

## **FINAL REPORT FOR:**

### **Preparation of Eastern Massasauga Tissues for Genetic Analysis**

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#### **JUSTIFICATION**

The Eastern Massasauga has suffered a range-wide decline in Illinois with perhaps only one extant population, Carlyle Lake. Since 1999 we have collected blood samples from the Carlyle population during annual surveys and have stored those samples for future conservation genetics studies. Currently the Illinois Natural History Survey (INHS) is building a demographic based Population Viability Analysis (PVA) for the species. They will also refine the PVA model with spatial ecological data collected for the Eastern Massasauga. The final step is to produce a robust PVA that includes population genetics. A robust PVA accounting for demographics, habitat, and genetics will provide information for more targeted conservation actions and accurate threat assessment. This project was the first step in a multi-step process to obtain the genetic information needed. The project's overall goal was to prepare samples for subsequent and genetic analysis.

#### **OBJECTIVES**

- 1) Extract DNA from the individual tissue samples collected since 1999 for the Carlyle Lake population.
- 2) Prepare and store extracted DNA samples for use in future genetic studies

#### **MATERIALS AND METHODS**

Throughout the monitoring project, INHS staff has taken blood samples from all Eastern Massasaugas captured around Carlyle Lake from 1999 – present. We stored tissue samples in a -80 °C freezer. For preparation of tissue samples, staff first subsampled from the stored blood, and then extracted DNA using Quiagen DNeasy Blood and Tissue kits. Once extracted staff stored the purified DNA in a -80 °C freezer and built a database of all individuals with extracted DNA for future primer screening, PCR optimization, and genetic studies.

#### **RESULTS AND DISCUSSION**

We extracted DNA from 280 Eastern Massasauga tissue samples (Appendix I) collected from 1999 – 2010. Continued demographic monitoring has provided samples from late 2010 through 2012. The lack of representative samples from 1999 – 2002 is the result of a previous collaboration with

Northeastern Illinois University (Dr. Richard King). Dr. King's lab maintains the raw and extracted DNA samples. The INHS will obtain aliquots of these samples as raw tissues or extracted DNA to complete the tissue library for Carlyle Lake. The next two steps in the project are dependent on future funding. First, we will be selecting candidate primers from the scientific literature, then screening the primers for variability using PCR protocols. The goal is to include 16 variable loci in future work. The next, and more costly step, is to establish optimized multiplex panels and screen all extracted DNA samples. Multiplexing affords the ability to screen multiple loci at the same time and thus is more cost effective. Once the multiplex screening process is complete, the results will provide the needed population genetic data.

### **ACKNOWLEDGEMENTS**

We thank G. Tatham, J. Birdsell, and J. Bunnell of the Illinois department of Natural Resources, J. Smothers and D. Baum of the U. S. Army Corps of Engineers without whom; we could not have accomplished much of this work. We thank Stephanie Zec and Stacy Beyer for all their hard work in the lab extracting the DNA samples. We thank the many fieldworkers and volunteers who have helped us capture snakes since 1999. Funding for this project has been provided from the Illinois Department of Natural Resources – Illinois Wildlife Preservation Fund. We conducted this project in accordance under the University of Illinois IACUC protocol #08019 and Illinois Department of Natural Resources T&E species permit #05-11S.

## APPENDIX I

Extracted tissue samples for Eastern Massasaugas from Carlyle Lake, Clinton County, Illinois by snake number (#), sex and collection date.

