



Facultative Scavenging by Southern Leopard Frog (*Rana sphenocephala*) Larvae

Lawrence G. Bassett, Andrea Villamizar-Gomez, and Michael R. J. Forstner

Department of Biology, Texas State University, San Marcos, Texas 78666, USA (lawrencebassett@gmail.com)

Facultative scavenging has gained some recognition as a stabilizing force within ecological communities and is thought to manifest in food webs as the number of scavenger-carrion relationships increases (Wilson and Wolkovich 2011; Beasley et al. 2019). Scavenger-carrion relationships are plentiful within temperate forest communities (e.g., Wilmers et al. 2003; Selva and Fortuna 2007; Gomo et al. 2020), but the trophic linkages that are typically given the most attention by research scientists are those existing between predators and their prey. Although some anuran species are known to act as facultative scavengers as larvae (e.g., Smith 2005; Street et al. 2013; Trivedi et al. 2018; Vazifdar et al. 2021), no such evidence exists for Southern Leopard Frog (*Rana sphenocephala* [= *Lithobates sphenocephalus*]) larvae.

Rana sphenocephala is a medium sized (maximum head-body length = 127 mm) ranid native to the southeastern and midwestern United States (Powell et al. 2016). This species is a habitat generalist that occupies many types of water bodies and remains active throughout most of the year (Tipton et al. 2012). *Rana sphenocephala* larvae are generally considered green algae feeders (Tipton et al. 2012; Dodd 2013) although laboratory experiments indicate larvae have accepted other foods that include lettuce, rabbit chow, and pelleted fish food (Babbitt 2001; Sparling et al. 2006). Herein we provide what is, to the best of our knowledge, the first report of *R. sphenocephala* larvae facultatively scavenging on a deceased vertebrate in the wild; more specifically, on a Houston Toad, *Bufo houstonensis* (= *Anaxyrus houstonensis*). We maintain recognition of the generic names *Bufo* and *Rana* over the newer *Anaxyrus* and *Lithobates*, respectively, for the sake of taxonomic stability (Pauly et al. 2009) and to discourage paraphyletic groupings (Yuan et al. 2016). Common names presented herein correspond with the standard English names presented by Crother (2017).

At 2343 h on 27 February 2021, while conducting an anuran survey on a private ranch in Bastrop County, Texas, USA, we observed four anuran larvae of identical appearance scavenging on the carcass of an adult *B. houstonensis* (Fig. 1).

This observation took place along the margin of a small pond in a densely wooded forest patch (30.2162 N, 97.2418 W; WGS 84). Although we are unaware of the exact cause of death, the toad might have been killed by a mesocarnivore (e.g., Schaaf 1970). After watching the larvae feed on the carcass for about one minute, we captured one of the larvae and collected a small tail clip for genetic identification (stored in 95% ethanol), then released it. Although we suspected that the observed larvae were *R. sphenocephala*, we felt that genetic identification was warranted given the difficulty associated with identifying anuran larvae in Texas based on morphological characters alone (Dixon 2013).

DNA was extracted from the tail clip using a Qiagen DNeasy Blood and Tissue Kit following the manufacturer's protocol. The mitochondrial gene cytochrome b (cytb) was PCR-amplified using the primers BM1 (5'-CCC CTG AGA ATG ATA TTT GTC CTC A-3') and BM2 (5'-CCA TCC AAC ATC TCA GCA TGA TGA AA-3') (Meece et al. 2005). PCR conditions included an initial denaturation cycle of 94 °C for 3 min, and 40 subsequent cycles at 94 °C, 54 °C, and 72 °C for 1 min each. Success of the PCR was assayed by gel electrophoresis and products were cleaned using an ExoSAP-IT enzymatic protocol (Affymetrix). Clean PCR products were cycle sequenced across 40 cycles, each consisting of 20 sec at 96 °C, 20 sec at 50 °C, and 4 min at 60 °C. Cycle sequencing products were cleaned using CENTRI-SEP spin columns (Princeton Separations) and underwent capillary electrophoresis using an ABI PRISM 3500 XL Genetic Analyzer.

