**Evaluating the Status of Cicadas in Illinois and their Importance as Indicators of Grassland Conservation**

Project T-119-R-1

**Final Report**

**Project Start Date: 1 October 2018**

**Project End Date: 30 September 2022**

Sam W. Heads (Principal Investigator)

Catherine E. Dana (Co-PI), M. Jared Thomas (Co-PI), Mark A. Davis (Co-PI), and Jonathan Tetlie (PhD Candidate)


Illinois Natural History Survey

Prairie Research Institute, University of Illinois

1816 South Oak Street

Champaign, IL 61820

## Table of Contents

## Executive Summary

Listed below are developments on the goals/aims outlined by the grant. Further information can be found in the expanded summary on the following pages.

*1) Continue development of habitat assessment tools from a previous project (T- 92-R-1);*

Due to COVID-19 restrictions, we moved data analysis from our previous state wildlife grant (T-92-R-1) online. Representative curated specimens (collected from T-92-R-1) will be deposited at the Illinois Natural History Survey Insect Collection.

*2) Utilize aforementioned tools to assess recent and long-term restorations of prairies;*

Per the No Cost Extension, additional field tests of the previous methods in 2022 were conducted. The NCE and additional hourly technicians have allowed us to continue work on this job and the previous.

*3) Identify current status of cicada species in Illinois, including potential Species of Greatest Conservation Need (SGCN);*

The map provided (**Figure 1**) indicates the field sites visited during the the project where specimens of: *Beameria venosa* (**Figure 2**)*, Cicadettana calliope calliope* (**Figure 4**)*, Diceroprocta vitripennis* (**Figure 6**)*, Magicicada septendecim* (**Figure 7**)*, Magicicada cassinii* (**Figure 8**)*, Magicicada septendecula* **(Figure 9)***, Megatibicen dorsatus* (**Figure 11**)*, Neotibicen auriferus* **(Figure 14)***, Neotibicen lyricen lyricen* (**Figure 16**)*, Neotibicen pruinosus pruinosus, linnei, and canicularis* (**Figure 17**)*, Neotibicen tibicen tibicen* (**Figure 18**)*, Megatibicen pronotalis walkeri* (**Figure 19**), *Megatibicen auletes* (**Figure 21**), *Neocicada hieroglyphica hieroglyphica* (**Figure 23**) and *Okanagana balli* (**Figure 24**) were captured (or DNA sampled) from frequently visited sites outlined in **Table 2**. In this report we present our recommendations for listing and habitat management for these species and others. The manuscript reviewing the cicada species of Illinois and covering two new state records (*Neotibicen auriferus***,** and *Beameria venosa*) is currently being reviewed by co-authors and will likely be ready for publication by Spring 2023. *Neotibicen auriferus* and *Beameria venosa* are currently only known to inhabit hill/clifftop prairies in southwestern Illinois, according to our research. Our team identified additional species by utilizing audio samples recorded by Wildlife Acoustics SM4 recorders placed at multiple sites during the project duration. We make recommendations on updating the current SGCN listings of several species of Illinois Cicadas (Table 1).

*4) Determine habitat connectivity between potentially isolated populations of cicadas found in prairies using population genetics and other measures;*

To assess the habitat connectivity of the prairie-associated cicada, *Megatibicen dorsatus* (**Figure 11**) samples (n=452) were submitted to the campus sequencing center in January 2021. Test results allowed us to evaluate the degree of gene flow present between prairies of varying condition (high quality, remnant, restored to/from minimally maintained) in Illinois. A computationally slow and expensive analysis due to the high number of reads obtained through NovaSeq has provided for a more robust study on this species. Interestingly, the analyses indicate that populations in the higher-quality nature preserves are isolated and that there is more gene flow along the railroad right-of-way (ROW) prairies. There is still uncertainty as to the geographic variables acting as barriers to isolate these populations.

*5) Develop cicada identification and monitoring resources for land managers and the general public;*

Throughout late May and early June 2021 was the emergence of Brood X 17-year periodical cicadas, which emerged in several counties in eastern Illinois. Since these cicadas created a bit of a buzz throughout local communities, we were able to present our lab's work on cicadas and stressed the ecological role and diversity of cicadas in Illinois through several news outlets.The "Presentations/Outputs/Outreach" section contains links to several of these interviews. High-quality images of all Illinois cicadas and cicada genitalia for use in publications and other outreach materials were obtained by our lab technicians.

## Expanded Summary

### Methods

#### Job 1 Methodology

This includes visual obstruction measurements of vegetation (methods based on Limb et al. 2007) for the habitat heterogeneity structural quality index and analysis as well as the Butterfly Quality Index and associated Coefficients of Conservatism described in Methods 1 and 2 below. Images, specimens, and data for this objective were collected between 2014 and 2017.

**Method 1:** Coefficients of Conservatism and Butterfly Quality Indices ($BQI^{-}_{a}$ and $BQI_{b}$)

Coefficients of Conservatism (CC) are values assigned to a species of butterfly based on a number of life history variables, including number of generations per year, overwintering strategy, host plant specificity, and habitat specificity. Based on these variables, figures are assigned to each species where lower values indicate low conservatism and higher values indicate high conservatism (ranging between values of 4 and 12). The Butterfly Quality Indices are calculated using two separate equations (based on Wallner et al. 2013a).

Equation 1 ($BQI_{a}$):

Where:

meanCC = Mean coefficient of conservatism value for all species encountered per sampling effort (e.g. site, transect, plot).

√Spp. = Square root transformation of total no. species encountered at the site sampled.

Equation 2 ($BQI\text{-}_{b}$):

Where:

$n_i$ = Total number of individuals for species *i*.

$N$ = Total number of individuals for all species.

$CC_i$ = Coefficient of conservatism for species *i*.

√Spp. = Square root transformation of total no. species encountered at site sampled.

We have applied our indices to two of the eleven sites that were visited between 2014 and 2016. Our initial results indicated that sites with high BQI values do not necessarily indicate higher quality botanical resources; thus other factors like physical structure of the habitat and proximity to other suitable surrounding habitat may be more relevant to butterfly diversity and abundance. Indices will be applied to the remaining sites to evaluate how this method fits with the habitat heterogeneity structural quality index and other measures (e.g. Floristic Quality Index) in Job 2.

**Method 2:** Habitat heterogeneity structural quality index (HHSQI) quantification and analysis.

The method is based on a paper by Limb et al. (2007) in Rangeland Ecology & Management: "Digital Photography: Reduced Investigator Variation in Visual Obstruction Measurements for Southern Tallgrass Prairie". Habitat structural diversity is important for a number of insect species (potentially butterflies, as described in Method 1, and cicadas, as described in Jobs 3 and 4) for mating displays, oviposition, and molting success (i.e. development).

For this method, 6 images were taken along a 50 meter transect at each site, using a 1 x 1.5 m tall white tarp as a backdrop. Using Photoshop, we can calculate the amount of vegetation (live/dead) of a 1 x 1 square meter plot by converting the image to black and white using the Threshold and Histogram tools. We will also calculate average vegetation height and other similar measures to correlate with species diversity of insect taxa. Along this same transect, insects were sweep-netted and sorted to order.

## Job 2 Methodology

Based on the finalized tools and analysis from Job 1 we assessed recent and long-term restorations of prairies as well as remnant prairies by looking at butterfly diversity and habitat structural diversity. Again, this includes the Coefficients of Conservatism (CC), the related Butterfly Quality Indices ($BQI^{-}_{a}$ and $BQI_b$), and the habitat heterogeneity structural quality index (HHSQI). These methods are described above in Job 1 as well as in greater detail in our final report for T-92-R-1. Previous sites will be assessed using these equations in Job 1 and for Job 2 we will compare our methods between sites and compare to any recent floristic quality indices (FQI) or other measures. This should

inform how predictive these measurements can be of other habitat quality and diversity measures.

## Job 3 Methodology: Identify critical sites and associated cicada species

Sites historically occupied by candidate cicada SGCN, as well as sites with viable habitat and/or historic/recent connectivity to assess contemporary cicada occupancy were identified. Analysis of compiled records allowed for the creation of a species emergence table (Table #). Insufficient evidence of *B. venosa* emergence times required the deployment of acoustic monitoring devices at Fults Hill Prairie Nature Preserve in order to record their calls to more accurately predict their emergence in subsequent years. To determine potential cicada presence at a site, historical presence/absence data is examined and sweep-netting is conducted for verification. If rare cicadas are present, a voucher pair will be collected and others may be sampled (leg clipping) and released. Cicada exuviae are also collected at the field sites for later identification based on morphological characters and DNA analysis using barcoding regions (COI).

Habitat-specialist cicadas in prairies are of particular interest. These include the grass cicada (*Cicadettana calliope calliope*, unlisted), the giant grassland cicada (*Megatibicen dorsatus,* SGCN), *Beameria venosa* (unlisted in Illinois, SGCN in Missouri), *Okanagana balli* (Illinois watchlisted), and *Diceroprocta vitripennis* (Illinois watchlisted). Compilation of historical range data for these species within Illinois was also completed. Locations for this project: Loda Cemetery Prairie Nature Preserve, Prospect Cemetery Nature Preserve, Henry A. Gleason Nature Preserve, Sand Prairie Scrub Oak Nature Preserve, Ballard Nature Center, Stephen A Forbes State Recreation Area, Twelve Mile Prairie, and Railroad ROW Prairies along US-45. Future work would benefit from additional site monitoring to compile better species distribution throughout the state.

## Job 4 Methodology: Genetic techniques to assess habitat connectivity of cicadas

Detailed information about the protocols can be found in the dissertation of Dr. Catherine Dana by using IDEALS https://www.ideals.illinois.edu/.

*Field Collection*

Cicadas were caught using 15-inch sweep nets then placed in mesh cages or on ice before being taken back to the lab. If legs were removed in the field (for later DNA analysis), scissors were flame-sterilized using 80-100% ethanol. Legs were placed dry in 1.5 mL tubes on ice and then transported back to the lab for long term dry storage at -20°C at the Illinois Natural History Survey.

For population genetics studies, *Megatibicen dorsatus* individuals were collected by insect net at prairie locations throughout the state (**Figure 13**) starting in 2015 and during the months of July and August. A voucher pair was taken whole to document populations and will be accessioned into long-term collections at the Illinois Natural History Survey (INHS). Given the large body size and desire to minimize impact on the population, one

hind leg was sufficient for DNA needs. Individual legs were clipped across the femur using flame-sterilized scissors before being released at the same prairie. Legs were placed dry in 1.5 mL centrifuge tubes and kept on ice until they could be stored at -20°C long-term at the INHS. Attempts were made to collect at least 12 individuals of equal male:female ratio from a site, but this was not always possible given the difficulty in collecting. Three sites were chosen for annual collections: a right-of-way prairie south of the town of Paxton Illinois (40.429590°, -88.108767°), Loda Cemetery Prairie Nature Preserve (40.527315°, -88.075882°), and Henry Allan Gleason Nature Preserve (40.379807°, -89.929928°). Sites were chosen based on records in the INHS Insect Collection, word of mouth, and audio surveys of railroad prairies.

*DNA Extraction, PCR, and Sanger Sequencing*

DNA was extracted from cicada legs using QIAGEN DNEasy® Blood and Tissue Kits. Several modifications were made to the QIAGEN protocol to optimize DNA yield.

*Barcoding for species confirmation*

In order to amplify the Cytochrome c oxidase I (COI) region of the mitochondria for barcoding, we used modified methodology from Hill et al. (2015) to amplify the full region in two parts using 2X GoTaq® DNA Polymerase Master Mix and PCR primers. PCR products were Sanger sequenced by Eurofins Genomics using their "crude PCR products" PrePaid plate service or by the Sanger Core Facility at the Roy J. Carver Biotechnology Center at the University of Illinois at Urbana-Champaign. COI sequences were assembled in Geneious Prime® 2021.2.2.

