Cryptic Diversity in Metropolis: Confirmation of a New Leopard Frog Species (Anura: Ranidae) from New York City and Surrounding Coastal Regions

Jeremy A. Feinberg, Catherine E. Newman, Gregory J. Watkins-Colwell, Matthew D. Schlesinger, Brian Zarate, Joanna Burger

Published: October 29, 2014 • DOI: 10.1371/journal.pone.0108213

Abstract

We describe a new cryptic species of leopard frog from the New York City metropolitan area and surrounding coastal similar to two largely parapatric eastern congeners, Rana sphenoccephala and R. pipiens. We primarily use bioacoustic species, but also examine other lines of evidence. This discovery is unexpected in one of the largest and most densely demonstrates that new vertebrate species can still be found periodically even in well-studied locales rarely associated species typically occurs in expansive open-canopied wetlands interspersed with upland patches, but centuries of loss for conservation concern. Other concerns include regional extirpations, fragmented extant populations, and a restricted type locality within New York City and report a narrow and largely coastal lowland distribution from central Connecticut data) and south to North Carolina (based on call data).

Figures


Editor: Helge Thorsten Lumbsch, Field Museum of Natural History, United States of America

Received: June 21, 2014; Accepted: August 18, 2014; Published: October 29, 2014

Copyright: © 2014 Feinberg et al. This is an open-access article distributed under the terms of the Creative Common unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All Supporting Information files.

Funding: Financial support for field work and analysis was primarily provided by a Rutgers Graduate School of Natural Science Fellowship and a New Jersey Department of Environmental Protection Conserve Wildlife Matching Grant to JAF, a Brookhaven National Laboratory, the Foundation for Ecological Research in the Northeast, and the Tiko Fund. Further
Introduction

In order to develop clear understandings of species and their ecologies, distributions, and conservation needs, they must be delimited [1]. Such efforts can be complicated, however, by the presence of cryptic species – species that, due to morphological similarity, are included with one or more other species under a single species classification [2]. Identifying cryptic species can be difficult in heavily altered environments and areas where insufficient numbers of individuals or populations for sampling. Nonetheless, a cryptic species discovery can have important conservation implications: amphibians can be sensitive to disease, contaminants, and environmental perturbations, and they are often highly fragmented and urbanized [9]. Also worrisome are enigmatic declines that have led to disappearances of species [10]–[13]; this includes some relatively non-urbanized coastal, suburban, and agricultural regions in so Connecticut (CT) [11], and presumably parts of northeastern Pennsylvania (PA) where they were reported historically.

Here, we expand upon the initial genetic results presented by Newman et al. [3] to name, diagnose, and describe the supporting evidence, but focus on bioacoustic signals and molecular data. We also provide a brief history of relevant taxonomic concepts across those years. This has led to numerous synonyms and conflicting species frameworks over time; for example, R. sphenocephala and R. pipiens, received lasting consideration and taxonomic recognition in the east [26], [29]. R. pipiens has a reported range from extreme southeastern NY to Florida (FL) and west from Texas to Iowa [30]. Rana pipiens, eastern Canada, New England, and the northern mid-Atlantic, west to the Pacific Coast states and British Columbia [3] along the US East Coast [29], [30], although Pace [26] reported one possible example of sympatry from Bronx County.

Taxonomic Overview

Although one of the most well-known and best-studied amphibian groups on earth, the R. pipiens complex has long been the subject of much debate in eastern North America [21]–[27]. Our work resolves some of this confusion. In this section we provide appropriate context for our discovery.

The unsettled taxonomic history of the R. pipiens complex spans several centuries and has been fueled largely by a lack of consistent species concepts across those years. This has led to numerous synonyms and conflicting species frameworks over time; for example, R. sphenocephala and R. pipiens, received lasting consideration and taxonomic recognition in the east [26], [29]. Rana pipiens has a reported range from extreme southeastern NY to Florida (FL) and west from Texas to Iowa [30]. Rana pipiens, eastern Canada, New England, and the northern mid-Atlantic, west to the Pacific Coast states and British Columbia [3] along the US East Coast [29], [30], although Pace [26] reported one possible example of sympatry from Bronx County.
Much of the historical discord and confusion surrounding the *R. pipiens* complex can be traced to the Northeast and greater New York City metropolitan area [11], [33], [34] (referred to hereafter as the NY/NJ-metro area and defined to New Jersey [NJ], and extreme eastern PA). This relatively small region has been associated with longstanding ambiguity in the locality of *R. pipiens* itself [7], [34], [35] and as many as five different species names over the past 250 years [7], [33].

In 1936, Kauffeld [35] attempted to reconcile some of this confusion. He did so by noting the possibility of a third, central species in the NY/NJ-metro area, between the recognized East Coast ranges of *R. sphenocephala* and *R. pipiens* at that time. Examinations with subspecies descriptions by Cope [36] and putative type localities for *R. pipiens* northernmost species – to his proposed central species (occupying much of the NY/NJ-metro area and mid-Atlantic region and west to Texas); *R. sphenocephala* was maintained as the southernmost species. Despite acknowledging the this could cause, Kauffeld [33] proposed these changes to reflect his conclusion that the type locality for *R. pipiens* fell reported central species occurred, not the northernmost species.

Kauffeld's three-species framework and taxonomic changes received some initial recognition [37]–[39] but did indeed to garner lasting support [23]–[25]. His proposals also provided the impetus for several studies that led to more concomitant predominant mid-20th Century single-species interpretation that classified all North American leopard frogs as *R. pipiens* based on inconsistent differences among purported species and successful cross-breeding experiments with frogs from decades later, relying primarily on morphology and bioacoustics, Pace [26] presented a detailed treatment of the *R. p.* arrangement in the eastern US, echoing arrangements prior to Kauffeld's work [43]–[45]. This included *R. sphenocephala* to the south, and *R. pipiens* to the north, with a species boundary centered in the NY/NJ-metro area. Pace's arrangement decades, particularly across the eastern US.

Occasional discussion of distinct populations, potential intergradation, and cryptic species in the NY/NJ-metro area co largely speculative [11], [46], [47]. More recently, however, advances in molecular methods utilizing nuclear and mitochondrial sophisticated species delimitations and analyses of phylogenetic and population genetic relationships. Initial molecular suggesting that an undescribed cryptic leopard frog lineage, termed *R. sp. nov.*, does indeed occur between population NY/NJ-metro area. They also reported mitochondrial data showing this species to be most closely related to the pick and readily identifiable species [29], rather than to *R. sphenocephala*, the species to which it had been included base regarding interspecific relationships were inconclusive.

