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No Evidence of Hybridization Between the Arizona Toad (*Anaxyrus microscaphus*) and Woodhouse's Toad (*A. woodhousii*) in New Mexico, USA

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Abstract.—**Hybridization, facilitated by habitat modification, poses a significant conservation risk to many amphibian species. The construction of water impoundments, which alter stream conditions, has facilitated the spread of Woodhouse's Toads (*Anaxyrus woodhousii*) and resulted in hybridization with Arizona Toads (*A. microscaphus*) in Arizona, Nevada, and Utah, USA. To date, there has been no evaluation or reported occurrence of hybridization between *A. microscaphus* and *A. woodhousii* in New Mexico. In New Mexico, the range of *A. microscaphus* encompasses an area of approximately 9,585 km^2, and extends primarily in the upper Gila River and upper Mimbres River basins. We conducted a morphological comparison using hybrid index scores of specimens collected over a 107-y period to determine whether hybridization has occurred and if it represents a threat to *A. microscaphus* in New Mexico. We found no evidence of hybridization between *A. microscaphus* and *A. woodhousii* in New Mexico; however, we uncovered numerous misidentified specimens. This finding resulted in a significant revision of the distribution of *A. woodhousii*, reducing the number of previously recognized areas of syntopy of the two species from 20 to two. We speculate that the construction of a proposed water diversion project along the Gila River in the Gila-Cliff Valley, one area where the two species occur within 2 km of each other, may facilitate the spread of *A. woodhousii*, increasing the likelihood of contact and hybridization with *A. microscaphus*. This research highlights the importance of museum specimens in assessment of conservation risks and validation of identifications to assess species distributions accurately.**

Key Words.—amphibians; conservation; Mogollon Rim; museum specimens

Introduction

The anthropogenic breakdown of ecological and habitat barriers can often result in contact and hybridization between species that were once allopatric (Rhymer and Simberloff 1996; Allendorf et al. 2001). The synergistic effects of habitat disturbance and hybridization may be especially acute when the parent species is rare or an ecological specialist and the invader is disturbance tolerant (Vogel and Johnson 2008; Walters et al. 2008; Schwaner and Sullivan 2009). Outcomes of hybridization are complex and can have negative or positive consequences in the resulting hybrids (Coyne and Orr 2004). Positive hybridization outcomes include instances where hybrids can exploit resources available to both parent species (Pfennig et al. 2007), have increased survival during droughts (Lamichhaney et al. 2016), or be less vulnerable to parasites (Goldberg et al. 1996). Negative outcomes to parent species include lower fitness of hybrids (Coyne and Orr 2004) and population declines, range reduction, and loss of native genotypes and genetic diversity of the parent species (Mooney et al. 2001; Ryan et al. 2009; Jensen et al. 2014; Todesco et al. 2016). In these ways, hybridization can increase conservation risk to pure lineage parent species in contact zones (Walters et al. 2008; Milko 2012). Hybridization further presents complex and unique policy and management challenges because the U.S. Endangered Species Act and state laws do not protect hybrid species (Fitzpatrick et al. 2015; vonHoldt et al. 2016). For species that are endangered, threatened, or otherwise at risk, understanding the extent and location of zones of hybridization is crucial to implementing effective conservation and management actions.

North American toads of the genera *Anaxyrus* and *Incilius* (*Bufo* by some authors) are especially vulnerable to congeneric hybridization facilitated by habitat alterations that benefit disturbance-tolerant, generalist species over disturbance-intolerant parent species (Hillis et al. 1984; Gergus et al. 1999; Sullivan 1995). The Arizona Toad, *Anaxyrus microscaphus*, is one such species at risk from the combination of habitat

Figure 1. Calling male (top) and amplectant pair (middle) of Arizona Toads (*Anaxyrus microscaphus*) from Little Creek in Catron County, New Mexico, USA, and breeding habitat along the West Fork of the Gila River near the Gila Cliff Dwellings, Catron County (bottom). (Photographed by Mason Ryan).

modification and hybridization (Sullivan 1986; Sullivan 1993) because it requires low gradient, clear, shallow rivers and streams for breeding (Fig. 1) in Arizona, New Mexico, Nevada, and Utah, USA (Dodd 2013). The disturbance tolerant Woodhouse's Toad, *Anaxyrus woodhousii*, breeds in a wide variety of natural and human made water bodies (e.g., cattle tanks, golf course ponds, drainage ditches, and reservoirs; Fig. 2). The adaptability of *A. woodhousii* and its ability to disperse into newly disturbed habitats has enabled the species to come into contact and hybridize with at least