*ddRADSeq library preparation*

Library preparation methods were modified from Clark et al. (2014) and an OpenWetWare protocol (2017). Extracted genomic DNA was double digested using the enzymes PstI and MspI. PstI and MspI adapters to identify individual samples were ligated to fragmented DNA. Samples were then pooled by plate and size selected to 370 to 700 bp. After size selection fragments were amplified using Illumina indexing primers to identify samples by plate. Cleaned PCR products of each plate were combined in equal ratios based on DNA concentration and the individual sample number (of cicadas) per plate.

*Sequencing*

To ensure even coverage across 96-well plates, a MiSeq titration run was completed prior to more in-depth sequencing. Based on this run, a new pool of DNA was created to have more evenness of sequencing depth for each sample. Quality control in the form of fragment size assessment using a Fragment Analyzer ensured that any primer sequences were removed. Sequencing was provided by the Carver Center (University of Illinois at Urbana-Champaign) using the Illumina NovaSeq 6000 S4 flowcell producing paired 150 bp reads.

*Sequence processing and analysis*

Due to the use of variable length MspI adapters, base pairs needed to be trimmed prior to use of the Stacks pipeline. Reverse reads were run through cutadapt 2.10 to remove any excess base pairs from the 5' end (i.e., any that were prior to the CGG cut site) from variable length adapters used in library preparation (Martin 2011). To identify common loci across samples for population genetics analysis I used Stacks v2.54 (Catchen et al. 2011; Catchen et al. 2013). In order to optimize the *de novo* assembly of loci in the Stacks program I selected the parameters based on trial runs using 15 randomly selected individuals to maximize the number of SNPs and loci available for further analysis (Paris et al. 2017; Rochette and Catchen 2017). Further filtering was done using VCFTools 0.1.16 (Danecek et al. 2011) and based on poor coverage (more than 50% missing data), an additional set of individuals was removed (Puritz et al. 2014a; Puritz et al. 2014b).

The maximum number of loci possible was output as a STRUCTURE file. Given the large size of the dataset, FastSTRUCTURE was used to get an idea of the best value for k (Raj et al. 2014). STRUCTURE 2.3.4 (Settings: Admixture Model, Length of Burnin Period: 1,000, Number of MCMC Reps after Burnin: 10,000) to determine k (clusters/populations) and better visualize data. Statistics were visualized using STRUCTURE HARVESTER (Earl and VonHoldt 2012). After output from Stacks, analysis using STRUCTURE, and output to vcf results results were visualized using R 4.2.1 in RStudio (2022) using multiple packages, including vcfR 1.13.0 (Knaus and Grünwald 2016; Knaus and Grünwald 2017) and poppr 2.9.3 (Kamvar et al. 2014; Kamvar et al. 2015). Pairwise $F_{ST}$ values were calculated using hierfstat 0.5-11 (Weir and Cockerham 1984). A Mantel test was performed in R Studio using adegenet 2.1.8 (Jombart 2008; Jombart and Ahmed 2011).

## Job 5: Create resources for land managers and the general public

**Results**

Creation of a printable guide to the cicadas of Illinois found in this report for ease of identification for land managers is in process and will be made available as soon as it is complete.

Creation of audio compilation of the cicadas of Illinois found in this report will be made available as soon as it is complete. Given the large file size of the audio files we are working with, we need to obtain a publicly accessible file repository for their storage. Audio recording of cicada songs can aid land managers and the general public with identification of species without having to catch cicada specimens.

Publication on the cicadas of Illinois featured in this project is forthcoming in the first quarter of 2023.

**Discussion**

## Population genetic structure of *Megatibicen dorsatus*

## Recommendations for listing and management

Due to the unknown impacts of land management practices and because of the fragmented landscape, an Illinois Wildlife Action Plan was created by the Wildlife Conservation and Restoration Program and the Illinois State Wildlife Grant Program that lists three cicadas as Species in Greatest Conservation Need (SGCN) or places them on the watchlist – *D. vitripennis*, *O. balli*, and *Me. dorsatus* (IWAP 2015, pages 49, 265, 273). We propose adding *Neot. auriferus* and *B. venosa* as SGCN species, as well (Table 1.3), due to their highly restricted range and rarity. *O. balli* also has an extremely restricted distribution (Figure 25) and should be considered for elevation to threatened in Illinois from the Illinois Watch List. Two additional county records were added during the course of this project on *Me. auletes,* which While *Me. auletes* is found in multiple counties throughout Illinois, and I was able to add more information on distribution with this study (Figure 1.11), more information is needed on population sizes given their primary association with oak trees and patchy distribution within surburban areas (Sanborn and Phillips 2013; Dana *pers. obs.*). Despite my efforts, I was unable to sample *Neoc. h. hieroglyphica* over the years of this study. Acoustic recordings of *Neoc. h. hieroglyphica* were obtained, but a larger effort to locate individuals to better assess their status is needed. Therefore, I suggest placing *Neoc. h. hieroglyphica* on the Illinois Watch List. Given that *O. r. rimosa* was not observed throughout the study, nor has it been reported in any known collection since prior to 1962, more information is needed. Given timing and logistical constraints, I was unable to sample to any large degree in Northern Illinois. Thus, I recommend placement of *O. r. rimosa* on the Illinois Watch List to determine if this species is indeed extirpated within Illinois.

In terms of general management recommendations for cicada conservation, land managers should do patchy burning, especially in smaller prairies like cemetery prairies where there may be no refuge. Additionally, any mowing efforts should consider oviposition phenology for a given species, as I have observed mowing in areas either during or directly after cicadas were observed ovipositing on plants in the area (both in grass parking lots and along roadsides). With these changes, I hope that conservation of cicadas, as ecologically important insects, will be ensured for many years to come.

As restrictions associated with the COVID-19 caused losses in lab and fieldwork during the grant period and thus we applied and secured a No Cost Extension to extend to the end of the grant by one year (2021 to 2022).

During the final quarter of 2020, work began on numerous reports, including the annual SWG report, the Illinois Nature Preserve Commission (INPC) permit annual report for 2020, and the Illinois Department of Natural Resources (IDNR) State Parks report (Job 6 – Write quarterly and annual reports. Present research at meetings and write manuscripts). During this time, technicians continued to assess data from a previous SWG (T-92-R-1).

In early 2021, DNA samples from the prairie cicada, *Megatibicen dorsatus*, were submitted to the campus sequencing center. These data will be used to assess habitat connectivity by evaluating genetic differences associated with geographically disparate populations. These analyses will assess the degree of gene flow between prairies across Illinois. Prairies that were sampled

included high-quality, remnant, restored, and minimally maintained right-of-way prairies (Job 4 – Use genetic techniques to assess habitat connectivity of cicadas).

Site scouting for the 2021 sampling season began in May, with full sampling efforts starting in June (Table 2). As mentioned previously, 2021 was unique in that the Brood X 17-year periodical cicadas emerged and were prevalent during June. In July, August, and September, specimens of: *Beameria venosa, Cicadettana calliope calliope, Diceroprocta vitripennis, Magicicada septendecim, Magicicada cassinii, Megatibicen dorsatus, Neotibicen auriferus, Neotibicen lyricen lyricen, Neotibicen pruinosus pruinosus, Neotibicen tibicen tibicen,* and *Okanagana balli* were captured from sites outlined in Table 2. Work has continued on a review paper concerning the cicadas of Illinois. This review paper includes a list of species, known distributions, status updates on species, and management recommendations. These recommendations and outreach will consist of photographs for identification and audio files/spectrograms of cicada calls.

During visits to Olin Nature Preserve (Madison County) and Fults Hill Prairie Nature Preserve (Monroe County), we discovered *Cicadettana calliope calliope* present. These are new site records and confirm a Monroe County record from 1956. Interestingly, the date it was found in 1956 is two weeks later than we found it in 2021. We will attempt to use audio data from Fults from 2019 and 2021 to determine the earliest and latest dates that *C. c. calliope* is present in this area as changing emergence and active periods for adult cicadas could be an indication of impacts from climate change data. This information is useful for the manuscript that we are preparing on a review of cicadas of Illinois.

In contrast to the 2020 field season – which was reduced due to COVID constraints – audio recorders were consistently utilized, which have proven to be critical monitoring resources regarding cicada presence and emergence timing. Starting in early fall 2021, DNA extractions were conducted on *M. dorsatus* samples collected during the 2021 field season. Currently, work is being done on extracting DNA from select samples of other species that have been sampled in previous years. Audio sampling of specimens likewise concluded in the fall, and audio files collected during the 2021 field season are being processed. These data have so far allowed us to determine the earliest and latest dates of *Cicadettana calliope calliope* (new site records at Olin Nature Preserve and Fults Hill Prairie Nature Preserve), which can be compared to historical records to determine potential phenological emergence shift that could be linked to climate change (Job 3 – Identify critical sites and associated cicada species).

**Deliverables**

*Presentations*

**Dana, C.E.** 2022. Cicadas of Illinois: New discoveries and our local soundscapes. Clifftop Meeting, Waterloo, IL. 9/2022. [Link to PPT]

**Dana, C.E.** 2021. Misadventures in cicada population genetics. Failures in Research: It Happens! (Part of a multi-speaker lecture.) Department of Entomology Colloquium, University of Illinois at Urbana-Champaign. Urbana, IL. 8/2021. [invited, presenter]

**Dana, C.E.**, Clancy, B.M., Thomas, M.J., Heads, S.W., and Davis, M.A. 2019. Of songs and SNPs: How landscape fragmentation has shaped Illinois' cicadas. Entomological Society of America Annual Meeting, St. Louis, MO. 11/2019.

**Dana, C.E.** 2019. Cicadas of the cemetery prairies. Annual Grand Prairie Friends Meeting. Urbana, IL. 4/2019.

**Dana, C.E.** 2016. Prairie cicada (Hemiptera: Cicadidae) natural history and distribution in Illinois. North American Prairie Association Meeting. Bloomington, IL. 6/2016.

*Posters*

**Dana, C.E** and S.W. Heads**.** 2019. The Soundscape of Nachusa Grasslands: Observations of Regional Variation in Dog-day Cicada Choruses. Friends of Nachusa Grasslands Science Symposium. 10/2019. **[presenter]**

*Media*

Ariel Parrella-Aureli, Book Club Chicago. 2 March 2021. 17-Year Cicadas Will Make This Summer Extra Loud for Illinois — But Chicago Will Be Spared for Now. [Link]

Morgan Greene, Chicago Tribune, Telegraph Herald, and Phys.org. 24 March 2021. Illinois is 'kind of the place to be with periodical cicadas,' researchers say as 17-year brood expected. [Link]

Host Matt Bubala, WGN Radio Chicago. 26 March 2021. 25 Minute Radio Interview.

Fox 32 Chicago TV. 26 March 2021. Cicadas to emerge this May after 17 years underground. [Link]

Greg and Dan Show, 100.3 FM WMBD Peoria. 16 April 2021. 10 Minute Radio Interview.