In retrospect, the long history of taxonomic and nomenclatural confusion in the NY/NJ-metro area was likely due to the occurring in close proximity to several similar congeners. For example, in the Philadelphia region – an area replete with among leopard frogs [26], [27], [48] – all four regional spotted congeners are now known to occur; *R. pipiens*, *R. palustris*, occur in succession along a narrow 90-km west-to-east transect between Berks County, PA and Burlington County, N.

**Materials and Methods**

**Ethics Statement**

The species described here was discovered during research activities conducted under an Institutional Animal Care at Rutgers University (#07-024). Additional field work and collection of the holotype specimen occurred under New York MDS) in compliance with Yale University IACUC protocol #2012-10681.

**Taxonomic Note**

We briefly point to an area of unresolved taxonomic debate within the herpetological community. This debate centers versus a recently proposed replacement name, *Lithobates*, which has been applied to a number of North American remains largely unsettled, we have followed the conservative taxonomic practice of continuing to use *Rana* for all Nort complex.
Morphology

Fieldwork to collect an adult male holotype was conducted in Richmond County, NY. The specimen was preserved in 70% ethanol and deposited at the Yale Peabody Museum of Natural History (YPM). We collected morphometric measurements on the holotype (YPM 13217) and 282 other museum specimens across four species (R. sp. nov., R. sphenoecephala, R. seven eastern states, and Quebec, Canada (Table S1). When genetic data were not available to confirm species identity, morphology and location were used to classify preserved specimens based on our knowledge of species habitat preferences and measurements were taken to the nearest 0.01 mm with Mitutoyo Digimatic calipers. We measured 13 characters, 11 (SVL; anterior end of snout to posterior end of urostyle), head length (HL; anterior end of snout to occiput), head width diameter (ED; at widest point of eye), tympanum diameter (TD; at widest point of tympanum), foot length (FOL; tip of (END; anterior eye to naris), naris to snout distance (NSD; naris to anterior end of snout), thigh length (THL; anterior k (IND; closest distance between nares), and interorbital distance (IOD; closest distance between the eyes). We also included Heyer et al. [52] and dorsal snout angle (DSA; [arcsine (HW/2)/HL] x2) following Lemmon et al. [6].

Figure 1. Leopard frog distributions in the Northeast and mid-Atlantic US.
Left: currently recognized IUCN (2012) range maps for R. pipiens (green) and R. sphenoecephala (red) with areas interpreted distributions for all three leopard frog species including R. kauffeldi. Symbols indicate known R. kauffeldi areas where our field work has confirmed the occurrence of R. kauffeldi. Yellow shading indicates areas of less kauffeldi may occur in these areas based on habitat and proximity to known populations. Potential sympatry is also R. sphenocephala (from Long Island southward), or R. pipiens (north and west of Long Island). The type locality doi:10.1371/journal.pone.0108213.g001

We looked for univariate differences in species morphology using boxplots and one-way ANOVAs followed by Tukey HSD post-hoc pairwise comparison among species, and then Tukey HSD post-hoc pairwise comparison to our DFA. All analyses were conducted in R, v. 2.15.2 and v. 3.0.2 [53], including package MASS.

We also examined color and patterning differences between leopard frog species. We compared dorsal spots (number between the new species and its closest morphological congener, R. sphenoecephala, following Platz [54]. For spot color (R. sp. nov., n = 22; R. sphenoecephala, n = 18) into ArcMap 10.0 [55] and digitized polygons representing the dorsum in order to calculate the proportion of the dorsal surface covered by spots. We examined both variables using boxplot differences. We also conducted several categorical comparisons between R. sp. nov. and R. sphenoecephala, including snout spot (present or absent), and 3) skin color (three color categories). We categorized a dorsal spot as ‘elongate’ its widest point, but excluded eyelid spots from this analysis because the curvature of the eye made them difficult to a the posterior dorsal surface of the femur (thigh) among specimens of R. sp. nov., R. sphenoecephala, and R. pipiens. distinguish leopard frogs in regions where R. sp. nov. occurs [24], [32]. We follow Moore [24] in referring to it as the "light (light ground color with dark spots)" or dark (dark ground color with light spots). All specimens used in spot and color photo vouchers were deposited at YPM.
Genetic Analysis

Following the methods described in Newman et al. [3], we extracted genomic DNA from a liver sample obtained from 12S–16S regions of the mitochondrial genome, including intervening and flanking tRNAs (1444 bp), and the nuclear gene (Tyr, 557–585 bp), Rag-1 (647–683 bp), seven-in-absentia (SIA, 362–393 bp), and chemokine receptor 4 (CXCR4, 555 Be). All sequences generated in this study were uploaded to GenBank (JX867559-JX867563). Data from the present study were added to the Newman et al. [3] data set, and Bayesian phylogenetic analyses were performed for each locus following the analyses described in Newman et al. [3] to verify the species identity of the holotype.

Bioacoustic Analysis

We recorded calls of the new species with an Olympus DS-40 digital voice recorder and Sennheiser MKE 400 directivity microphone, and 16-bit sampling size. We converted files to .wav format using Roxio Sound Editor (Sonic Solutions, Novato, CA, US) using the following settings: spectrogram FFT length 2048, Hanning window size 1024, amount of overlap between calls from 2048. We analyzed calls from four populations (two in Richmond County, NY; one in Bergen County, NJ). For calls from four congeners using these same methods unless otherwise stated (Table S2); these included R. sphenoeulis acoustically similar species outside the leopard frog complex, R. sylvatica. We examined two populations of R. sphenoeulis, one population of R. pipiens (Columbia Co., NY), one population of R. palustris (Suffolk Co., NY), and three Suffolk Co., NY, and Larimer Co., Colorado). We did not collect frogs used in our call analysis, but deposited call voucher specimens in the Peabody Museum.

We measured seven variables: call length (CL; time from beginning to end of a single call), call rate (CR; based on time from call start to maximum amplitude), call duty cycle (CDC; call length/tcall length + time to next call from 821; pulse rate (PR; based on time between start of first and last pulse), and dominant frequency (DF; frequency of the pulse). We used the following parameters and terminology from Cocroft and Ryan [59] but follow Lemmon et al. [6] for CDC and PN. We derived these variables from individual unless otherwise noted (Table S2). For the purposes of this study, we examined only the primary mating call by Heyer et al. [52]. This approach provided a clear means for comparing species and minimized confusion between the call types. We considered secondary repertoire to fall outside the scope of our objectives and were not analyzed here. We measured the same univariate and multivariate statistical procedures described for our morphological analyses. Call rate and call temperature, so we adjusted these two parameters to a common water temperature of 14°C for our statistical analysis. Regression equations from R. sp. nov. in place of R. pipiens and R. palustris because both species were recorded at various temperatures.

