

*Lithobates catesbeianus*, *L. clamitans*, and *Pseudacris crucifer*) simultaneously present at the site.

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**ANAXYRUS MICROSCAPHUS (Arizona Toad). DIET, VERTEBRATE PREY.** *Anaxyrus microscaphus* occurs along riparian habitats from 365–2700 m elevation in Arizona, Nevada, New Mexico, and Utah, USA (Blair 1955. *Am. Mus. Novit.* 1722:1–37; Degenhardt et al. 1996. *Amphibians and Reptiles of New Mexico*. University of New Mexico Press, Albuquerque, New Mexico. 431 pp.). Little is known about its diet or type of prey consumed (e.g., Schwaner and Sullivan 2005. *In* Lannoo [ed.], *Amphibian Declines: The Conservation Status of United States Species*, pp. 422–424. University of California, Berkeley, California). Previous descriptions of prey for *A. microscaphus* are limited to the stomach contents of five individuals from Utah that included sand crickets, beetles, true bugs, moth larvae, and ants (Tanner 1931. *Trans. Utah Acad. Sci.* 8:159–198). We report two vertebrate prey items from the stomachs of two individuals from New Mexico. The stomach contents were discovered in a review of specimens at the Museum of Southwestern Biology at the University of New Mexico. The discovery of these novel prey from museum specimens highlights the value of museums in elucidating the diet composition of little-known species.

We found the forearm and hand of a lizard in the stomach of an adult female (SVL = 78.1 mm) *A. microscaphus* collected on 18 July 1970 (MSB 23147) from near the Gila Cliff Dwellings, Grant Co., New Mexico, USA (33.1760°N, 108.2050°W, WGS 84; 1689 m elev.). We determined the forearm and hand to be that of an *Aspidoscelis* sp. based on scutellation (enlarged postantibrachial scales) and shape of the fingers, but we cannot determine the species of the lizard. The forearm measured 22 mm in length, and there were no other discernible fragments in the stomach or gut. Because *A. microscaphus* is not known to forage during the day when *Aspidoscelis* species are active (Degenhardt et al. 1996, *op. cit.*), this was unexpected.

We found skin and small bone fragments in an adult male (SVL = 70.1 mm) *A. microscaphus* collected on 4 April 2013 (MSB 94983) from Hells Hole, Catron Co., New Mexico, USA (33.7875°N, 108.6941°W, WGS 84; 1915 m elev.). The male was collected during the breeding season along the Tularosa River at night. We determined that the skin was from a toad species based on dorsal texture, presence of tubercles, spotting, and pigmentation. The skin was approximately 1 mm thick and possessed small and large dark pigmented brown spots and splotches, consistent with *A. microscaphus*. One other toad species, *A. punctatus*, occurs in the area but we ruled this species out as a prey item. The fragment of skin had few widely spaced dorsal warts opposed to the numerous and densely spaced warts characteristic of *A. punctatus*, suggesting the skin was from *A. microscaphus*. In addition, in three years of sampling at Hells Hole (2013–2015) we have not observed *A. punctatus* in April and the species is reported to breed in mid- to late May in New Mexico (Degenhardt et al. 1996, *op. cit.*). Therefore, we infer the skin fragments are from *A. microscaphus*, suggesting an instance of cannibalism. The presence of small bone fragments and the small size of the outer-metatarsal fragment suggest this was from the consumption of a smaller conspecific and not dermatophagy. Cannibalism is relatively common in other toad species (Crump 1992. *In* Elgar and Crespi [eds.] *Cannibalism: Ecology and Evolution in Diverse*

*Taxa*, pp. 256–276. Oxford University Press, Oxford; Pizzato and Shine 2008. *Behav. Ecol. Sociobiol.* 63:123–133), but has not been reported in *A. microscaphus*. These observations expand the known diet of this species to lizards and conspecifics.

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**ANAXYRUS MICROSCAPHUS (Arizona Toad). ECTOPARASITES.**

Although there is information on the helminth parasites of *A. microscaphus* (Goldberg et al. 1996. *Great Basin Nat.* 56:369–374), little is known about their arthropod parasites (Schwaner and Sullivan 2005. *In* Lannoo [ed.], *Amphibian Declines: The Conservation Status of United States Species*, pp. 422–424, University of California Press, Berkeley, California). One report (Duszynski and Jones 1973. *Int. J. Parasitol.* 3:531–538) of ectoparasites on *A. microscaphus* noted an infestation of *Hannemania* spp. from New Mexico, but the mites were not identified to species. Notably, the Arizona Toad is a New Mexico Department of Game and Fish Species of Greatest Conservation Need under the State's Comprehensive Wildlife Conservation Strategy. Here we report a new host and distributional record for a larval mite (chigger) from *A. microscaphus* in New Mexico.