Tom Kacich, The News-Gazette. 30 April 2021. Tom's #Mailbag: Cicada Show. [Link]

Ryan Pankau, The News-Gazette. 8 May 2021. In the garden – Spring brooding. [Link]

Matt Troher, Daily Illini. 10 May 2021. Cicadas return, specific brood emerges in Illinois counties. [Link]

Reginald Hardwick, The 21st Show on Illinois Public Media. 27 May 2021. Cicadas Are Waking Up In Eastern Illinois But Many More Arrive In 2024. [Link]

Morgan Greene, Chicago Tribune. 20 June 2021. It's cicada season: Ready for bulging red eyes, lots of screeching, and a light dousing of sticky bug juice? [Link]

## Figures and Tables

**Table 1.** Recommended changes to Illinois Wildlife Action Plan (IWAP 2015) Species Greatest Conservation Need (SGCN). Older nomenclature is indicated in the "Synonym" column when appropriate (i.e. when the species name has changed). Current species names are based on Hill et al. (2015), Sanborn and Heath (2016), and Marshall and Hill (2017).

| Current Species Name | Synonym | Common Name | Current Status in IL | Proposed Status |
|---|---|---|---|---|
| *Beameria venosa* | - | Concealed-tymbal cicada | NA** | Illinois SGCN |
| *Cicadettana calliope calliope* | *Cicadetta calliope* | Southern Grass Cicada | NA | Illinois Watch List |
| *Diceroprocta vitripennis* | - | Green Winged Cicada | Illinois Watch List | Illinois Watch List |
| *Megatibicen auletes* | *Tibicen auletes* | Northern Dusk-Singing Cicada | NA* | Illinois Watch List |
| *Megatibicen dorsatus* | *Tibicen dorsatus* | Giant Grassland Cicada | Illinois SGCN | Illinois SGCN |
| *Neocicada hieroglyphica hieroglyphica* | - | Hieroglyphic Cicada | NA | Illinois Watch List |
| *Neotibicen auriferus* | | | NA | Illinois SGCN |
| *Okanagana balli* | - | NA | Illinois Watch List | Threatened |
| *Okanagana rimosa rimosa* | - | Say's Cicada | NA | Illinois Watch List |

**Megatibicen* auletes is on the Connecticut SGCN list (CWAP 2015).

***Beameria venosa* is on the Missouri SGCN list.

**Table 2.** Frequently visited sites throughout the project duration

| Audio Data | Site Description | Latitude | Longitude | City | County |
|---|---|---|---|---|---|
| | 12-mile Prairie (along US37) | 38.814751 | -88.795057 | Multiple | Multiple (Effingham, Marion, Fayette) |
| | Beadles Barrens Nature Preserve | 38.352199 | -88.126372 | Ellery | Edwards |
| | Eldon Hazlet State Park | 38.667732 | -89.324938 | Carlyle | Clinton |
| | Forest Glen Preserve | 40.008753 | -87.570736 | Westville | Vermilion |
| YES | Fults Hill Prairie Nature Preserve | 38.158146 | -90.191337 | Prairie du Rocher | Monroe |
| YES | Henry A. Gleason Nature Preserve | 40.378370 | -89.927142 | Topeka | Mason |
| | Herschel Workman Pheasant Area | 40.461273 | -87.920212 | Butler Township | Vermilion |
| | Illinois Ozarks Nature Preserve | 38.286584 | -90.302765 | Valmeyer | Monroe |
| | Kennekuk Cove County Park | 40.193378 | -87.716952 | Danville, IL | Vermilion |
| YES | Loda Cemetery Prairie Nature Preserve | 40.527125 | -88.076185 | Loda | Iroquois |

| Audio Data | Site Description | Latitude | Longitude | City | County |
|---|---|---|---|---|---|
| | Long Branch Sand Prairie Nature Preserve | 40.227314 | -90.053285 | Havana | Mason |
| | Meredosia Hill Prairie Nature Preserve | 39.856387 | -90.464523 | Arenzville | Morgan |
| YES | Nachusa Grasslands Nature Preserve | 41.882542 | -89.359092 | Nachusa Township | Lee |
| | Olin Nature Preserve | 38.916003 | -90.224507 | Godfrey Township | Madison |
| | Pellsville Cemetery Prairie | 40.461001 | -87.924490 | Rankin | Vermilion |
| | Prospect Cemetery Nature Preserve | 40.444858 | -88.097316 | Paxton | Ford |
| | Rankin Right-of-Way Prairie | 40.472686 | -87.829045 | Butler Township | Vermilion |
| YES | Revis Hill Prairie Nature Preserve | 40.152862 | -89.852320 | Easton | Mason |
| | Ridgetop Hill Prairie Nature Preserve | 40.652414 | -89.161154 | Secor | Woodford |
| | Russel M. Duffin Woods Nature Preserve | 40.000328 | -87.535810 | Danville, IL | Vermilion |
| | Salt Lick Point Land and Water Reserve | 38.303980 | -90.307747 | Valmeyer | Monroe |

| Audio Data | Site Description | Latitude | Longitude | City | County |
|---|---|---|---|---|---|
| | Sand Prairie Scrub Oak Nature Preserve | 40.190246 | -90.074421 | Bath Township | Mason |
| | Sand Ridge State Forest | 40.391279 | -89.872003 | Forest City | Mason |
| | South of Buckley Right-of-Way Prairie | 40.581729 | -88.044895 | Buckley | Iroquois |
| | South of Ludlow Right-of-Way Prairie | 40.357046 | -88.139563 | Ludlow Township | Champaign |
| | South of Paxton Right-of-Way Prairie | 40.429704 | -88.108996 | Paxton | Ford |
| | Stephen A. Forbes State Recreational Area | 38.722212 | -88.772616 | Kinmundy | Marion |
| | Vermilion River Observatory | 40.059779 | -87.564859 | Danville | Vermilion |
| | War Buff Valley Sanctuary | 37.445882 | -88.492191 | Lusk | Pope |
| YES | Weston Cemetery Prairie Nature Preserve | 40.747006 | -88.614788 | Chenoa | McLean |
| | Wildcat Hollow State Habitat Area | 38.995783 | -88.618410 | Mason | Effingham |
| | Woodford State Fish and Wildlife Area | 40.878619 | -89.446638 | Low Point | Woodford |

**Figure 1.** Map of sites visited during the project duration.

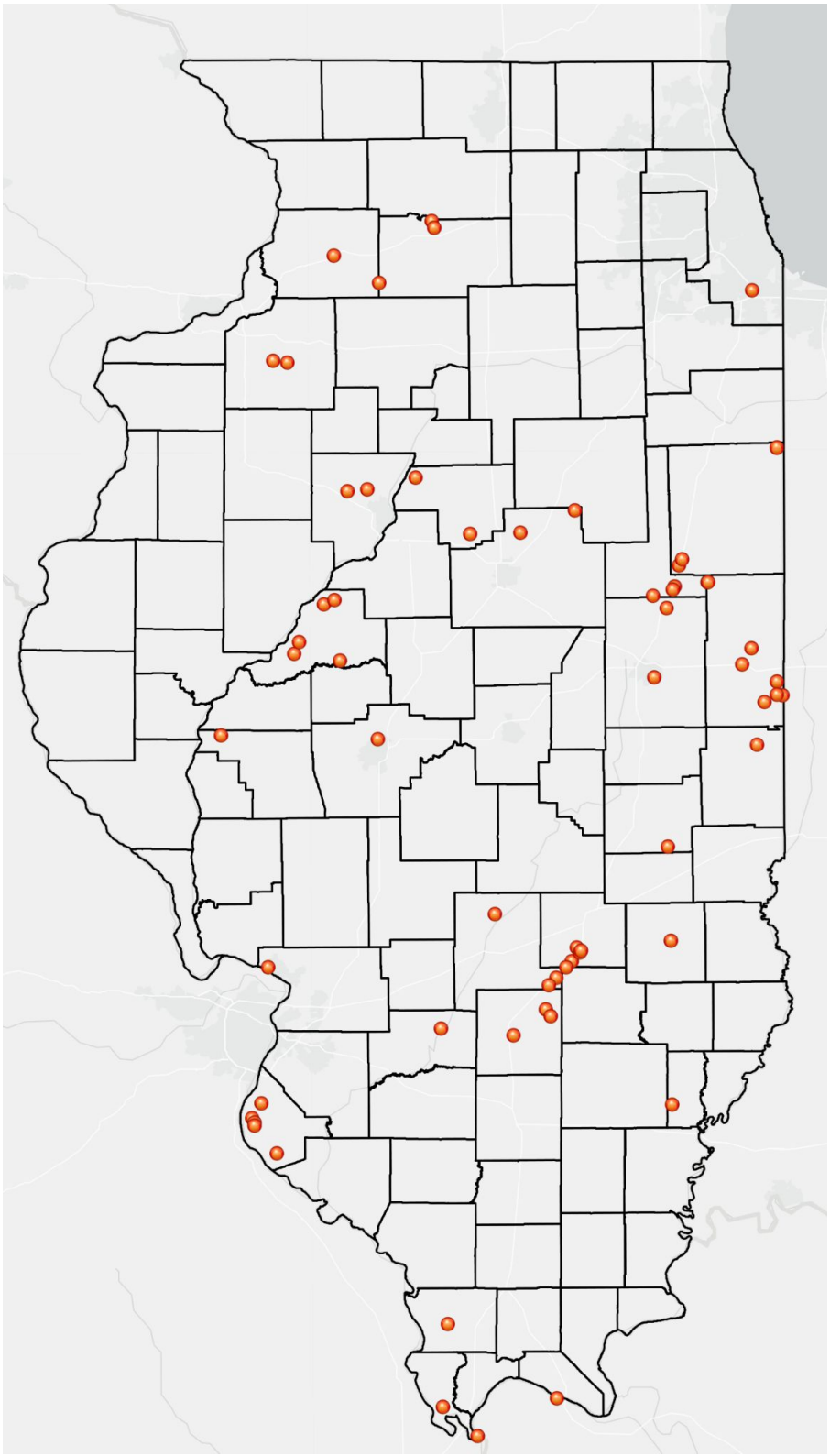


**Figure 2.** *Beameria venosa* (Uhler, 1888), (Dana Collection BV200107M) ♂, USA: IL: Monroe County: Valmeyer. Salt Lick Land and Water Reserve, "Newman Prairie". 38.30252961°, -90.3087846°. 15.vii.2020. C.E. Dana.

