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Cover: Dwarf Mexican treefrog, Tlalocohyla smithii, Zapopan, Jalisco, Mexico. Photograph by Israel Salcido-Rodríguez.

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Clandestine Biodiversity: The Discovery of Eastern Narrow-mouthed Toads (*Gastrophryne carolinensis*) at LaRue-Pine Hills, Shawnee National Forest, Union County, Illinois

John G. Palis¹, Jill A. Schumacher² and Jeremy J. Schumacher²

Outside of the breeding season, Engystoma *is seldom found, although one is occasionally found by chance*... Dickerson, 1906, referring to the Eastern Narrow-mouthed Toad.

Abstract

The LaRue-Pine Hills/Otter Pond Research Natural Area, Shawnee National Forest, is an extremely biodiverse area in northwestern Union County, Illinois. The first herpetofaunal list generated for the area in 1960 included 11 species of anurans. Two more frog species have since been added. With the recent detection of Eastern Narrow-mouthed Toads, anuran diversity of the area is now known to include 14 species.

Introduction

Established in 1991, the LaRue-Pine Hills/Otter Pond Research Natural Area (LPH), Shawnee National Forest, is an extremely biodiverse area comprising approximately 1137 hectares in northwestern Union County, Illinois. As a result of its geographic location and diversity of habitats — ranging from spring-fed perennial wetlands (e.g., LaRue Swamp and Otter Pond) to xeric limestone bluffs (Pine Hills) — LPH contains more plant species than any area of comparable size in the midwestern United States (Hutchison et al., 1987). Western, eastern, northern, and southern faunal and floral components intermix in this unique area (Evers and Page, 1977). Rossman (1960), the first to publish the results of a herpetofaunal survey of LPH, noted that 21 amphibians and reptiles from the area have geographic affinities centered to the south, west and north.

Rossman (1960) reported 11 anuran species from LPH (scientific nomenclature follows Phillips et al., 2022): Blanchard's Cricket Frog (Acris blanchardi), American Toad (Anaxyrus americanus), Fowler's Toad (Anaxyrus fowleri), Bird-voiced Treefrog (Hyla avivoca), Cope's Gray Treefrog (Hyla chrysoscelis), Green Treefrog (Hyla cinerea), Spring Peeper (Pseudacris crucifer), Trilling Chorus Frog (Pseudacris triseriata complex), American Bullfrog (Rana catesbeiana), Bronze Frog (Rana clamitans), and Southern Leopard Frog (Rana sphenocephala). In a follow-up survey, Ballard (1994) added Wood Frogs (Rana sylvatica) to the anuran fauna of LPH based on an anecdotal report of a single specimen observed by Ronald Brandon, herpetologist at Southern Illinois University Carbondale, sometime between 1968 and 1971. Palis et al. (2019) shared recent (2017 and 2018) observations of Wood Frogs at LPH, and reported a previously undetected species, Plains Leopard Frog (Rana blairi), increasing the anuran diversity for the area to 13 species. Here, we report observations of another previously undetected anuran species at LPH, Eastern Narrow-mouthed Toad (Gastrophryne carolinensis), raising the anuran faunal diversity to 14 species.

Eastern Narrow-mouthed Toads are small frogs, generally about 2.2–3.2 cm long (Powell et al., 2016). They are fossorial,

sheltering by day in soil crevices, root channels, and animal burrows, or beneath cover objects such as logs, rocks, and leaf litter (Dodd, 2023). Surface activity is typically nocturnal and is often stimulated by rainfall (Dodd, 1995, 2023). Breeding in Illinois extends from April into August (Phillips et al., 2022). The breeding call of the male is a brief (0.5–4.0 seconds) buzz with little carrying power (Wright and Wright, 1949).

Narrow-mouthed Toad Observations

<u>Observation 1</u>: At 1846 CT on 12 October 2022, JAS and JJS observed a small frog hop across the dirt road between the south gate of Forest Road 345 (also known as Snake Road) and the north end of Otter Pond about 10 minutes after heavy rain. Initially thought to be a Blanchard's Cricket Frog (including by passersby), closer inspection by JJS revealed it to be a juvenile Eastern Narrow-mouthed Toad. JJS captured a voucher photo (Figure 1) and submitted the record to HerpMapper (record #397731). Illinois Department of Natural Resources biologist Scott Ballard and Illinois Natural History Survey biologist Mike Dreslik, provided independent confirmation of the frog's identity.

<u>Observations 2–4</u>: After 3.5 months (April through mid-July) of below-normal precipitation, pre-dawn thunderstorms passing



Figure 1. Juvenile Eastern Narrow-mouthed Toad observed crossing dirt road at LPH after a heavy rain, 12 October 2022. Image by Jeremy J. Schumacher,

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Figure 2. Eastern Narrow-mouthed Toad vocalizing location in denselyvegetated pool near LaRue Swamp, 19 July 2023. Image by John G. Palis.

through southernmost Illinois on 19 July 2023 dropped substantial rain. Because heavy rains are known to stimulate breeding by Eastern Narrow-mouthed Toads (Anderson, 1954; Dodd, 1995; Wright, [1932] 2002), JGP visited LaRue Swamp in the hopes of locating Eastern Narrow-mouthed Toad breeding sites. During this outing the sky was overcast, the air temperature was 22 °C, and rain fell intermittently.