Forward and reverse sequences were bidirectionally aligned using MUSCLE (Edgar 2004) implemented in Geneious v.8.1.9. The resulting consensus sequence was utilized in a BLAST search of the NIH genetic sequence database (GenBank) for initial taxonomic confirmation. Our BLAST search revealed that the highest percentage identity of the larva cytb sequence (307 bases total length; GenBank OM128140.1) was 99.34% to a *R. sphenocephala* sequence (GenBank KX269321.1).

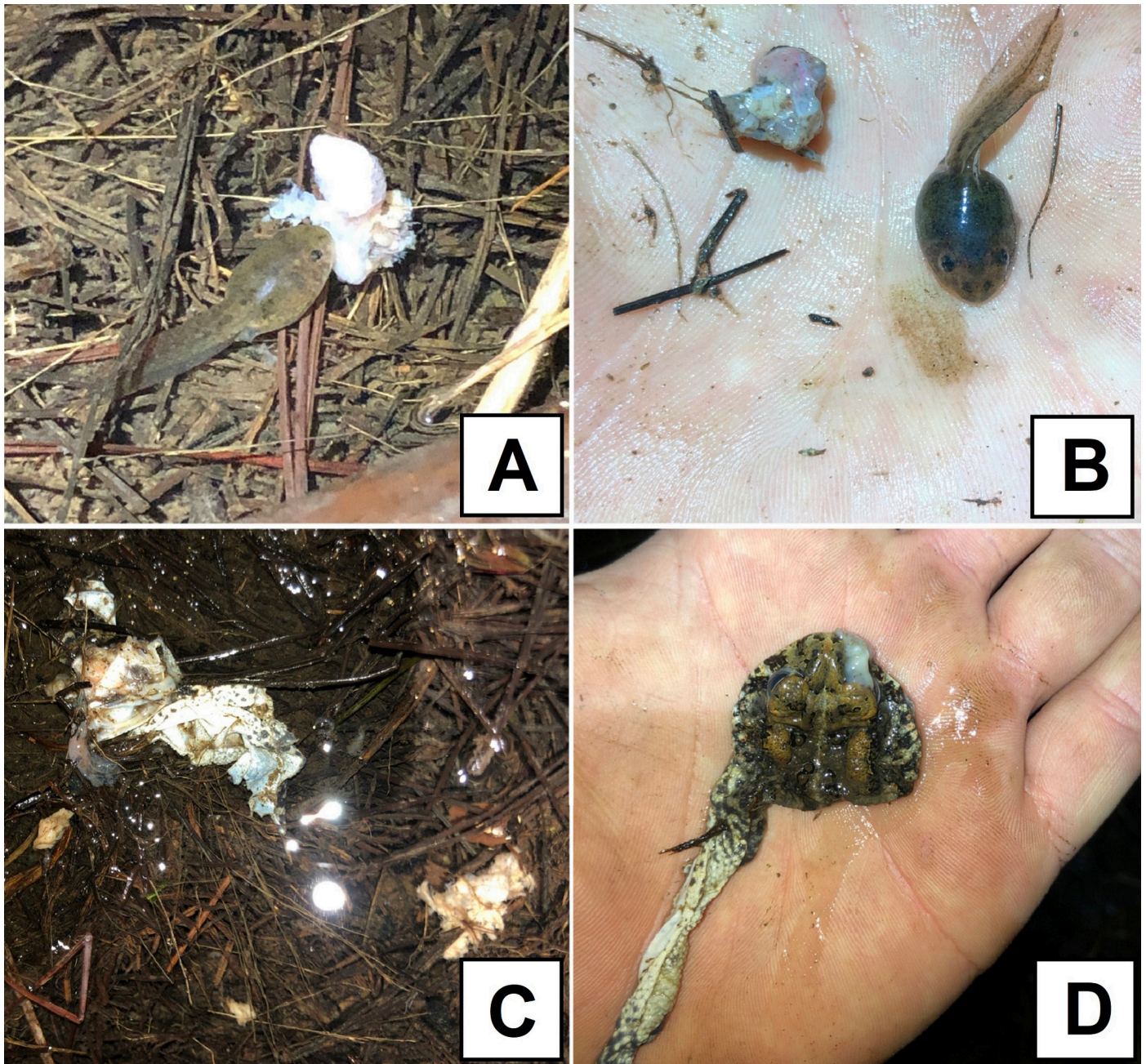


Fig. 1. Southern Leopard Frog (*Rana sphenoccephala* [= *Lithobates sphenoccephalus*]) larva scavenging on tissue from a Houston Toad (*Bufo houstonensis* [= *Anaxyrus houstonensis*]) carcass (A); *Rana sphenoccephala* larva in hand upon capture along with the *B. houstonensis* tissue it was scavenging (B); The bulk of the *B. houstonensis* carcass located immediately adjacent to the tissue upon which the *R. sphenoccephala* larva was scavenging (C); The anterior portion of the *B. houstonensis* carcass with the following distinctive morphological characters exhibited: bean-shaped paratoid glands, thickened postocular cranial crests, relatively reduced parietal crests, heavily tuberculated dorsal skin, and dorsal spotting that lacks clear borders (D). All photographs were taken on 27 February 2021 at the Griffith League Ranch in Bastrop County, Texas, USA (30.2162 N, 97.2418 W; WGS 84) by Lawrence G. Bassett.

We constructed a phylogeny of the resident anuran species occurring on the Griffith League Ranch (Brown et al. 2011) to confirm the taxonomic identity of the scavenging larva. We downloaded cytb sequence data from GenBank for *R. sphenoccephala* (n = 1) and known syntopic anuran taxa with indirect development: the American Bullfrog (*Rana catesbeiana* [= *Lithobates catesbeianus*]) (n = 3), Hurter's Spadefoot (*Scaphiopus hurterii*) (n = 1), the Green Treefrog (*Hyla cine-*

rea) (n = 3), and the Gulf Coast Toad (*Bufo nebulifer* [= *Incilius nebulifer*]) (n = 3). Cytochrome b sequence data for some resident anuran taxa (e.g., *Acris blanchardi* [Blanchard's Cricket Frog], *Hyla versicolor* [Gray Treefrog], and *B. houstonensis*) were unavailable. We substituted available cytb sequences of *Acris crepitans* (Eastern Cricket Frog) (n = 3) for *A. blanchardi*. A cytb sequence for *Xenopus laevis* (African Clawed Frog), our chosen monophyletic outgroup (Hay et