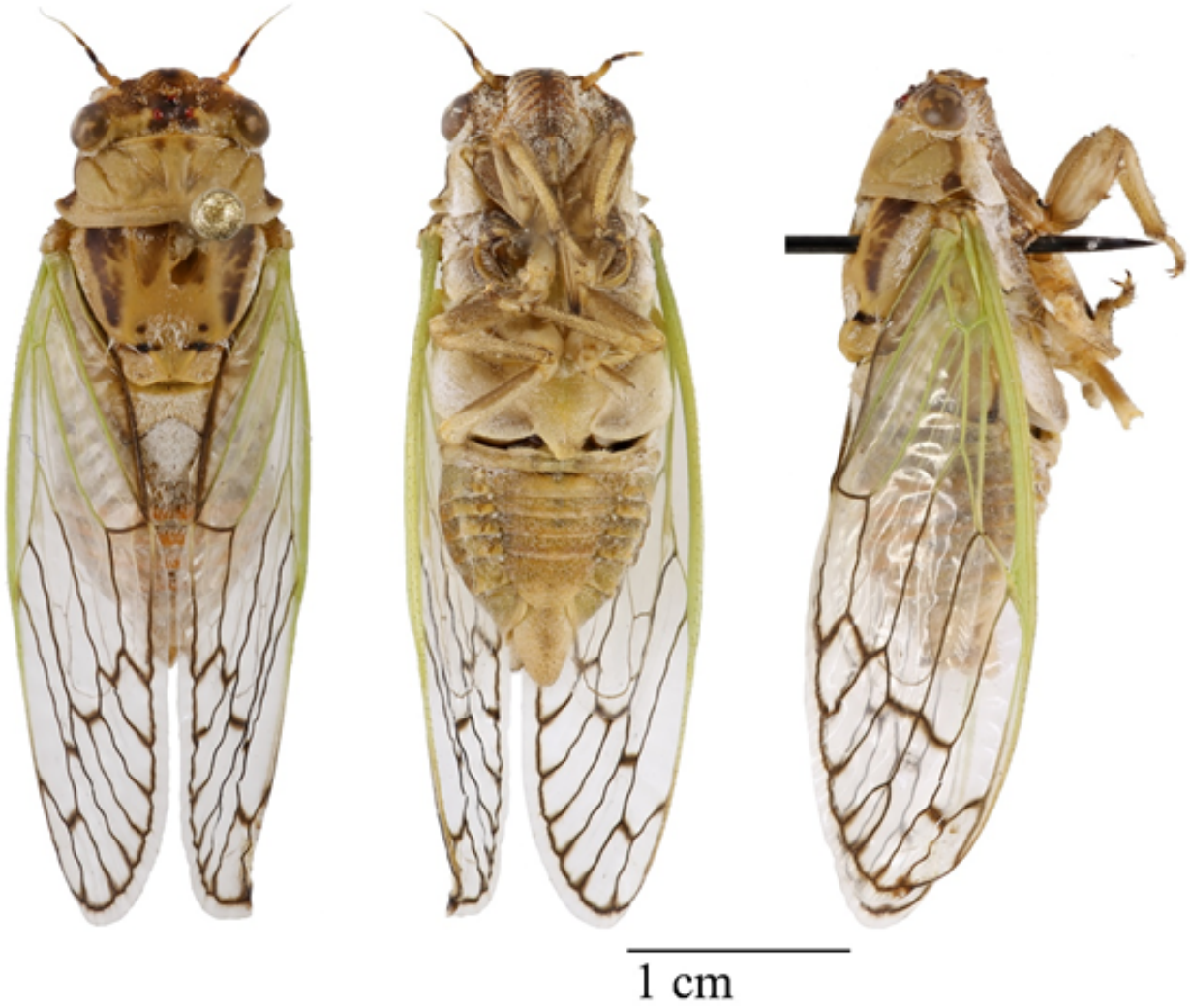


**Figure 3.** Distribution and study sites (hill prairies) of *Beameria venosa* (Uhler, 1888) in Monroe County, IL

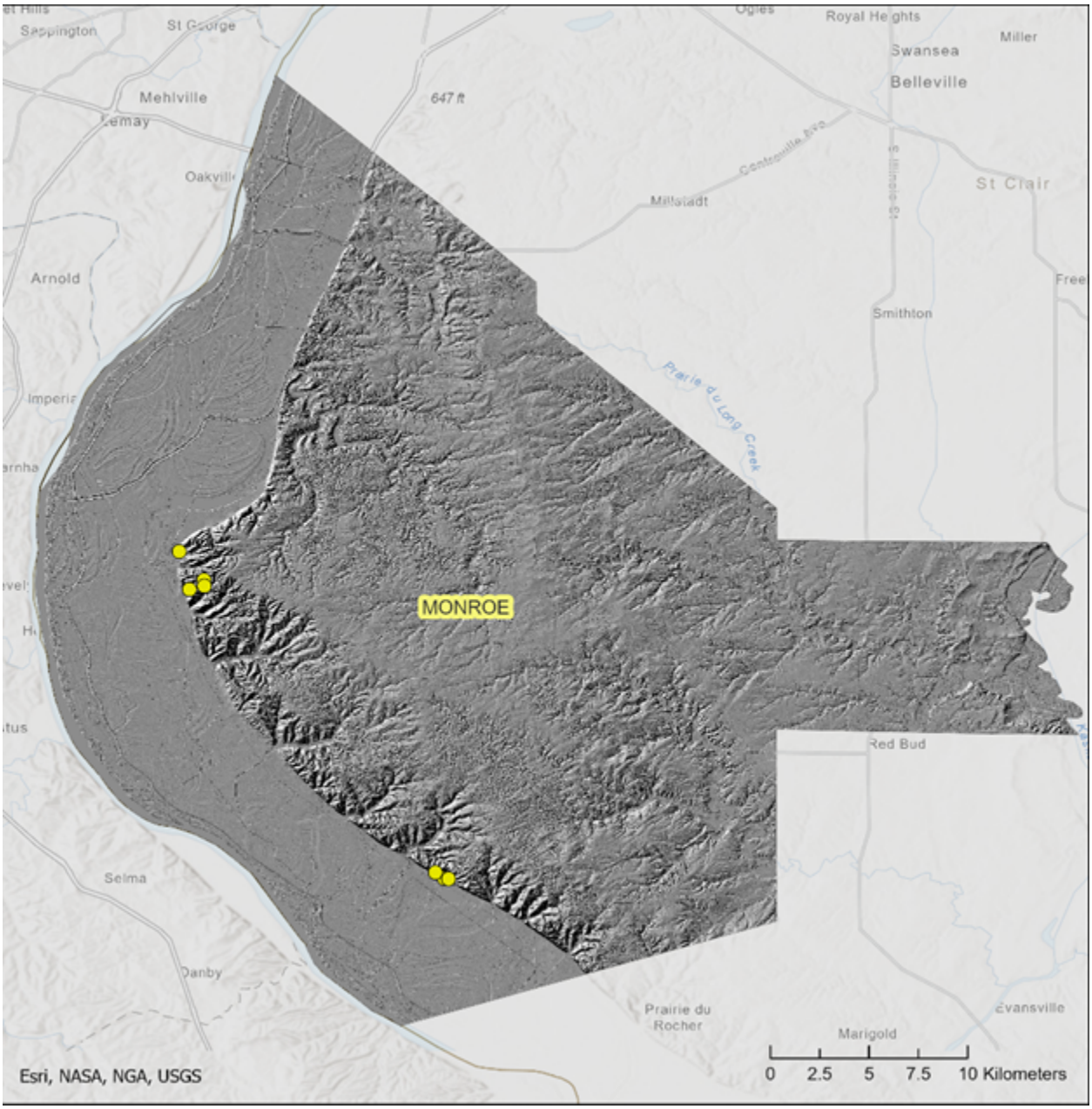


**Figure 4.** *Cicadettana calliope calliope* (Walker, 1850), (Dana Collection CC21036M), (INHS Insect Collection 1,001,485) ♂. USA: IL: Morgan County: Meredosia. Meredosia Hill Prairie Nature Preserve. 39.85330486°, -90.46565664°. 23-vi-2021. J.R. **Tetlie, M. Keeley.**

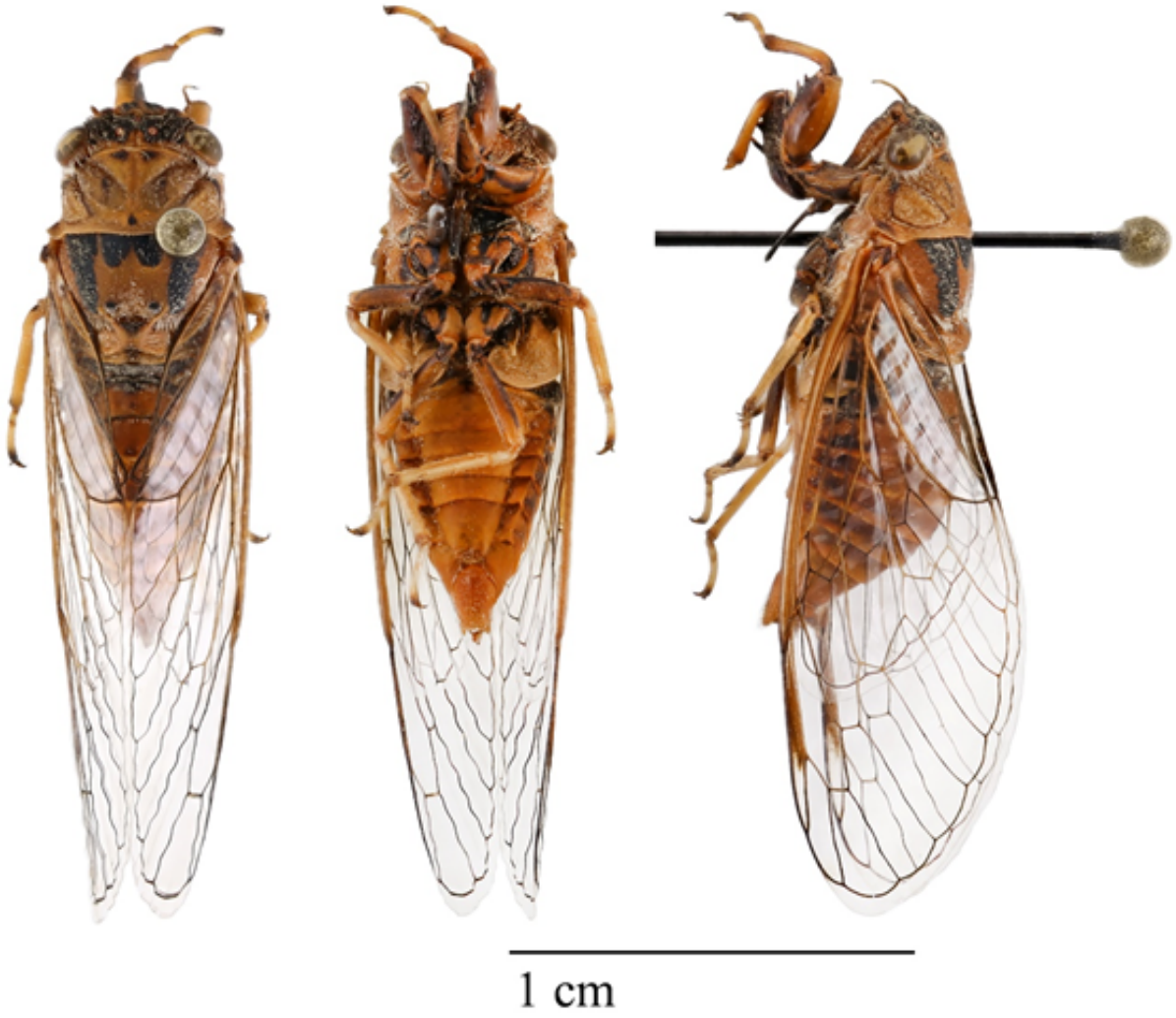


**Figure 5.** County map showing the distribution of *Cicadettana calliope calliope* (Walker, 1850) in Illinois. Grey counties are those based on previous records and black counties are those newly added based on specimens collected during the duration of this study.

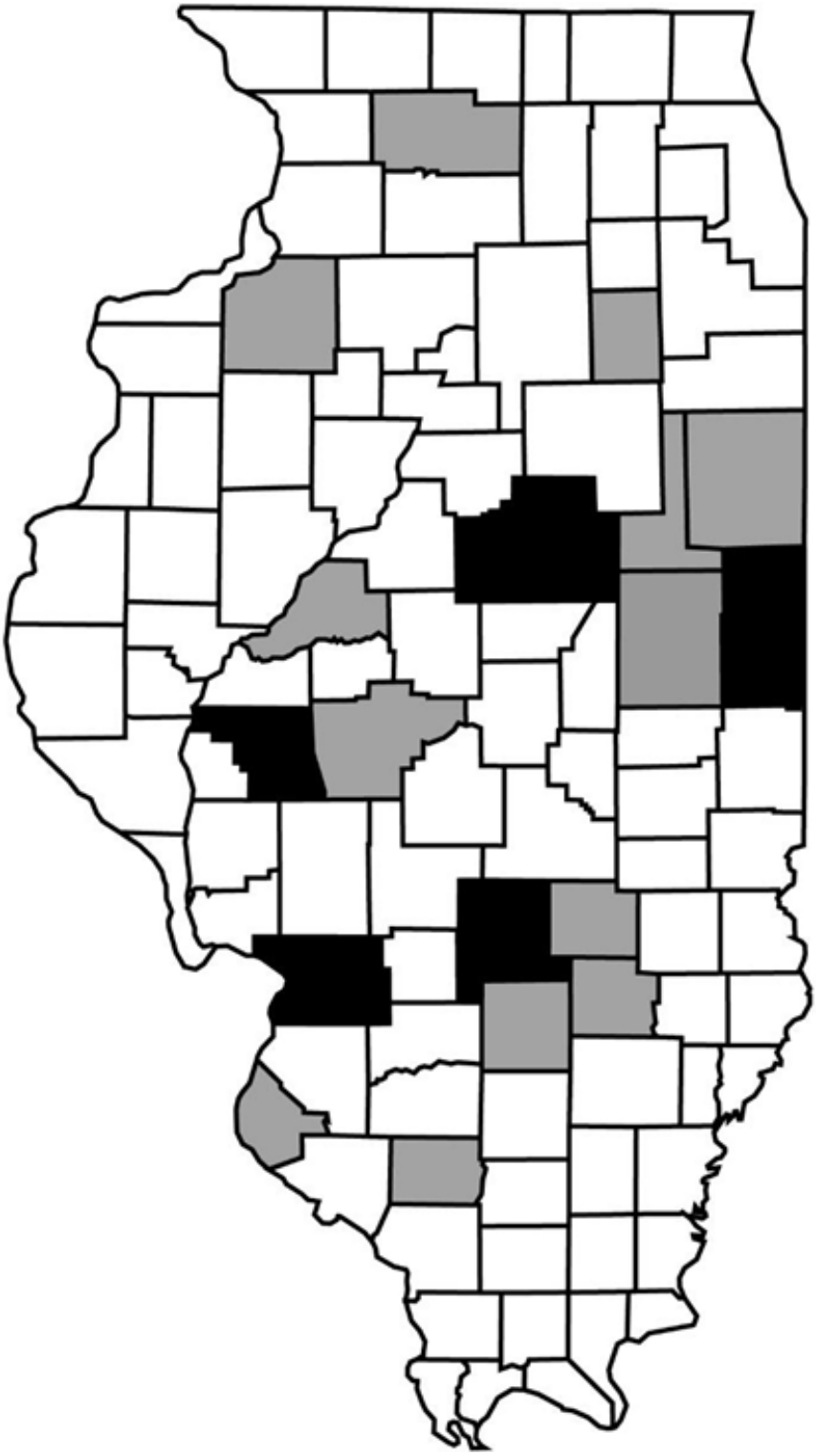


**Figure 6.** *Diceroprocta vitripennis* (Say, 1830), (Dana Collection DV200005M) ♂, USA: IL: Mason County: Topeka. Henry Allan Gleason Nature Preserve. 40.378118°, -89.92856°. 24-vii-2020. C.E. Dana.