JGP first investigated a relatively large (90 m \times 14 m when filled to capacity), shallow, wetland basin near the edge of the swamp. The basin was approximately 1/3 full of water. From approximately 25 m away, JGP heard the distinctive buzz of a



Figure 4. Adult male Eastern Narrow-mouthed Toad adjacent to log in pool near LaRue Swamp, 19 July 2023. Image by John G. Palis.



Figure 3. Eastern Narrow-mouthed Toad vocalizing location beneath rank grass at edge of LaRue Swamp, 19 July 2023. Image by John G. Palis.

single Eastern Narrow-mouthed Toad at 0921 CT. The frog was vocalizing from a cryptic position about mid-basin amid a dense growth of cutgrass (*Leersia* sp.), monkey flower (*Mimulus alatus*), and ditch stonecrop (*Penthorum sedoides*; Figure 2). The frog's call was recorded on a cell phone. The cracked soil on the floor of the basin indicated that the wetland had been completely dry before the day's rain. Due to the density of the vegetation, the frog could not be located.

A second Eastern Narrow-mouthed Toad was detected about 150 m away from the first individual at 1000 CT, vocalizing near the swamp edge in a protected location in shallow (< 5 cm deep) water beneath a rank growth of 1.5-m tall southern wild millet (*Echinochloa walteri*; Figure 3). The presence of the frog was documented by recording its call on a cell phone. The substrate was cracked, revealing that this part of the swamp had been dry prior to the storm system. As with the first frog, JGP was unable to locate this individual.

While re-approaching the first basin, JGP again heard a single Eastern Narrow-mouthed Toad calling, this time from approximately 50 m away. The frog was found on the edge of shallow water (about 1 cm deep) at 1025 adjacent to a large log where the herbaceous vegetation was not as dense (Figure 4).

At 1328, JGP heard and recorded four Eastern Narrow-mouthed Toads calling at the edge of the swamp approximately 800 m from the two first two sites. Like the other swamp-edge location, the water level in this part of the swamp had recently risen. The frogs were calling from cryptic locations amid dense grass and

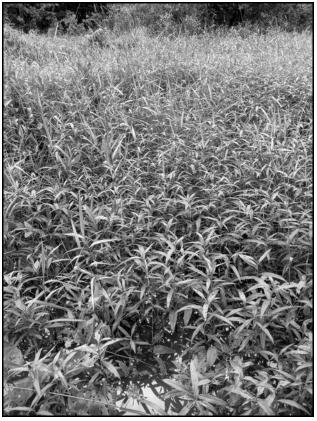


Figure 5. Eastern Narrow-mouthed Toad vocalizing location beneath rank herbaceous vegetation at edge of LaRue Swamp, 19 July 2023. Image by John G. Palis.

smartweed (*Polygonum* sp.) preventing visual detection (Figure 5). Kevin Enge, herpetologist with the Florida Fish and Wildlife Conservation Commission, verified that the calls in all three recordings are those of Eastern Narrow-mouthed Toads.

Discussion

The discovery of a previously undetected anuran species at LPH is remarkable considering frequent visitation to the area by professional and amateur herpetologists since at least the 1940s (Cagle, 1942). Eastern Narrow-mouthed Toads were not previously detected at LPH despite: 1) two general herpetofaunal surveys of the area (Rossman, 1960; Ballard, 1994); 2) a multiple-year study of the reproductive biology of Green

Treefrogs conducted at Otter Pond (Garton and Brandon, 1975); 3) two Bird-voiced Treefrog vocalization surveys of LPH (Redmer et al., 1999; Palis, 2008); 4) our frequent visits (> 700) to LPH since 1996 (principally on or near Snake Road during road closure periods); and 5) visits by large numbers of nature enthusiasts who walk Snake Road during the spring and fall road closure periods. How could Eastern Narrow-mouthed Toads go unnoticed at LPH for over 60 years? We suggest that their small size, cryptic behavior, and subdued call prevented earlier detection.

Eastern Narrow-mouthed Toads are small frogs, generally fossorial by day, and typically surface-active at night. Therefore, they tend to go unnoticed. In addition, their relatively quiet call does not carry very far (Wright and Wright, 1949) and is often drowned out by the louder voices of other summer-breeding frogs (Bridges and Dorcas, 2000). Known breeding sites at LPH are more than 220 m from Snake Road; it is unlikely a human on the road would detect vocalizing Eastern Narrow-mouthed Toads from that distance. Moreover, detection of Eastern Narrowmouthed Toad calls from Snake Road is probably impeded by the attenuating effect of forest vegetation (MacLaren et al., 2018). Although Snake Road has become a popular ecotourism destination-warranting its own snake field guide (Vossler, 2021) and Facebook page (Facebook.com/groups/laruepinehills)relatively few people visit during the summer breeding season of Eastern Narrow-mouthed Toads (JGP, personal observations).

The discovery of Eastern Narrow-mouthed Toads could be considered an example of hidden biodiversity, defined as "flora and fauna that, despite being present and often prevalent in an area, go undetected or unnoticed" (Luhring, 2008: p. 372). Astonishingly, Eastern Narrow-mouthed Toads have apparently eluded detection at LPH for more than 60 years. Although recent colonization of LPH cannot be ruled out, we suggest that the clandestine nature of Eastern Narrow-mouthed Toads is a more parsimonious explanation for their previous lack of detection.