Six adult *A. microscaphus* (mean  $\pm$  1SD SVL = 71.5  $\pm$  7.4 mm, range 59–79 mm) were collected by hand on 12 March 2015 at



FIG. 1. Adult *Anaxyrus microscaphus* showing red sores, encapsulated mites, (arrows) indicative of *Hannemania bufonis* infestation. The bolder arrow on the underside of the left foreleg shows an active infestation.

Indian Tank (N = 5), Catron Co. (33.4325°N, 108.0334°W, WGS 84; 2175 m elev.) and Black Canyon (N = 1), Sierra Co., New Mexico, USA (33.1856°N, 108.0326°W, WGS 84; 2045 m elev.). Toads were euthanized, fixed in 10% neutral buffered formalin, and their tissues later examined for ectoparasites. Red spheroidal lesions (chiggers were mostly encapsulated) measuring 0.5–1.5 mm were noted on the venter and legs of *A. microscaphus* (Fig. 1). Mites were excised with dissecting scissors and fine forceps, placed in 70% ethanol, cleared in lactophenol, slide-mounted in Hoyer's medium (Walter and Krantz 2009. *In* Krantz and Walter [eds.], *A Manual of Acarology*, pp. 83–96, Texas Tech University Press, Lubbock, Texas) and identified using appropriate guides (Loomis 1956. *Univ. Kansas Sci. Bull.* 37:1195–1443; Loomis and Welbourn 1969. *Bull. South. California Acad. Sci.* 68:161–169; Brennan and Goff 1977. *J. Parasitol.* 63:554–566). A voucher specimen was deposited in the General Ectoparasite Collection in the Department of Biology at Georgia Southern University (accession no. L3798). Two host voucher specimens were deposited in the Museum of Southwestern Biology (MSB) of the University of New Mexico, Albuquerque, New Mexico (MSB 96363, 96377). Four fixed specimens, not accessioned (field tags: JTG 370, MJR 626, MJR 632, MJR 633), from Indian Tank were shipped to the Florida Fish and Wildlife Conservation Commission's Fish and Wildlife Research Institute, St. Petersburg, Florida for necropsy and pathology assays. Specimens were dissected to obtain tissues for processing by routine paraffin embedded histology, and then sectioned slides were stained with hematoxylin and eosin (H&E) or thionin.

Three of six (50%) *A. microscaphus*, two from Indian Tank (MSB 96377, male, SVL = 63 mm, detected by slide mounted specimen; MJR 631, male, SVL = 68 mm, detected by histological specimen) and the one specimen from Black Canyon (MSB 96363, male, SVL = 65 mm, detected by slide mounted specimen), were found to be infested with larval mites that fit the description of *Hannemania bufonis* (Loomis and Welbourn) in having one genuala II and III and lacking femoralae (traits also shared by *Hannemania hylae* [Ewing]), having parasubterminala I branched, tarsala I longer and narrower than tarsala II, and the unique shape of the scutum (Loomis and Welbourn 1969, *op. cit.*). No gene sequences of *Hannemania* spp. are available for molecular species determinations. Histologically, the parasite was found in the left side of the thoracic skin of two specimens, MJR 631 and MJR 633 (Fig. 2, MJR 631), and in the pectoral skin (180 × 340; 184 × 284; 224 × 332 μm; N = 3 per sectioned slide). Associated congestion, hemorrhage, and leucocytic infiltrates surrounded the larval mites, causing host inflammation (Figs. 2a, b). The organism was separated from the host by a capsule (Figs. 2c, d) and possessed mouth-parts, possibly chelicerae (seen in section) presumably composed of chitin-like structures (Figs. 2e, f). In the left anterior leg of the toad, severe congestion and hemorrhaging as well as leucocytic infiltrates surrounded the parasites (N = 1 per