Figure 2. Calling male Woodhouse's Toad (*Anaxyrus woodhousii*) at a cattle tank (top) and cattle tank habitat (bottom) from eastern Grant County, New Mexico, USA. (Photographed by Mason Ryan).

eight other bufonid species: *Anaxyrus microscaphus*, American Toads (*A. americanus*), Great Plains Toads (*A. cognatus*), Fowler's Toads (*A. fowleri*), Houston Toads (*A. houstonensis*), Red-spotted Toads (*A. punctatus*), Sonoran Desert Toads (*Incilius alvarius*), and Gulf Coast Toads (*I. nebulifer*); often posing a conservation risk to these species (Hillis et al. 1984; Gergus et al. 1999; Sullivan and Lamb 1988; Lannoo 2005). In the case of *A. microscaphus*, contact with *A. woodhousii* often occurs in modified riparian habitats, and hybridization and introgression is unidirectional with female *A. woodhousii* mating with male *A. microscaphus* (Malmos et al. 2001). The resulting hybrids are fertile and, over decades, hybrids with *A. woodhousii* alleles replace and dominate *A. microscaphus* alleles in populations within contact zones (Schwaner and Sullivan 2009; Sullivan et al. 2015). This introgression has led to population declines of *A. microscaphus* in Arizona and Utah following dam construction, which facilitated the spread of *A. woodhousii* (Blair 1955; Sullivan and Lamb 1988; Sullivan et al. 2015).

Although there are several investigations on the extent and conservation risk of hybridization between *A. microscaphus* and *A. woodhousii* in Arizona and

Table 1. Data sets used to identify syntopic localities of the Arizona Toad (*Anaxyrus microscaphus*) and the Woodhouse's Toad (*Anaxyrus woodhousii*) within the upper Gila River Basin, New Mexico. The data sets are from the Global Biodiversity Information Facility. 2014. http://api.gbif.org/v1/occurrence/download/request/0000204-140325112802170.zip. We accessed 378 records on 29 March 2014 from the seven datasets.

Data Sets
5df38344-b821-49c2-8174-cf0f29f4df0d
7a25f7aa-03fb-4322-aaeb-66719e1a9527
9587f32a-f762-11e1-a439-00145eb45e9a
bd2feca8-ec39-4480-9dad-e353ab6a506d
cece4fc2-1fec-4bb5-a335-7252548e3f0b
76dd8f0d-2daa-4a69-9fcd-55e04230334a
8483c6a8-f762-11e1-a439-00145eb45e9a

Utah (Schwaner and Sullivan 2009; Sullivan et al. 2015), there have been no investigations on occurrence and extent of hybridization in New Mexico. In New Mexico, *A. microscaphus* is restricted to the relatively unaltered upper Gila River Basin, including the Gila and San Francisco rivers (Propst et al. 2008), the upper Mimbres River Basin, and a disjunct population in the Rio Grande Basin east of the continental divide in the San Mateo Mountains (Degenhardt et al. 1996; Jennings et al. 2010). *Anaxyrus microscaphus* and *A. woodhousii* have been reported to be syntopic at 20 localities within the upper Gila River Basin (Degenhardt et al. 1996; Jennings et al. 2010). Because of declining population trends of *A. microscaphus*, and threats to this species from hybridization and habitat modification in neighboring states, *A. microscaphus* is currently listed as a Species of Greatest Conservation Need (SGCN) in New Mexico (New Mexico Department of Game and Fish 2006). However, the SGCN status of *A. microscaphus* was assigned without an assessment of hybridization and it is not known how pervasive hybridization is in New Mexico, or if it is the cause of population declines (Charlie Painter, pers. comm.). Future conservation actions and management of *A. microscaphus* in New Mexico will be best informed with an accurate evaluation of the distribution of pure and hybrid populations (e.g., Ervin et al. 2013).