Acknowledgments

JGP thanks Melanie Kasson for alerting him to JAS's and JJS's discovery, Kevin Enge for confirming Eastern Narrowmouthed Toad recordings and Chris Benda for confirming some plant identifications. JAS and JJS thank Scott Ballard and Mike Dreslik for providing independent confirmation of their Eastern Narrow-mouthed Toad identification.

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Minutes of the CHS Board Meeting, September 12, 2023

A meeting of the CHS board of directors was called to order via Zoom at 7:34 P.M. Board members Kyle Houlihan and Margaret Ann Paauw were absent. Caitlin Monesmith also attended. Minutes of the August 15 board meeting were read and accepted.

Officers' reports

Treasurer: Rich Crowley presented the august financial report.

Membership secretary: Mike Dloogatch reported no nonrenewals from the previous month.

Media secretary: Gabrielle Evans reported Instagram is our principal engagement website, but also slow and steady growth on Tumblr. She is looking into Bluesky as another possibility.

Sergeant-at-arms: Tom Mikosz said that the August Junior

Herpers meeting been canceled. Only six persons attended the August general meeting.

New business

It was suggested that we create a QR code to facilitate donations at shows. Rich Crowley said that he should be able to take care of it.

Gail Oomens has written an open letter to the CHS membership, specifying the areas in which volunteers are needed. The wording was discussed. Rich and Gail will tweak things a bit, and then distribute the letter via email and posts on social media.

The meeting adjourned at 9:11 P.M.

Respectfully submitted by recording secretary Gail Oomens

Notes on the Herpetofauna of Western Mexico 31: Herpetofauna from Sierra de Tesistán, Zapopan, Jalisco, Mexico

Israel Salcido-Rodríguez¹, Francisco Isaac Hernández-Valadez¹, Adriana Elizabeth Castillo-Franco¹, Daniel Cruz-Sáenz², Edgar Emmanuel Hernández-Juárez², David Lazcano³,

Lydia Allison Fucsko⁴ and Larry David Wilson⁵

Abstract

We present a list of amphibian and reptile species from the locality known as Sierra de Tesistán, which is located in the municipality of Zapopan in the Mexican state of Jalisco. After four years of sampling, 65 species are recorded, of which 18 are amphibians and 47 are reptiles. Ten amphibians and 28 reptiles are endemic to Mexico; four amphibians and 18 reptiles come under some category of protection of NOM-059 SEMARNAT-2010.

Resumen

Presentamos un listado de especies de anfibios y reptiles de la localidad conocida como sierra de Tesistán la cual se encuentra en el municipio de Zapopan en el estado mexicano de Jalisco. Después de 4 años de muestreo se registraron 65 especies de las cuales 18 son anfibios y 47 son reptiles. Diez anfibios y 28 reptiles son endémicos de México y además cuatro anfibios y 18 reptiles se encuentran bajo alguna categoría de protección de la NOM-059 SEMARNAT-2010.

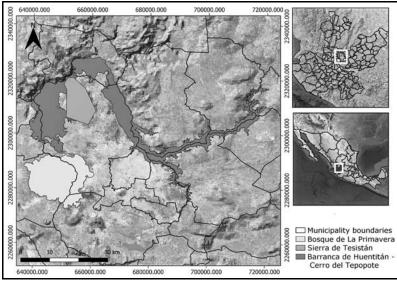
Introduction

The state of Jalisco in the western part of Mexico is home to 223 species of amphibians and reptiles (Cruz-Saenz et al., 2017). This high diversity of herpetofaunal species is in great part explained because four physiographic provinces converge in the state (INEGI, 1981), resulting in a high variety of ecosystems and, therefore, a high variety of habitats available for the herpetofauna. So, the study of its sierras is vital task, because

these areas could be the last source of herpetological diversity (Barrera Rodríguez and Zaragoza Vargas, 2023).

Background of the study site

Sierra de Tesistán is a mountain range reasonably close to the Metropolitan Area of Guadalajara (MAG), extending from Cerro del Tepopote to the Santiago River Canyon. This area receives a great deal of pressure from real estate activities because of the



Location of the Sierra de Tesistán.

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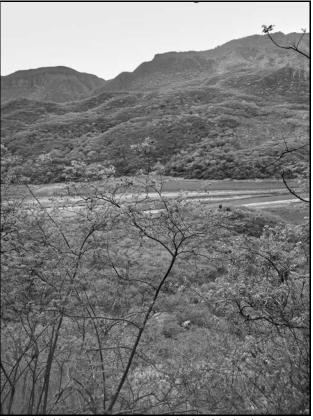
Photographs by Israel Salcido-Rodríguez.



Gallery forest in a ravine that reaches the Santiago River.



Oak forest found in an area known as El Col.



Tropical deciduous forest adjacent to the banks of the Santiago River.



Secondary forest in Rancho El Cimarrón.



Agalychnis dacnicolor on a rock in a tropical deciduous forest at Rancho El Tescalame. Photograph by Israel Salcido-Rodríguez.

growth of the MAG. The highest elevation is Cerro del Col at 2200 masl; its lowest point is at the bottom of Santiago River Canyon at 774 masl. Sierra de Tesistán lies in the Central Plateau physiographic province (Cruz-Sáenz et al., 2017).

