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A new species of leopard frog (Anura: Ranidae) from the urban northeastern US

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ABSTRACT

Past confusion about leopard frog (genus *Rana*) species composition in the Tri-State area of the US that includes New York (NY), New Jersey (NJ), and Connecticut (CT) has hindered conservation and management efforts, especially where populations are declining or imperiled. We use nuclear and mitochondrial genetic data to clarify the identification and distribution of leopard frog species in this region. We focus on four problematic frog populations of uncertain species affiliation in northern NJ, southeastern mainland NY, and Staten Island to test the following hypotheses: (1) they are conspecific with *Rana sphenocephala* or *R. pipiens*, (2) they are hybrids between *R. sphenocephala* and *R. pipiens*, or (3) they represent one or more previously undescribed cryptic taxa. Bayesian phylogenetic and cluster analyses revealed that the four unknown populations collectively form a novel genetic lineage, which represents a previously undescribed cryptic leopard frog species, *Rana* sp. nov. Statistical support for *R.* sp. nov. was strong in both the Bayesian (pp = 1.0) and maximum-likelihood (bootstrap = 99) phylogenetic analyses as well as the Structure cluster analyses. While our data support recognition of *R.* sp. nov. as a novel species, we recommend further study including fine-scaled sampling and ecological, behavioral, call, and morphological analyses before it is formally described.

1. Introduction

Leopard frogs of the *Rana pipiens* (=*Lithobates pipiens*) complex are widespread and common throughout much of the United States, but species delimitation and the associated taxonomy of the group have been challenging and contentious (Brown, 1973; Pace, 1974; Moore, 1975; Brown et al., 1977, 1990; Zug et al., 1982; Hillis, 1988; Frost et al., 2006, 2008, 2009; Pauly et al., 2009). While studies of range-wide phylogeography and systematics at the genus and species level are common (e.g., Pace, 1974; Hillis et al., 1983; Pytel, 1986; Hoffman and Blouin, 2004; Hillis and Wilcox, 2005; Oláh-Hemmings et al., 2010; Newman and Rissler, 2011), relatively little attention has been focused on taxonomic status and conservation needs of local or regional populations or subspecies (but see Di Candia and Routman, 2007; Hekkala et al., 2011). As is true for any group, appropriate conservation measures cannot be identified and implemented in the face of uncertain taxonomy (Köhler et al., 2005).

The species composition of leopard frogs in parts of the mid-Atlantic and northeastern US—hereafter the Tri-State area, including New Jersey (NJ), New York (NY), and Connecticut (CT)—has been questioned by biologists over the past several decades (Kauffeld, 1937; Yeaton, 1968; Schlauch, 1971; Pace, 1974; Klemens et al., 1987; Klemens, 1993). Currently, two species are recognized in the region (Conant and Collins, 1998). *Rana pipiens*, the northern leopard frog, is widely distributed across New England and the Great Lakes region, including the western two-thirds of CT and central and northern NY. From NJ, Long Island (NY), and southern mainland NY to the south, it is replaced by *R. sphenocephala* (=*L. sphenocephalus*), the southern leopard frog. While natural history collection data suggest the two species have a narrow zone of overlap in southern NY (Fig. 1), no area of sympatry has been directly identified. Some earlier studies based on morphological data suggested the possibility of intergradation (Schlauch, 1971), whereas others speculatively discussed a putative third species in this region (Kauffeld, 1937; Klemens, 1993).

Although widespread and often common at the continental scale (Fig. 1), leopard frog populations have been severely declining in certain regions, resulting in extirpation from some portions

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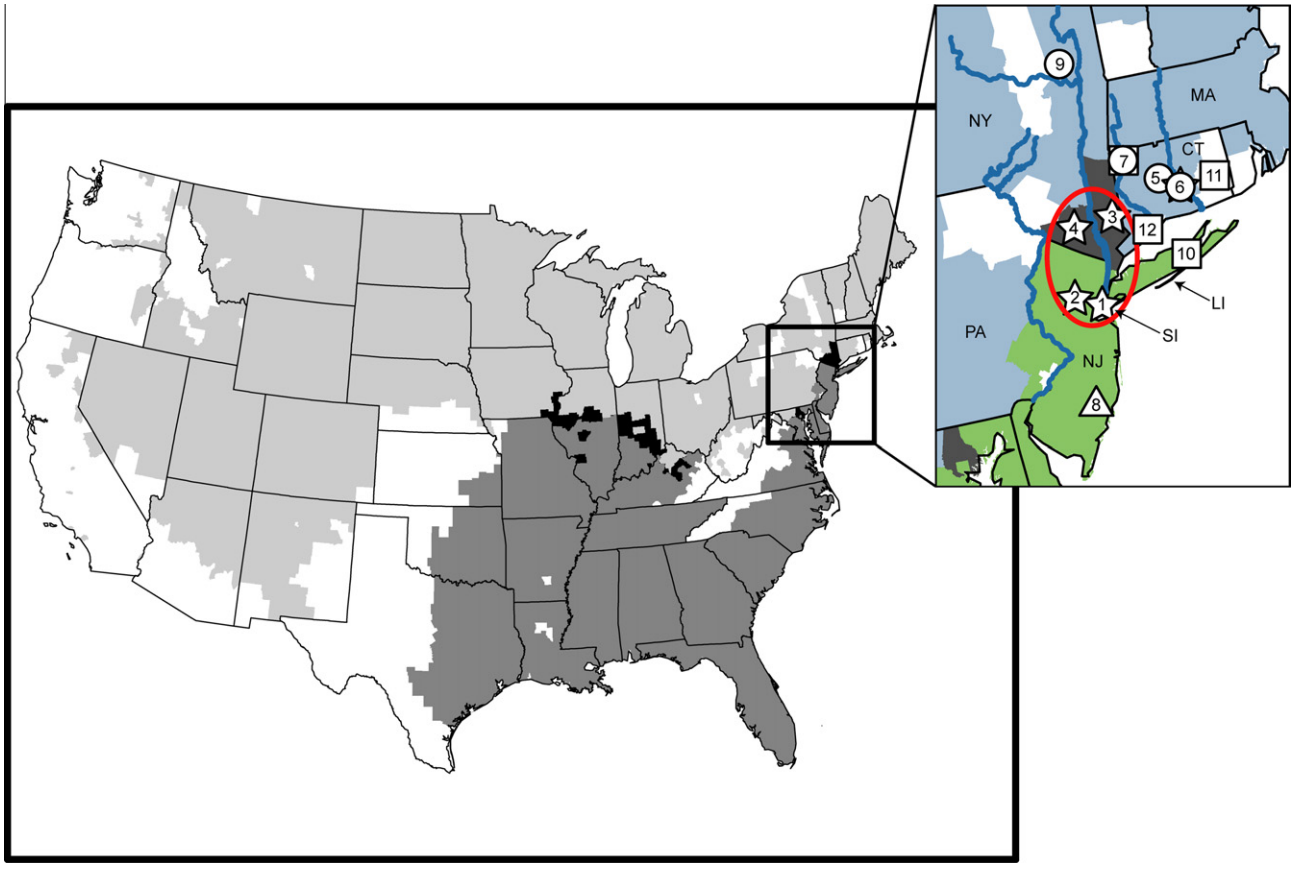