Motivated by the lack of quantitative information from New Mexico, we reviewed museum specimens to evaluate the extent of hybridization between *A. microscaphus* and *A. woodhousii* in the eastern Mogollon Rim of New Mexico. Conservation of species at risk from hybridization requires delimiting the geographic extent of hybrid zones using robust and reliable methods (Allendorf et al. 2001; Allendorf et al. 2013). To assess the present extent of hybridization we: 1) reviewed specimens of both species from the eastern Mogollon Rim to verify specimen identification; 2) identified morphological intermediates that would indicate the presence of hybrids using the morphological hybrid index of Blair (1955) and Sullivan (1986), which has been used to document hybridization between these species in Arizona, Utah, and Nevada (Schwaner and Sullivan 2009; Sullivan et al. 2015; Bradford et al. 2005); and 3) identified locations of hybrid contact zones. This review is timely considering the proposed diversion project for the upper Gila River in New Mexico (Gori et al. 2014a; Wiseman et al. 2016), which could further facilitate and/or increase hybridization between the two species in New Mexico. Furthermore, in July 2015 the U.S. Fish and Wildlife Service (USFWS) issued a 90-day Notice of Petition Findings and Initiation of Status Reviews for *A. microscaphus* to receive protection under the Endangered Species Act (U.S. Fish and Wildlife Service 2015). As of this writing (July 2017), no decision has been issued by the USFWS 90-day petition.

Materials and Methods

We first reviewed 641 *A. microscaphus* and 176 *A. woodhousii* adult, juvenile (≤ 45 mm snout-vent length; SVL), and tadpole lots of specimens collected from the upper Gila River and upper Mimbres River basins in New Mexico between 1908 and 2015 to verify the accuracy of species identification. We used the identification keys of Degenhardt et al. (1996), Powell et al. (2012), and Altig and McDiarmid (2015) to verify identification of tadpole, juvenile, and adult specimens. We used georeferenced museum locality records from seven online museum datasets (Table 1) to identify specific areas of syntopy (i.e., from the same collection locality), and areas of potential syntopy, which we define as species collection records within 2 km of each other. We chose 2 km as a cut-off based on the best available information movement data for both species; there is scant information on the movement distances of *A. microscaphus*, but the closely related A. californicus has been reported to move 1 km along streams (e.g., Dodd 2013) and *A. woodhousii* has been reported to move 1.9 km (King 1960). We used these data on movement to estimate the distance toads can potentially come into contact at what we consider syntopic locations. We obtained the specimens from the Museum of Southwestern Biology at The University of New Mexico (MSB), Western New Mexico University's Gila Center for Natural History (WNMU), New Mexico State University's Center for Natural History Collections (NMSU), Carnegie Museum of Natural History (CM), and Yale University's Peabody Museum (YPM).

Next, we used the four-character hybrid index method of Blair (1955) and Sullivan (1986) to identify morphological hybrids between the two species. We scored only adult specimens (i.e., > 45 mm SVL)

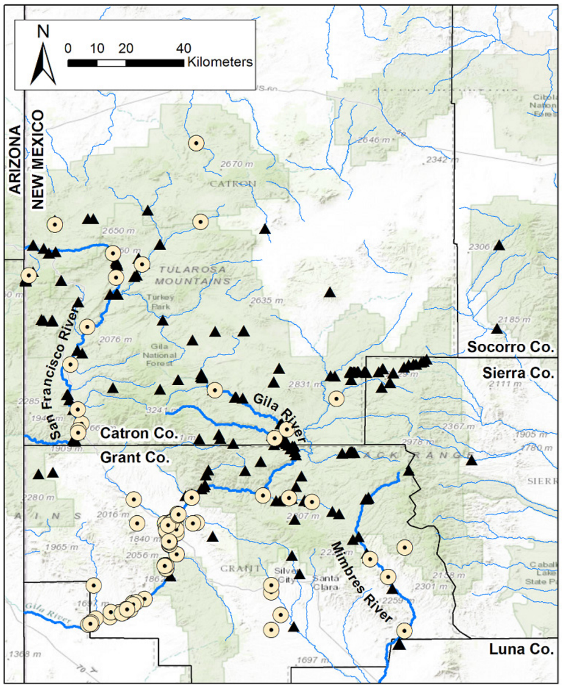

Figure 3. Distribution of the Arizona Toad (*Anaxyrus microscaphus*; black triangles) and the Woodhouse's Toad (*Anaxyrus woodhousii*; sand circles) in upper Gila River Basin (including Gila and San Francisco rivers) and upper Mimbres River Basin, New Mexico, based on museum specimens and before identification revisions in this study. Bold blue lines indicate major rivers (identified) and minor blue lines represent tributaries. Areas in green indicate U.S. Forest Service managed lands. Map sources used were from Esri, Inc. (Redlands, California, USA).