The area includes the following vegetation communities:

Oak forest: The predominant tree species are oaks; also common are agaves, shrubs, sections of secondary vegetation, grasslands, and riparian vegetation. The oak forests include a large number of streams, ponds and rivers.

Thorny forest or secondary vegetation: Composed of grasslands, huizaches, papelillo trees, and some shrubs; these are located in ravine areas; oak-pine forests have been deforested for livestock raising or some other land use.

Oak-pine forests: Composed primarily of oaks, with a few pines; also found are agaves (*Agave guadalajarana*), small sections of secondary vegetation, streams and natural or human-excavated ponds.

A number of canyons in the sierra contain secondary vegetation: grasslands, huizaches, fruit trees such as plums, guamuchiles, mangoes and some others. Agaves, mammilarias and cacti are found in large quantities. It is also common to find agave monocultures, nopal and fruit trees. These plants are commonly associated with streams, ponds, and rivers.

Results

After four years of nonsystematic sampling of herpetofauna, 64 species have been recorded, of which 18 are amphibians and 46 are reptiles. Of these species, 10 amphibians and 28 reptiles



Tlalocohyla smithii on a leaf next to a pond in secondary vegetation in the town of Palo Gordo. Photograph by Israel Salcido-Rodríguez.



Dryophytes eximius found in secondary vegetation in the population center known as Las Mesitas. Photograph by Israel Salcido-Rodríguez.

are endemic to Mexico. Four amphibian species and 17 reptile species are under some category of protection of NOM-059 (SEMARNAT, 2010) (Table 1).

The amphibians are included in one order, six families, 13 genera, and 18 species. Ten species are endemic to Mexico (55.5%), with only four species protected by Mexican environmental law (22.2%). Otherwise, on the basis of the environmental vulnerability score (EVS), eight species have low risk, seven medium risk, and three high risk. Most of the species are assessed as LC (least concern) under the IUCN risk classification (Table 1).

Reptiles are included in two orders, 19 families, 38 genera, and 46 species. Sierra de Tesistán presents high endemism with 28 species endemic to Mexico (60.9%). Eighteen species are protected by Mexican environmental laws (39.1%), and 35 species assessed as least concern (LC) by the IUCN. Based on the EVS, 13 species are low risk, 14 species are medium risk, and 17 species are high risk (Table 1).

Of importance is that three species are invasive within the Sierra de Tesistán. These are the green iguana (*Iguana iguana*), the pond slider (*Trachemys scripta*), and the common house gecko (*Hemidactylus frenatus*). Two of these species have natural distributions in Mexico but are not native to this site.

Discussion and Conclusions

Sierra de Tesistán is a very important area, experiencing



Hypopachus variolosus found in secondary vegetation at Rancho El Cimarrón. Photograph by Israel Salcido-Rodríguez.

Photographs by Israel Salcido-Rodríguez.



Phyllodactylus lanei found on a rock wall in a tropical deciduous forest at Rancho El Tescalame.



Sonora mutabilis found in a tropical deciduous forest at Cerro del Coyote.



Pituophis deppei found in secondary vegetation on the outskirts of Ejido Copalita.



Oxybelis microphthalmus found in a tropical deciduous forest in the town of Palo Gordo.



Micruroides euryxanthus in found in a tropical deciduous forest within the population center known as Las Mesitas.



Crotalus basiliscus found in an oak forest at Rancho El Cimarrón.

enormous pressure due to the growth of the Metropolitan Area of Guadalajara. But more important could be the fact that it is a natural biological corridor within the Santiago River Canyon. It is of vital importance to preserve this site, not only because of its herpetological richness but also because it is a natural corridor.

Environmental education is essential to understand the need for the conservation of different ecosystems and within them their herpetofauna. Teaching fundamental facts about conservation will create an important positive feedback among the human population that surrounds and visits this area. This will reduce without doubt the unfortunate and unnecessary slaughtering of both amphibians and reptiles that doesn't help conservation programs at all.

Sierra de Tesistán is an area with large tracts of forest that are in the process of being fragmented by population growth and land use. This activity has caused the herpetofauna to undergo population decrease caused by the destruction of their ecosystem and the multiple myths that cause these animals to be misunderstand and massacred on sight. In addition, species introduced to forests or rivers near urbanized populations can be found. This study is essential to understanding the relationships between herpetofaunal diversity and human needs, in order to support a sustainable process of urbanization.

Reflection

Why do we need biological corridors? We know they can be narrow or spacious strips of land that have different dominant vegetation types. They serve as traveling avenues for biological diversity to move on between two similar yet fragmented habitat areas, and provide important sources of food and cover for many species, as in our case (Challenger, 1998; Valdez et al., 2006; Conrada et al., 2012; Liu et al., 2018; Moreno and Guerrero-Jimenez, 2019; Zellmer and Goto, 2022). Even plant communities are involved in this positive process. As animals use them in different ways, this helps the dispersion in form of seeds, to let these plant communities continue growing in these biological corridors.