Fig. 1. Range maps for *Rana pipiens* (light gray shading) and *R. sphenocephala* (dark gray shading) in the US. Black indicates range overlap. Inset: sampling localities for genetic analyses. Numbers correspond to Table 1. Green: *R. sphenocephala* range, blue: *R. pipiens* range, dark gray: range overlap. Red oval contains the four focal populations in this study. NY: New York, PA: Pennsylvania, NJ: New Jersey, CT: Connecticut, MA: Massachusetts, SI: Staten Island, LI: Long Island. Range maps were downloaded as ESRI shapefiles from the IUCN Red List spatial data collection (2011). Colors are available in the online version. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of their historical range (Lannoo, 2005), including coastal regions and islands north and east of Long Island, NY (Ditmars, n.d.; Latham, 1971; Klemens, 1993; Feinberg, et al., unpublished data). Leopard frogs are also believed to be extirpated from highly developed areas including Long Island, NY (Kiviat, 2010; Feinberg et al., unpublished data); New Haven, CT; and Providence, Rhode Island (Klemens, 1993). While the exact causes of these declines are unclear, environmental pesticides and endocrine disruptors (Hayes et al., 2003; Lannoo, 2008), disease (Carey et al., 1999; Greer et al., 2005; Davis et al., 2007; Searle et al., 2011), habitat loss and alteration (Lannoo, 2005), and over-harvesting for use as laboratory specimens (Hillis, 1988; Klemens, 1993; Lannoo, 2005) have all been identified as contributing factors, particularly regarding *R. pipiens*. *Rana sphenocephala*, in contrast, remains relatively abundant throughout most of its range to the south, including coastal islands south of Long Island. However, near its northern range limit, it is listed as a Species of Special Concern in NY (NY Department of Environmental Conservation) and as endangered in Pennsylvania (PA) (Pennsylvania Fish and Boat Commission).

To gain a better understanding of the status and distributions of leopard frog populations in the Tri-State area, we analyzed mitochondrial and nuclear gene sequences from four focal populations of unknown leopard frog species composition in northern NJ, southeastern mainland NY (two populations), and Staten Island, NY (one of the five boroughs of New York City). Direct observations by one of us (JAF) showed that these four populations exhibited several unique characteristics, including an advertisement call distinct from both *R. pipiens* and *R. sphenocephala*. We also analyzed three CT populations from localities within the traditionally accepted geographic range of *R. pipiens*. We evaluated three possible interpretations of the status of leopard frogs in the Tri-State area: (1) the four focal populations are conspecific with either *R. pipiens* or *R. sphenocephala*, (2) the populations are hybrids between *R. pipiens* and *R. sphenocephala*, or (3) the populations represent a previously undescribed leopard frog lineage distinct from *R. pipiens* and *R. sphenocephala*.

2. Materials and methods

2.1. Study area and sample collection

Our study region was focused on the Tri-State area of the northeastern US, including NY, NJ, and CT—a total area of roughly 40,000 km^2 (Fig. 1). The region includes an area of putative range overlap between *R. sphenocephala* and *R. pipiens* according to range maps downloaded from the IUCN [IUCN Red List of Threatened Species 2011.1 (http://www.iucnredlist.org)]. Our study included four focal populations of unknown leopard frog species composition: Great Swamp (NJ), Staten Island (NY), Putnam County (NY), and Orange County (NY) (Fig. 1). The Great Swamp and Staten Island sites fall within the geographic range of *R. sphenocephala* and outside the range of *R. pipiens*, whereas the Putnam and Orange sites fall in the overlap zone of the two species' ranges. Leopard frog species composition in CT has also been questioned (Klemens, 1993), so we collected samples from three sites across CT to include in the analyses (Fig. 1).