Distribution and Conservation Status of the Newly Described Species of Leopard Frog in the Coastal Northeast

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RCN funds requested: $99,764

Timeline: January 2014 to December 2015

Project Description: The recent discovery of a cryptic species of leopard frog in the Northeast means that nine states (CT, DE, MA, MD, NJ, NY, PA, RI, VA) may have to redefine their faunal checklists and/or lists of Species of Greatest Conservation Need. The new leopard frog appears to be of conservation concern in at least portions of its range, and until the states are able to address some basic information gaps, conservation efforts in the Northeast will be challenged with uncertain taxonomic statuses, potentially flawed bases for species and/or site prioritizations, and misappropriations of limited resources for strategic inventory, research, and/or management action. Through a multi-agency collaborative effort, we will 1) Determine conclusively which leopard frog species occur presently and occurred historically in the nine states; 2) Refine the northeastern distribution of the new species relative to the two other leopard frogs; 3) Contrast multi-level habitat associations among the three species; and 4) Refine the separation of species via field characters (calls, morphology) to facilitate future inventory, monitoring, and status assessments of the new species without reliance on genetic testing. Extensive bioacoustic surveys in 2014 will define the ranges of each species and identify sites for an intensive survey effort of occupied sites in 2014-2015 to characterize habitat associations, obtain tissue for genetic testing, and examine suspected rangewide morphological differences. The proposed study will provide northeastern states with a better capacity to implement sound, well-informed conservation strategies for native amphibians and their habitats.

Background and Need

Over a century of taxonomic confusion regarding the leopard frogs of the East Coast was resolved in 2012 with the publication of a genetic analysis (Newman *et al.* 2012) confirming that a third, cryptic species of leopard frog (*Rana* [= *Lithobates*] sp. nov.) occurs in southern New York, northern New Jersey, and western Connecticut. The molecular evidence strongly supported the distinction of this new species from the previously known northern (*R. pipiens* [= *L. pipiens*]) and southern (*R. sphenocephala* [=*L. sphenocephalus*]) leopard frogs. The new species' formal description, which highlights differences in vocalizations, morphology, and habitat affiliation (Feinberg *et al.* in preparation), is nearing submission for publication. This manuscript also presents bioacoustic evidence of the frog's occurrence in southern New Jersey, Maryland, Delaware, and as far south as the Virginia/North Carolina border, thereby raising uncertainty about which species of leopard frog occur(s) presently and historically throughout the region.

The elucidation of this cryptic species in the Northeast means that each state from Virginia to Massachusetts may have to redefine its faunal checklist and/or list of Species of Greatest Conservation Need (SGCN). Leopard frogs currently are granted some form of official status in five northeastern states and are listed as SGCN in four of them (Table 1). States that historically recognized just one species of leopard frog within their borders may now need to recognize a different or second species, and some states, including New York, New Jersey, Pennsylvania, Maryland, and Virginia, may need to recognize all three species. Given the very recent discovery of the new leopard frog species, each of the aforementioned northeastern states is