Acknowledgments

We thank the inhabitants of Sierra de Tesistán and all the people who collaborated in helping out with this study, without whose help this work would not have been possible. Special thanks go to Marco Antonio Léon-Dávila, who made it possible to enter the mountain sites and who contributed with the tools to facilitate this research.

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Table 1. Species of herpetofauna recorded from Sierra de Tesistán, Zapopan Jalisco, Mexico. NOM = protection status under NOM-ECOL-059-2010 (SEMARNAT, 2010): Pr = protección especial (special protection); A = amenazada (threatened). IUCN = protection status according to the International Union for Conservation of Nature (IUCN, 2017): LC = least concern; VU = vulnerable; EN = endangered; NT = near threatened; DD = data deficient; NE = not evaluated. EVS = Environmental Vulnerability Score *sensu* Wilson et al. (2013a, b): L = low risk (3–9); M = medium risk (10–13); H = high risk (14–20).

Family	Species (* = endemic to Mexico)	NOM-059	IUCN	EVS
Bufonidae	Incilius occidentalis*	-	LC	11 (M)
Bulonidae	Rhinella horribilis	-	NE	3 (L)
	Craugastor augusti	-	LC	8 (L)
Craugastoridae	Craugastor hobartsmithi*	-	EN	15 (H)
	Craugastor occidentalis*	-	DD	13 (M)
	Agalychnis dacnicolor*	-	LC	13 (M)
	Dryophytes arenicolor	-	LC	7 (L)
II-J.J.	Dryophytes eximius*	-	LC	10 (M)
Hylidae	Smilisca fodiens	-	LC	8 (L)
	Tlalocohyla smithii*	-	LC	11 (M)
	Exerodonta smaragdina*	Pr	LC	12 (M)
x . 1 . 1 ¹ 1	Leptodactylus melanonotus	-	LC	6 (L)
Leptodactylidae	Eleutherodactylus modestus*	Pr	VU	16 (H)
Microhylidae	Hypopachus variolosus	-	LC	4 (L)
-	Lithobates forreri	Pr	LC	3 (L)
Ranidae	Lithobates neovolcanicus*	А	NT	13 (M)
	Lithobates psilonota*	-	DD	14 (H)
Scaphiopodidae	Spea multiplicata	-	LC	6 (L)
Anguidae	Elgaria kingii*	Pr	LC	10 (M)
Anolidae	Anolis nebulosus*		LC	13 (M)
Gekkonidae	Hemidactylus frenatus	-	-	-
Helodermatidae	Heloderma horridum*	А	LC	14 (H)
Terrenides	Ctenosaura pectinata*	А	NE	15 (H)
Iguanidae	Iguana iguana	Pr	LC	12 (M)
	Sceloporus horridus*	-	LC	11 (M)
Dharman and the	Sceloporus melanogaster*	-	-	-
Phrynosomatidae	Sceloporus melanorhinus	-	LC	9 (L)
	Sceloporus nelsoni*	-	NE	15 (H)

Family	Species (* = endemic to Mexico)	NOM-059	IUCN	EVS	
	Sceloporus scalaris*	-	LC	12 (M)	
	Sceloporus spinosus*	-	LC	11 (M)	
	Sceloporus torquatus*	-	LC	11 (M)	
	Sceloporus utiformis*	-	LC	15 (H)	
	Urosaurus bicarinatus*	-	LC	12 (M)	
Phyllodactylidae	Phyllodactylus lanei*	-	LC	15 (H)	
Quinci la c	Plestiodon callicephalus	-	LC	12 (M)	
Scincidae	Plestiodon bilineatus	-	LC	13 (M)	
T 1	Aspidoscelis communis*	Pr	LC	14 (H)	
Teiidae	Aspidoscelis gularis	-	LC	9 (L)	
Xantusiidae	Xantusia sanchezi*	Р	LC	16 (H)	
Boidae	Boa sigma	А	NE	10 (M)	
	Drymarchon melanurus	-	LC	6 (L)	
	Drymobius margaritiferus	-	NE	6 (L)	
	Lampropeltis polyzona	-	NE	11 (M)	
	Leptophis diplotropis*	А	LC	14 (H)	
	Masticophis mentovarius	А	LC	6 (L)	
Colubridae	Oxybelis microphthalmus	-	NE	5 (L)	
	Pituophis deppei*	А	LC	14 (H)	
	Senticolis triaspis	-	LC	6 (L)	
	Sonora mutabilis*	-	LC	14 (H)	
	Tantilla bocourti*	_	LC	9 (L)	
	Trimorphodon tau*	-	LC	13 (M9	
	Coniophanes lateritius*	-	DD	13 (H)	
	Imantodes gemmistratus*	Pr	NE	6 (H)	
N 111	Hypsiglena torquata*	Pr	LC	8 (L)	
Dipsadidae	Leptodeira maculata	Pr	LC	7 (L)	
	Leptodeira splendida*		LC	14 (H)	
	Rhadinaea hesperia*	Pr	LC	10 (M)	
Elapidae	Micruroides euryxanthus	А	LC	15 (H)	
Leptotyphlopidae	Rena humilis	-	LC	8 (L)	
•	Storeria storerioides*	-	LC	11 (M)	
Natricidae	Thamnophis cyrtopsis	-	LC	7 (L)	
	Thamnophis eques	А	LC	8 (L)	
Viperidae	Crotalus basiliscus*	-	LC	18 (H)	
Emydidae	Trachemys scripta	-	-	-	
Kinosternidae	Kinosternon integrum*	Pr	LC	11 (M)	

Table 2. Species richness and numbers of Mexican protected and endemic species from Sierra de Tesistán compared to surrounding areas
in Jalisco that have been studied in the past.