**Figure 7.** 17-year periodical cicada, *Magicicada septendecim* (Linnaeus, 1758), (Dana Collection MC190077M) ♂, Brood VIII. Penn State Beaver Campus Athletic Fields, USA: PA: Beaver County: Monaca. 40.681083 -80.296715. 8-vi-2019. C.E. Dana, M.J. Thomas, J.R. Dana.

**Figure 8.** 17-year periodical cicada, *Magicicada cassinii* (Fisher, 1852), (Dana Collection MC190093M) ♂, Brood VIII. Settler's Cabin Park, parking lot west of Settler's Cabin Park Wave Pool, USA: PA: Allegheny County: Pittsburgh. 40.433928°, -80.154878°. 9.vi.2019. C.E. Dana, M.J. Thomas, J.R. Dana.

**Figure 9.** 17-year periodical cicada, *Magicicada septendecula* (Alexander & Moore, 1962), (Dana Collection MC210432M) ♂, Brood X, USA: IL: Vermillion County: Oakwood. 630 E Rd. Collected on Solter property with permission. 40.08984°, -87.822605°. 11.vi.2021. C.E. Dana.

**Figure 10.** 17-year periodical cicada brood distribution by Illinois county.

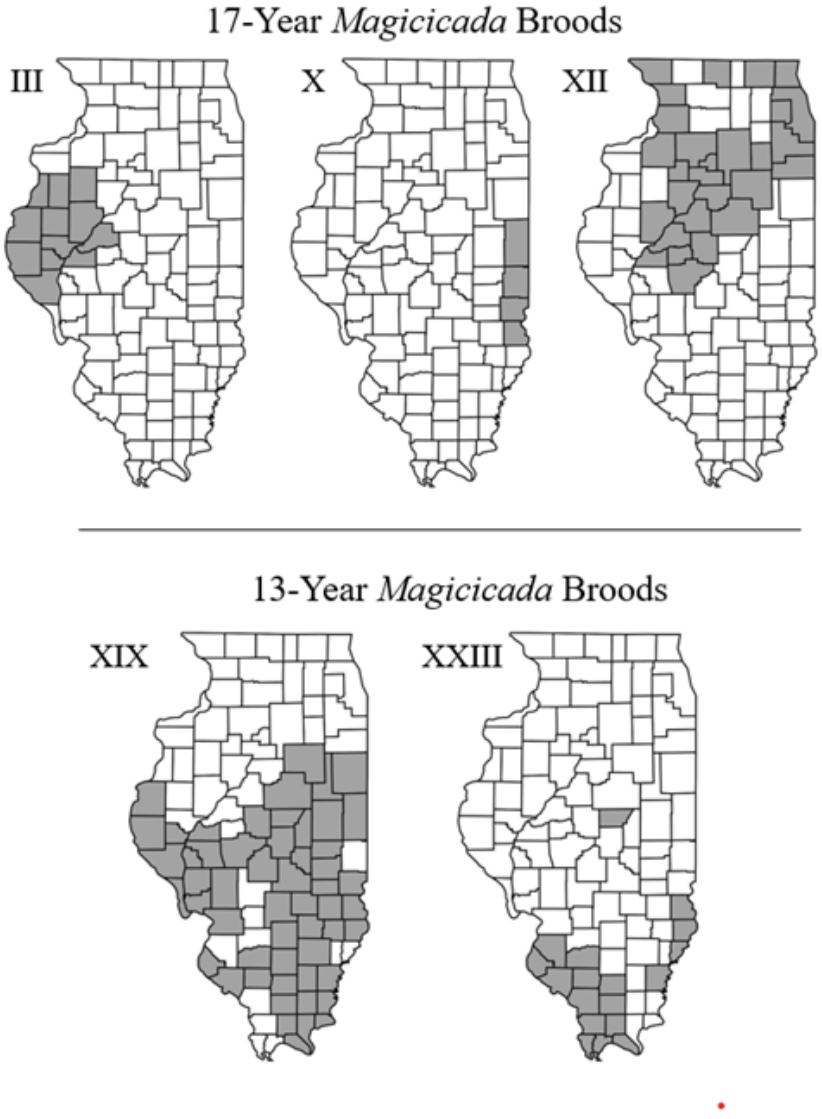


**Figure 11 (and report cover image).** *Megatibicen dorsatus* (Say, 1825), (Dana Collection NDor170028M) ♂, USA: IL: Iroquois County: Buckley. Right-of-Way Prairie South of Buckley. 40.58152°, -88.044949°. 10-viii-2017. C.E. Dana.

**Figure 12.** County map showing distribution of *Megatibicen dorsatus* (Say, 1825) in Illinois. Grey counties are those based on previous records and black counties are those newly added based on specimens collected during the duration of this study.

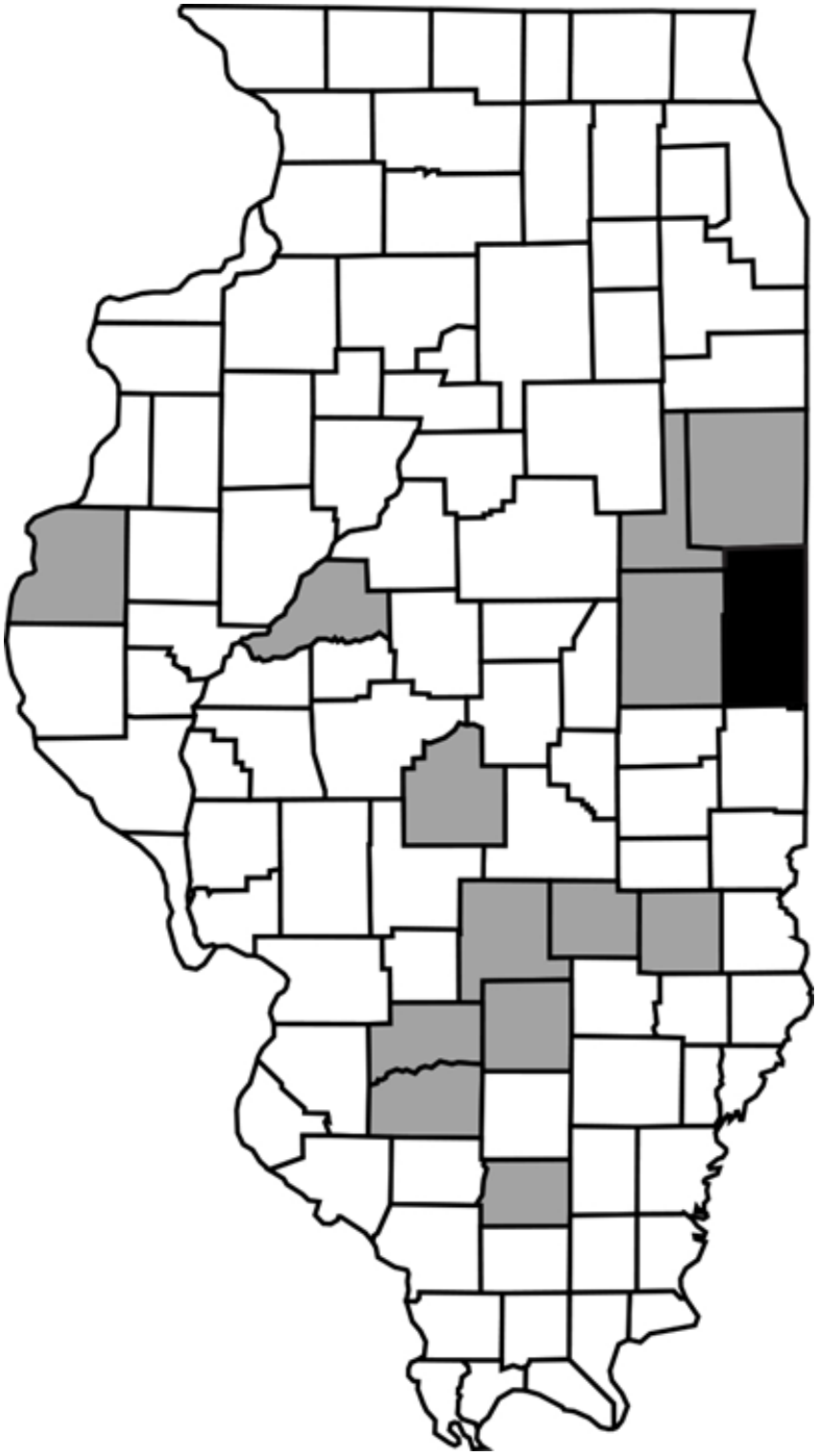


**Figure 13.** Partial map of Illinois showing collection locations of *Megatibicen dorsatus* (red dots). Loda Cemetery Prairie Nature Preserve (Loda CPNP), Prospect Cemetery Nature Preserve (Prospect CPNP), Ballard Nature Center (Ballard NC) and Henry A. Gleason Nature Preserve are indicated on the map as well. Railroad rights-of-way (ROWs) that follow US 45 are indicated in dashed lines north of Champaign. Railroad ROWs that follow IL-37 (12 Mile Prairie) prairies are indicated in dashed lines south of Effingham.