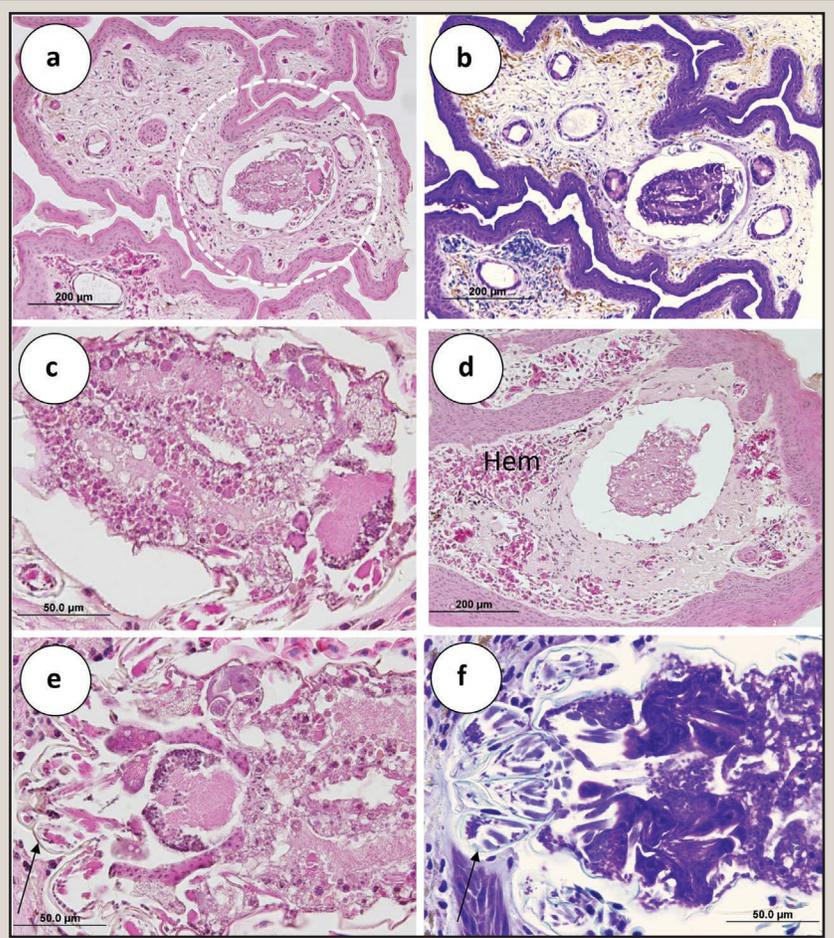


FIG. 2. Histology of *Anaxyrus microscaphus* (MJR 631) exhibiting skin lesion from Indian Tank. a) Skin tissue from thoracic area infested with a mite and (circled) associated host inflammatory response (H&E). b) Same sectioned view of (a) stained with thionin. c) Enlarged view of (a) (H&E). d) Host inflammatory response as hemorrhage (hem) and leucocytic infiltrates surrounding the mites at the left anterior leg (H&E). e) Enlarged view of larval mite mouthparts, possibly chelicerae (arrow) from pectoral skin area (H&E). f) Same sectioned view of (e) stained with thionin.

sectioned slide; 185 × 250 μm; Fig. 2d). No skin tissue from the left side of the pelvic skin was sectioned. However, capillaries at the myosepta in the skeletal muscle tissue were congested. The second histologic specimen (MJR 633) also revealed mites (460 × 730 μm; N = 1 per sectioned slide) in the left side of the pelvic skin dermis, and was infiltrated with leucocytes (e.g. fibrocytes). Parasites were found in the left side of the abdominal skin (256 × 470 μm; N = 1 per sectioned slide) and again, were associated with congestion. No mites were detected on the third histological specimen (MJR 626), but the capillaries of the thoracic skin and pelvic skin dermis were congested, suggesting parasitism elsewhere and not in the skin section assayed.

*Hannemania bufonis* has been previously reported only from *A. punctatus* (Red-spotted Toad) from western North America in Arizona, California, western Texas, southern Utah and Sonora, México (Loomis and Welbourn 1969, *op. cit.*; Walters et al. 2011. *Fac. Publ. Harold W. Manter Lab. Parasitol.* 697:1–183). Unlike some other *Hannemania* species (Loomis 1956, *op. cit.*; Walters et al. 2011, *op. cit.*), *H. bufonis* appears to be fairly host-specific and has only been reported from *A. punctatus*, *A. mazatlanensis* (Sinaloa Toad; Goldberg et al. 2002. *Herpetol. Rev.* 33:301–302),

and *A. microscephus* (this study). The histological findings of *Hannemania* spp. infesting anurans in New Mexico (Duszynski and Jones 1973, *op. cit.*; Grover et al. 1975. *J. Parasitol.* 61:382–384) resemble our findings in morphology, encapsulation, and the associated host cellular response (i.e. infiltration of fibroblasts). The geographic location also coincides with Duszynski and Jones (1973, *op. cit.*) in that the anuran samples infested with chiggers were also collected from Sierra Co., New Mexico. Duszynski and Jones (1973, *op. cit.*) also suggested that prevalence of the infestation positively correlates with altitude. They found a high prevalence of mite infestation in *Hyla arenicolor* at 1829–2743 m above sea level, similar to the elevation in our cases (2175–2181 m). We document a new host record as well as the first report of *H. bufonis* from New Mexico.