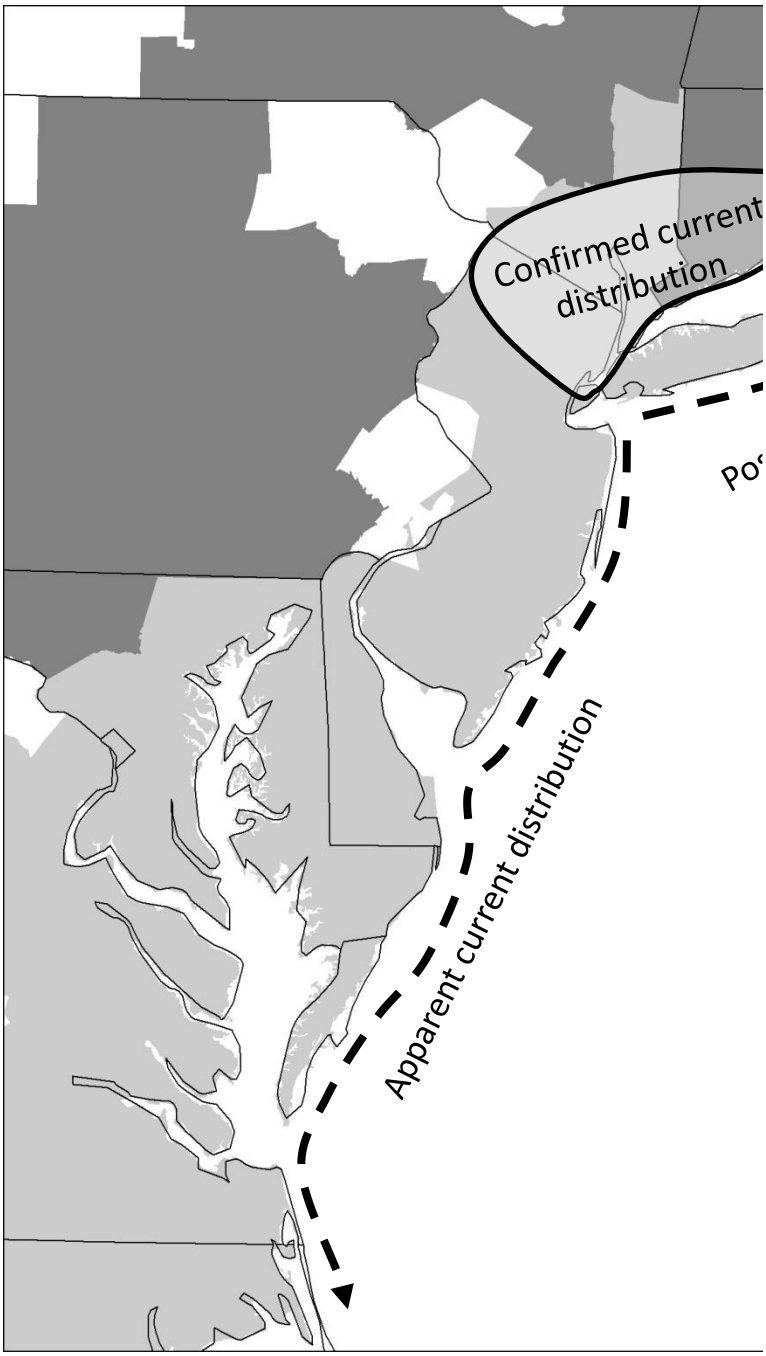

Figure 1. The distribution of *Rana pipiens* (dark gray), *R. sphenocephala* (light gray), and *R.* sp. nov. (black outline, with dotted lines representing potential distribution along the coast) in the northeast U.S. Adapted from Newman et al. (2012).

faced with the most fundamental of information gaps: 1) Which species of leopard frog are extant in the state? 2) Which species occurred historically in the state? 3) What is the conservation status of each species? 4) How can we easily distinguish among species for future status assessments and conservation planning? Until the states are able to address, at minimum, those four information gaps, conservation of amphibian diversity in the Northeast will be challenged with uncertain taxonomic statuses, potentially flawed bases for species and/or site prioritizations, and misappropriations of limited resources for strategic inventory, research, and/or management action.

There is reason to believe this newly identified leopard frog is a species of conservation concern and may merit listing at the state and/or federal levels. First, the distribution of this species appears to be one of the smallest of any U.S. anuran (Lannoo 2005). Second, leopard frogs have disappeared from many parts of their range for unknown reasons (Schlauch 1978, Klemens 1993, Kiviat *et al.* 2011, Newman *et al.* 2012, J. Feinberg, unpubl. data). Third, as a primarily coastal species, occurring sometimes in brackish water close to the ocean, this leopard frog may be especially susceptible to sea-level rise resulting from climate change and saltwater intrusion from extreme storm events. Fourth, this leopard frog species occurs in a highly urbanized region of the U.S. and further loss and/or degradation of habitat could eliminate local populations. However, the species apparently tolerates highly altered wetlands (the holotype comes from an industrial wetland on Staten Island [Feinberg et al. in preparation], and New Jersey's Meadowlands contains a sizeable population [Kiviat 2012]) and may have lessons for conservation of biodiversity in urban environments.

Table 1. Current status of leopard frogs in nine northeastern states and presence/absence in each state based on the updated taxonomy.