Nomenclatural Acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature and is available under that Code from the electronic edition of this article. This published work and the references contained herein are available under that Code from the electronic edition of this article. The electronic edition of this work was published and is available from the following digital repositories: PubMed Central and LOCKSS.

Results

Diagnosis and Description

*Rana kauffeldi* sp. nov.

urn:lsid:zoobank.org:pub:2E7F07A6-19B1-4352-B5B7-A227A93A37CD. The electronic edition of this work was published and is available from the following digital repositories: PubMed Central and LOCKSS.
YPM 13217, adult male (Fig. 2, Table 1), collected from Bloomfield region, Richmond County (Staten Island), NY, Unit Curry.

Figure 2. Photographs of Rana kauffeldi sp. nov. holotype (YPM 13217).
Male frog presented live: (a) whole body, dorsolateral view and (b) dorsal view; and preserved: (c) dorsal view and BRC (a), BZ (b), and GWC (c–d).
doi:10.1371/journal.pone.0108213.g002

Table 1. Mean morphological parameters for four species of Rana.
doi:10.1371/journal.pone.0108213.t001

Paratypes.

YPM 13559, subadult male (paragenotypes: GenBank accession numbers JN227403, JN227458, JN227127, JN227128; collected from Wangunk Meadows in Portland, CT by T. Mahard and M. Blumstein on 15 September 2010; genetically holotype [3].

Referred material.

YPM 13560, adult male (paragenotypes: GenBank accession numbers JN227404, JN227459, JN227128, JN227181, collected from Wangunk Meadows in Portland, CT by T. Mahard and M. Blumstein on 15 September 2010; genetically holotype [3].
YPM 13920, juvenile (GenBank accession numbers JN227377, JN227432, JN227102, JN227155, JN227209, JN2273
Feinberg from the type locality on 27 March 2009 (hatched in captivity and raised in situ within a field enclosure on L
o project); genetically confirmed within the same clade as the holotype [3]. AMNH 121857–121858, juveniles; collected · Warny and E. Johnson.

Etymology.

The specific epithet is a patronym in recognition of Carl F. Kauffeld who studied the R. pipiens complex in the NY/NJ-r
species, including an undocumented central species, occurred there.

Common Name.

We propose the common name ‘Atlantic Coast Leopard Frog’ for this species.

Synonymy.

Given the complex nomenclatural history of leopard frogs in the NY/NJ-metro area, we searched for potential synonymy
assigning a binomial and identified five candidates: R. pipiens Schreber [60], R. halecina Daudin [61], R. utricularius I
and R. brachycephala Cope [36] as elevated to species rank by Kauffeld [33]. Based on our review and commentary
determined that none of these candidates has clear unequivocal support or the precise locality information or type spe
the new species. Most recently, Frost et al. [50] proposed Lithobates pippins as a systematic replacement for Rana I
and, as noted earlier, disagreements in the herpetological community as to the utility and appropriateness of Lithobate

We include R. pipiens as a synonym because its type locality has been restricted to various parts of the NY/NJ-metro
[63], [64]. However, given the lack of precision, geographic consensus, or a physical type specimen, Pace [26] design
central NY (UMMZ 71365). We follow Pace, and thus consider R. pipiens to be removed from further geographic con
Pace [26] that the frog illustrated by Schreber [60] most resembles the northernmost species, not the species descrit
gographic range of R. pipiens is unwarranted and, despite the confusion and numerous synonymies from the NY/NJ-
warrants resurrection. We also refer briefly to Lavilla et al. [62] and point out that R. halecina was introduced to tran
a scientific name. Further, it comes only from an observation and lacks an explicit type locality or type specimen.

Diagnosis.

Rana kauffeldi is morphologically similar to R. sphenocophala and R. pippins, but distinguishable by 1) advertisement
genetics [3], 3) habitat (see Distribution), 4) geographic distribution (Fig. 1), and 5) a combination of morphological c

Figure 3. Primary (advertisement) calls of five Rana species from the study region.
Species include R. kauffeldi (column 1), R. sphenocophala (column 2), R. pippins (column 3), R. palustris (column
individuals were recorded within 8°C of each other at 10.0, 11.0, 18.0, 15.0, and 10.1°C, respectively. Row 1 show
(12 s scale) (note: R. pippins contains secondary grunts). Rows 2 and 3 show single-call waveforms and spectroç
shows power spectra for each single call. Numbers assigned to waveforms in row 1 indicate and identify different et al. [6].
doi:10.1371/journal.pone.0108213.g003

| Table 2. Mean primary (advertisement) call parameters for five species of Rana. |
|------------------|------------------|------------------|------------------|------------------|
| Variable         | R. kauffeldi     | R. sphenocephala | R. pipiens       | R. palustris     | R. sphenocephala |
|                  | [cm] (n=13)      | [cm] (n=12)      | [cm] (n=13)      | [cm] (n=11)      | [cm] (n=12)      |
| CL (cm)          | 53.81±10.68      | 53.45±11.08      | 105.42±35.45     | 142.9±137.24     | 25.89±16.27      |
| range            | 64.5±15.29       | 64.5±15.29       | 142.9±137.24     | 25.89±16.27      | 25.89±16.27      |
| CF (kHz)         | 1.34±0.46        | 1.38±0.59        | 0.37±0.07        | 0.19±0.06        | 1.72±0.77        |
| range            | 3.7±0.25         | 3.6±0.36         | 0.6±0.06         | 0.59±0.07        | 4.09±1.70        |
| fH (kHz)         | 51.92±3.74       | 52.64±13.81      | 129.61±23.79     | 159.4±21.87      | 168.6±13.70      |
| range            | 16.5±4.72        | 21.3±6.16        | 56.5±12.67       | 85.3±21.67       | 95.3±21.67       |
| C (ms)           | 0.12±0.03        | 0.14±0.04        | 0.16±0.05        | 0.16±0.06        | 0.16±0.06        |
| range            | 0.35±0.10        | 0.36±0.29        | 0.53±0.16        | 0.50±0.16        | 0.50±0.16        |
| H (ms)           | 1.08±0.69        | 7.93±1.20        | 34.6±3.73        | 41.9±6.16        | 32.5±3.73        |
| range            | 1.00             | 6.3±0.90         | 29.3±4.83        | 47.3±7.69        | 32.5±3.73        |
| Pr (pulses)      | 1                 | 1                 | 1                 | 1                 | 1                 |
| range            | 1                 | 1                 | 1                 | 1                 | 1                 |
| F' (kHz)         | 1303.11±1114.41  | 1204.81±220.09   | 1179.91±133.91   | 1204.81±220.09   | 1425.72±214.89   |
| range            | 1371.33±1315.22  | 1371.33±1315.22  | 1371.33±1315.22  | 1371.33±1315.22  | 1371.33±1315.22  |

