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# RESURRECTING ILLINOIS' BIOLOGICAL HERITAGE, REINTRODUCTION OF A LOCALLY EXTIRPATED STONEFLY

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## INTRODUCTION

Many species of aquatic insects have been extirpated from Illinois waters. We know this only because their historic presence was amply documented by the scientists who came before us. The past distribution of stoneflies in Illinois is known through the efforts of Theodore H. Frison, former Chief of the Illinois Natural History Survey (INHS) in the 1930s and 1940s. He completed statewide monographs of winter-emerging stoneflies (1929) and for the entire state (1935) and then focused his attention on regional and national treatments. His legacy at the INHS includes much of the 22,000 records and 113,000 stonefly specimens in our insect collection. These specimens have been digitized and their identification is up-to-date, providing the best documented stonefly assemblage anywhere in the world. Webb (2002) documented declines in the so-called "winter stoneflies" and DeWalt et al. (2005) used 5,000 Illinois records to determine changes in the fauna throughout the 20$^{th}$ Century. The latter found that 22 of 77 species ever known from the state have been extirpated or extinguished (2 endemics). Those experiencing declines were mostly the large, predatory, long-lived species, many of which were widely distributed within the state and the region. The till plain of Illinois experienced the greatest loss of species. Most losses are correlated with a time of massive, indiscriminate DDT usage across the state (Favret & DeWalt 2002), although there may be myriad other causes for their decline.

One species lost from most of its original Illinois range was *Acroneuria frisoni* Stark & Brown, 1991 (Plecoptera: Perlidae) (Fig. 1) (DeWalt et al. 2005), a cryptic species that was not separated from other similar species until recently. This species is predicted to occur mostly in eastern Illinois, with a preference for permanent water in glaciated regions of the state (DeWalt et al. 2009). This distribution is widened westward across the uplifted areas of the Shawnee Hills. Its historic range in Illinois, based on Pre-1950 records from the INHS Insect Collection database, totaled 46,660 km$^2$ (Fig. 2). Its range has shrunk dramatically since then to approximately 920 km$^2$, its only refuge in Illinois being the Shawnee Hills (DeWalt et al. 2008). Recent sampling suggests that this species has experienced similar range reduction in glaciated areas of Indiana and Ohio. The closest extant populations in neighboring states include SW Missouri, NE and central Indiana, and W Kentucky. Replacement of lost historical populations would mean adult or larval dispersal of up to 300 km over poor habitat and through waters that are moderately to severely polluted (DeWalt et al. 2008). To date there has been no evidence of natural recolonization of this or any other stonefly species lost from Illinois. Returning this or other aquatic insects to parts of their former range in Illinois will likely require that they be physically transplanted.

Translocation of extirpated species should not take place without adequate knowledge of population genetic structure of original and of potential source populations. This information is critical if one wishes to reintroduce populations that are pre-adapted to local climate and other environmental conditions and that mirror historical populations genetically. Because much of Illinois has experienced multiple glacial events, population genetic structure of its species may well be complex. For instance, most of Illinois has been glaciated by the Illinoisan glacial advance. Another 45% has been scraped flat by the Wisconsinan glaciers. Still, there are two large areas that were not buried under ice sheets; although, they may have experienced cold, dry, and windy conditions associated with glaciers. These areas include the Shawnee Hills of southern Illinois and the Driftless area in the northwest corner of the state. Because of this complex glacial history, populations of some species are relatively new (10-15 thousand yr. bp., such as those in the till and drift plains of the north), while others could be older (100 thousand to over a million yr. bp, Southern Till Plains), and others older still (unglaciated sections). Add to this the close proximity of two major glacial refugia (western flanks of the Appalachians and Ozarks of Missouri and Arkansas) and several migration corridors leading to Illinois (Tennessee, Cumberland, Ohio, and Mississippi rivers and Cincinnati Arch) and one could possibly have very complex genetic population structures that make the choice of source populations for reintroduction efforts a difficult proposition. A single phylogeographic study on an aquatic insect in eastern North America has been conducted (Heilveil & Berlocher 2006); hence, no generalizations and few predictions can be made as to how *A. frisoni* recolonized Illinois after the last glacial maximum.

The major goal of this project is to reintroduce *A. frisoni* to parts of eastern Illinois from where it has been extirpated since the early 1960s. A candidate stream in the region is the Middle Fork of the Vermilion River, a National Scenic River. This stream is of the best water quality in the region (Sangunett 2005) and is surrounded by much public property above the Interstate 74 corridor. Much preparation is necessary to begin such a reintroduction. Tasks include the following: (1) update what is known of the historical and contemporary distribution of this species, (2) conduct a rangewide screening of the population genetic structure of the species, (3) select source populations for reintroduction from the genetic screening results, (4) reintroduce the species, and (5) conduct follow-up studies to determine if the reintroduction was successful.

## METHODS

*Updating Distribution Information.*-Specimens of *A. frisoni* were examined from those found in the INHS Insect Collection (INHS) and from all regional research collections including the Field Museum of Natural History (FMNH), Cleveland Museum of Natural History (CMNH), Purdue University Entomological Research Collection (PERC), Michigan State University (MI SU), Southern Illinois University Carbondale Insect Collection (SIUC), Royal Ontario Museum (ROM), University of Notre Dame (UND), University of Quelph, Ontario (DEBU), and the Canadian National Collection (CNC). All specimens were examined and identifications updated using the keys of Stark (2004) and Poulton & Stewart (1991). Using the updated distribution data, additional sampling was conducted within Illinois and nearby states to determine if historical locations still held the species and to collect specimens for genetic work. Adults were attracted to UV lights and nymphs were collected using dipnets. Up to 10 individuals were collected when encountered. All new specimens were preserved in 95% EtOH. Voucher specimens reside in the INHS.

*Population Genetic Structure and Selection of Source Populations.*- The sequence divergence of the entire *Cytochrome oxydase subunit I* (COI) mitochondrial gene was used as a marker to determine population genetic structure of *A. frisoni*. This gene accrues mutations at a relatively rapid rate, fast enough to track changes in the populations of *A. frisoni* over the past 10,000 to 100,000 years. Up to 10 individuals from populations throughout the species' range were sampled and sequenced. Vouchers for molecular work were deposited in INHSIC.