State	Prior taxonomy		Updated taxonomy		
	pipiens	*sphenocephala*	*pipiens*	*sphenocephala*	sp. nov.
MA	S3S4*	-	X		?
RI	S2*‡	-	X		?
CT	S2‡	-	X		X
NY	S5	S1S2*‡	X	?	X
NJ	-	S5	?	X	X
PA	S2S3	S1*†	X	?	X
DE	-	S5		X	X
MD	S4	S4S5	?	X	X
VA	-	S4	?	X	X

* Species of Greatest Conservation Need; † Endangered; ‡ Species of Concern or Special Concern

Objectives

To help determine whether this new species is an appropriate candidate for SGCN listing, we propose to fill key information gaps regarding its distribution and conservation status. Our specific objectives are to

1) Determine conclusively which leopard frog species occur presently and occurred historically in the nine states (CT, DE, MA, MD, NJ, NY, PA, RI, VA);
2) Refine the northeastern distribution of the new species relative to the two other leopard frogs;
3) Refine the conservation status in areas where the new species is already known to be of concern;

4) Contrast multi-level habitat associations among the three species; and
5) Refine the separation of species via field characters (calls, morphology) to facilitate future inventory, monitoring, and status assessments of the new species without reliance on genetic testing.

Our proposal primarily addresses RCN Topic 3 (Identify NE Species of Greatest Conservation Need Data Gaps, Design Data Collection Protocols, and Collect Data).

Methods and Timeline

This two-year study will take place in two phases: an extensive survey phase in winter/spring 2014 and an intensive site-based phase in spring/summer 2014 and 2015.

Phase 1: Extensive surveys. Extensive surveys will be used to meet Objectives 1 and 2 and to identify sampling sites for additional data collection and sampling in Phase 2 (below). We will use an occupancy modeling framework (Mackenzie *et al.* 2006) using repeated visits to inform estimates of regional rarity and serve as a rigorous baseline for monitoring. In winter and spring 2014, we will conduct bioacoustic (calling) surveys at up to 200 wetland sites determined to have high potential for *Rana* sp. nov., including all known locations (n = 19; Feinberg et al. in prep.), as well as up to 40 sites with known presence of *R. sphenocephala* or *R. pipiens*. Survey effort will be intensified in some areas where leopard frogs are known to be of concern (PA, NY, CT, MA) to meet Objective 3. Sites targeted for *R.* sp. nov. may include those along North American Amphibian Monitoring Program routes at which leopard frogs or wood frogs (*R. sylvatica*), which sound similar to *Rana* sp. nov. (Feinberg *et al.* in preparation), were reported. These broad-brush surveys will be conducted by professional biologists in all states and trained citizen scientist volunteers in MA, CT, NJ, MD, and VA at minimum, all employing a standard protocol. Surveys will be conducted at each wetland site twice in March, twice in April, and twice in May, using guidelines for survey timing and weather conditions from NAAMP (Weir *et al.* 2005, 2009). All anuran species detected will be documented, and observers will make recordings of all species encountered for expert confirmation and comparison of overall species occupancy across sites and regions.

Phase 2: Photography, tissue collection, and habitat description. These site-specific surveys will be used to meet Objectives 4 and 5 and will serve to follow up on suspected rangewide morphological and genetic patterns identified by Feinberg et al. (in preparation, and unpublished data) for a portion of the region. Specifically, we will catch and photograph frogs, collect tissue for genetic analysis, and characterize basic habitat conditions. Because of the precise nature of photography desired and the care needed to take tissue samples, we will keep to a minimum the number of people performing these surveys. Observers will traverse occupied sites and each leopard frog caught will be photographed from multiple angles. In addition, a toe clip will be taken from each captured frog and preserved for genetic analysis. We will obtain all necessary permits and follow standard protocols for preventing the spread of chytrid fungus and for care of individual frogs.

We will also analyze archival tissue samples from museum specimens when available (and where DNA has not been destroyed by formalin) from areas where we do not encounter extant populations of *Rana* sp. nov. to determine whether the species was present historically. Likely sources of samples include MA, RI, and Long Island, NY.