al. 1995; Gissi et al. 2006; Zhang et al. 2013), was likewise downloaded from GenBank. All sequences were aligned with the larva cytb sequence using the Geneious alignment tool v.8.1.9 (Biomatters Ltd, 2013) with 65% similarity. The resulting alignment was then manually trimmed to eliminate hanging ends. Nucleotide substitution models were evaluated with Bayesian information criteria (BIC) using MEGA X v.10.2.6 (Kumar et al. 2018). The model with the lowest BIC score was Hasegawa-Kishino-Yano (Hasegawa et al. 1985) with a proportion of 0.58 invariant sites. We conducted maximum likelihood phylogenetic analysis using MEGA X v.10.2.6 with the aforementioned model parameters. Bootstrap values were estimated from a heuristic search for 10,000 maximum likelihood iterations and the resulting tree was rooted with the *X. laevis* sequence data.

Our phylogenetic analysis placed the larval sequence as sister to the reference *R. sphenoccephala* sequence acquired through GenBank with 100% bootstrap support (Fig. 2). This clade was placed sister to another clade containing all *R. catesbeiana* sequences (Fig. 2). Together, the results of our BLAST search and phylogenetic analysis provide strong evidence that the scavenging larvae were *R. sphenoccephala*.

The consumption of carrion on behalf of *R. sphenoccephala* larvae suggests that the larval diet of this taxon is broader than previously described, reaching beyond the confines of

strict algivory (Tipton et al. 2012; Dodd 2013). Anuran larvae, in general, are often characterized as microphagous, feeding on small organisms such as algae, bacteria, and protozoans (Stebbins and Cohen 1995). However, a growing body of literature demonstrates that anuran larvae from a diversity of families will consume carrion present in aquatic environments (Smith 2005; Street et al. 2013; Alvarez et al. 2021). Continued research is needed to better understand the breadth and composition of larval anuran diets, as well as the frequency at which carnivory and facultative scavenging occur. Continually reporting otherwise undescribed scavenger-carrion relationships such as this is necessary for complete food web characterization and enriches our collective understanding of nutrient cycling within relevant communities (Beasley et al. 2019).

