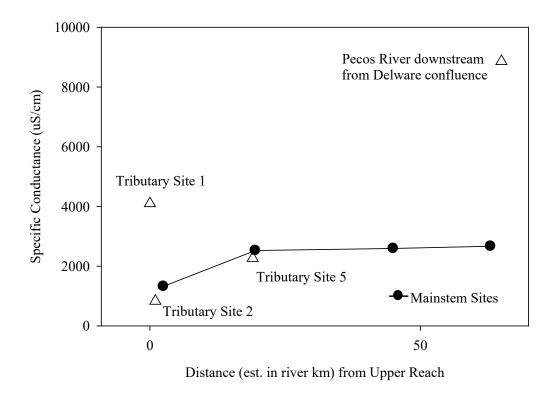


Figure 2. Spatial trends (upstream to downstream) trends in groundwater contributions to surface flows as estimated by specific conductance.

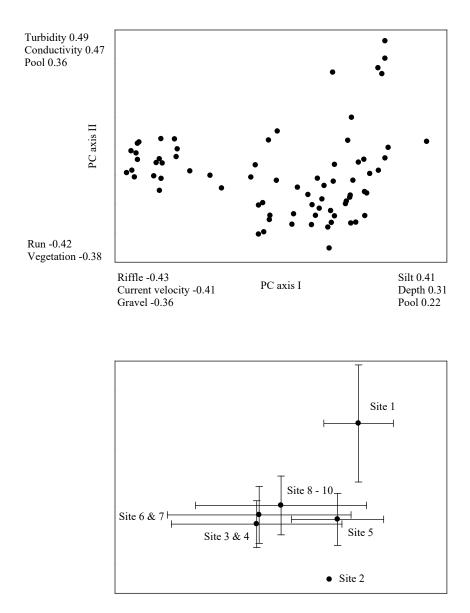


Figure 3. Individual sample scores (top panel) and mean  $\pm$  1 SD of sample scores by site (bottom panel) for principal components axes I and II. Habitat parameter loadings are provided next to the strongest parameters.

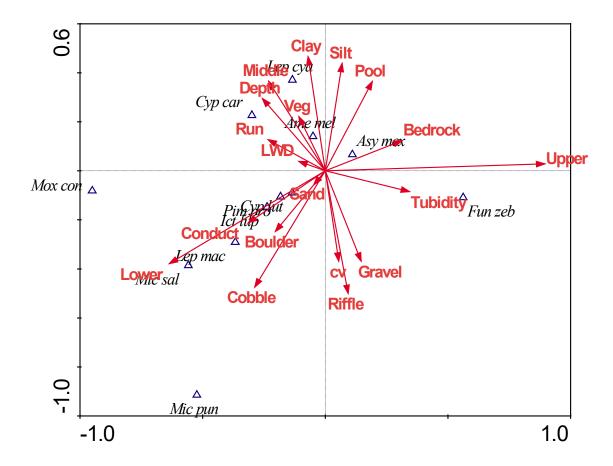


Figure 4. Fish-habitat associations among three reaches of the Delaware River taken May through August 2016. Fishes are identified by the first three letters of the genus and specific names (see Table 2).

## Appendix 1.

Twenty-nine tissue samples or whole specimens (014-0024 – 0052) were preserved from field identified Headwater Catfish (HC: *Ictalurus lupus*) or Channel Catfish (CC: *I. punctulatus*) collected (8/2016) from the Delaware River (Culberson Co., TX). Total nucleic acids were isolated from each sample. The Cytochrome B gene (CytB) was amplified and sequenced bidirectionally using the primers MT11 and MT29. Trimmed and assembled bi-directional sequences were aligned (~950 bases) and used to construct a maximum likelihood phylogeny (Figure 1).

Three of the 29 sampled fishes were identical in sequence to a HC-haplotype A from McClure-Baker et al. (2010), indicating that their mitochondrial genetic background is HC (Figure 1). The remaining sampled fishes comprised the CC clade along with reference samples of CC from McClure-Baker et al. (2010), indicating that their mitochondrial genome is derived from CC.

Our assessment of the catfish populations in the Delaware River were similar to the assessment of McClure-Baker et al. (2010). Specifically, a majority (90%) of the individual's mitochondria originated from CC and a minority (10%) originated from HC. McClure-Baker et al. (2010) further stated "...that interspecific genetic interaction occurs wherever HC and CC are found together". Given that HC and CC mitochondrial genomes are found in the Delaware River, it is likely that the three sampled specimens in mitochondrial HC-Clade A are introgressed at some level with CC and not pure HC.

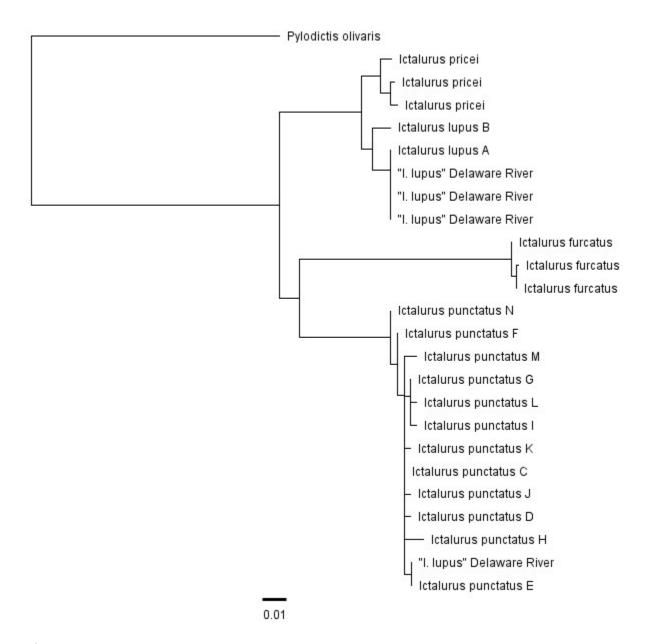


Figure 1. Maximum likelihood phylogeny of representative Cytochrome B haplotypes from collected Delaware River samples, reference taxa, and an outgroup.