DNA was isolated in the laboratory of Dr. Rosanna Giordano at the INHS and sequence data produced at the University of Illinois' Keck Center. Sequences were aligned manually and unique sequence (haplotypes) frequencies were generated using Collapse (Posada & Crandall 1998). To display the evolutionary relationships between individual sequences, haplotype networks were built using the program TCS (Templeton et al. 1992). These networks consist of lines drawn between related haplotypes in relation to geography and helped to determine from where extirpated populations are likely to have migrated. Inferring the genetic structure of historical populations was necessary since isolation of DNA from old museum specimens was not entirely successful. Pinned specimens from Vermilion River basin (Wabash drainage) from 1938 and 1943 were not successfully sequenced. However, sequences from the far western Shawnee Hills were obtained from specimens captured in 1986 and stored at room temperature in 70-80% EtOH.

Sequences were aggregated within six subregions (Ozarks, S IL, IN/OH, C KY, C TN, E TN) to test for within and between group genetic differentiation using an analysis of molecular variation (AMOVA) and Mantel Test. These analyses test the hypothesis that there is significant population structure across the subregions. Pie charts of the relative frequency of haplotypes in each subregion were produced and displayed on a map of the region to clarify the relationships between them. The uniqueness of the haplotype frequency will help to determine the most appropriate source location for reintroduction.

*Reintroduction of A. frisoni*.-Two source locations in Indiana were chosen: Owen Co., Rattlesnake Creek, 1.6 km W Cuba, 39.37886, -86.81607 and Lawrence Co., Leatherwood Creek, 5.5 km NE Bedford, 38.8992, -86.4133. Eggs are the best life stage for transplantation since they may be collected in much larger numbers than either nymphs or adults. Females of *A. frisoni* readily come to UV lights, often holding egg batches at the tip of their abdomen that contain up to 300 eggs. Eggs were removed from the females and deposited separately in a Whirlpac™ bag with stream water. Females were preserved in 95% EtOH for later genetic studies. Eggs were returned to Illinois that evening, counted, and deposited in the stream as early the next day as practicable.

Three locations were chosen at the Middle Fork Vermilion River to deposit eggs: Collison bridge, Possum Lake canoe put-in, and Glenburn Creek Road. The number of eggs deposited at these locations was increased with distance downstream with the hope that a test of the number of eggs needed to successfully start a population could be conducted.

As with all streams in Illinois during the spring and early summer of 2009, water levels were high in the Middle Fork of the Vermilion River. Since the eggs of *A. frisoni* lack a means to adhere to substrates, a cylinder was used to slow the current speed of the area where eggs were deposited (Fig. 3). This allowed the eggs to settle into the substrate before the cylinder was removed. Egg batches were placed at least 3 m from each other to avoid disturbance of previously deposited batches.

*Evaluation of Success of Reintroduction*-.The life cycle of *A. frisoni* is univoltine, with eggs hatching within one month after being deposited in the stream (Ernst and Stewart 1985) Individuals approach 10 mm length by November, making them easy to detect and identify once collected. Hence, nymphs were sampled on two separate occasions in November, 2009, using dipnets. Additional nymph sampling is planned for April, 2010 and again in June, 2010 using UV light traps for adults.

## RESULTS

*Updating Distribution Information.*-This project resulted in the examination of 2,503 specimens. Most of the 1,136 unique records (a site/date event) were from the INHS collection or newly collected, but a large number of records were derived from the Dr. Joseph A. Beatty stonefly collection (donated by SIUC Zoology Chairman to INHS), Southern Illinois University, Purdue University, the Canadian National Collection, University of Guelph (Ontario), and the Royal Ontario Museum (Fig. 4).

Regionally, this species is distributed from eastern Oklahoma through the Ozarks, Tennessee, Kentucky, Pennsylvania, and north to southern Ontario and central Michigan (Fig. 5). Historically, it was abundant in high gradient streams throughout the region, even venturing onto the prairie where bottom substrates were course enough to provide them refuge from predators and places from which to ambush prey. Recent sampling has demonstrated that there are now large areas of the till and drift plain that no longer support this species. Presumably this is because once glaciated areas are flatter and inviting to agriculture. Indeed, the only locations where the species still resides in Illinois and Indiana are areas that are too steep to farm. It is probably that this environmentally sensitive species, and many like it, were the victims of indiscriminant DDT usage in the 1950s (DeWalt et al. 2005). Most sensitive stoneflies and aquatic insects declined precipitously after this time frame.

Within Illinois, this species has a long historical record, having been collected from 55 unique locations and as early as 1887 from the city of Chicago (Table 2). Historically, there were locally abundant populations in several locations in Coles, Champaign, Kankakee, Vermilion and Will counties in the north. In addition, smaller populations occurred in Indian Creek (LaSalle Co.), the Fox River (Kane Co.), and the Illinois River (at Ottawa). These northern populations have been extirpated since at least the mid-1950s, but in most cases much earlier.

South of Charleston, where the landform drops in elevation and stream bottoms become sand, this species drops out until near the Shawnee Hills. In this area, the species is locally abundant in some years, and rare in others depending upon the availability of water; it cannot tolerate complete drying of the stream bed. In the eastern Shawnee Hills, Lusk Creek, Gibbons Creek, and Big Grand Pierre support the largest populations of this species. Small numbers of individuals have also been collected from Bay Creek in the fast flowing section that runs through Bell Smith Springs. The central portion of the Shawnee Hills appears to be too narrow to produce streams large enough and with coarse enough substrates to support this species. Once one gets to Anna, streams again have large enough drainages to support this species. The most consistently large populations in the west are supported in a small tributary to the Big Muddy River at a location locally known as the Clear Springs Picnic Area (no such designation can be found on a map). Small populations are also present in Clear Creek, Hutchins Creek, and Indian Creek.

*Population Genetic Structure and Select Source Populations.*-A total of 1,511 base pairs (bp) of the mtDNA COI gene was sequenced for a total of 30 populations and 223 individuals (Table 2). Across the range of *A. frisoni* there were 121 unique haplotypes found, although the mean nucleotide diversity was consistently low across the range. Tajima’s D, a measure of selection pressure, indicates that populations were under selection and rapidly expanding.