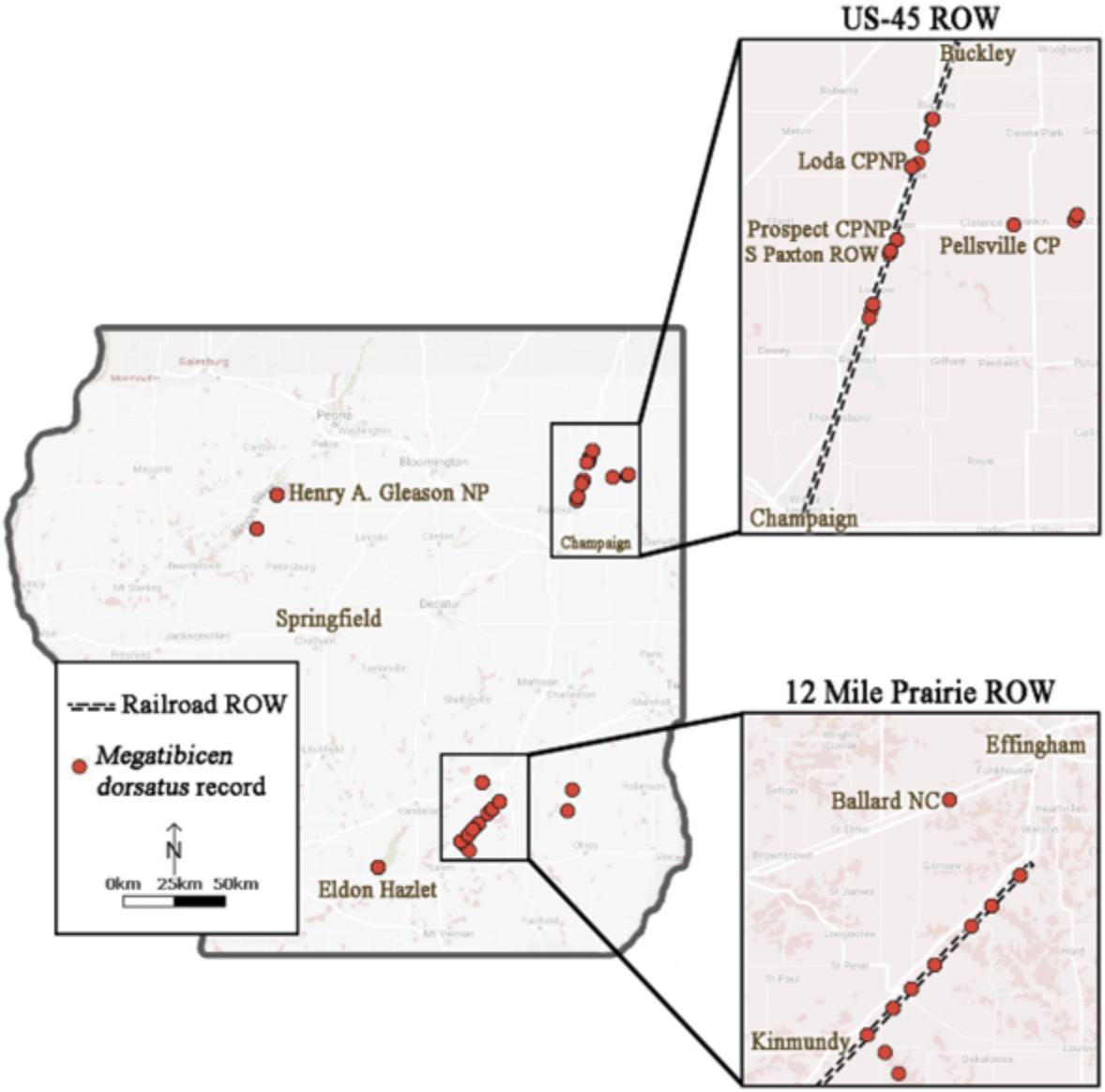


**Figure 14.** Image of *Neotibicen auriferus* (Say, 1825), (Dana Collection NAur210007M), USA: IL: Monroe County: Valmeyer. Salt Lick Land and Water Reserve, “Boyscout Prairie”. 38.30809486°, -90.30398494°. 12.ix.2021. M.J. Thomas, G.M. Lewis, J.R. Tetlie.

**Figure 15.** Distribution and study sites (hill prairies) of *Neotibicen auriferus* (Say, 1825).

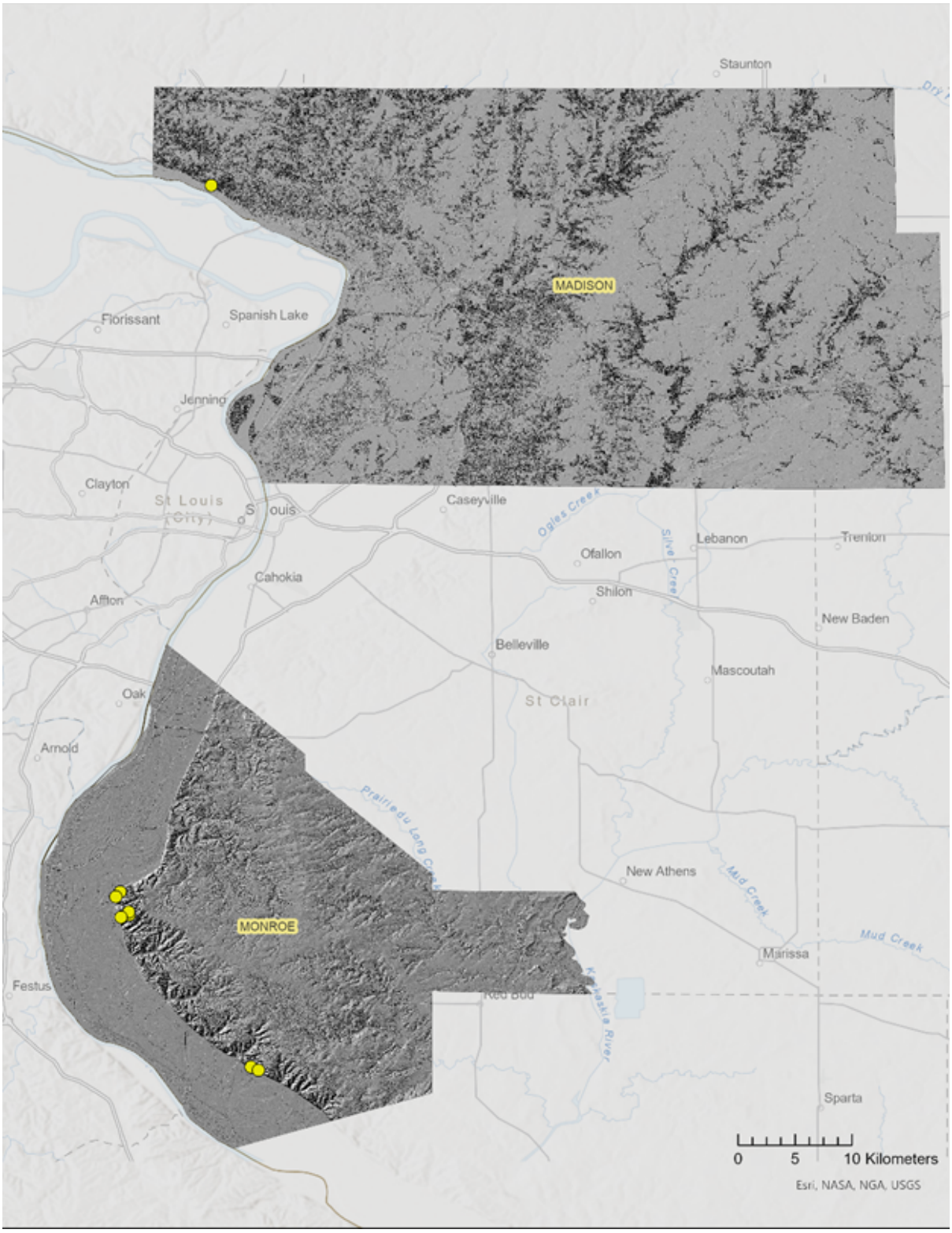


**Figure 16.** *Neotibicen lyricen lyricen* (De Geer, 1773), (Dana Collection NLyr1704M) ♂, USA: FL: Alachua County: Gainesville. Alfred A. Ring Park. 29.671720°, -82.347323°. 12-vi-2017. T. Hedlund.

**Figure 17.** *Neotibicen linnei* (Smith & Grossbeck, 1907), *Neotibicen pruinosus pruinosus* (Say, 1825), and *Neotibicen canicularis* (Harris, 1841) with specimen information**.**

**Figure 18.** *Neotibicen tibicen tibicen* (Linnaeus, 1758). (Dana Collection NTT200010M) ♂, USA: IL: Fayette County: La Clede Township. 12 Mile Prairie Tract 4. 38.842177°, -88.7602°. 13-viii-2020. C.E. Dana.

**Figure 19.** *Megatibicen pronotalis walkeri* (Metcalf, 1955), (Dana Collection MPro170001M), (INHS Insect Collection 1,001,494) ♂, USA: IL: Union County: Dongola. Dongola Gas Station. 37.368600°, -89.157475°. Caught at gas station lights in the evening. 20-viii-2017. C.E. Dana.

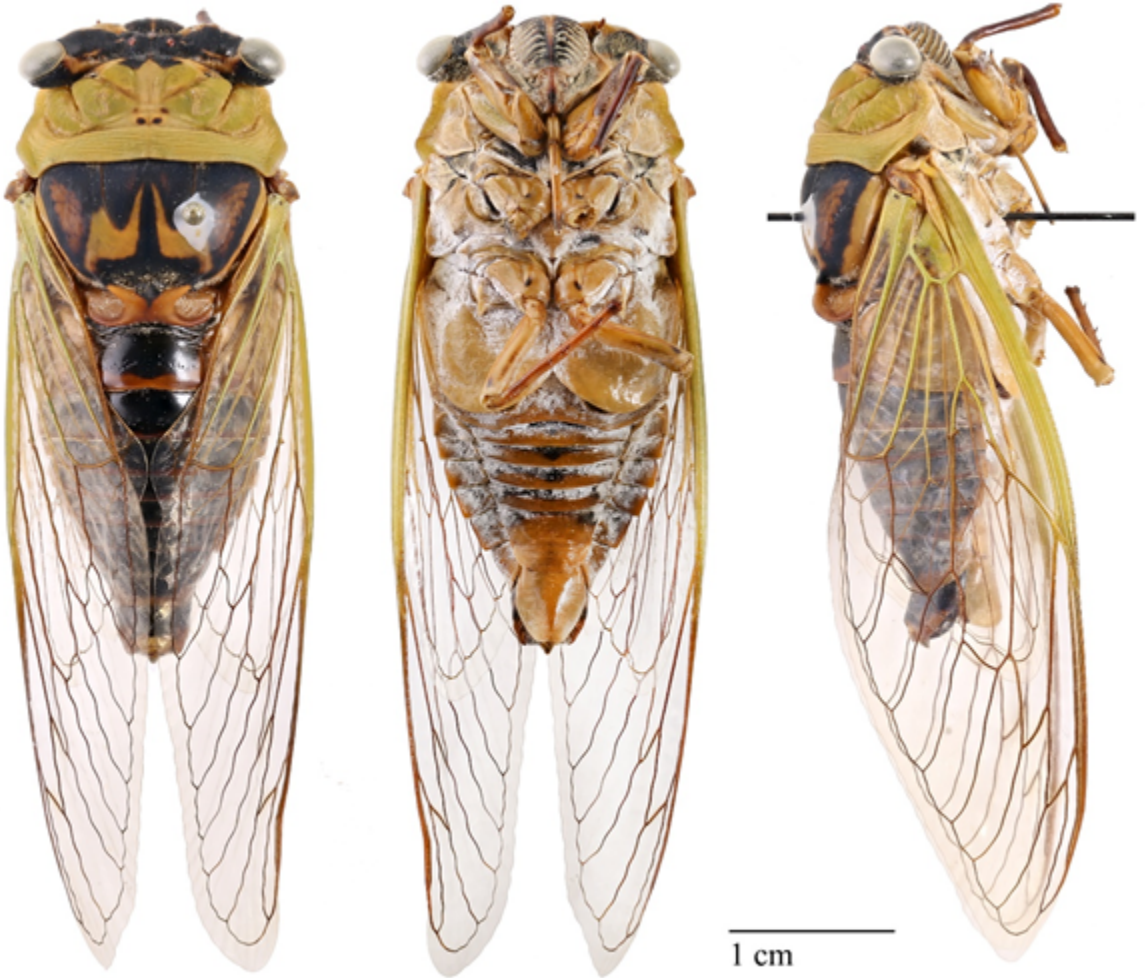


**Figure 20.** County map showing distribution of *Megatibicen pronotalis walkeri* (Metcalf, 1955) in Illinois. Grey counties are those based on previous records and black counties are those newly added based on specimens collected during the duration of this study.