Acknowledgments

We thank Dr. Andrew R. MacLaren and Dr. Devin B. Preston for their thoughtful and meticulous review of an earlier version of this manuscript. All work was authorized by a Texas Parks and Wildlife Department Scientific Permit for Research (SPR-0102-191) and a United States Fish and Wildlife Service Permit (TE039544-0) both issued to Michael R.J. Forstner. This research was approved by the Texas State University Institutional Animal Care and Use Committee

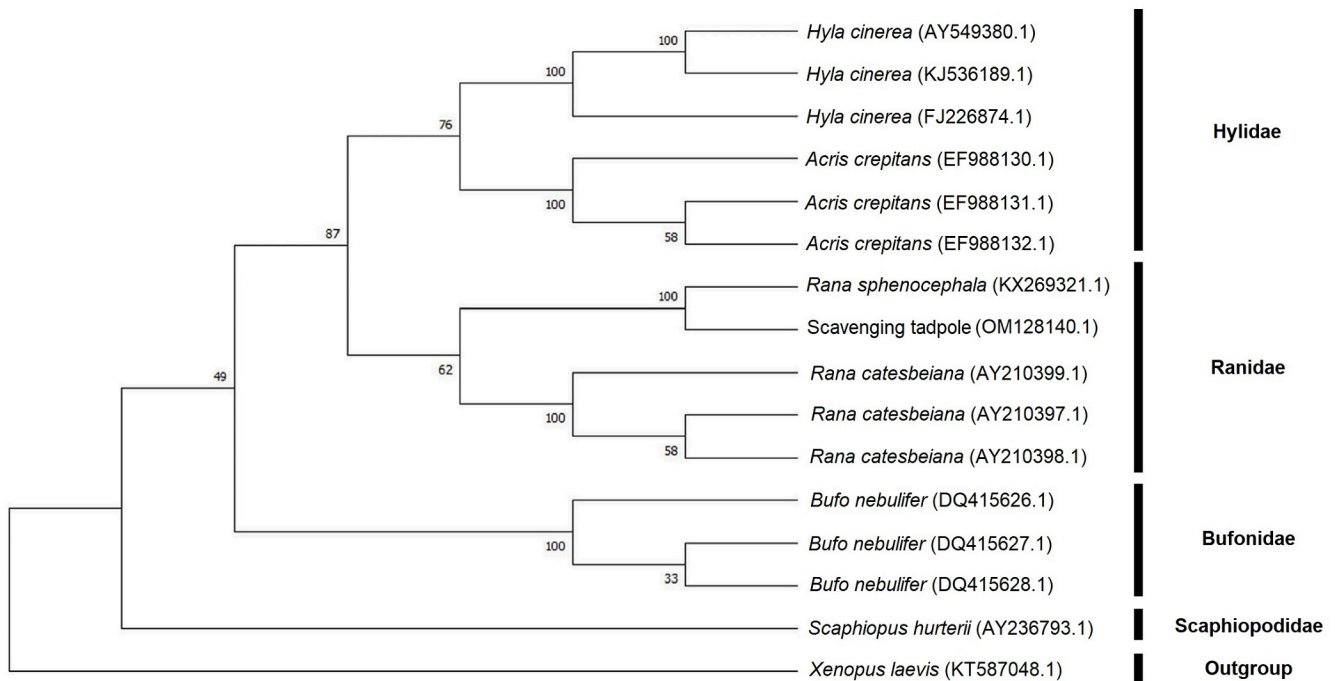


Fig. 2. Cladogram of indirect-developing anurans known to inhabit the Griffith League Ranch in Bastrop County, Texas, USA, including the scavenging tadpole. Eastern Cricket Frog (*Acris crepitans*) sequences were used in lieu of Blanchard’s Cricket Frog (*Acris blanchardi*) sequences due to an absence of the latter on GenBank. GenBank accession numbers are provided in parentheses adjacent to each taxonomic unit. Cladogram produced from a bootstrapped maximum likelihood analysis (replication = 10,000) of the mitochondrial cytochrome b gene. Analysis conducted in MEGA X v.10.2.6 using the Hasegawa-Kishino-Yano model of nucleotide evolution. African Clawed Frog (*Xenopus laevis*) was designated as a monophyletic outgroup. The number adjacent to each node indicates the percentage of bootstrap replicates which generated a tree containing that node. Note that the tadpole scavenger forms a monophyletic clade with the reference Southern Leopard Frog (*Rana sphenoccephala* [= *Lithobates sphenoccephalus*]) sequence.