Sequences formed four independent networks (Fig. 6); their relationships cannot at this time be resolved. The network with the largest number of individuals and haplotypes includes southern Illinois, Indiana, central Kentucky, and central TN. This network was composed of two frequently occurring haplotypes that spawned a large number of haplotypes with from one to three bp differences between them. The data at hand suggest that central Tennessee populations gave rise to Illinois populations and all populations north and east of the Ohio River.

A second major grouping consists of sequences derived from Arkansas and Missouri Ozark Mountains specimens with a sizeable contingent from southern Illinois. These haplotypes are many steps away from the Indiana/Ohio and central Tennessee and Kentucky networks, a sign that these populations were separated from eastern ones a very long time ago and have evolved largely independently. An exception to this occurs in the Shawnee Hills, which appears to be a zone of secondary contact between eastern and western refugia. Two small networks, both contained within central and eastern Tennessee, did not link to others. The relationship of these small networks will likely resolve themselves once more specimens and populations are sequenced from Kentucky and Tennessee.

Pie charts of the distribution of haplotypes suggest that the Ozarks are an evolutionary lineage of the species that has been separated from eastern populations for many tens of thousands of years (Fig. 7). Some leakage of Ozark haplotypes has occurred into the Shawnee Hills, as have some eastern haplotypes. The Indiana/Ohio subregion appears to be most closely aligned with central Tennessee and Kentucky. An AMOVA demonstrated that most genetic differentiation occurred between (63%) subregions ($p<0.001$). The Mantel Test found significant differences between all subregions with the exception of Kentucky and central Tennessee. The southern Illinois subregion had the highest nucleotide diversity, due to it being a zone of secondary contact between east and west subregions.

*Reintroduction of A. frisoni*.-Nothing is known of the egg production of this species and this effort only adds a little information. This species appears to require outdoor, humid conditions to batch eggs, conditions that could not be adequately replicated for females in the laboratory. Females reared from nymphs and mated in the laboratory did not produce eggs within a week of confinement. Therefore, egg production data is biased since one could not determine if an egg batch from the field is the first or any subsequent batch and it could not be determined if the female would shed all her eggs. It is assumed that this species produces multiple egg batches since museum specimens with dates from the end of June held but a few eggs in the abdomen.

Thirty-five egg batches and a total of 7,325 eggs were deposited into the Middle Fork of the Vermilion River at three locations during June of 2009 (Table 3). The locations for egg release are presented in the order added to the stream and spanning a period of nearly two weeks. This is possibly why there is a large discrepancy between average egg counts between the three locations. The females at the second and third sites were probably producing small, second or third egg batches.

*Evaluation of Success of Reintroduction*-.No nymphs were recovered from the Middle Fork of the Vermilion River during our November sampling, despite a total of 18 person hrs dipnetting. This drainage had received record rainfall and cool weather throughout the growing season. On the two dates of sampling, the river was clear, but nearly twice its average volume for the season. Sampling later in March or April may yield better conditions. Extensive light trapping in early to mid-June may also yield specimens that confirm that the reintroduction was successful.

## CONCLUSIONS

*Acroneuria frisoni* is an aquatic insect with a wide distributional range (Fig. 5); unfortunately, there is ample evidence that its range has shrunk dramatically since 1950 (DeWalt et al. 2008). Because of this, DeWalt et al. (2005) suggested that in Illinois this species should have a NatureServe rating of S2 based on it being known from relatively few extant locations and those locations being restricted to the Shawnee Hills. In addition, DeWalt et al. (2008) found that for this species and many others, natural recolonization would have to take place over distances of up to 300 km across agricultural lands and along moderately to severely polluted waterways.

The Middle Fork of the Vermilion River supported environmentally sensitive aquatic insects in the past, has undergone degradation from agricultural runoff and coal mining activities, and has again improved in quality. The adjacent Salt Fork drainage supported *A. frisoni* as late as 1940s and it is probably that it also occurred in the Middle Fork of the Vermilion River. Since then, coal mining has ceased, the state has purchased large tracts of land adjacent to the stream to protect it against agricultural runoff and to provide habitat. Its water quality has improved dramatically, placing it far above regional water quality rating (Sangunett 2005). The stream has been a National Scenic River for decades.

Reintroduction of *A. frisoni* to the Middle Fork of the Vermilion River has begun. The source population was determined by state-of-the-art population genetics from throughout the species' range. Extant populations in southern Illinois were too diverse genetically to be used as a source for east-central Illinois reintroductions. Populations in central Indiana were chosen as the appropriate source because there was but one refuge contributing to these populations. In June, 7,325 eggs were released into the stream at three locations (Table 3). Although no nymphs of this species have been recovered from the Middle Fork of the Vermilion, future efforts in spring and summer may produce them. This would be a wonderful way to reclaim some of Illinois' lost biological heritage.

Recurrent funding has been secured to study the reintroduction of this species and other aquatic insects to the Middle Fork of the Vermilion River. While these funds are small, they will help to pay for trips to collect more eggs and to help finish the molecular work. Perhaps within the next five years Illinois can claim to have reintroduced 2-3 species of stoneflies that maintain robust populations.