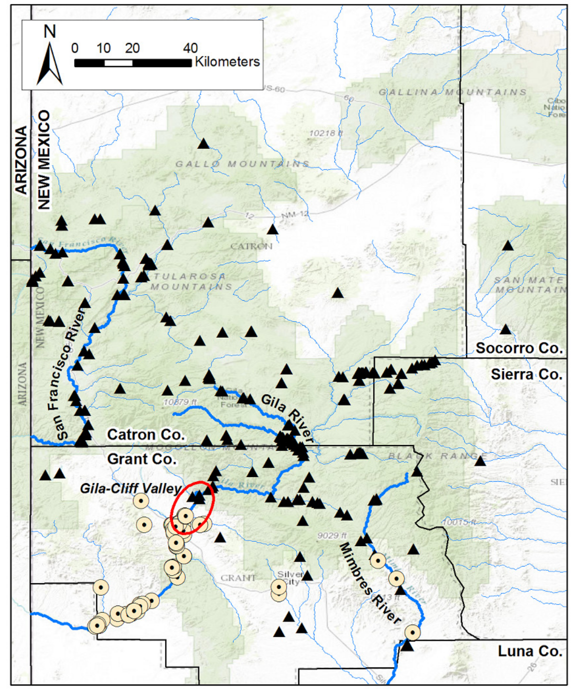

Figure 4. Revised distribution after identification revisions of the Arizona Toad (*Anaxyrus microscaphus*; black triangles) and the Woodhouse's Toad (*Anaxyrus woodhousii*; sand circles). The red circle identifies the general location of the proposed water diversion project and a potential area of syntopy in the Gila-Cliff Valley. Areas in green are U.S. Forest Service managed lands. Map sources used were from Esri, Inc. (Redlands, California, USA). major rivers (identified) and minor blue lines represent tributaries. Areas in green indicate U.S. Forest Service managed lands. Map sources used were from Esri, Inc. (Redlands, California, USA).

to determine the presence of hybrids from museum collections (*A. microscaphus*: n = 202; *A. woodhousii*: n = 83), and avoided examining juvenile toads because of trait ambiguities associated with ontogenetic changes (Sullivan 1986). We recorded the presence of ventral spotting, cranial crests, mid-dorsal stripe, and pale bar across the eyelids using ordinal values: P = present, W = weakly present, VW = very weakly present, A = absent. We converted these values to numerical scores following Sullivan (1986) and Sullivan and Lamb (1988): we scored ventral spotting, mid-dorsal stripe, and cranial crest as: P = 3, W = 2, VW = 1, A = 0; whereas we scored pale bar across the eyelids conversely: P = 0, W = 1, VW = 2, A = 3. We then summed numerical scores to calculate a hybrid index from 0 to 12, with low scores representing *A. microscaphus*, high scores representing *A. woodhousii*, and intermediate scores representing hybrids (Blair 1955; Sullivan 1986). We used logistic regression ($\alpha = 0.05$) to compare hybrid index scores between *A. microscaphus* and *A. woodhousii* with all specimens pooled together; and then repeated the logistic regression for Grant and Sierra counties where the two species co-occur. We did not repeat this analysis for Catron County due to previous errors with species identifications.

Results

We found that all previously identified *A. woodhousii* specimens from 17 localities within the upper Gila River and San Francisco River watersheds in Catron County (n = 2 adults, n = 56 juveniles, n = 3 tadpole lots) were misidentified and should be assigned to *A. microscaphus* (Table 2). This finding substantially reduces the range of *A. woodhousii* in western New Mexico, and indicates the species is absent, with no sites of syntopy, in Catron County (Fig. 4). In addition, we found one juvenile specimen previously identified as *A. woodhousii* from Grant County (WNMU 12428) that we now assign to *A. microscaphus* (Table 2) and three adult *A. microscaphus* specimens from Grant County that we assigned to *A. woodhousii* (WNMU 12422, male; 12425, 13271, females). The revised species distribution now indicates that *A. microscaphus* and *A. woodhousii* are found within 2 km of each other at only two localities, one in Grant County along the Gila River in the Gila-Cliff