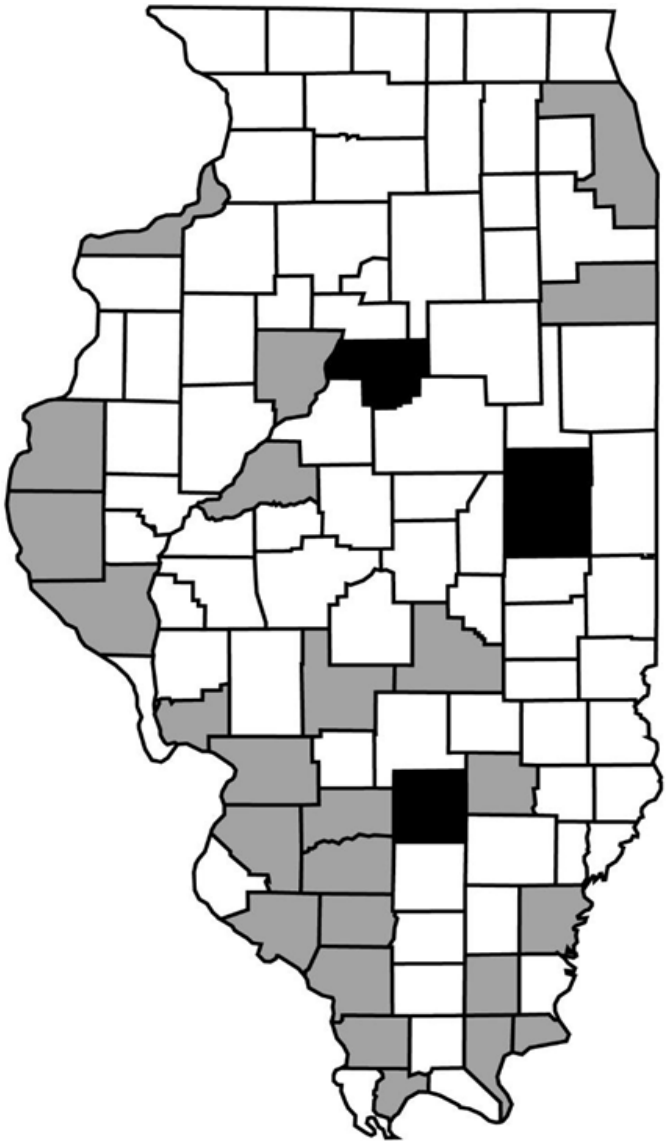


**Figure 21.** *Megatibicen auletes* (Germar, 1834). ♂ (left): (Dana Collection MAu1901M), (INHS Insect Collection 1,001,492), USA: IL: Marion County: Salem. Bryan Memorial Park. 38.637800°, -88.946921°. 31-vii-2019. C.E. Dana. ♀ (right): (Dana Collection MAu1801F), USA: IL: Marion County: Kinmundy. Stephen A. Forbes State Recreation Area. 38.715155°, -88.752477°. 2-viii-2018. C.E. Dana.

**Figure 22.** County map showing distribution of *Megatibicen auletes* (Germar, 1834) in Illinois. Grey counties are those based on previous records and black counties are those newly added based on specimens collected during the duration of this study.

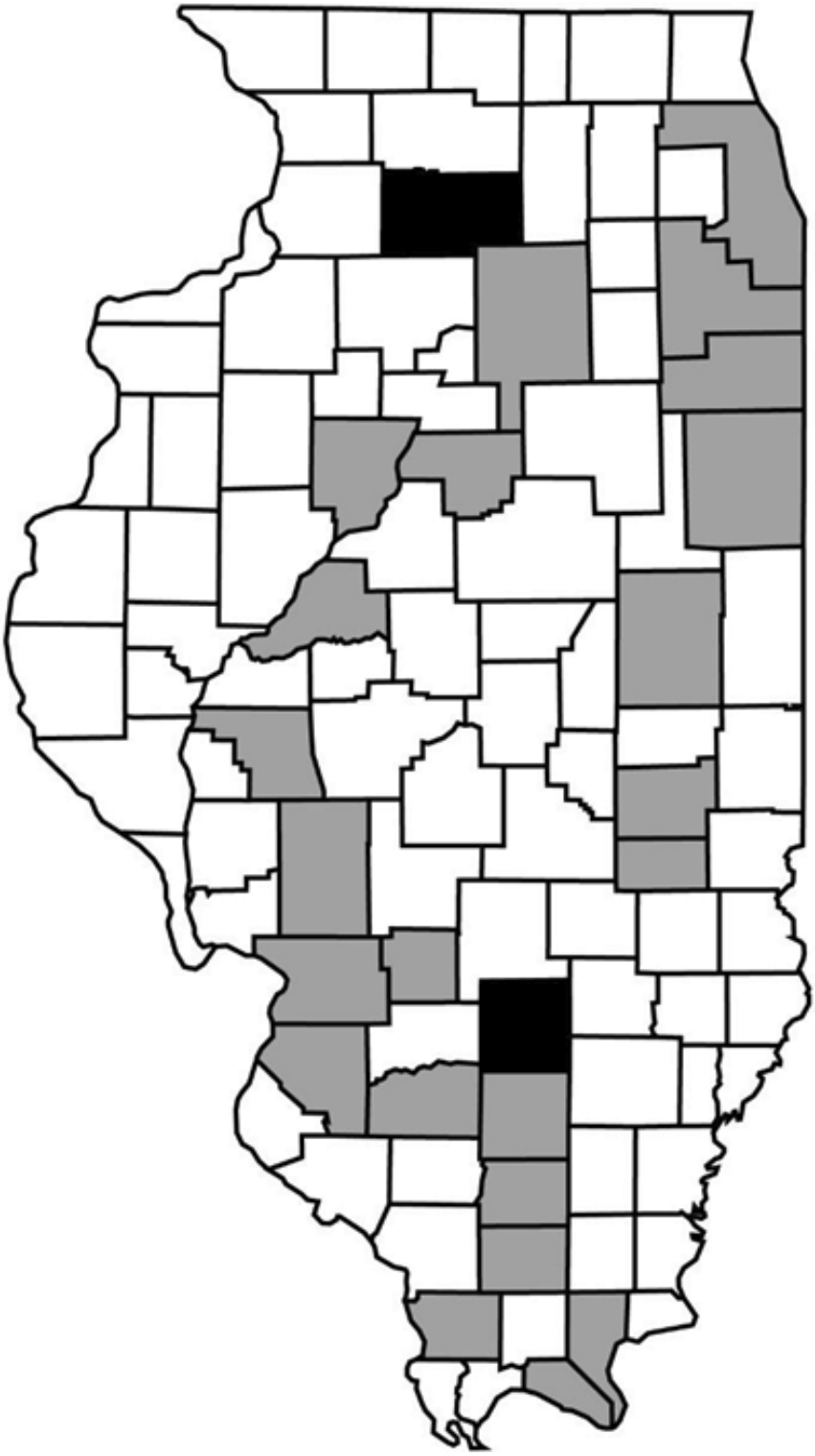


**Figure 23.** *Neocicada hieroglyphica hieroglyphica* (Say, 1830), (Dana Collection NH17001M) ♂. USA: MO: Shannon County: Fremont. Peck Ranch Conservation Area. 37.041452°, -91.163398°. 18-vii-2020. J.R. Tetlie.

**Figure 24.** *Okanagana balli* (Davis, 1919), (Dana Collection DOK200001M) ♂, USA:.IL: McLean County: Chenoa. Weston Cemetery Prairie Nature Preserve. 40.746767°, -88.614269°. 7-vii-2020. C.E. Dana.

**Figure 25.** Known distribution of *Okanagana balli* (Davis, 1919). Grey counties are those based on previous records and black counties are those newly added based on specimens collected during the duration of this study.

**Figure 26.** *Okanagana rimosa rimosa* (Say, 1830), (Field Museum Specimen 418846) ♂, USA:MI: Marquette County. 1-x-1956. H.S. Dybas.

**Figure 27.** Known historical distribution of *Okanagana rimosa rimosa* (Say, 1830), grey counties are those based on previous records.

**Figure 28.** Adult activity periods of a select group of Illinois cicada species based on findings from this study as well as museum specimens.

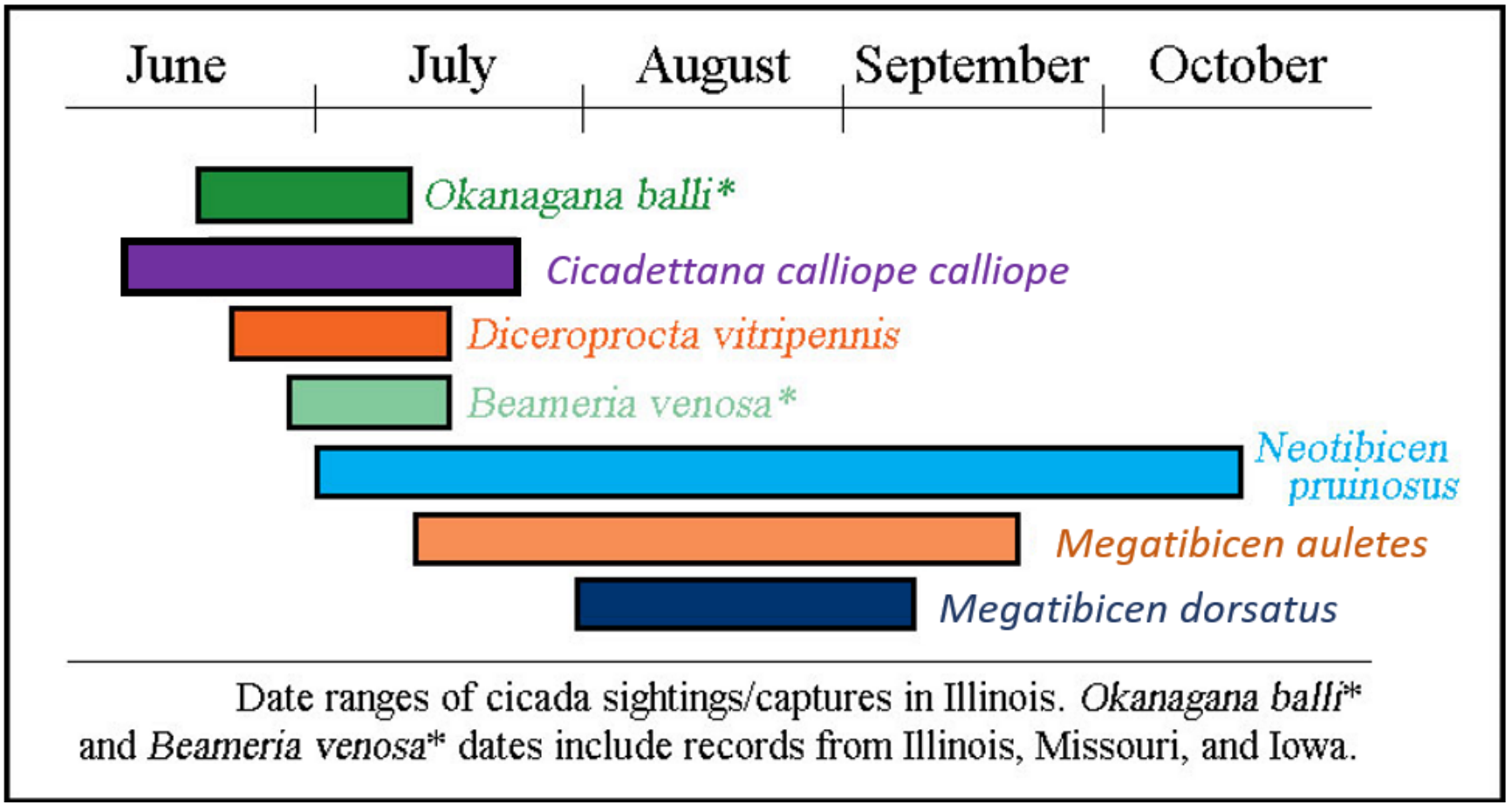


**Figure 29.** Map of *Megatibicen dorsatus* sampling locations and structure (k=6) plots by location. Colors on map indicate the population that the location best fits within (highest proportion). Colors correspond to groups from Table 2.4: R (light blue, 12-mile group), S (red, Rankin group), T (yellow, Gleason group), U (lilac, Loda group), V (green, southern half US-45 group), and W (dark blue, US-45 Buckley group).