Locality	Species richness	Protected species	Endemic species	Reference
Huaxtla	36	10	19	Cruz-Sáenz et al., 2011
Bosque La Primavera	56	17	13	Reyna-Bustos et al., 2007
Hostotipaquillo, Jalisco	61	21	28	Flores-Covarrubias et al., 2012
Volcán de Tequila	31	18	22	Rojo-Gutiérrez et al., 2018
Arcediano	44	21	24	Cruz-Sáenz et al., 2009
Palo Gordo	55	16	34	Rojo-Gutiérrez et al., 2022
El Diente	33	8	21	Cortés-Vásquez et al., 2022
Sierra de Tesistán	65	22	38	This paper

Notes on Reproduction of Mountain Chorus Frogs, Pseudacris brachyphona (Anura: Hylidae)

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Abstract

I conducted a histological examination of gonadal material from 29 *Pseudacris brachyphona* consisting of 17 adult males, 8 adult females and 4 subadults. The smallest mature male (abundant sperm in lumina of seminiferous tubules) measured 23 mm SVL. The smallest mature female (in spawning condition) measured 28 mm SVL. As previously reported for *P. brachyphona*, reproduction occurs in the early part of the year. Two *P. brachyphona*, one each from May and June contained postovulatory follicles, evidence of a recent spawning (*sensu* Redshaw, 1972). Both contained concurrent vitellogenic (yolking) follicles suggesting they would have spawned a second time during the same reproductive period.

The mountain chorus frog Pseudacris brachyphona (Cope, 1889) occurs from southwestern Pennsylvania through southern Ohio and West Virginia to central Kentucky, central Tennessee and northern Alabama (Frost, 2023). Reproduction occurs from late winter to midsummer, depending on latitude and elevation; males remain at breeding sites throughout the breeding season whereas females visit for only one night to mate (Dodd, 2023). Ethier et al. (2021) reported breeding occurred February to June. Habitats include temporary pools, roadside ditches, mountaintop bogs, furrows in plowed fields, seepages and woodland springs (Mitchell and Pauley, 2005). Deforestation, urbanization and loss of flood-plain pools have resulted in reduced habitat for P. brachyphona (Green et al., 2013). The biology of P. brachyphona is summarized in Hoffman (1980). In this paper, I present data from a histological examination of P. brachyphona gonadal material in a combined sample from Kentucky, Ohio, Pennsylvania and West Virginia. Utilization of museum collections for obtaining reproductive information avoids removing additional animals from the wild.

A sample of 29 *P. brachyphona* from Kentucky (N = 5), Ohio (N = 6), Pennsylvania (N = 16), West Virginia (N = 2), USA (Appendix) collected 1941 to 1985 consisting of 17 adult males (mean snout–vent length, SVL = 26.9 mm \pm 1.4 SD, range = 23–29 mm), 8 adult females (mean SVL= 30.4 mm \pm 2.0 SD, range = 28–34 mm), and four subadults (mean SVL = 18.8 mm \pm 1.3 SD, range = 17–20 mm) was examined from the herpetology collection of the Carnegie Museum (CM), Pittsburgh, Pennsylvania, USA.

A small incision was made in the lower part of the abdomen and the left testis was removed from males and a piece of the left ovary from females. Gonads were embedded in paraffin, sections were cut at 5 μ m and stained with Harris hematoxylin followed by eosin counterstain (Presnell and Schreibman, 1997). Histology slides were deposited at CM. An unpaired *t*-test was used to test for differences between male and female SVLs.

Testes of *P. brachyphona* are surrounded by black pigment as previously reported for *Pseudacris* (as *Hyla*) crucifer by Rugh (1941) and Oplinger (1966). Otherwise, the testicular morphology of *P. brachyphona* is similar to that of other anurans as described in Ogielska and Bartmañska (2009a). Within the seminiferous tubules, spermiogenesis occurs in cysts which are closed until the late spermatid stage is reached; cysts then open and differentiating sperm reach the lumina of the seminiferous tubules (Ogielska and Bartmañska, 2009a). There was typically a tangled mass of spermatozoa in the lumen of each seminiferous tubule. All 17 adult males exhibited spermiogenesis: March (N = 1), April (N = 6), May (N = 7), June (N = 3). There was no discernible monthly variation in the testis cycle. The smallest mature male of *P. brachyphona* measured 23 mm SVL (CM 21668) and was from May. Wright and Wright (1949) reported *P. brachyphona* adult males ranged from 24–32 mm. One smaller *P. brachyphona* male from March (CM 23810) that measured 20 mm SVL contained small sperm clusters in some seminiferous tubules. The quantity of sperm was far less than that of larger males and I doubt it would joined the breeding population. I thus considered it to be a subadult.

Three other very small unsexed subadults of *P. brachyphona* were (CM 89272, SVL = 20 mm; CM 26788, SVL = 18 mm; CM 89266, SVL = 17 mm). I cannot speculate when they have might have reached maturity.