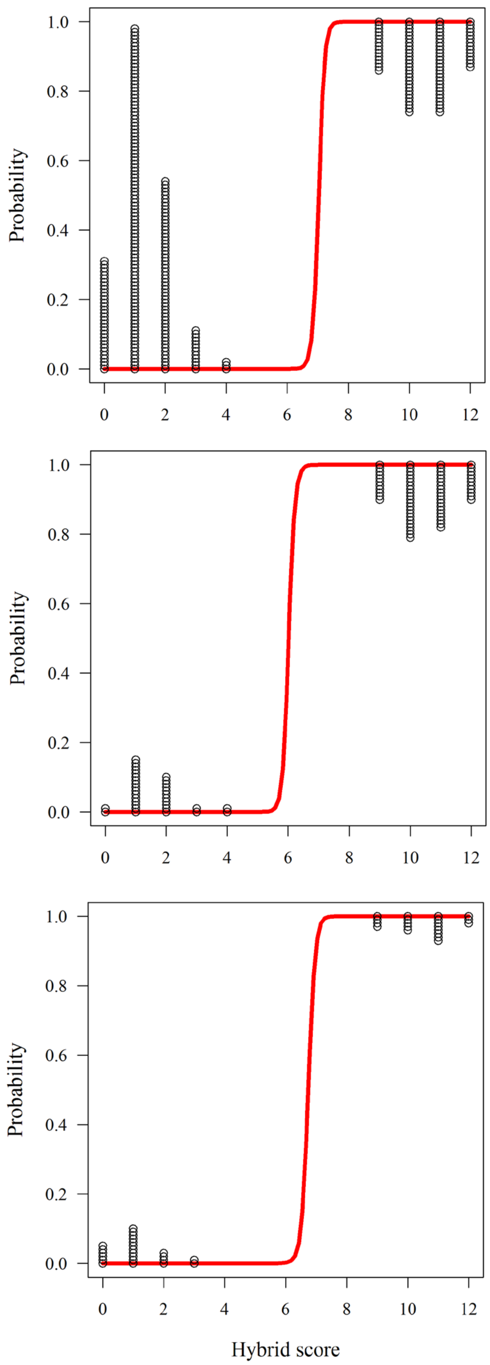

Figure 5. Fitted logistic regression curves (red line) indicating the probability of specimen assignment to the Woodhouse's Toad (*Anaxyrus woodhousii*) based on hybrid index scores. Each dot represents a specimen with the observed score: (A) all specimens of the Arizona Toad (*Anaxyrus microscaphus*; scores 0–4) and *A. woodhousii* (scores 8–12); (B) *Anaxyrus microscaphus* and *A. woodhousii* from Grant County; (C) *Anaxyrus microscaphus* and *A. woodhousii* from Sierra County.

Valley and one in Luna County along the Mimbres River (Fig. 4). Specimen records from the eastern edge of the range of *A. microscaphus* indicate no areas of syntopy in Sierra and Socorro counties and, according to currently known occurrence records, the two species are separated by more than 40 km.

Our hybrid index scores provided no evidence of morphological hybridization between *A. microscaphus* and *A. woodhousii* in any adult specimens in the upper Gila River and upper Mimbres River basins in New Mexico (Table 3). There was a strong separation between *A. microscaphus* and *A. woodhousii* specimen hybrid index scores in the counties encompassing the upper Gila River and upper Mimbres River basins (χ^2 = 348.04; $P < 0.001$; Estimate = -6.46; Fig. 5A), Grant County (χ^2 = 123.55; $P = 0.001$; Estimate = -7.21; Fig. 5B), and Sierra County (χ^2 = 59.40; $P < 0.001$; Estimate = -5.82; Fig. 5C). Given that the single *A. microscaphus* record from Luna County is a photo voucher, we could not include it in our regression analyses.

After identification and distribution corrections, we found no evidence of hybrid specimens at the five localities along the Gila River in the Gila-Cliff Valley, Grant County, where records of *A. microscaphus* and *A. woodhousii* are separated by 2 km or less. However, the sample sizes of adult specimens from the Gila-Cliff Valley were too small (one *A. microscaphus*, five *A. woodhousii*) to perform a separate statistical analyses. We did review the juvenile specimens of both species from the Gila-Cliff-Valley (*A. microscaphus*, n = 12; *A. woodhousii*, n = 28) and they did not show evidence of hybridization.

Discussion

We found a lack of hybridization between *A. microscaphus* and *A. woodhousii* based on museum specimens; corrected misidentified specimen records, which redraws the known range of *A. woodhousii*. We also revised the number of localities within the upper Gila River (Gila and San Francisco rivers) and the upper Mimbres River basins where the two species have the potential to come into contact due to close proximity. Prior to this study, the two species appeared to be syntopic at 20 localities, where we expected hybrids to occur (Degenhardt et al. 1996; Jennings et al. 2010). We determined that the purported occurrences of *A. woodhousii* at 17 localities throughout Catron County along the Gila and San Francisco rivers to be in error, or reflected earlier taxonomy that has subsequently been corrected in this paper. The continued recognition of these erroneous records could potentially mislead conservation assessments by over-representing the risk to *A. microscaphus* from hybridization with *A. woodhousii*.