(Protocol No. IACUC 7993). The Capitol Area Council of the Boy Scouts of America provided us with access to their private property where we made this observation, and we appreciate their continuing support of our research and their stewardship of the Houston Toad.

Literature Cited

- Alvarez, J.A., J.T. Wilcox, and J. Jelincic. 2021. Cannibalism and scavenging by *Hyliola regilla* Baird and Girard, 1852 tadpoles in California. *Herpetology Notes* 14: 1163–1165.
- Babbitt, K.J. 2001. Behaviour and growth of southern leopard frog (*Rana sphenoccephala*) tadpoles: effects of food and predation risk. *Canadian Journal of Zoology* 79: 809–814. <https://doi.org/10.1139/z01-040>.
- Beasley, J.C., Z.H. Olson, N. Selva, and T.L. DeVault. 2019. Ecological functions of vertebrate scavenging, pp. 125–157. In: P.P. Olea, P. Mateo-Tomás, and J.A. Sánchez-Zapata (eds.), *Carion Ecology and Management*. Springer Nature, Cham, Switzerland.
- Brown, D.J., T.M. Swannack, J.R. Dixon, and M.R.J. Forstner. 2011. Herpetofaunal survey of the Griffith League Ranch in the Lost Pines Ecoregion of Texas. *Texas Journal of Science* 63: 101–112.
- Crother, B.I. 2017. Scientific and standard English names of amphibians and reptiles of North America north of Mexico, with comments regarding confidence in our understanding. *SSAR Herpetological Circular* 43: 1–102.
- Dixon, J.R. 2013. *Amphibians and Reptiles of Texas: with Keys, Taxonomic Synopses, Bibliography, and Distribution Maps*. Texas A&M University Press, College Station, Texas, USA.
- Dodd, C.K. 2013. *Frogs of the United States and Canada*. The Johns Hopkins University Press, Baltimore, Maryland, USA.
- Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with accuracy and high throughput. *Nucleic Acids Research* 32: 1792–1797. <https://doi.org/10.1093/nar/gkh340>.
- Gissi, C., D.S. Mauro, G. Pesole, and R. Zardoya. 2006. Mitochondrial phylogeny of Anura (Amphibia): A case study of congruent phylogenetic reconstruction using amino acid and nucleotide characters. *Gene* 366: 228–237. <https://doi.org/10.1016/j.gene.2005.07.034>.
- Gomo, G., L. Rød-Eriksen, H.P. Andreassen, J. Mattisson, M. Odden, O. Devineau, and N.E. Eide. 2020. Scavenger community structure along an environmental gradient from boreal forest to alpine tundra in Scandinavia. *Ecology and Evolution* 10: 12860–12869. <https://doi.org/10.1002/ece3.6834>.
- Hasegawa, M., H. Kishino, and T. Yano. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* 22: 160–174. <https://doi.org/10.1007/BF02101694>.
- Hay, J.M., I. Ruvinsky, S.B. Hedges, and L.R. Maxson. 1995. Phylogenetic relationships of amphibian families inferred from DNA sequences of mitochondrial 12S and 16S ribosomal RNA genes. *Molecular Biology and Evolution* 12: 928–937. <https://doi.org/10.1093/oxfordjournals.molbev.a040270>.
- Kumar, S., G. Stecher, M. Li, C. Knyaz, and K. Tamura. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35: 1547–1549. <https://doi.org/10.1093/molbev/msy096>.
- Meece, J.K., C.E. Reynolds, P.J. Stockwell, T.A. Jenson, J.E. Christensen, and K.D. Reed. 2005. Identification of mosquito bloodmeal source by terminal restriction fragment length polymorphism profile analysis of the cytochrome b gene. *Journal of Medical Entomology* 42: 657–667. <https://doi.org/10.1093/jmedent/42.4.657>.