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Table 1. Historic and contemporary locations for Acroneuria frisoni within Illinois. Date1 and Date2 correspond to first and last dates collected at a given location. Number of records indicates the number of unique site/date events that this species was collected. The number of specimens is provided as a qualitative index of abundance.

| County | Locality | Parks | Stream | Lat. | Long. | Date1 | Date2 | #Records | #Specimens |
|---|---|---|---|---|---|---|---|---|---|
| Champaign | 2 km N Homer | | Salt Fk. Vermilion R. | 40.05600 | -87.95800 | 07/19/1924 | 06/11/1931 | 104 | 213 |
| Champaign | 5 mi NNE Mahomet | | Sangamon R. | 40.26470 | -88.37920 | 09/01/1928 | 09/01/1928 | 1 | 2 |
| Champaign | Champaign | | Boneyard Cr. | 40.11200 | -88.23700 | 6/12/1888 | 06/26/1944 | 22 | 22 |
| Coles | 3 .5 km SE Charleston | | Embarras R. | 39.45700 | -88.14700 | 03/22/1931 | 03/28/1932 | 17 | 23 |
| Coles | 6 km NE Charleston at IL-16 | | Embarras R. | 39.51030 | -88.11330 | 12/06/1930 | 12/06/1930 | 1 | 1 |
| Coles | Ashmore | | Polecat Cr. | 39.52900 | -88.01500 | 07/01/1955 | 07/31/1955 | 1 | 1 |
| Coles | | | | 39.52200 | -88.28400 | 07/07/1958 | 07/07/1958 | 1 | 1 |
| Cook | Chicago | | | 41.85000 | -87.65000 | 9/29/1881 | 9/29/1881 | 1 | 1 |
| Gallatin | | Pounds Hlw Rec. | Robinette Cr.? | 37.63000 | -88.26860 | 06/14/1990 | 06/14/1990 | 1 | 1 |
| Gallatin | Shawneetown | | | 37.69694 | -88.13667 | 06/21/1927 | 06/21/1927 | 1 | 1 |
| Hardin | 0.3 mi upstr confl. Goose Cr. | | Big Cr. | 37.49806 | -88.32750 | 02/28/1976 | 02/28/1976 | 1 | 1 |
| Hardin | 2.4 km NE Elizabethtown | | Peters Cr. | 37.48800 | -88.25600 | 09/08/1974 | 09/13/1980 | 3 | 6 |
| Hardin | 3.8 mi S Karbers Ridge | | Big Cr. | 37.52300 | -88.32300 | 06/03/1980 | 04/06/1992 | 2 | 6 |
| Hardin | 4 mi NNW Elizabethtown | | Big Cr. | 37.47400 | -88.33500 | 10/31/2002 | 10/31/2002 | 2 | 2 |
| Hardin | 5.2 mi WNW Cave In Rock | | Peters Cr. | 37.46600 | -88.25800 | 05/06/1972 | 05/06/1972 | 2 | 2 |
| Hardin | Elizabethtown | | Ohio R. | 37.44500 | -88.30500 | 05/05/1972 | 05/05/1972 | 2 | 3 |
| Jackson | Clear Springs Picnic Area | Shawnee Natl For. | | 37.62530 | -89.42805 | 05/07/1971 | 01/01/2010 | 14 | 84 |
| Jackson | Murphysboro | | Big Muddy R. | 37.76500 | -89.32300 | 08/26/2000 | 08/26/2000 | 2 | 2 |
| Jackson | 2.5 mi NE Makanda | | Indian Cr. | 37.65612 | -89.17973 | 05/29/1972 | 05/29/1972 | 1 | 1 |
| Jefferson | Belle Rive | | | 38.23276 | -88.74062 | 10/24/2000 | 10/24/2000 | 1 | 1 |
| Johnson | 2.5 mi E Simpson | | Bay Cr. | 37.46100 | -88.71200 | 04/05/1975 | 04/05/1975 | 1 | 1 |
| Kane | Aurora | | Fox R. | 41.75800 | -88.31700 | 07/17/1927 | 07/17/1927 | 1 | 1 |
| Kankakee | Kankakee | | Kankakee R. | 41.12000 | -87.86100 | 04/28/1931 | 06/29/1939 | 8 | 21 |
| Kankakee | Momence | | Kankakee R. | 41.16700 | -87.66300 | 06/04/1932 | 10/28/1938 | 7 | 27 |
| Kankakee | | | | 41.13100 | -87.99100 | 06/01/1901 | 06/01/1901 | 1 | 1 |
| La Salle | Ottawa | | | 41.34570 | -88.84320 | 06/12/1901 | 06/12/1901 | 1 | 1 |
| La Salle | 3.5 km W Serena at US-52 | | Indian Cr. | 41.48500 | -88.77400 | 05/16/1938 | 05/16/1938 | 1 | 1 |
| LaSalle | Oglesby Bailey Falls | | Vermillion R. | 41.30210 | -89.03760 | 07/02/1946 | 07/02/1946 | 1 | 1 |
| Pope | 0.8 km N Herod | | Gibbons Cr. | 37.58420 | -88.44220 | 04/12/2008 | 04/12/2008 | 5 | 34 |
| Pope | 2.5 km NNE Eddyville | | Lusk Cr. | 37.51900 | -88.57500 | 10/01/1994 | 10/01/1994 | 1 | 1 |
| Pope | 2.5 mi SE Eddyville | | Lusk Cr. | 37.47270 | -88.54760 | 03/01/1983 | 05/30/1983 | 1 | 1 |
| Pope | 2.8 km SSE Herod | | Big Grand Pierre Cr. | 37.55764 | -88.42266 | 11/15/2008 | 11/15/2008 | 1 | 8 |