Table 2. Museum specimens from the eastern Mogollon Rim, New Mexico, USA of the Arizona Toad (*Anaxyrus microscaphus*) and the Woodhouse's Toad (*Anaxyrus woodhousii*) that were misidentified with revised identifications. These were the only misidentifications we found among the New Mexico specimens we reviewed. The asterisk (*) indicates no additional date available for this specimen. Museum abbreviations are MSB = Museum of Southwestern Biology and WNMU = Western New Mexico University and stage abbreviations are A = adult, J = juvenile, T = tadpole, F = female, and M = male.

Specimen examined	Original Identification	Revised Identification	Stage	County	Latitude	Longitude	Collection Date
MSB 733	*A. woodhousii*	*A. microscaphus*	J	Catron	33.45	-108.91	7 August 1943
MSB 631−635	*A. woodhousii*	*A. microscaphus*	J	Catron	34.14	-108.51	3 September 1948
MSB 661−675	*A. woodhousii*	*A. microscaphus*	J	Catron	33.80	-108.77	5 September 1948
MSB 5801−5826	*A. woodhousii*	*A. microscaphus*	J	Catron	33.89	-108.95	6 September 1948
WNMU 724−726	*A. woodhousii*	*A. microscaphus*	J	Catron	33.90	-108.50	3 September 1948
MSB 727	*A. woodhousii*	*A. microscaphus*	J	Catron	33.72	-108.76	4 September 1948
MSB 732	*A. woodhousii*	*A. microscaphus*	J	Catron	33.37	-108.91	4 September 1949
MSB 739−740	*A. woodhousii*	*A. microscaphus*	J	Catron	33.77	-108.68	20 August 1949
MSB 3676	*A. woodhousii*	*A. microscaphus*	J	Catron	33.27	-108.87	24 August 1959
MSB 3677	*A. woodhousii*	*A. microscaphus*	A, F	Catron	33.25	-108.88	13 June 1959
MSB 3678	*A. woodhousii*	*A. microscaphus*	J	Catron	33.25	-108.71	13 June 1959
MSB 3679	*A. woodhousii*	*A. microscaphus*	J	Catron	33.31	-108.88	13 June 1959
WNMU 12428	*A. woodhousii*	*A. microscaphus*	J	Grant	32.63	-108.28	27 April 1968
WNMU 12429	*A. woodhousii*	*A. microscaphus*	A, M	Catron	33.35	-108.08	19 March 1976
MSB 43330	*A. woodhousii*	*A. microscaphus*	T	Catron	33.24	-108.88	29 March 1984
MSB 43333	*A. woodhousii*	*A. microscaphus*	T	Catron	33.57	-108.85	7 May 1984
MSB 43339	*A. woodhousii*	*A. microscaphus*	T	Catron	33.22	-108.27	9 June 1984
WNMU 13264	*A. woodhousii*	*A. microscaphus*	A, F	Catron	33.26	-108.23	15 July 1989
WNMU 12422	*A. microscaphus*	*A. woodhousii*	A, M	Grant	32.92	-108.59	27 April 1980
WNMU 12425	*A. microscaphus*	*A. woodhousii*	A, F	Grant	32.85	-107.97	8 April 1978
WNMU 13271	*A. microscaphus*	*A. woodhousii*	A, F	Grant	32.96	-108.61	1989*

We found that 8% of *A. microscaphus* and *A. woodhousii* specimens held in the museum collections we assessed had been misidentified. The majority of misidentified toad specimens was from Catron County and consisted of 56 juveniles collected between 1943 and 1949, prior to the recognition of *A. microscaphus* as a valid species (Stebbins 1951). The taxonomy and species status of *A. microscaphus* was unresolved prior to 1949, until Shannon (1949) recognized specimens from southern Nevada, southwestern Utah, and western Arizona as the subspecies *Bufo woodhousii microscaphus*. Prior to 1954, *A. microscaphus* was not recorded from New Mexico (Stebbins 1954); therefore we infer that collectors from before 1954 assigned many toads collected in southwestern New Mexico to *A. woodhousii* in accordance with the taxonomy of that era, and that these specimens were not reviewed following subsequent taxonomic changes. We found other misidentifications with tadpoles, which are notoriously difficult to identify and require careful scrutiny (Altig and McDiarmid 2015). Our review of field notes associated with these tadpole lots indicates that they came from an ichthyology course trip in 1984, and these tadpoles were identified in the field but not reviewed at the time of institutional cataloging. Thus, our revised identifications revealed no areas of syntopy and indicate there is currently low likelihood for hybridization in the Gila and San Francisco rivers in Catron County.