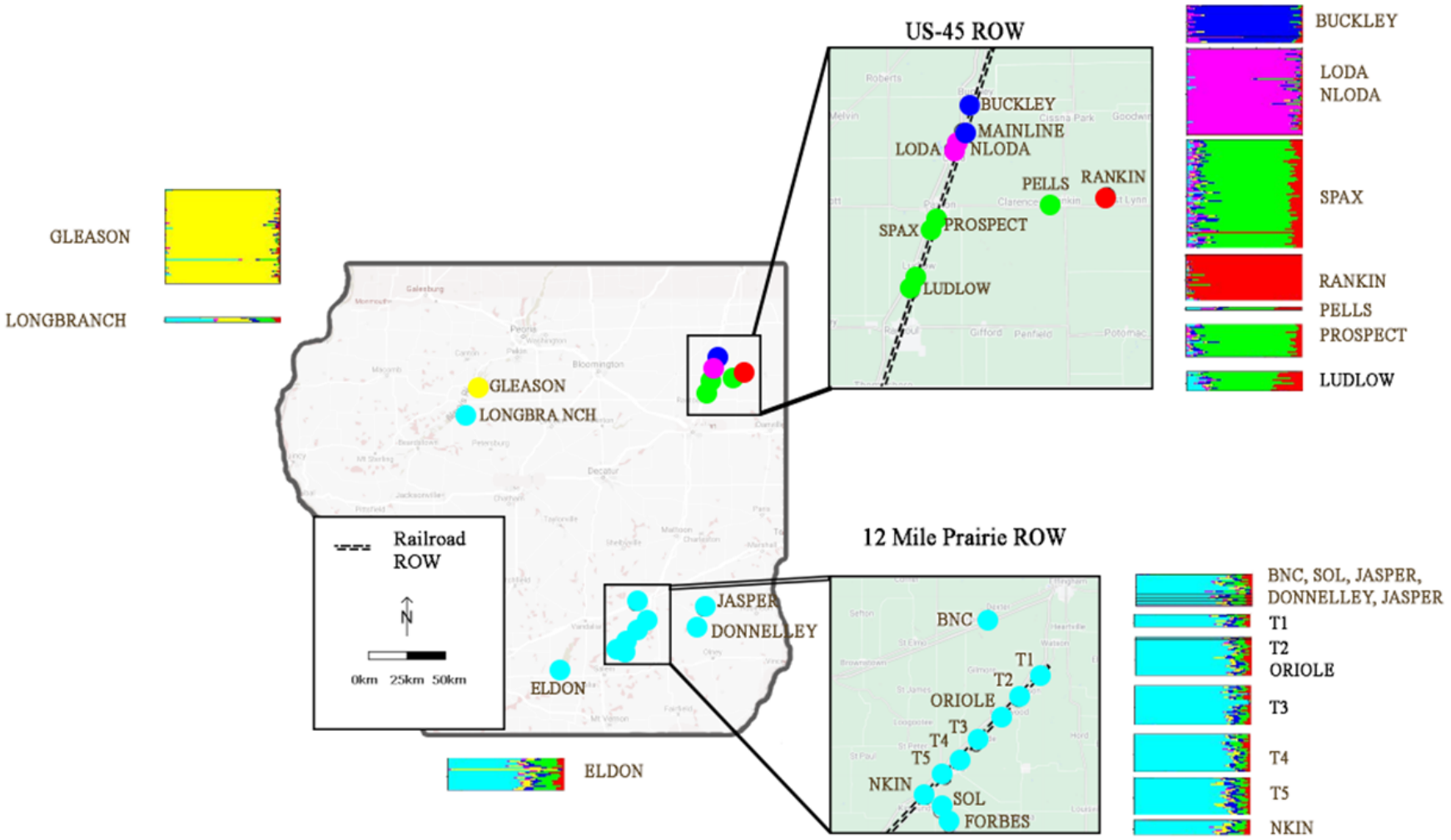
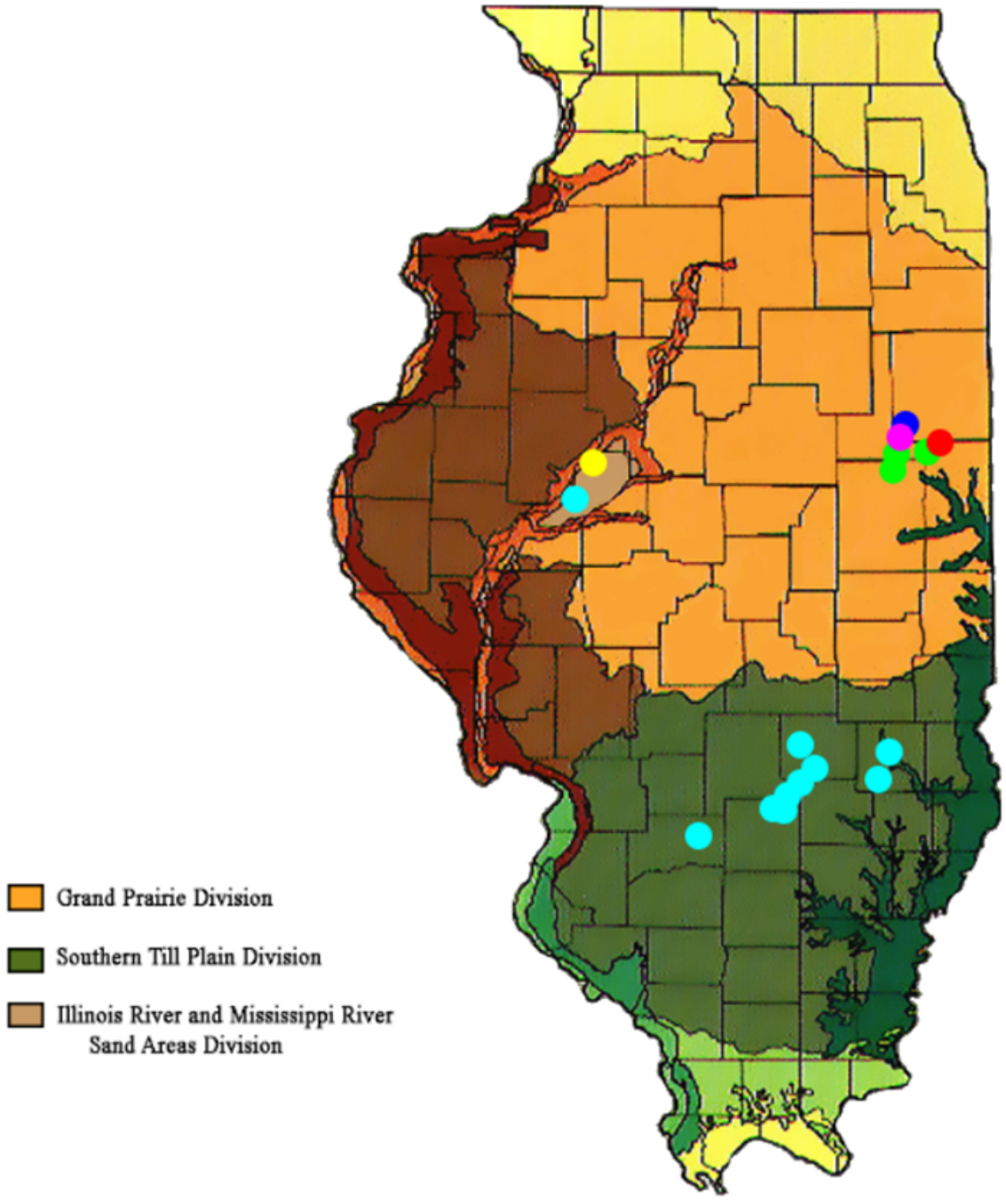


**Figure 2.16.** Overlay of population assignments (k = 6) on Schwegman's Natural Divisions of Illinois (Schegman 1997). 12 Mile Prairie ROW points fall within the Southern Till Plain Division, US-45 ROW points in Grand Prairie Division, and Gleason and Longbranch within the Illinois River and Mississippi River Sand Areas Division.



**References**


Catchen, J.M., Amores, A., Hohenlohe, P., Cresko, W., and J.H. Postlethwait. 2011. Stacks: building and genotyping loci *de novo* from short-read sequences. G3: Genes, Genomes, Genetics 1(3): 171-182.

Catchen, J., Hohenlohe, P.A., Bassham, S., Amores, A., and W.A. Cresko. 2013. Stacks: an analysis tool set for population genomics. Molecular Ecology 22(11): 3124-3140.

Clark, L.V., Brummer, J.E., Głowacka, K., Hall, M.C., Heo, K., Peng, J., Yamada, T., Yoo, J.H., Yu, C.Y., Zhao, H. and S.P. Long. 2014. A footprint of past climate change on the diversity and population structure of *Miscanthus sinensis*. Annals of Botany 114(1): 97-107.

Earl, D.A. and B.M. VonHoldt. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4(2): 359–361.

Hill, K.B., Marshall, D.C., Moulds, M.S., and C. Simon. 2015. Molecular phylogenetics, diversification, and systematics of *Tibicen* Latreille 1825 and allied cicadas of the tribe Cryptotympanini, with three new genera and emphasis on species from the USA and Canada (Hemiptera: Auchenorrhyncha: Cicadidae). Zootaxa 3985: 219–251.

Jombart, T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24: 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Jombart T., and I. Ahmed. 2011. adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. Bioinformatics 27(21): 3070–3071. https://doi.org/10.1093/bioinformatics/btr521

Kamvar, Z.N., Tabima, J.F., and N.J. Grünwald. 2014. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:e281. https://doi.org/10.7717/peerj.281

Kamvar, Z.N., Brooks, J.C., and N.J. Grünwald. 2015. Novel R tools for analysis of genome-wide population genetic data with emphasis on clonality. Frontiers in Genetics 6(208): 1–10. https://doi.org/10.3389/fgene.2015.00208

Knaus, B.J. and Grünwald, N.J., 2016. VcfR: a package to manipulate and visualize VCF data in R. Molecular Ecolpgy Resources. https://doi.org/10.1101/041277.

Knaus, B.J., and N.J. Grünwald. 2017. vcfr: a package to manipulate and visualize variant call format data in R. Molecular Ecology Resources 17(1): 44–53.

Limb, R.F., Hickman, K.R., Engle, D.M., Norland, J.E., and S.D. Fuhlendorf. (2007). Digital photography: reduced investigator variation in visual obstruction measurements for southern tallgrass prairie. Rangeland Ecology and Management 60(5): 548

Martin, M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet. journal 17(1): 10-12. https://doi.org/10.14806/ej.17.1.200

Paris, J.R., Stevens, J.R. and J.M. Catchen. 2017. Lost in parameter space: a road map for Stacks. Methods in Ecology and Evolution 8(10): 1360–1373.

Puritz, J.B., Hollenbeck, C.M., and J.R. Gold. 2014. dDocent: a RADseq, variant-calling pipeline designed for population genomics of non-model organisms. PeerJ 2:e431 https://doi.org/10.7717/peerj.431

Puritz, J.B., Matz, M.V., Toonen, R.J., Weber, J.N., Bolnick, D.I., and C.E. Bird. 2014. Demystifying the RAD fad. Molecular Ecology 23: 5937-5942. https://doi.org/10.1111/mec.12965

Rochette, N. and J. Catchen. 2017. Deriving genotypes from RAD-seq short-read data using Stacks. Nature Protocols 12: 2640–2659. https://doi.org/10.1038/nprot.2017.123

Sanborn, A.F. and Phillips, P.K. 2013. Biogeography of the cicadas (Hemiptera: Cicadidae) of North America, north of Mexico. Diversity 5(2): 166-239.

Sanborn, A.F. and M.S. Heath. 2016. *Megatibicen* n. gen., a new North American cicada genus (Hemiptera: Cicadidae: Cicadinae: Cryptotympanini). Zootaxa 4168(3): 577-582.

Wallner, A.M., Molano-Flores, B., and C.H. Dietrich. (2013). Using Auchenorrhyncha (Insecta: Hemiptera) to develop a new insect index in measuring North American tallgrass prairie quality. Ecological Indicators 25: 58-64.

Weir, B.S., and C.C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38(6): 1358–1370.