| County | Locality | Parks | Stream | Lat. | Long. | Date1 | Date2 | #Records | #Specimens |
|---|---|---|---|---|---|---|---|---|---|
| Pope | 2.9 mi SE Eddyville | | tributary Lusk Cr. | 37.48640 | -88.53740 | 02/20/1982 | 03/07/1982 | 1 | 7 |
| Pope | 3.4 km ENE Eddyville | Shawnee Natl For. | Bear Branch | 37.51610 | -88.55010 | 06/30/2003 | 06/30/2003 | 1 | 1 |
| Pope | 4 mi NW Eddyville | Bell Smith Springs | Bay Cr. | 37.51767 | -88.65719 | 12/19/2009 | 12/19/2009 | 1 | 3 |
| Pope | 4.8 km SE Eddyville | | Lusk Cr. | 37.47260 | -88.54750 | 05/24/1940 | 06/21/1993 | 4 | 5 |
| Pope | 7 km S Eddyville | Shawnee Natl For. | Quarrel Cr. | 37.43540 | -88.58500 | 07/06/2003 | 07/06/2003 | 1 | 1 |
| Pope | 7.4 km ENE Eddyville | | Little Lusk Cr. | 37.52830 | -88.50640 | 07/10/2003 | 07/10/2003 | 1 | 1 |
| Pope | 8 km ENE Eddyville | Shawnee Natl For. | E. Fk. Little Lusk Cr. | 37.52460 | -88.49970 | 07/07/2003 | 07/07/2003 | 1 | 4 |
| Pope | Bell Smith Springs | Bell Smith Springs | Spring Cr. at Bay Cr. | 37.51700 | -88.65570 | 09/02/1990 | 09/02/1990 | 2 | 7 |
| Pope | Glendale at IL-145 | | Hayes Cr. | 37.45500 | -88.67000 | 09/19/1998 | 09/20/2000 | 2 | 7 |
| Pope | Golconda | | Mouth of Lusk Cr. | 37.36720 | -88.48640 | 05/13/1932 | 05/13/1932 | 1 | 1 |
| Pope | 7 mi NNE Golconda at IL-146 | | Big Grand Pierre Cr. | 37.45890 | -88.43010 | 09/11/2001 | 09/11/2001 | 1 | 1 |
| Union | 3 mi E Wolf Lake | | Clear Cr. | 37.50167 | -89.37667 | 04/13/1977 | 09/29/1991 | 3 | 4 |
| Union | Anna | | | 37.46028 | -89.24694 | 06/08/1907 | 06/08/1907 | 1 | 1 |
| Union | Hutchins Creek | | Hutchins Cr. | 37.54364 | -89.39955 | 10/15/1984 | 10/15/1984 | 1 | 3 |
| Vermilion | 2 mi W Oakwood | | Stony Cr. | 40.11300 | -87.83300 | 05/27/1905 | 09/28/1955 | 6 | 18 |
| Vermilion | 3.4 km S Oakwood | | Salt Fk. Vermilion R. | 40.08277 | -87.78061 | 06/16/1925 | 06/04/1948 | 9 | 13 |
| Vermilion | Danville | | Salt Fk. Vermilion R. | 40.10370 | -87.71700 | 04/05/1936 | 09/22/1943 | 2 | 5 |
| Vermilion | Ogden | | Salt Fk. Vermilion R. | 40.11400 | -87.95600 | 06/14/1930 | 06/17/1933 | 2 | 2 |
| Vermilion | Old Oakwood Dam | | Salt Fk. Vermilion R. | 40.08300 | -87.74600 | 04/12/1933 | 07/07/1936 | 3 | 5 |
| Will | New Lenox | | Hickory Cr. | 41.51485 | -87.96548 | 10/05/1940 | 10/05/1940 | 1 | 3 |
| Will | Ritchie | | Fk.ed Cr. | 41.25500 | -88.10600 | 04/10/1935 | 04/10/1935 | 1 | 1 |
| Will | Wilmington | | Kankakee R. | 41.30610 | -88.15100 | 06/12/1931 | 06/06/1935 | 2 | 2 |
| Williamson | 7.2 km E Creal Springs | | Grassy Cr. | 37.61500 | -88.74500 | 09/20/1998 | 09/20/1998 | 1 | 1 |

Table 2. Summary variables for mtDNA COI sequences.

| Variables | Values |
|---|---|
| COI Length | 1511 |
| Populations | 30 |
| Individuals | 223 |
| Haplotypes | 121 |
| Mean Haplotype Diversity | 0.956 |
| Mean Nucleotide Diversity | 0.0029 |
| Range | 0.0015-0.0045 |
| Tajima's D[1] | -2.102 |

[1]Measure of "neutrality", or lack of selection, indicates expansion of population, significant at $p<0.05$.

Table 3. Number of *Acroneuria frisoni* egg batches released into three locations in the Middle Fork of the Vermilion River during June, 2009.

| Locations | Mean | SD | Total | N |
|---|---|---|---|---|
| **Collison BR** | 281.2 | 53.54 | 1406 | 5 |
| **Possum L** | 198.7 | 69.29 | 1987 | 10 |
| **Kick SP** | 188.6 | 52.77 | 3932 | 20 |
| **Total** | | | 7325 | 35 |