This work demonstrates the value of specimen-based natural history collections for conservation assessments (e.g., Ervin et al. 2013; Cook et al. 2014; Rocha et al. 2014). Inaccurate distributions based on misidentified specimen records or unverified photographic vouchers can lead to misallocated funds and hinder conservation efforts (Ervin et al. 2013; Goodwin et al. 2015), which in turn can skew conservation status and risk assessments (International Union for Conservation of Nature 2014; Brown 2015). Access to physical specimens allowed us to correct the range of *A. microscaphus* and *A. woodhousii* and more precisely evaluate the risk from hybridization in New Mexico. The continued recognition of these erroneous records might mislead future conservation actions and management for *A. microscaphus* in New Mexico by over-representing the risk to *A. microscaphus* from hybridization with *A. woodhousii*.

Table 3. Museum specimens from the eastern Mogollon Rim, New Mexico, USA of the Arizona Toad (*Anaxyrus microscaphus*) and the Woodhouse's Toad (*Anaxyrus woodhousii*) that were misidentified with revised identifications. These were the only misidentifications we found among the New Mexico specimens we reviewed. The asterisk (*) indicates no additional date available for this specimen. Museum abbreviations are MSB = Museum of Southwestern Biology and WNMU = Western New Mexico University and stage abbreviations are A = adult, J = juvenile, T = tadpole, F = female, and M = male.

	A. microscaphus			*A. woodhousii*		
	Mean ± SD	Range	Sample Size	Mean ± SD	Range	Sample Size
All Specimens						
Dorsal Stripe	0.16 ± 0.44	0–3	202	3.00 ± 0.00	0	85
Throat Spots	0.20 ± 0.46	0–2	202	1.57 ± 0.91	0–3	85
Cranial Crest	0.73 ± 0.46	0–2	202	2.91 ± 0.27	2–3	85
Pale-bar	0.18 ± 0.41	0–2	202	3.00 ± 0.00	0	85
Hybrid Index	1.29 ± 0.87	1–4	202	10.49 ± 0.98	9–12	85
Catron County						
Dorsal Stripe	0.17 ± 0.43	0–3	123	NA	NA	NA
Throat Spots	0.23 ± 0.61	0–4	123	NA	NA	NA
Cranial Crest	0.69 ± 0.46	0–2	123	NA	NA	NA
Pale-bar	0.17 ± 0.39	0–1	123	NA	NA	NA
Hybrid Index	1.21 ± 0.84	0–4	123	NA	NA	NA
Grant County						
Dorsal Stripe	0.23 ± 0.60	0–3	34	3.00 ± 0.00	0	63
Throat Spots	0.20 ± 0.47	0–2	34	1.52 ± 0.94	0–3	63
Cranial Crest	0.88 ± 0.40	0–2	34	2.95 ± 0.21	2–3	63
Pale-bar	0.32 ± 0.53	0–2	34	3.00 ± 0.00	0	63
Hybrid Index	1.64 ± 1.01	1–4	34	10.47 ± 0.98	9–12	63
Sierra County						
Dorsal Stripe	0.04 ± 0.20	0–2	23	3.00 ± 0.00	3	20
Throat Spots	0.30 ± 0.55	0–3	23	1.65 ± 0.81	0–3	20
Cranial Crest	0.65 ± 0.48	0–1	23	2.85 ± 0.36	2–3	20
Pale-bar	0.08 ± 0.28	0–1	23	3.00 ± 0.00	3	20
Hybrid Index	1.08 ± 0.90	0–3	23	10.50 ± 1.00	9–12	20

Although it is difficult to confirm the absence of a species, we feel confident that *A. woodhousii* does not currently occur in the upper Gila River Basin in Catron County based on multiple lines of evidence. First, since the 1940s, there have been no collections of verified or purported *A. woodhousii* specimens from Catron County (other than the misidentified tadpole specimens from 1984) despite 30 y of regional amphibian research and monitoring (Degenhardt et al. 1996; Jennings et al. 2010). Second, we conducted 350 breeding-season call surveys (weekly in March and April) at 49 localities in Catron County, including the 17 localities that were the source of the misidentified *A. woodhousii* specimens in 2013 (n = 111 surveys), 2014 (n = 116 surveys), and 2015 (n = 123 surveys), and only detected *A. microscaphus* (unpubl. data). In addition to the call surveys, we collected and scored 103 adult toads in 2013–2015 from Catron County, and we identified all of these specimens as *A. microscaphus*. The single record of *A. microscaphus* from the Mimbres River in Luna County is from a photo voucher, which was the first record of the species in Luna County (Watson 2012). This area merits further investigation because both species may occur in relative close proximity, and to date there is a dearth of specimens of both species from this area.