ID	Sex	Date Collected	ID	Sex	Date Collected	ID	Sex	Date Collected	ID	Sex	Date Collected	ID	Sex	Date Collected
1	♀	4/1/1999	289	♀	4/9/2002	343	♂	7/11/2002	439	♀	3/30/2006	511	♀	3/25/2007
9	♀	4/12/1999	290	♀	4/9/2002	345	♂	7/13/2002	448	♀	4/5/2006	512	♂	3/25/2007
13	♀	4/11/1999	291	♀	4/9/2002	349	♂	8/19/2002	448	♂	4/9/2006	513	♂	3/25/2007
21	♂	4/7/1999	292	♂	4/10/2002	350	♂	8/3/2002	450	♀	4/1/2006	514	♂	3/25/2007
35	♀	4/12/1999	293	♀	4/11/2002	352	♀	8/19/2002	455	♀	3/31/2006	515	♂	3/25/2007
50	♀	4/14/2002	294	♀	4/11/2002	353	♀	8/3/2002	455	♀	4/15/2006	516	♀	3/24/2007
54	♀	4/12/2002	295	♀	4/11/2002	354	♀	8/3/2002	455	♀	3/25/2007	517	♂	3/26/2007
58	♀	4/11/2002	297	♀	4/11/2002	356	♀	8/4/2002	456	♂	4/17/2008	518	♀	4/18/2008
76	♂	4/11/2002	299	♀	4/11/2002	357	♂	8/6/2002	458	♀	3/24/2007	519	♀	3/26/2007
86	♂	5/19/2002	300	♂	4/11/2002	358	♂	8/12/2002	466	♂	4/9/2006	520	♀	3/26/2007
94	♂	4/16/2002	301	♀	4/11/2002	361	♂	8/18/2002	469	♂	3/14/2007	521	♀	3/26/2007
95	♂	4/16/2002	302	♀	4/12/2002	362	♂	8/18/2002	473	♀	4/16/2008	522	♀	3/26/2007
96	♂	4/16/2002	303	♂	5/14/2003	363	♂	8/18/2002	477	♂	3/23/2006	523	♂	3/27/2007
111	♂	4/12/2002	304	♀	5/14/2003	364	♂	8/22/2002	478	♀	3/25/2006	524	♂	3/27/2007
112	♂	4/4/2003	306	♀	4/13/2002	365	♀	8/22/2002	479	♀	3/26/2006	525	♀	3/27/2007
113	♀	4/14/2002	307	♀	4/13/2002	369	♂	9/2/2002	480	♂	3/26/2006	526	♀	3/27/2007
118	♀	4/22/2001	308	♀	4/13/2002	370	♂	9/4/2002	480	♂	4/15/2006	527	♀	3/27/2007
118	♀	4/12/2006	309	♀	4/13/2002	383	♂	4/17/2003	481	♀	3/26/2006	528	♀	3/28/2007
131	♀	4/1/2002	310	♀	4/13/2002	386	♂	4/28/2003	482	♂	3/31/2006	529	♀	3/28/2007
138	♀	4/11/2002	311	♀	4/13/2002	395	♀	3/30/2004	483	♀	4/4/2006	530	♂	3/28/2007
140	♀	4/1/2002	312	♂	4/13/2002	397	♂	3/24/2003	484	♂	4/1/2006	531	♀	4/28/2007
145	♀	4/9/2002	313	♀	4/13/2002	406	♀	4/1/2003	484	♂	4/23/2008	532	♀	3/28/2007
150	♂	3/30/2004	314	♂	4/14/2002	407	♂	4/1/2003	485	♀	4/7/2006	533	♀	3/28/2007
151	♂	4/14/2002	315	♂	4/14/2002	409	♀	4/1/2003	485	♀	4/9/2006	534	♀	3/28/2007
153	♀	4/19/2002	316	♂	4/16/2002	410	♀	4/1/2003	486	♀	4/9/2006	535	♀	3/29/2007
159	♀	4/11/2002	317	♂	4/16/2002	411	♂	4/2/2003	487	♂	4/9/2006	536	♀	4/1/2007
161	♀	4/10/2002	318	♀	4/16/2002	412	♂	4/2/2003	489	♂	4/9/2006	536	♀	4/7/2008
162	♀	4/12/2002	319	♀	4/16/2002	413	♂	4/2/2003	490	♂	4/15/2006	537	♀	3/29/2007
164	♀	4/8/2001	320	♀	4/17/2002	414	♀	4/1/2003	491	♀	4/15/2006	538	♀	3/29/2007
167	♂	4/9/2001	321	♀	4/19/2002	415	♀	4/11/2003	492	♀	4/24/2007	539	♀	3/29/2007
174	♀	4/16/2001	322	♀	4/18/2002	419	♀	4/14/2003	493	♂	6/1/2006	540	♂	3/30/2007
181	♀	4/13/2001	323	♀	4/21/2002	420	♂	4/23/2003	494	♂	3/13/2007	541	♀	3/30/2007
182	♂	4/12/2002	324	♀	4/18/2002	421	♂	4/30/2003	495	♀	3/13/2007	542	♂	3/30/2007
187	♀	3/26/2007	325	♀	4/20/2002	422	♀	3/17/2004	496	♂	3/13/2007	543	♀	3/31/2007
191	♀	5/26/2002	327	♂	4/23/2002	423	♀	4/2/2004	497	♂	3/13/2007	544	♂	3/31/2007
193	♀	7/13/2002	328	♀	4/25/2002	424	♀	3/18/2004	498	♀	3/21/2007	545	♀	3/31/2007
195	♀	4/12/2002	330	♂	5/4/2002	425	♀	3/29/2004	499	♀	3/21/2007	546	♂	3/31/2007
196	♂	4/15/2004	331	♂	5/2/2002	425.5	♂	3/29/2007	500	♂	3/24/2007	547	♂	3/31/2007
244	♀	4/11/2002	332	♀	5/2/2002	426	♂	3/30/2004	501	♂	3/24/2007	548	♂	4/1/2007
270	♂	4/11/2002	333	♀	5/8/2002	429	♂	4/5/2003	502	♂	3/24/2007	549	♀	4/1/2007
274	♀	10/20/2001	335	♀	5/21/2002	430	♂	4/5/2003	503	♂	3/24/2007	550	♀	4/3/2007
275	♂	10/20/2001	337	♀	5/25/2002	431	♂	4/5/2003	504	♂	3/24/2007	551	♂	4/3/2007
276	♂	10/26/2001	337	♀	3/31/2006	431	♂	3/24/2007	505	♂	3/24/2007	552	♀	4/3/2007
278	♀	11/14/2001	338	♂	5/27/2002	432	♂	4/6/2002	506	♀	3/24/2007	553	♂	4/2/2007
283	♂	4/8/2002	339	♀	6/13/2002	433	♂	4/6/2004	507	♀	3/24/2007	554	♂	4/2/2007
284	♀	4/4/2002	340	♀	6/19/2002	434	♂	4/6/2004	508	♂	3/25/2007	555	♀	4/11/2007
285	♀	4/8/2002	341	♂	6/28/2002	435	♀	4/1/2004	509	♀	3/25/2007	556	♂	4/6/2007
287	♀	4/9/2002	342	♀	8/16/2002	436	♂	4/15/2004	510	♀	3/25/2007	557	♀	4/23/2007