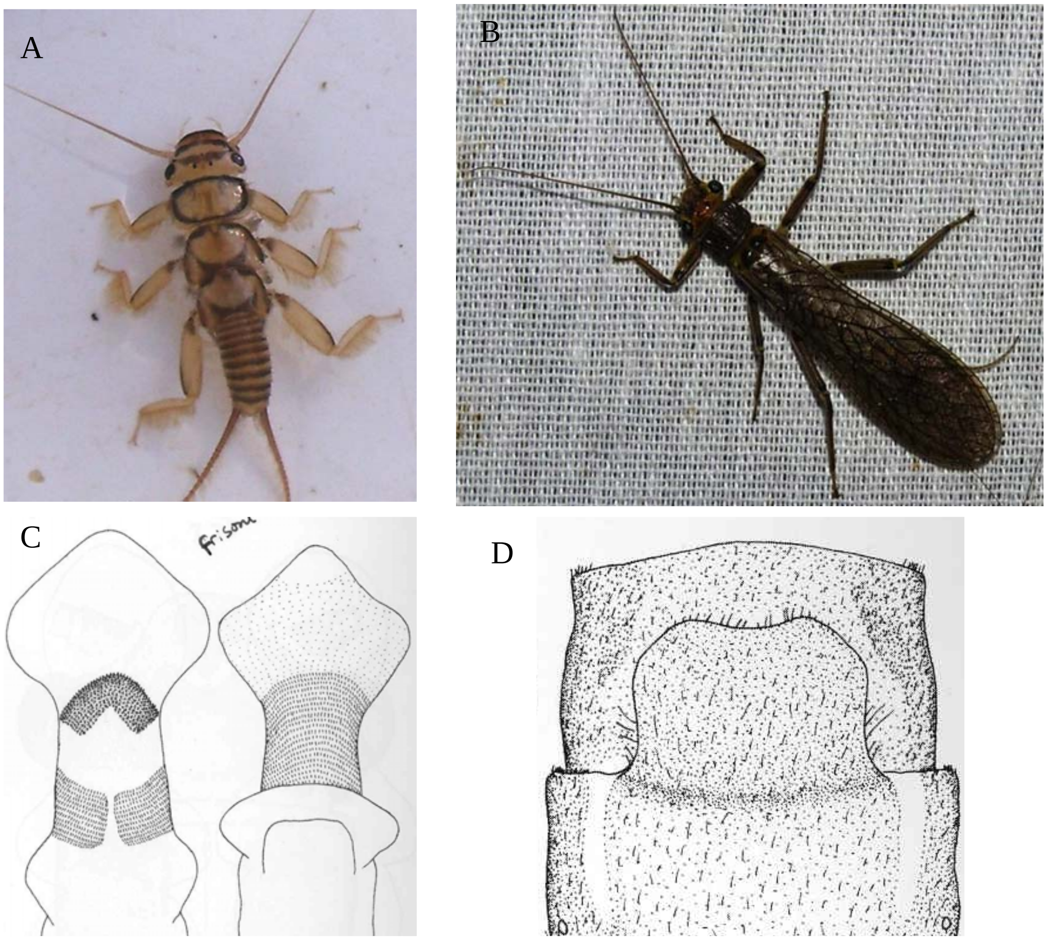


Fig. 1. The Illinois Stone, *Acroneuria frisoni* Stark & Brown, 1991. A. Nymph, B. Adult at UV light trap, C. male internal reproductive structure, aedeagus, with species specific pattern of spinules, D. female eighth abdominal segment, e.g., subgenital plate. Credits: A. Ember Chabot, B. R. Edward DeWalt, C & D from Stark et al. 2004. Size of full grown nymph is 25-40 mm, excluding antennae and cercae, adults 30-45 mm head to tip of wings.

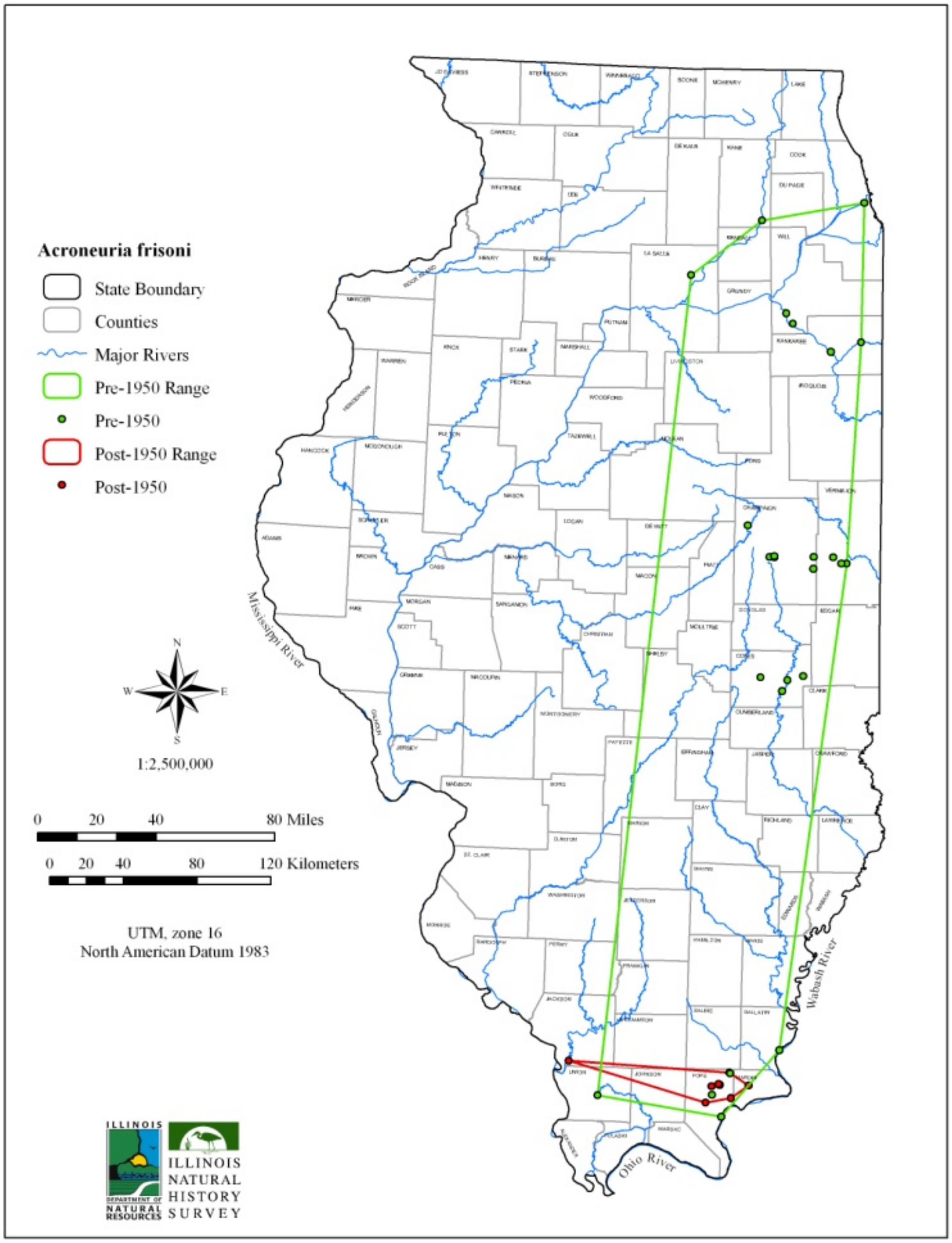


Fig. 2. Minimum convex polygons of Pre-1950 and Post-1950 distributions from the INHS Insect Collection database. From DeWalt et al. 2008.

Fig. 3. Release of eggs of *Acroneuria frisoni* into Middle Fork of the Vermilion River, June, 2009.