Additional diagnostic characters are listed as follows: call rate (PR), call rate (SP), call rate (RSP), pulse number (PN), pulse rate (PR), and dominant frequency (DF). When available, the standard deviation (SD) was noted. All data were collected at a common temperature of 14°C. Following Lamont et al. [5], all character states were scored against the mean of all samples by scale intervals in all other taxonomic analyses. The diagnostic value of this character may be limited to northern populations of Rana sphenocephala in which leopard frogs predominantly exhibit a dark reticulum across portions of the Southeast where R. sphenocephala is

The advertisement call is a single-noted unpulsed ‘chuck’ (Video S1) that is distinct from the pulsed ‘ak-ak-ak’ of R. sp puipeps and R. palustris. The quivering ‘quack’ of R. sylvatica is superficially similar but consists of discrete bouts of 2 accompanied by secondary ‘groans’ as occasionally emitted by R. kauffeldi. Although sympatric with R. kauffeldi, R. distinct and typically calls from smaller canopied wetlands and forested pools whereas R. kauffeldi usually calls from

Adult male R. kauffeldi possess very large, laterally paired external vocal sacs that distinguish them from all similar c

Additionally, R. kauffeldi has a dark femoral reticulum (Fig. 4a) whereas northeastern populations of R. sphenocephalai (Fig. 4b). This diagnostic was 100% consistent in R. kauffeldi from NY and NJ (n = 27) and R. pipiens from the north88.6% consistent in R. sphenocephala from NJ (n = 35). The diagnostic value of this character may be limited to norntl that leopard frogs predominantly exhibit a dark reticulum across portions of the Southeast where R. sphenocephala is

Figure 4. Reticulum shading patterns. Examples include (a) dark state, Rana kauffeldi (YPM 14143); (b) light state, R. sphenocephala (YPM 14097); (c) R. kauffeldi green variant (YPM 14025). Photographs taken by E. Kiviat (a), M. Cram (b), and BRC (c, d).
Rana kauffeldi may be further distinguished from R. sphenocephala by a tympanic spot that is typically duller, less well-defined; from R. pipiens by a light spot in the center of the tympanum that is often small and faint (but occasionally present); inner thighs without deep yellow coloration and round, unaligned dorsal spots.

**Description.**

Body moderate and robust; head longer than wide. Dorsal outline of snout acuminate; lateral snout profile round. Naras around two-thirds closer to tip of snout than anterior corner of eye. Canthus rostralis distinct and angular; loreal region protuberant; diameter slightly less than combined eye-to-naris and naris-to-snout distances. Internarial distance nearly distinct and relatively large (>65% diameter of the eye); bordered dorsally and posteriorly by faint supratympanic fold. Forearms relatively short and robust; unwebbed fingers; relative length without expansion; subarticular tubercles small, round, and moderately prominent. No palmer tubercles appear present; all other fingers slender. Hindlimbs relatively long, moderately robust; thigh and shank length nearly equal. Rounded tips without expansion; subarticular tubercles small, round, and prominent. Inner tarsal fold connects tarsus to metatarsal tubercle. Indistinct, small outer metatarsal tubercle faintly evident. Toe IV very long and slender; toe V slig

webbing formula $I_{1} – 2I_{1}^{1} – 2I_{1}^{2} – I_{2}^{3} – 3IV_{3} – 1V$ following Savage [66]. Skin on dorsum smooth with several raised dorsolateral folds. Flanks, thighs, and shanks smooth. Ventral surface mostly smooth with papillae-like granulation on external vocal sacs.

**Color in life.**

In photographs taken before preservation, dorsal ground color of holotype varies from mint-gray in bright lighting (Fig. 2b) to medium to dark brown spots irregularly distributed across dorsum and lateral body; more elongate or barreled spots encompass dorsal and posterior tympanum along the supratympanic ridge. Labial margins slate grey with light mottled brown color; terminates under the tympanum (continues to anterior forearm in females). Dark canthal band runs from snout of dorsolateral fold; terminates above the arm. On snout, inner edge of canthal band is paralleled by light brown band encompassing dorsal and posterior tympanum along the supratympanic ridge. Labial margins slate grey with light mottled brown color; terminates under the tympanum (continues to anterior forearm in females). Dark canthal band runs from snout to pelvic insertion of femur. Forearms relatively short and robust; unwebbed fingers; relative length without expansion; subarticular tubercles small, round, and moderately prominent. No palmer tubercles appear present; all other fingers slender. Hindlimbs relatively long, moderately robust; thigh and shank length nearly equal. Rounded tips without expansion; subarticular tubercles small, round, and prominent. Inner tarsal fold connects tarsus to metatarsal tubercle. Indistinct, small outer metatarsal tubercle faintly evident. Toe IV very long and slender; toe V slig

webbing formula $I_{1} – 2I_{1}^{1} – 2I_{1}^{2} – I_{2}^{3} – 3IV_{3} – 1V$ following Savage [66]. Skin on dorsum smooth with several raised dorsolateral folds. Flanks, thighs, and shanks smooth. Ventral surface mostly smooth with papillae-like granulation on external vocal sacs.

**Color in preservative.**

Generally similar to that in life with several notable distinctions. Ground color dark olive green in holotype (Fig. 2c) but more yellowish in preservative (as in paratypes YPM 13559 and 13560). Colored flecks and mottles in life appear white in preservative. Lower flank of holotype pale with light yellowish-green hues and smaller, lighter spots and mottles; thigh bone-white (Fig. 4a), light yellow (Fig. 4c) or green (Fig. 4d) in some individuals. Ventral limbs of holotype pinkish-gray; tarsal fold and outer metatarsal tubercle are bright white against a dark brown tarsal background; webbing pale gray.

**Genetics**

Holotype (YPM 13217) falls within the R. kauffeldi clade (R. sp. nov. in Newman et al. [3]) in the mitochondrial phylogeny; nuclear haplotypes are identical to other R. kauffeldi samples. As reported by Newman et al. [3], R. kauffeldi is gene
distinguished spotted ranid frogs (R. sphenocephala, R. pipiens, and R. palustris). The mitochondrial phylogeny suggests that R. k Aaverage pairwise mitochondrial sequence divergence (uncorrected p) is similar to genetic divergences between other complex (Newman et al. [3]).