The mean SVL of P. brachyphona females was significantly larger than that of males (t = 4.9, df = 23, P < 0.0001). The ovaries of P. brachyphona are similar to those of other anurans in being paired organs lying on the ventral sides of the kidneys; in adults the ovaries are filled with diplotene oocytes in various stages of development (Ogielska and Bartmañska, 2009b). Mature oocytes are filled with yolk droplets; the layer of surrounding follicular cells is thinly stretched. Three stages were noted in the ovarian cycle of P. brachyphona (Table 1): Stage 1 (Spawning condition) in which mature oocytes predominated: Stage 2 (Vitellogenic) in which follicles were accumulating volk, and concurrent postovulatory follicles (sensu Redshaw, 1972) were present; Stage 3 (Not in spawning condition) in which previtellogenic oocytes predominated. The smallest mature P. brachyphona female (spawning condition) in my sample measured 28 mm SVL (CM 46218) and was from March. Wright and Wright (1949) reported adult females of P. brachyphona ranged from 27 to 34 mm.

Vitelligenic (yolking) follicles were noted concurrently with postovulatory follicles in both CM 54414A from May and CM 20924E from June (Table 1), suggesting these females would have spawned a second time during the same reproductive

Month	n	Spawning condition	Vitellogenesis (yolking follicles and postovulatory follicles)	Not in spawning condition (previtellogenic)
March	4	1	0	3
April	1	1	0	0
May	1	0	1*	0
June	2	1	1*	0

 Table 1. Three monthly stages in the ovarian cycle of 8 adult female Pseudacris

 brachyphona. * = Postovulatory follicles sensu Redshaw (1972).

period. Postovulatory follicles form when the ruptured follicle collapses after ovulation; the follicular lumen disappears and proliferating granulosa cells are surrounded by a fibrous capsule (Redshaw, 1972). Postovulatory follicles are short-lived in most anuran species and are resorbed after a few weeks (Redshaw, 1972).

Four of the eight (50%) of the adult female P. *brachyphona* in my sample contained atretic oocytes. Atresia is a widespread process occurring in the ovaries of all vertebrates (Uribe Aranzábal, 2009). It is common in the amphibian ovary (Saidapur, 1978) and is the spontaneous digestion of a diplotene oocyte by its own hypertrophied and phagocytic follicle cells which invade the follicle and eventually degenerate after accumulating dark pigment (Ogielska and Bartmañska, 2009b). See Saidapur and Nadkarni (1973) and Ogielska et al. (2010) for a detailed description of the stages of follicular atresia in the frog ovary. Atresia may influence the number of ovulated oocytes (Uribe Aranzábal, 2011) and can remove females from the breeding population (Goldberg, 2019).

In conclusion, my data supports previous studies indicating *P. brachyphona* reproduces in winter–spring (Table 2). The presence of vitellogenic (yolking) follicles for a subsequent spawning and concurrent postovulatory follicles (from a recent spawning) in the same ovary, suggests that some *P. brachy-phona* females may spawn more than once in the same reproductive period. This was also noted in *Pseudacris streckeri* (Goldberg, 2020), *P. fouquettei* (Goldberg, 2021) and *P. feriarum* (Goldberg, 2022). Histological examination of additional *Pseudacris* species are warranted to ascertain if they can also spawn a second time during the same reproductive period.

Acknowledgments

I thank Jennifer Sheridan (CM) for permission to examine *P*. *brachyphona* and Stevie Kennedy-Gold (formerly at CM) for facilitating the loan: 2022-08.

Locality	Breeding period	Source
Alabama	December-January to April	Guyer and Bailey, 2023
Carolinas and Virginia	February to April	Beane et al., 2010
Georgia	late winter to early spring	Jensen et al., 2008
Kentucky	February, March to August	Barbour, 1953, 1971
Maryland	April to June	Cunningham and Nazdrowicz, 2018
North Carolina	call February to April	Dorcas et al., 2007
Ohio	March to June	Walker, 1967
Pennsylvania	April and May	Hulse et al., 2001
Southeast	call February through spring	Dorcas and Gibbons, 2008
Tennessee	February to May	Niemiller and Reynolds, 2011
West Virginia	start in February	Green and Pauley, 1987
No specific locality	March–July	Wright and Wright, 1949

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Appendix

Twenty-nine *P. brachyphona* examined (by county) from the herpetology collection of the Carnegie Museum (CM), Pittsburgh, Pennsylvania, USA., * = no county available for CM 89272 from Ohio. **KENTUCKY: Breathitt** CM 44588; **Wolfe** CM 46218; **Rowan** CM 54403, 54414A; **Bell** CM143186; **OHIO: Scioto** CM 54392; **Ross** CM 54493; **Athens** CM 89258, 89266; **Hocking CM** 89259; (89272*); **PENNSYLVANIA: Beaver** CM 23810–23813, 26095C, 26788; **Fayette** CM 21666-21668, 31762; **Clearfield** CM 36562; **Westmoreland** CM 32091, 32096, 35300, 113366; **Allegheny** CM 155830; **WEST VIRGINIA: Nicholas** CM 20924E; **Gilmer** CM 61488.