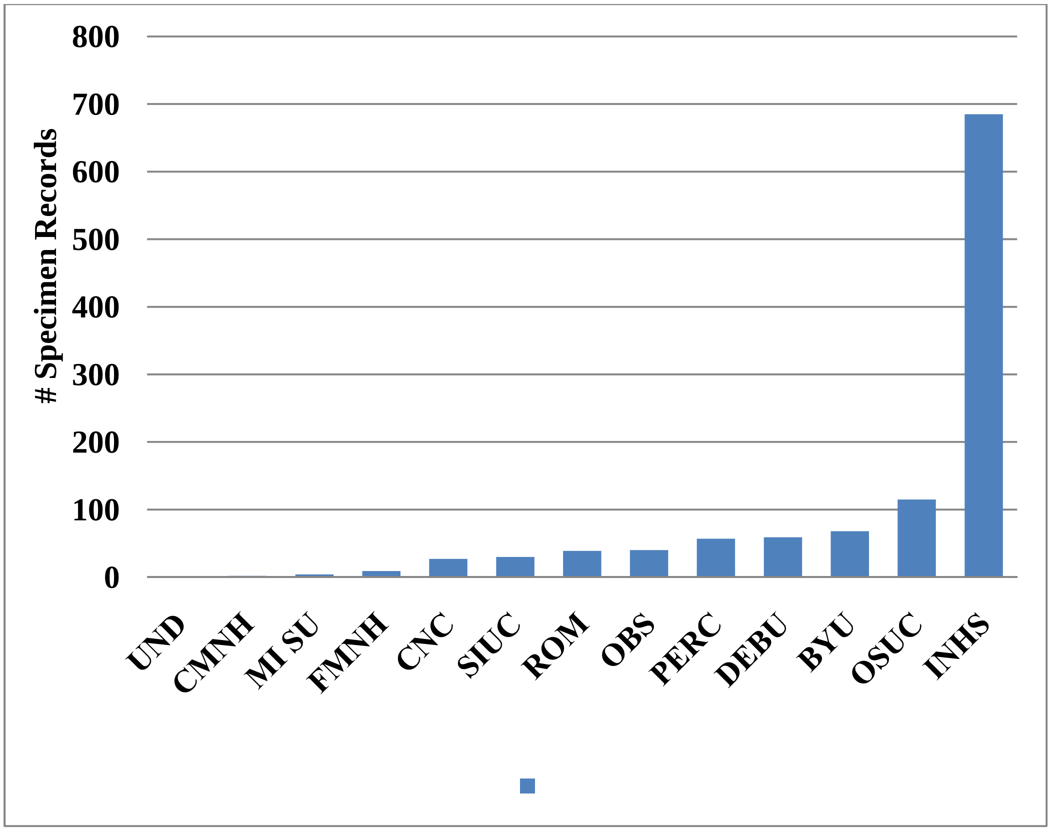


Fig. 4. Number of specimen records and their institutional affiliation for *Acroneuria frisoni*.

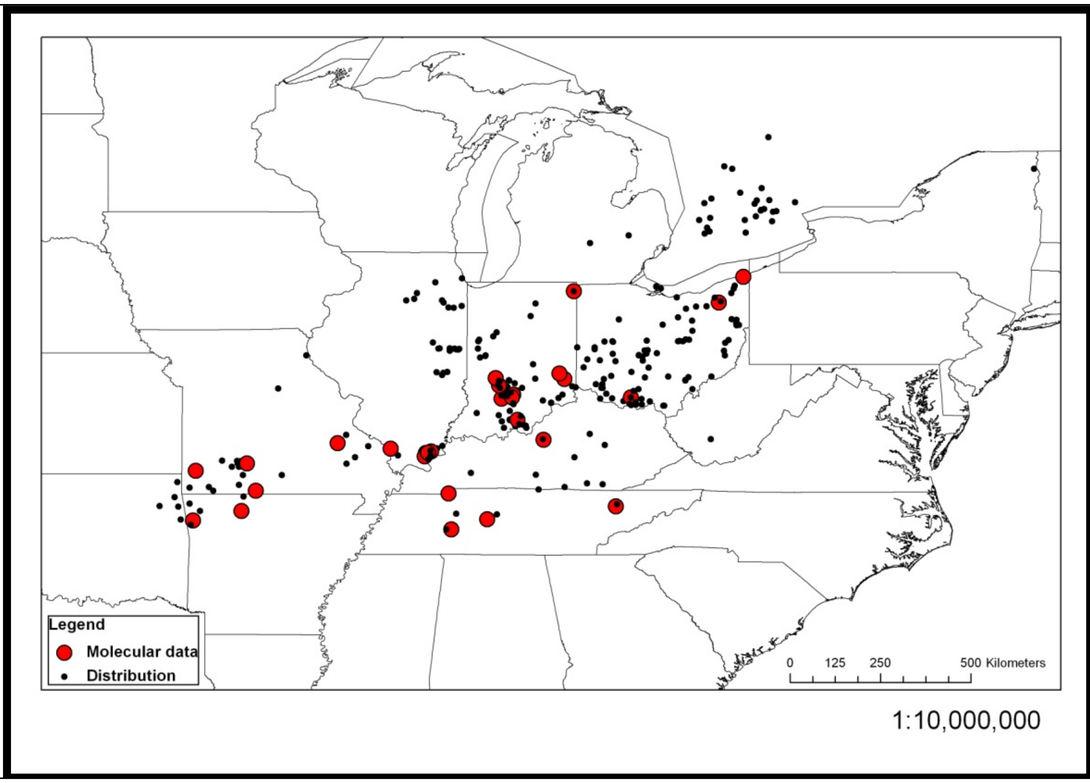


Fig. 5. Distribution of *Acroneuria frisoni*. Red dots include locations of specimens used in molecular analyses.