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**COLOSTETHUS FRATERDANIELI** (Santa Rita Rocket Frog, *Rana Cohete*). **ANUROPHAGY.** *Colostethus fraterdanieli* is endemic to Colombia, distributed along the western flank of the Cordillera Central and along both the western and eastern flank of the Cordillera Occidental from Antioquia to Nariño, at elevations between 1000 and 2500 m (Silverstone 1971. *Los Angeles Co. Mus. Contrib. Sci.* 215:1–8; Grant and Castro 1998. *J. Herpetol.* 32:378–392; Sánchez et al. 2010. *Phyllomedusa* 9:133–139). *Colostethus fraterdanieli* is a leaf litter dwelling species (Grant and Castro 1998, *op. cit.*) that preys primarily on arthropods, like other members of the genus (Hoyos-Hoyos et al. 2012. *S. Am. J. Herpetol.* 7:25–34; Blanco-Torres et al. 2013. *Herpetol. Rev.* 44:493–494; Blanco-Torres et al. 2014. *Herpetol. Rev.* 45:476). Herein we present the first records of anurophagy in *C. fraterdanieli* adults from both Ecoparque Los Alcazares Arenillo (5.06508°N, 75.5329°W, WGS 84; 1893 m elev.) and Ecoparque Recinto del Pensamiento (5.0393°N, 75.4465°W, WGS 84; 2154 m elev.), Manizales, Caldas, Colombia.

We stomach-flushed 57 individuals *C. fraterdanieli* from 19–27 January 2016, between 1000 and 1400 h, in leaf litter. Of the 57 individuals examined, four (7%) contained anuran prey items besides arthropods. Three individuals from Ecoparque Recinto del Pensamiento, two females (mean SVL = 25.5 mm, range =



FIG. 1. Anuran prey items in the stomachs of adult *Colostethus fraterdanieli*. A) Eggs of *Pristimantis* sp.; B) Female of *C. fraterdanieli* swallowing a juvenile *Pristimantis achatinus*; C) Juvenile *P. achatinus* consumed by *C. fraterdanieli* female.

25–26 mm) and one male (SVL = 26 mm) consumed eggs (N = 28, range = 2–3 mm; Fig. 1A) of an undetermined *Pristimantis* species. One female (SVL = 29 mm) from Ecoparque Los Alcazares Arenillo contained a juvenile of *P. achatinus* (SVL = 11 mm), which was ingested headfirst (Fig. 1B–C).

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**HYLA CHRYSOSCELIS** (Cope's Gray Treefrog), *H. AVIVOCA* (Bird-voiced Treefrog), and *H. CINEREA* (Green Treefrog). **EGG AND TADPOLE MORTALITY.** Three examples are given that show the consumption or other loss of frog eggs and small tadpoles by either ciliated protozoans or an alga. An egg film of *Hyla chrysoscelis* was collected (14 April 2015; 8 km SW Starkville, Oktibbeha Co. Mississippi, USA) by letting it flow into a container tipped slightly below the water surface. Numerous ciliate protozoans were noticed in the culture container, and the population increased rapidly in the lab for the next 3 days. When a tadpole at Gosner stage 25 died during a photographic session, numerous large protozoans (Ciliophora: *Tetrahymena* sp., identified by Eleni Gentekaki, Mae Fah Luang University, Thailand) consumed the entire tadpole except the skin within about 12 h, and this kind of event was observed several times. The ciliates did not interact with living tadpoles or with tadpoles infected with an oomycete water mold. These ciliates have a cytostome with large cilia at the opening but no biting apparatus, so they apparently consume particles as they are sloughed from the carcass. These large protozoans were also associated with egg films of *Gastrophryne carolinensis* but not with the submerged egg strings of *Anaxyrus fowleri* in the same pool at the same time or with degrading