Tissue samples will be sent to the Shaffer lab at UCLA where DNA will be extracted, amplified via PCR, and sequenced according to the methods described in Newman et al. (2012). We will generate nucleotide sequence data from up to 100 individuals for seven loci including two mitochondrial DNA (mtDNA) fragments (*ND2*, *12s-16s*), and five nuclear DNA (nuDNA)

fragments including *NTF3*, *Tyr*, *Rag-1*, *SIA*, and *CXCR4*. These new data will be combined with existing GenBank sequences and analyzed using phylogenetic and population assignment methods to confirm the identity of each sample as belonging to *Rana* sp. nov.

Although a full treatment of habitat differences among the species of leopard frogs is beyond the scope of this project, we intend to collect basic habitat data at each occupied site. Habitat descriptions will be conducted at three levels: landscape, site, and capture location. Landscape-level habitat descriptions will include GIS analysis of surrouding vegetation, urbanization, geology, and soils. Site-level descriptions will include characteristics of the wetland, such as dominant vegetation, hydrology, and associated amphibian species. Descriptions of capture locations will include water chemistry, temperature, and surrounding vegetation, taken at the point of capture of a leopard frog.

Products and Outcomes

A final report and journal article (or series of articles) will document results of rangewide bioacoustic surveys, genetic sampling, and definition of field characters, provide updated range maps for the new leopard frog species, and make recommendations for SGCN status and state listings where applicable. The project director will present preliminary results to the Northeast Wildlife Diversity Technical Committee in September 2014 and final results to the Northeast Fish and Wildlife Conference after the conclusion of the project in 2016. Taken together, these products will provide northeastern states with a better capacity to implement sound, well-informed conservation strategies for native amphibians and their habitats.

Literature Cited

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Budget

Grant funds are requested for NY Natural Heritage salary and benefits; travel for field work; contracts with Jeremy Feinberg, Nate Nazdrowicz, the Shaffer Lab at the University of California, Los Angeles, and Hudsonia, Ltd.; and supplies. Non-federal cash and in-kind matching funds will be provided from salary and benefits expenses for university faculty and state biologists, volunteer citizen scientist time and mileage provided by partners, and donations

of time by professional consultant biologists. See Appendix for detailed accounting of match expenses.

EXPENSES	Federal	Non-federal	Total
Salary	$32,598	13,737	46,335
Fringe Benefits	14,196	7,188	21,384
Contractual services	21,000	0	21,000
Travel	8,250	0	8,250
Supplies	200	0	200
Other (Meetings, technical services)	2,934	0	2,934
Indirect	20,586	5,440	26,026
In-kind		78,561	78,561
Total	99,764	104,926	204,690

Qualifications of Individuals and Organizations

Dr. Matthew Schlesinger has conducted field studies of frogs since 1996 and has studied New York's leopard frogs since 2007. He is a co-author of the taxonomic description manuscript.

John Bunnell is the Chief Scientist for the New Jersey Pinelands Commission and has over 23 years experience researching the effect of land use on fish and anuran assemblages in the Pinelands, including a large population of southern leopard frogs.

Dr. Joanna Burger is a world-renowned conservation biologist who has studied New Jersey's fauna for much of her career. She serves as Jeremy Feinberg's major advisor. She was a co-author of the paper that separated the leopard frog species and is a co-author of the taxonomic description manuscript.

Jenny Dickson is Supervising Wildlife Biologist for the Connecticut Department of Environmental Protection. She is responsible for overseeing and managing many wildlife projects in the state.

Jeremy Feinberg has studied reptile and amphibian ecology and conservation since 1996 and accumulated 17 years of experience across a diverse professional history that includes work in the private sector as well as the federal government (USFWS) and academia. He has studied leopard frogs in New York and New Jersey since 2002. He was a co-author of the paper that separated the leopard frog species and is lead author of the taxonomic description manuscript.

Dr. James P. Gibbs is lead author of "The Amphibians and Reptiles of New York State" (Oxford University Press) and co-author on numerous publications on amphibian biology and conservation in the northeastern United States.

Dr. Erik Kiviat is Executive Director of Hudsonia, a nonprofit research institute. Wetland ecology, herpetology, and urban biodiversity are among his research areas. Erik co-authored a 1987 paper on New York's leopard frogs and studied the undescribed leopard frog in northeastern New Jersey in 2006 and 2012, including sound recording and water quality measurements.