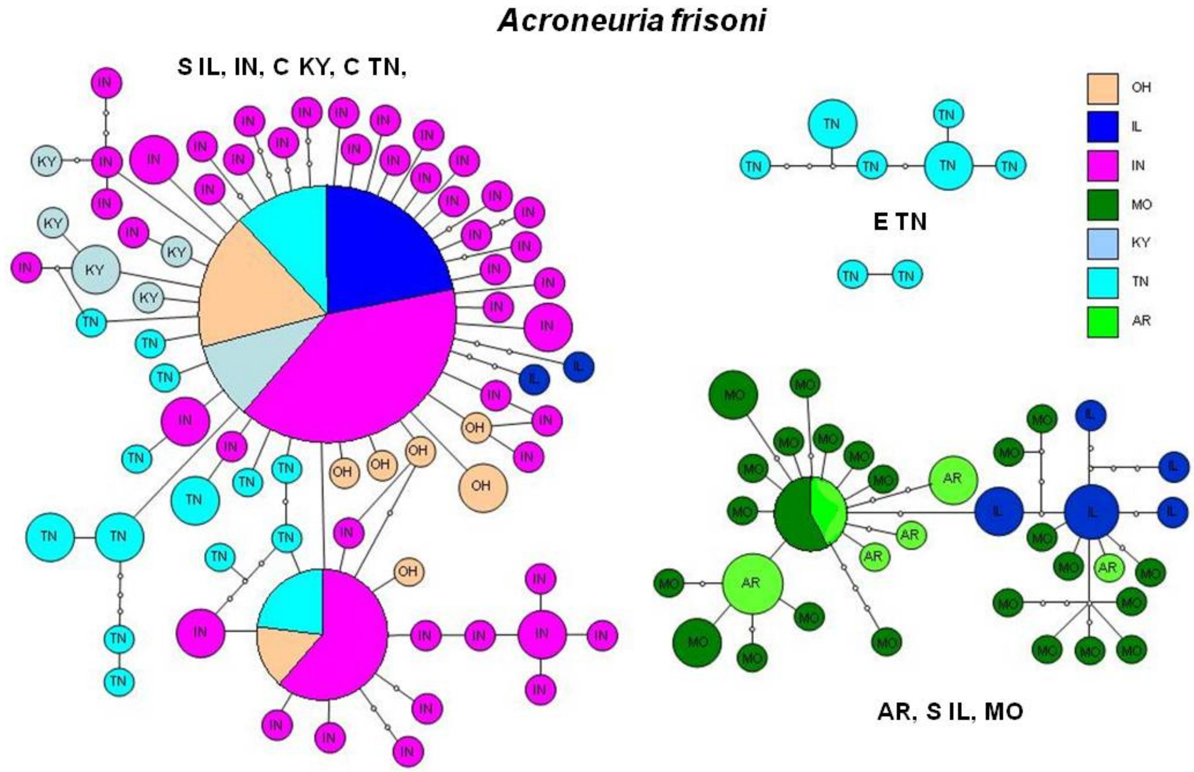


Fig. 6. Network diagram demonstrating relative frequency, evolutionary relationship, and distribution of mtDNA COI haplotypes found for *Acroneuria frisoni*. Each circle is a separate haplotype and colors indicate from which Midwest state that sequence came. The frequency of a haplotype in the entire dataset is indicated by the relative size of the circle. Relationships of one haplotype to another are indicated by lines between them. Each node on the line represents one additional base pair difference between one haplotype and another.

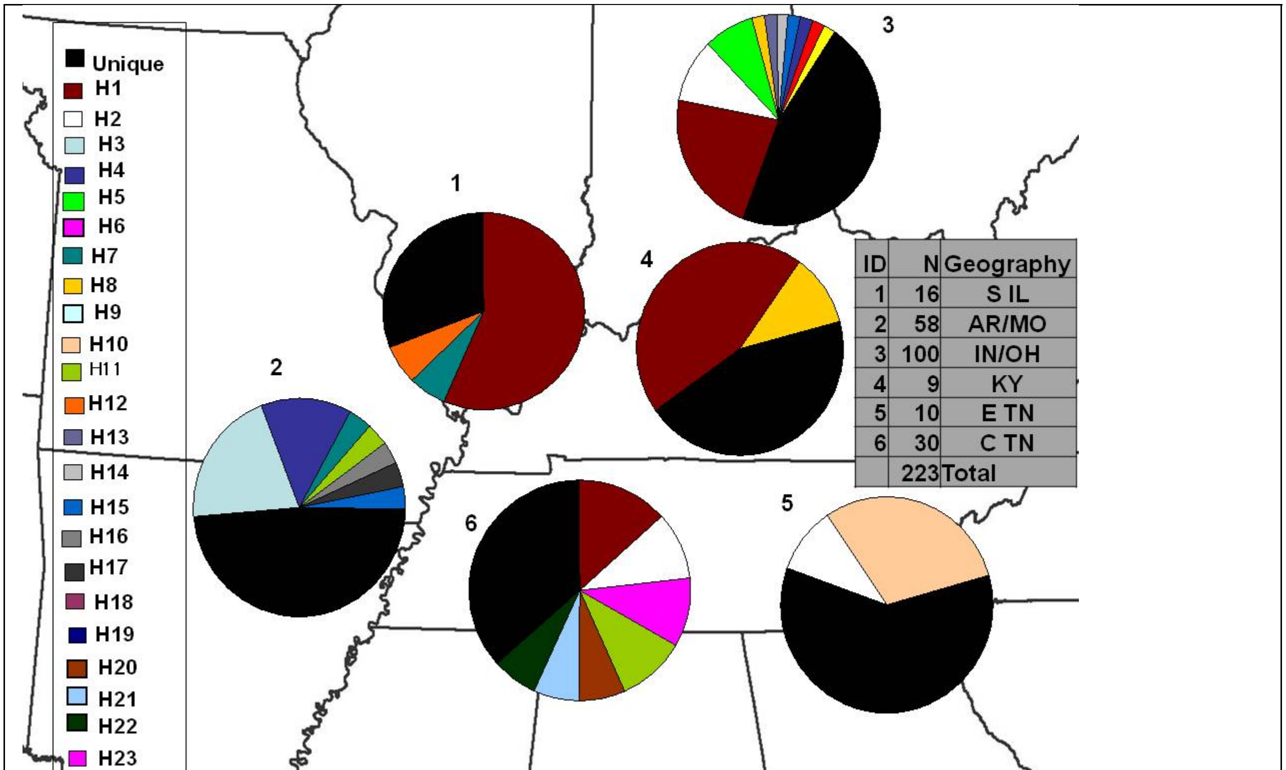


Fig. 7. Pie diagrams of haplotype frequency in six subregions for *Acroneuria frisoni*. Pie segment in black consists of individuals with unique haplotypes, other color represent shared haplotypes between subregions. S IL is apparently a contact zone between two sources of haplotypes.