**Distribution**


**Rana kauffeldi** is known from three states (CT, NY, NJ) based on genetic samples [3] and seven states (NY, NJ, PA, I and North Carolina [NC]) based on bioacoustic sampling reported here. The estimated range from these samples is a central CT to northeastern NC (Fig. 1). The range is narrow, however, east-to-west, occurs almost entirely within the most if not all other ranid frogs along the eastern North American seaboard. Within the presented range, we depicthe gaps in genetic and bioacoustic information were filled by other lines of evidence (e.g., specimens, photographs) **Rana kauffeldi** appears to occur parapatrically in this core area. Beyond the core area, we depict an extended area based on habitat features and proximity to known bioacoustic confirmations in DE, MD, VA, and NC. Within the yellow sympathy with **R. sphenoccephala** (in the south) and **R. pipiens** (in the north) based on genetic, bioacoustic, and specific

**Rana kauffeldi** has a mesic distribution that is wider in the north and narrows from Trenton, NJ, to the Delmarva Peninthe Delaware River floodplain and the Atlantic Fall Line – the geologic interface between the relatively xeric Atlantic cc and more interior and upland regions to the west – where **R. pipiens** occurs. This species is usually abundant where it area tend to be disjunct and isolated from one another and often occur in highly fragmented landscapes with limited cc **kauffeldi** was generally included within the range of **R. sphenoccephala** prior to its discovery, but northern mainland po may have been included within **R. pipiens** instead (Fig. 1, yellow shading).

We also consider **R. kauffeldi** to have previously occurred within parts of an apparent extirpation zone that includes m We used multiple lines of evidence to inform this conclusion, including historical locality information [11], [33], photogra personal communications (A. Sabin and F. C. Schlauch), and museum specimens (Table S1). Our assessment of musifrom Long Island (n = 27) and Bronx County, NY (n = 7). Based on our examination, 29 of these 34 frogs were **R. kauf of Long Island, NY (Suffolk County), appeared to be **R. sphenoccephala** (AMNH 125956, 176153). The remaining thre106549, 106550) came from the Bronx County site previously noted by Pace [26] and Klemens et al. [31], where spes106551–106554) were also collected historically. The third was a lone individual from western Long Island, in Queens (specimens (n = 9) from two presumably extirpated sites in southeastern CT (New Haven County) (Table S1). All were located within a bottomland riparian floodplain where **R. kauffeldi** would be expected to occur.

### Morphological Evidence

Univariate analysis recovered significant differences among 11 of 12 size-corrected characters between **R. kauffeldi** a **palustris** (Fig. S1). **Rana kauffeldi** had 1) the shortest eye-to-naris distance (F$_{3,279}$ = 28.41, p<0.0001), 2) shortest th shortest Shank length (F$_{3,279}$ = 27.95, p<0.0001) of the four species examined. **Rana kauffeldi** had 4) narrower eyes (F$_{3,279}$ = 14.59, p<0.0001), 6) and longer interorbital distance (F$_{3,279}$ = 35.02, p<0.0001) than **R. sphenoccephala** and shorter head than **R. sphenoccephala** and a longer head than **R. pipiens**, (F$_{3,279}$ = 16.00, p<0.0001), 8) a longer interr shorter internarial distance than **R. pipiens** (F$_{3,279}$ = 8.48, p<0.0001), 9) a larger tympanum diameter than **R. pipiens** 10) a shorter naris-to-snout distance (F$_{3,279}$ = 19.92, p<0.0001) than **R. pipiens**, and 11) a wider snout angle than R. The unadjusted summary data for all 13 morphometric characters are also presented (Table 1).

In multivariate space using DFA, we found considerable morphological overlap among all four species examined (Fig. .detected (F$_{3,280}$ = 120.0, p<0.0001). The DFA correctly classified 78.0% of specimens (Table S3). Post-hoc Tukey's l be significantly different from one another (p<0.0001) except for **R. sphenoccephala** and **R. palustris** (p = 0.9966). The $p$ 58.4% of the variation in the data with tympanum diameter loading most heavily, while the second function accounted for distance having the greatest load (Table S4).

Previous studies report fewer and smaller dorsal spots among leopard frogs from areas where **R. kauffeldi** occurs [2]has fewer dorsal spots than **R. sphenoccephala** (mean = 13.18±3.22 SD vs. 20.44±4.10 SD, respectively) (t = −4.32, covered by spots (mean = 13.56%±3.29 vs. mean = 22.13%±7.76, respectively) (t = −6.12, two-tailed p<0.0001) (Fig 35.71% (n = 42) of **R. kauffeldi** had one or more elongated spot compared to 61.16% (n = 67) of **R. sphenoccephala** 32.86% (n = 70) of **R. kauffeldi** versus 16.88% (n = 77) of **R. sphenoccephala**. Lastly, we found considerable categori75) (74.7% = dark olive to mint-gray, 24.0% = green to light brown, and 1.3% = bright green) and **R. sphenoccephala** 39.4% = green to light brown, and 13.8% = bright green). Multi-colored frogs were categorized by their lightest color.

### Bioacoustic Evidence
The unpulsed advertisement call of *R. kauffeldi* is typically emitted in evenly spaced, repeated series that can include recorded at multiple locations within the type locality. Five males (YPM 14137–14140; Table S2) were recorded at the heard calling and collected (but not recorded). These frogs were recorded between 2028 and 2042 h on 15 March 20 following mean characteristics: call length 60.55 ms (54.00–71.25±6.74 SD), call rate 1.10 calls/s (0.90–1.33±0.15), call duty cycle 0.07 (0.05–0.10±0.02), pulse rate 1.00 (1.00±0.00), pulse rate 0, and dominant frequency 1296.30 from one of these frogs (YPM 14137 and 14172) were used to represent temporal and spectral features for *R. kauffeldi*, *R. palustris*, and *R. sylvatica* in Fig. 3.

We compared summary data for all *R. kauffeldi* to the four other species (Table 2). Frogs were recorded opportunisti to 25.6°C (Table S2), reflecting the different geographies and phenologies among species. The temperature range we averaged by species: *R. kauffeldi* (12.56°C±2.87 SD), *R. sphenocophala* (18.30°C±7.80), *R. pipiens* (18.00°C±0), *R. palustris* (9.68°C±0.94).

Our univariate analysis revealed significant differences among species in 6 of 7 call parameters (Fig. S4). *Rana kauffeldi* has only one pulse per call. The first discriminant function accounted for 61.0% of the variation in Fig. 3.

We found clear separation in call parameters among all species (Fig. S2). The DFA accounted for 61.0% of the variation in Fig. 3. The only classification errors were two *R. sylvatica* classified as *R. kauffeldi* (Table S5). Post-hoc comparisons to be significantly different from one another (p<0.001) except for *R. kauffeldi* and *R. sylvatica* (**p** = 0.99 because *R. kauffeldi* has only one pulse per call. The first discriminant function accounted for 61.0% of the variation in Fig. 3.