- Pauly, G.B., D.M. Hillis, and D.C. Cannatella. 2009. Taxonomic freedom and the role of official lists of species names. *Herpetologica* 65: 15–128. <https://doi.org/10.1655/08-031R1.1>.
- Powell, R., R. Conant, and J.T. Collins. 2016. *Peterson Field Guide to Reptiles and Amphibians of Eastern and Central North America*. Houghton Mifflin Harcourt, Boston, Massachusetts, USA.
- Schaaf, R.T. 1970. Raccoon predation on the American toad, *Bufo americanus*. *Herpetologica* 26: 334–335.
- Selva, N. and M.A. Fortuna. 2007. The nested structure of a scavenger community. *Proceedings of the Royal Society B* 274: 1101–1108. <https://doi.org/10.1098/rspb.2006.0232>.
- Smith, K.G. 2005. Effects of nonindigenous tadpoles on native tadpoles in Florida: evidence of competition. *Biological Conservation* 123: 433–441. <https://doi.org/10.1016/j.biocon.2005.01.005>.
- Sparling, D.W., S. Krest, and M. Ortiz-Santaliestra. 2006. Effects of lead-contaminated sediment on *Rana sphenoccephala* tadpoles. *Archives of Environmental Contamination and Toxicology* 51: 458–466. <https://doi.org/10.1007/s00244-005-0243-0>.
- Stebbins, R.C. and N.W. Cohen. 1995. *A Natural History of Amphibians*. Princeton University Press, Princeton, New Jersey, USA.
- Street, K.B., J.S. Parmerlee, and R. Powell. 2013. Adventitious scavenging by Cuban treefrog tadpoles, *Osteopilus septentrionalis* (Anura: Hylidae). *Herpetology Notes* 6: 33–34.
- Tipton, B.L., T.L. Hibbitts, T.D. Hibbitts, T.J. Hibbitts, and T.J. LaDuc. 2012. *Texas Amphibians: A Field Guide*. University of Texas Press, Austin, Texas, USA.
- Trivedi, K., B. Nadolski, and P. Suwanwaree. 2018. Opportunistic scavenging by larval Spot-legged Treefrogs, *Polypedates megacephalus* (Anura: Rhacophoridae). *Reptiles & Amphibians* 25: 156–157. <https://doi.org/10.17161/randa.v25i2.14284>.
- Vazifdar, N., M.A. Khalid, and M. D'Costa. 2021. Larval Indian Bullfrogs (*Hoplobatrachus tigerinus*) scavenging on an Indian Tiger Centipede (*Scolopendra hardwickei*). *Reptiles & Amphibians* 28: 339–340. <https://doi.org/10.17161/randa.v28i2.15561>.
- Wilmers, C.C., D.R. Stahler, R.L. Crabtree, D.W. Smith, and W.M. Getz. 2003. Resource dispersion and consumer dominance: scavenging at wolf- and hunter-killed carcasses in Greater Yellowstone, USA. *Ecology Letters* 6: 996–1003. <https://doi.org/10.1046/j.1461-0248.2003.00522.x>.
- Wilson, E.E. and E.M. Wolkovich. 2011. Scavenging: how carnivores and carrion structure communities. *Trends in Ecology and Evolution* 26: 129–135. <https://doi.org/10.1016/j.tree.2010.12.011>.
- Yuan, Z.-Y., W.-W. Zhou, X. Chen, N.A. Poyarkov, H.-M. Chen, N.-H. Jang-Liaw, W.-H. Chou, N.J. Matzke, K. Iizuka, M.-S. Min, S.L. Kuzmin, Y.-P. Zhang, D.C. Cannatella, D.M. Hillis, and J. Che. 2016. Spatiotemporal diversification of the true frogs (genus *Rana*): a historical framework for a widely studied group of model organisms. *Systematic Biology* 65: 824–842. <https://doi.org/10.1093/sysbio/syw055>.
- Zhang, P., D. Liang, R.-L. Mao, D.M. Hillis, D.B. Wake, and D.C. Cannatella. 2013. Efficient sequencing of anuran mtDNAs and a mitogenomic exploration of the phylogeny and evolution of frogs. *Molecular Biology and Evolution* 30: 1899–1915. <https://doi.org/10.1093/molbev/mst091>.