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A 44-year-old Red Milksnake (Lampropeltis triangulum syspila)

This is a follow-up report on the longevity of a red milksnake, *Lampropeltis triangulum syspila*, that I maintained in my personal collection. See *Bulletin of the Chicago Herpetological Society*, Volume 55, Number 6, June 2020, for my first article concerning this snake.

Since my earlier article, changes in its health took place that eventually led to its death. Sometime in late October 2022, the snake refused to eat small live mice, a rare occurrence. Upon examining the snake more closely, I noticed it had Thomas (Tom) Zaremba 7972 Dublin Road Camp Douglas, WI 54618 zartenfour@mwt.net



Photograph by Tom Zaremba, 15 August 2022.

what appeared to be two separate bulges within its body. Since it did not seem that the snake was suffering, I decided to observe the snake more often and let nature take its course. The snake spent more time coiled under the small floodlight I provided each day during daytime hours. No other changes in the snake's behavior were noticed; however, the two bulges were getting larger. The snake continued to refuse all offered food (small mice), with the exception of one small mouse on 1 March 2023. The snake failed to eat again after this date.

- During the year 2020 it ate 54 small mice and shed its skin three times.
- During the year 2021 it ate 108 small or baby mice and shed its skin three times.
- During the year 2022 it ate 86 small mice and shed its skin three times.
- During the year of 2023 it ate one small mouse and shed its skin once.

On 21 April 2023 I discovered the snake dead under its shelter, an overturned clay flowerpot tray. This snake, a male, thrived for 44 years, seven months and eleven days while in my care. It attained the length of 32+ inches while living in a 10-gallon glass aquarium for the majority of its life.

My experience with this milksnake taught me that snakes are capable of living longer lives in captivity than I ever would have thought possible.

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Are you tech savvy? Do you know your way around the web? Do you have some expertise to help update our websites? If you are interested, email <u>president@chicagoherp.org</u>.

Social Media

We are looking for volunteers to help with engagement through our media platforms. One person cannot do it alone and we can use help with sharing content, answering questions/replies, and uploading photos.

Do you have a few minutes to check on a media site to post or comment? If you are interested, email president@chicagoherp.org.

Membership

We need more members.

Do you know someone interested in reptiles? Encourage them to join: http://chicagoherp.org/membership.

Activities

We are looking for volunteers to assist in organizing different activities for members to participate in. Events that bring our members together include: picnics, field trips, herping trips, "fun things" at the general meetings, or something totally innovative!

Do you have an "in" somewhere? Is there somewhere you would like to go/see? Are you knowledgeable on a "how to"? Could you present/teach a group about it? If you are interested, email president@chicagoherp.org and meeting@chicagoherp.org.

Shows

We are looking for volunteers to show off pet reptiles to help educate newcomers to the field of herping. We have different venues to go to all over the Chicagoland area. Some monthly, some yearly. Nothing complicated — just talking about your own reptile. Most current shows run from 10 A.M. to 3 or 4 P.M. You can help for the full show or for a couple of hours.

Do you like talking about your pets? Helping a child or adult learn to not be afraid of our critters? Let us know if you would like to be put on the email list for upcoming shows. If you are interested, email showrequest@chicagoherp.org.

New Board Members

We are always looking for new ideas and insights. a new direction for a concept. As a member-atlarge or any other board position, you are helping shape the Society for the future.

What would you like to pass on/share with the next generation? Even if you aren't ready for a board position, you can participate in board meetings, even mentor under a board member! If you are interested, email president@chicagoherp.org.

Sincerely,

The Chicago Herpetological Society board of directors

UPCOMING MEETINGS

Monthly meetings of the Chicago Herpetological Society begin at 2:00 P.M. on the third Sunday of each month. Please try to join us online or *in person* at the Notebaert Nature Museum, 2430 N. Cannon Drive, Chicago. The next meeting will take place on October 15. **Rachel Bladow**, last year's CHS vice-president, will be present in person to speak at the October 15 meeting. Rachel is a biologist at the American Zoo Association (AZA) Population Management Center at Lincoln Park Zoo. She will speak about "Population Management in AZA Zoos and Aquariums."

The program for the November 19 meeting has not yet been conirmed.

Please check the CHS website or Facebook page each month for information on the program. Information about attending a Zoom webinar can be found here:

https://support.zoom.us/hc/en-us/articles/115004954946-Joining-and-participating-in-a-webinar-attendee-

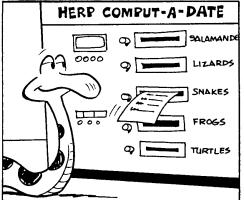
Board of Directors Meeting

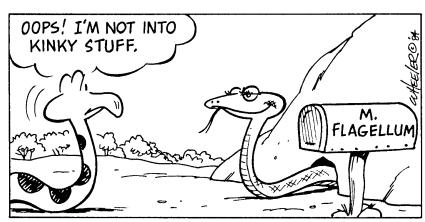
Are you interested in how the decisions are made that determine how the Chicago Herpetological Society runs? And would you like to have input into those decisions? The next board meeting will be held online. If you wish to take part, please email: rcrowley@chicagoherp.org.

NEW CHS MEMBERS THIS MONTH

Emma Erdman Ethan Fannin Matt & Blaise Filippini

THE ADVENTURES OF SPOT





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