Ecology, Behavior, and Natural History

*Rana kauffeldi* inhabits a restricted range of mesic lowland habitats that primarily includes coastal freshwater wetland riparian valley floodplains. This species is typically associated with large wetland complexes composed of open-canoc systems with ample open upland and early-successional habitats. Aquatic conditions are usually clear, shallow, and stands such as cattail, *Typha spp.*, or the invasive common reed, *Phragmites australis*.

*Rana kauffeldi* begins breeding around the same time as *R. sylvatica* and *R. sphenocophala* and slightly in advance observed migratory activity on rainy nights with above-average temperatures in early February, and have documented above-average temperatures in early-to-mid March. Choruses are most consistent nocturnally, with air temperatures and nocturnal chorusing is common early in the season and through the initial 2–3 week peak breeding period (late March). Thereafter, chorusing tapers to a more episodic nocturnal and precipitation-based regime from mid-April and observed opportunistic mid-summer chorusing as we and others [26], [71] have for *R. sphenocophala*, but we have our own with the onset of cooler autumn temperatures and precipitation (late August through November).

Individuals may exhibit a limited degree of color change around a general base color that can vary widely between frogs. Leopard frogs (sensu lato) tend towards darker nocturnal shading and brighter, more vivid diurnal colors (as degree of seasonal color change also appears to exist in *R. kauffeldi*; we often observed frogs with darker, drabber colors in spring, and more vivid and varied overall color and brighter, more defined tympanic spots later in the season.

During breeding, males congregate in concentrated groups, or possible leks [26], that typically include five or more frogs. Males call while floating in shallows with emergent vegetation and as little as 20 cm of water. As stated by Mathewson the range cast much farther. This is especially apparent in the presence of louder, higher pitched sympatric species like spring peepers (*P. pipiens*) from Philadelphia and NJ, respectively, that we consider *R. kauffeldi*.

Little is known about non-breeding activity or dispersal in *R. kauffeldi*, but leopard frogs have been described as being work, we observed individuals on land later in the season, but also noted periods, typically in summer and early fall, w
is not specifically known, but is presumably similar to those reported for other regional leopard frog species.

Discussion

Hidden Diversity in a Well-Documented Urban Region

The description of *R. kauffeldi* brings the current number of New World leopard frogs to 19 (excluding *R. palustris*) ar from the US mainland and Canada to 30 [7]. Despite the vast size of this area, new frog discoveries north of Mexico are significant. For example, *R. kauffeldi* and the Cajun chorus frog, *P. fouquettei*, [6] are the only newly described anurans from the US mainland and Canada to 30 [7], and *R. kauffeldi* is the first anuran from the US Atlantic coast since the New Jersey recognized (as a subspecies) in 1955 [7].

The specific region where *R. kauffeldi* was first identified, the New York City metropolitan area (with a type locality less than 10 kilometers from the type locality) is significant. It provides an example of new species discovery, not from a tropical biodiversity hotspot or poorly documented within one of the largest population centers on earth [8] spanning eight eastern US states and remarkable. As a point of comparison, we consider another cryptic species group from the eastern US, the gray tree frog, *Acris crepitans*. Despite being arboreal, smaller, and less conspicuous than leopard frogs, these two congeners were recognized as subspecies earlier (in 1966) by differences in their calls [7], [80].

In part, the sustained concealment of *R. kauffeldi* may have been due to its narrow and fragmented range, short and (less audible) call. Repeated acoustic misidentification may have also played a concealing role; many colleagues with experience working with frog populations now known to be *R. kauffeldi*. Some attributed these calls to *R. sylvatica* in unusual habitats; others attributed them to *R. sphenosephala*. Given these examples and the generally stereotyped and species-specific nature of frog calls [4], [9] in identifying species, we encourage greater scrutiny and examination of aberrant calls elsewhere, especially when encountering populations or regions. Such efforts may reveal additional diversity, especially in areas of systematic uncertainty or co-occurrence with other species.

Biogeography and Distributional Relationships with Close Congeners

New species can have important biogeographic implications, particularly when they occur within intricate species groups. In the case of *R. kauffeldi*, its discovery from the Northeast and mid-Atlantic US has direct consequences for three species that are entirely from two cryptic congeners, *R. sphenosephala* and *R. pipiens*. Thus, the recognized distributions of both congeners need to be reconsidered. The distribution of *R. kauffeldi* occurs alone. These changes will refine certain ecological understandings and distributional patterns too. For example, the statewide distribution in NJ, *R. sphenosephala* is now exclusively restricted to xeric habitats such as the Pine Barrens from a previous range over a wide variety of habitats and geologies to a newly defined range that conforms to the co-occurrence of two species.

Distributional relationships vary between *R. kauffeldi* and its close congeners. The general distributions of *R. kauffeldi* reported in Newman et al. [3] overlap broadly [29], [30], though we did not find them together in the field and noted differences in their calls. Conversely, the distribution of *R. kauffeldi* is generally parapatric with *R. sphenosephala*. Sympatry do exist with both species. Newman et al. [3] provided genetic evidence of sympatry without hybridization with both species. In the future, additional potential sympathy in northwestern
AMNH 13114, 35139). We also identified areas of sympathy between *R. kauffeldi* and *R. sphenoecephala* in southeast American Amphibian Monitoring Program), and suspect additional overlap in southern locales. Lastly, based on museum are now extirpated, we note several isolated examples of possible *R. sphenoecephala* from xeric eastern Long Island, Counties, NY (Table S1). Historical species composition in these areas remains unclear, however. These sparse samplings (and potential areas of overlap with *R. kauffeldi*) or possible human introductions; isolated geographic records can subsequently urban areas. Thus, we excluded both urban *R. pipiens* occurrences from Fig. 1.

Delineating Complicated Historical Ranges in Heavily Modified Landscapes

Determining the distribution of new species is essential to the process of identifying and interpreting their broader biogeographic patterns, identifying regional compositions and reassigning museum specimens can be challenging but important, especially in situations of extinction or species overlap. In our work, leopard frogs were simply unavailable across vast landscapes due to habitat fragmentation, differentiating similar-looking congeners was difficult. To overcome such challenges, several strategies involving genetic and bioacoustic methods at sites where new species and their cryptic congeners still occur to delineate hybridization; 2) using genetics and morphology to identify subtle physical differences, if any, between species; and 3) using archival specimens at sites where new species and their cryptic congeners still occur to delineate hybridization, are key priorities. We also leave open the possibility that *R. kauffeldi* may extend farther south.