John Kleopfer has been the lead biologist for the Virginia Department of Game and Inland Fisheries for the last eight years, overseeing all reptile and amphibian conservation programs in the Commonwealth.

Jacob Kubel has several years of experience conducting and administering amphibian inventory and research contracts in the Northeast, including handling and processing of amphibians for DNA samples. He is responsible for leading all amphibian inventory, research, and other conservation activities for the Massachusetts Division of Fisheries and Wildlife.

Nate Nazdrowicz is a Ph.D. student at the University of Delaware. He independently observed differences among leopard frogs in the Delmarva Peninsula.

Holly Niederriter is a Non-game Wildlife Biologist for Delaware's Natural Heritage and Endangered Species Program. She manages the Delaware Amphibian Monitoring Program.

Christopher Raithel is a Wildlife Biologist for the Rhode Island Department of Environmental Management. He has written extensively on Rhode Island's herpetofauna.

Dr. Alan Richmond curates the amphibian and reptile collection at the University of Massachusetts, Amherst. He has written extensively on Massachusetts's herpetofauna.

Dr. H. Bradley Shaffer is one of the foremost authorities on amphibian genetics in the world. He was a co-author of the paper that separated the leopard frog species and is also a co-author of the taxonomic description manuscript.

Scott Smith is a Wildlife Ecologist for the Maryland Department of Natural Resources and coordinates herpetological research and monitoring in the state.

Tom Tyning is a Professor of Environmental Science at Berkshire Community College. He has written extensively on Massachusetts's herpetofauna.

Chris Urban is Chief of the Natural Diversity Section of the Pennsylvania Natural Heritage Program and oversees much of the state's wildlife research and monitoring efforts.

Brian Zarate is a Senior Zoologist for the New Jersey Division of Fish and Wildlife, where he oversees much of the state's herpetological research and monitoring. He is a co-author of the taxonomic description manuscript.

Appendix: Detailed explanation of match

Cash match – non-federal sources

State	Source	Details	2014	2015	Total
NY	SUNY ESF	James Gibbs, 5% of summer & academic year salary, benefits and indirect@26% MTDC	$9,550	$9,631	$19,181
NY	NY Natural Heritage Program	Two weeks of survey time in NY State Parks; salary, benefits, indirect at 15.7% MTDC, and unrecovered indirect at 26% MTDC	$3,131	$4,053	$7,184
					$26,365

Third-party cash and in-kind – non-federal sources

State	Source	Details	2014	2015	Total
CA	University of California, Los Angeles	Shaffer lab, labor for processing tissue samples	0	$3,000	$3,000
CT	Volunteers	Volunteer citizen scientist time and mileage	$8,575	$8,575	$17,150
MA	MA Natural Heritage and Endangered Species Program	Volunteer citizen scientist time and mileage	$2,113	0	$2,113
MD	Department of Natural Resources	Staff time (Scott Smith) salary and benefits, plus volunteer citizen scientist time and mileage	$4,000	$4,0000	$8,000
NJ	New Jersey Division of Fish and Wildlife	Staff time (Brian Zarate) – salary and benefits	$2,000	$2,000	$4,000
NJ	New Jersey Division of Fish and Wildlife	Volunteer citizen scientist time and mileage	$1,955	$1,955	$3,910
NJ	Rutgers University	Joanna Burger, 5% of summer & academic year salary and benefits	$5,220	$5,220	$10,440
NJ	New Jersey Division of Fish and Wildlife	Contractual expense for annual field surveys	$3,500	$3,500	$7,000
NY	Office of Parks, Recreation, and Historic	Two weeks each of survey time in each year (Jesse Jaycox and Ariana Newell) – salary and	$6,474	$6,474	$12,948

	Preservation	benefits			
NY	Hudsonia, Ltd.	Grant for work in Meadowlands region – salary and benefits	$2,000		$2,000
RI	Chris Raithel	Expert volunteer time, mileage	$3,000	$3,000	$6,000
VA	Department of Game and Inland Fisheries	Staff time (J.D. Kloepfer) – salary and benefits	$500	$500	$1,000
VA	Department of Game and Inland Fisheries	Volunteer citizen scientist time and mileage	$500	$500	$1,000
					$78,561