None of the toad specimens reviewed in this study showed evidence of hybridization. Our hybrid index analyses included specimens of both species collected over 107 y from 83 localities, in two major river basins, upper Gila River and upper Mimbres River, encompassing an area of 9,585 km^2 and covering the range of *A. microscaphus* in New Mexico. Hybrid index scores for Catron, Grant, and Sierra counties are consistent with pure *A. microscaphus* and *A. woodhousii* populations as reported from Arizona (Sullivan et al. 2015), and we conclude that hybridization does not currently appear to be a threat to *A. microscaphus* in New Mexico. This result suggests that observed population declines are due to other factors (New Mexico Department of Game and Fish 2006), which may include disease (Ryan et al. 2014) or climate change (Mason Ryan et al., unpubl. report).

Outside of New Mexico, hybrids of *A. microscaphus* and *A. woodhousii* occur at virtually all localities near dams and reservoirs where the two species co-exist, and hybrids can even extend 45–64 km upstream (Sullivan 1995; Sullivan and Lamb 1988; Schwaner and Sullivan 2009). The major streams and rivers of the upper Gila River and upper Mimbres River basins in New Mexico are relatively unaltered and lack major water diversions or impoundments (Propst et el. 2008), and currently conditions facilitating hybridization are absent. We have identified one area along the Gila River in the Gila-Cliff Valley, Grant County, where contact and hybridization may occur if modifications that alter water flow are enacted. The Gila-Cliff Valley is a wide, flat valley that runs north to south, and is one area of potential syntopy. The two species are currently separated by approximately 2 km, where *A. woodhousii* is restricted to the south end of valley near the agricultural fields near the towns and Gila and Cliff, and *A. microscaphus* is restricted to the relatively undisturbed north end of the valley. Modifications to the river here will likely increase likelihood of contact.

There is currently a proposal to construct a diversion dam along a portion of the Gila River in the Gila-Cliff Valley near the towns of Cliff and Gila (Gori et al. 2014a, 2014b). The proposed diversion project is expected to alter the natural stream flow regimes, and include one to multiple reservoirs scattered within the Gila-Cliff Valley (AECOM. 2016. New Mexico Unit of the Central Arizona Project: Phase I – Concept Development and Selection Report. Final Report to New Mexico Interstate Stream Commission, Project 60504989. Available from nmawsa.org/ongoing-work/aecom/draft-phase-i-report/at_download/file. [Accessed 10 December 2016]). Gori et al. (2014b) evaluated the potential impacts of the proposed diversion on hydrology, ecohydrologic processes, and riparian and aquatic species; however, effects on *A. microscaphus* and *A. woodhousii* were not explicitly considered. A single or multiple diversion dams and reservoirs would convert the current lotic conditions to lentic conditions for some distance upstream. This change would provide favorable habitat for nonnative aquatic species, including Northern Crayfish (*Orconectes virilis*), American Bullfrog (*Lithobates catesbeianus*), and several species of nonnative fish (Stefferud et al. 2011; Gori et al. 2014a). *Anaxyrus woodhousii* could potentially spread dozens of kilometers upstream along the Gila River and its tributaries in the Gila-Cliff Valley (Gori et al. 2014b), coming into contact with currently pure *A. microscaphus* populations. The hydrological alterations associated with the proposed diversion project would likely increase both the distributional overlap and potential for hybridization between the two species in New Mexico. The U.S. Fish and Wildlife Service is currently conducting a review of *A. microscaphus* for protection under the Endangered Species Act, and the impetus for this action was based on natural or manmade factors affecting its continued existence (U.S. Fish and Wildlife Service [USFWS] 2015). The current lack of hybridization in New Mexico presented in this paper would suggest that New Mexican populations of *A. microscaphus* are beyond the purview of the USFWS review. Yet, the lack of water impoundments along the Gila River in New Mexico is not guaranteed in the years to come, and the proposed diversion project is likely to result in hybridization (i.e., Schwaner and Sullivan 2009). Furthermore, there is evidence that *A. microscaphus* has experienced declines and local population extirpations in New Mexico from yet unidentified factors (Mason Ryan et al., unpubl. report). Thus, the USFWS review, and all other conservation actions, of *A. microscaphus* in New Mexico will require careful consideration of how future development actions will alter the current status of the species.

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