The discovery of *R. kauffeldi* has several broad conservation implications. For one, it reaffirms that refined taxonomic proper conservation measures [2], [3]. It also reinforces the critical role that basic natural history and alternative methods in distinguishing potentially rare cryptic species. Lastly, it demonstrates that undocumented species can still reside in xeric and mesic habitats, where these areas harbor significant biodiversity and, with proper management, simultaneous educational opportunities to urban communities. The United Nations Environment Programme and US Fish and Wildlife both focused recent efforts on protecting urban biodiversity and enhancing the value and scope of urban wildlife refuges. Important observation to the growing consensus that we must protect sensitive species where they occur, not just in potentially valuable areas. For example, in urban communities.

The overall conservation status of *R. kauffeldi* awaits further definition of distribution and habitat use and should be considered a classification system. On-the-ground assessments, coupled with genetic and bioacoustic data, will be critical to this a broader picture of the status of *R. kauffeldi* and its cryptic congeners in some of these impacted areas. It may also provide further opportunity to inve boundaries throughout different parts of the range. This may be challenging, however, especially in states where leopard frog protections and in areas where multiple species are found to be sympatric. Thus, reliable, field-ready characters that distinguish different species, identifying regional compositions and reassigning museum specimens can be challenging but important, especially in situations of extinction or species overlap. In our work, leopard frogs were simply unavailable across vast landscapes due to habitat fragmentation, differentiating similar-looking congeners was difficult. To overcome such challenges, several strategies involving genetic and bioacoustic methods at sites where new species and their cryptic congeners still occur to delineate hybridization; 2) using genetics and morphology to identify subtle physical differences, if any, between species; and 3) using archival specimens at sites where new species and their cryptic congeners still occur to delineate hybridization, are key priorities. We also leave open the possibility that *R. kauffeldi* may extend farther south.

The overall conservation status of *R. kauffeldi* awaiting further definition of distribution and habitat use and should be considered a classification system. On-the-ground assessments, coupled with genetic and bioacoustic data, will be critical to this a broader picture of the status of *R. kauffeldi* and its cryptic congeners in some of these impacted areas. It may also provide further opportunity to investigate boundaries throughout different parts of the range. This may be challenging, however, especially in states where leopard frog protections and in areas where multiple species are found to be sympatric. Thus, reliable, field-ready characters that distinguish different species, identifying regional compositions and reassigning museum specimens can be challenging but important, especially in situations of extinction or species overlap. In our work, leopard frogs were simply unavailable across vast landscapes due to habitat fragmentation, differentiating similar-looking congeners was difficult. To overcome such challenges, several strategies involving genetic and bioacoustic methods at sites where new species and their cryptic congeners still occur to delineate hybridization; 2) using genetics and morphology to identify subtle physical differences, if any, between species; and 3) using archival specimens at sites where new species and their cryptic congeners still occur to delineate hybridization, are key priorities. We also leave open the possibility that *R. kauffeldi* may extend farther south.

The overall conservation status of *R. kauffeldi* awaiting further definition of distribution and habitat use and should be considered a classification system. On-the-ground assessments, coupled with genetic and bioacoustic data, will be critical to this a broader picture of the status of *R. kauffeldi* and its cryptic congeners in some of these impacted areas. It may also provide further opportunity to investigate boundaries throughout different parts of the range. This may be challenging, however, especially in states where leopard frog protections and in areas where multiple species are found to be sympatric. Thus, reliable, field-ready characters that distinguish different species, identifying regional compositions and reassigning museum specimens can be challenging but important, especially in situations of extinction or species overlap. In our work, leopard frogs were simply unavailable across vast landscapes due to habitat fragmentation, differentiating similar-looking congeners was difficult. To overcome such challenges, several strategies involving genetic and bioacoustic methods at sites where new species and their cryptic congeners still occur to delineate hybridization; 2) using genetics and morphology to identify subtle physical differences, if any, between species; and 3) using archival specimens at sites where new species and their cryptic congeners still occur to delineate hybridization, are key priorities. We also leave open the possibility that *R. kauffeldi* may extend farther south.

The overall conservation status of *R. kauffeldi* awaiting further definition of distribution and habitat use and should be considered a classification system. On-the-ground assessments, coupled with genetic and bioacoustic data, will be critical to this a broader picture of the status of *R. kauffeldi* and its cryptic congeners in some of these impacted areas. It may also provide further opportunity to investigate boundaries throughout different parts of the range. This may be challenging, however, especially in states where leopard frog protections and in areas where multiple species are found to be sympatric. Thus, reliable, field-ready characters that distinguish different species, identifying regional compositions and reassigning museum specimens can be challenging but important, especially in situations of extinction or species overlap. In our work, leopard frogs were simply unavailable across vast landscapes due to habitat fragmentation, differentiating similar-looking congeners was difficult. To overcome such challenges, several strategies involving genetic and bioacoustic methods at sites where new species and their cryptic congeners still occur to delineate hybridization; 2) using genetics and morphology to identify subtle physical differences, if any, between species; and 3) using archival specimens at sites where new species and their cryptic congeners still occur to delineate hybridization, are key priorities. We also leave open the possibility that *R. kauffeldi* may extend farther south.
A. Feinberg and J. Burger, unpublished data). Counterintuitively, *R. kauffeldi* persists in several locales within New Yoι Meadowlands. These sites are heavily industrialized and have endured severe long-term anthropogenic impacts and *R. australis*. Most offer large habitat areas, however, which may provide an important clue to survival. The surprising per landscapes, while not completely understood, is encouraging and may have implications for management and restorat

We finish with a cautionary note regarding reintroductions, repatriations, and translocations. Moving species to restore conservation and management practice, but one that can have unintended risks and consequences. For example, had on Long Island before the 2007 discovery of extant populations on nearby Staten Island (that were later found to be *F. sphenocephala*) would have been moved from known populations farther to the south that harbor *R. sphenocephala*, systematics and population genetics at both donor and recipient site ends is critical to responsibly conducting any suc

**Conclusions**

In diagnosing, describing, and defining the Atlantic Coast leopard frog, *R. kauffeldi*, we add a new and potentially at-r northeast and mid-Atlantic US fauna. *Rana kauffeldi* can be characterized as 1) potentially vulnerable with highly splot locally abundant where present, but often only occurring in isolated and scattered locales; 3) having a restricted distrit regions; and 4) having suffered extirpations from certain areas. Concerns over habitat loss and degradation continue t disease, contaminants) that may pose additional future challenges.