Date			Date			Date			Date			Date		
ID	Sex	Collected	ID	Sex	Collected									
558	U	4/23/2007	572	♀	4/9/2008	582	♀	4/19/2008	611	♂	8/29/2008	678	♀	9/8/2009
559	♀	4/22/2007	574	♀	4/18/2008	583	♂	4/19/2008	615	♀	10/20/2009	679	U	9/14/2009
560	♀	4/24/2007	575	♀	4/18/2008	586	♀	4/24/2008	621	♀	3/16/2010	680	U	9/14/2009
563	♀	4/4/2008	576	♀	4/18/2008	593	♂	4/30/2008	625	♀	3/16/2010	683	♀	9/14/2009
566	♀	4/5/2008	577	♀	4/18/2008	595	♂	5/4/2008	666	♂	9/16/2009	685	♀	9/14/2009
567	♂	4/5/2008	578	♀	4/19/2008	599	♀	5/17/2008	675	♀	9/18/2009	686	♂	9/14/2009
569	♀	4/6/2008	580	♀	4/19/2008	609	♂	9/8/2008	676	♂	9/8/2009	687	♀	9/15/2009
570	♂	4/9/2008	581	♂	4/19/2008	610	♂	9/2/2008	677	♂	9/8/2009	688	♀	9/18/2009

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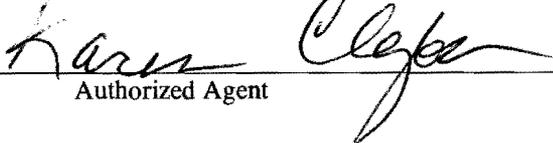
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Dressel, M. Preparation of Eastern Massasauga tissues for genetic analysis-\$1,683 (12-020W)

Taylor, S.J.; A. Yanahan Ground beetle assemblages on Illinois' significant slopes: A rare habitat threatened by climate change-\$1,958 (12-016W)

Anderson, B. INAI update of select areas of the state-\$30,000 (12-L20W)  
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