**Supporting Information**
**Figure S1.**

Box and whisker plots comparing the size-corrected residuals of 12 morphological characters among four *Rana* species. Species include *R. kauffeldi* (kauf), *R. palustris* (palu), *R. pipiens* (pipi), and *R. sphenocephala* (sphe). For whisker plots, black bars = median, boxes = 25th–75th quartiles, whiskers = minimum and maximum values but exclude outliers (represented by open circles). For each character, species whose measurements differed significantly (\(P\))
doi:10.1371/journal.pone.0108213.s001

**Figure S2.**

Discriminant function analyses (DFA). Left: DFA using 12 size-corrected morphological characters measured from 45 frogs examined across five *Rana* species. Species include *R. kauffeldi* (triangles), *R. pipiens* (plus signs), *R. palustris* (x-crosses), and *R. sylvatica* (red squares). Morphological characters Bioacoustic characters include all variables from Figure S4, except pulse rate. Black symbols twice as large in the m

doi:10.1371/journal.pone.0108213.s002

**Figure S3.**

Box and whisker plots comparing spot features between *Rana kauffeldi* (kauf) and *R. sphenocephala* (sphe). proportion of dorsal surface covered by spots. For whisker plots, black bars = median, boxes = 25th–75th quartiles, v exclude outliers (represented by open circles). Side notches in boxes indicate significantly different medians.
doi:10.1371/journal.pone.0108213.s003

**Figure S4.**

Box and whisker plots comparing seven bioacoustic characters among five *Rana* species. Species include *R. l* (pipi), *R. sphenocephala* (sphe), and *R. sylvatica* (sylv). For whisker plots, black bars = median, boxes = 25th–75th c values but exclude outliers (represented by open circles). For each character, species whose measurements differed denoted with different letters atop the plot. Call length and call rate were temperature-corrected.
doi:10.1371/journal.pone.0108213.s004

**Table S1.**

List of *Rana* specimens examined.
doi:10.1371/journal.pone.0108213.s005

**Table S2.**

List of *Rana* primary (advertisement) calls measured for bioacoustic data.
doi:10.1371/journal.pone.0108213.s006

**Table S3.**

Classification matrix for four *Rana* species using discriminant function analysis on morphometric variables.
doi:10.1371/journal.pone.0108213.s007
Table S4.
Coefficients for three discriminant functions (from four species of *Rana*) for each of 12 morphological characters: diameter (ED), tympanum diameter (TD), foot length (FOL), eye-to-naris distance (END), naris-to-snout distance (NSD), interorbital distance (IOD), shank length (SL), and dorsal snout angle (DSA).
doi:10.1371/journal.pone.0108213.s008

Table S5.
Classification matrix for five *Rana* species using discriminant function analysis on bioacoustic variables.
doi:10.1371/journal.pone.0108213.s009

Table S6.
Coefficients for four discriminant functions (from five species of *Rana*) for each of six bioacoustic characters: (CRT), call duty cycle (CDC), pulse number (PN), and dominant frequency (DF).
doi:10.1371/journal.pone.0108213.s010

Table S7.
Underlying (raw) morphometric data.
doi:10.1371/journal.pone.0108213.s011

Table S8.
Underlying (raw) bioacoustic data.
doi:10.1371/journal.pone.0108213.s012

Table S9.
Underlying (raw) data for color and pattern analyses.
doi:10.1371/journal.pone.0108213.s013

Video S1.
A male *Rana kauffeldi* emitting its primary (advertisement) call in foreground with several other males calling *Pseudacris crucifer*.
doi:10.1371/journal.pone.0108213.s014

Acknowledgments

We extend thanks to Chris Camacho, Erik Kiviat, Ellen Pehek, Susan Stanley, Ed Johnson, Jay Westerveld, Karena D Gruner for critical field assistance; John Burnley, Frederick C. Schlauch, Erik Kiviat, Eric Klaastad, Robert Zappalorti, feedback on distributions and natural history for Long Island and the NY/NY-metro area; and Tim Green, Michael Goc logistical and technical support. We acknowledge John Bunnell, Jason Tesauto, Nate Nazdrowicz, John F. White Jr., a unusual attributes or the potential for cryptic species among regional leopard frogs in the years prior to or during our v Nazdrowicz, Scott Bush, Michael Toroco, Jennifer Tennessen, Holly Niederriter, Kyle Loucks, Dave Golden, Chris Hob for sending audio recordings. We also extend thanks to Julie Lockwood for editorial assistance and David Wake, Davi thoughtful reviews and helpful comments. We are grateful for assistance provided by the following museums and their of Drexel University (Ned Gilmore, Ted Daeschler), American Museum of Natural History (David Kizirian, Darrel R. Fro Natural History (Stephen P. Rogers), and Sam Noble Oklahoma Museum of Natural History (Jessa Watters, Janlee P. Philip Kuchuk at Yale Peabody Museum of Natural History for photographic assistance.
Author Contributions

Conceived and designed the experiments: JAF CEN GJWC MDS BZ HBS JB. Performed the experiments: JAF CEN MDS. Contributed reagents/materials/analysis tools: JAF GJWC MDS BZ BRC JB. Wrote the paper: JAF CEN GJWC

References

   View Article • PubMed/NCBI • Google Scholar

   View Article • PubMed/NCBI • Google Scholar

   View Article • PubMed/NCBI • Google Scholar

   View Article • PubMed/NCBI • Google Scholar

   View Article • PubMed/NCBI • Google Scholar


   View Article • PubMed/NCBI • Google Scholar

   View Article • PubMed/NCBI • Google Scholar

   View Article • PubMed/NCBI • Google Scholar

25. Wright AH, Wright AA (1949) Handbook of frogs and toads of the United States and Canada. 3rd edition. Ithac

   View Article • PubMed/NCBI • Google Scholar

27. Brown LE, Smith HM, Funk RS (1977) Request for the conservation of Rana sphenocephala Cope, 1886, and
   View Article • PubMed/NCBI • Google Scholar

   View Article • PubMed/NCBI • Google Scholar


    View Article • PubMed/NCBI • Google Scholar

   View Article • PubMed/NCBI • Google Scholar

32. Porter KR (1941) Diploid and androgenetic haploid hybridization between two forms of Rana pipiens, Schreber
33. Kauffeld CF (1937) The status of the leopard frogs, *Rana t*


38. Bragg AN (1941) Some observations on Amphibia at and near Las Vegas, New Mexico. Great Basin Nat 2: 10


48. Harlan R (1826) Descriptions of several new species of batrachian reptiles, with observations on the larvae of View Article • PubMed/NCBI • Google Scholar


View Article PubMed/NCBI Google Scholar


View Article PubMed/NCBI Google Scholar
