VERIFICATION OF IDENTIFICATIONS OF CYPRINID SPECIMENS FROM THE COLORADO RIVER BASIN, TEXAS

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Final Report to U.S. Fish & Wildlife Service

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ABSTRACT

Numerous published reports indicate that records of occurrence of Sharpnose Shiner, *Notropis oxyrhynchus*, in the Colorado River basin of Texas are the result of an introduction, though the species is clearly native in the adjacent Brazos River basin. We discovered previously mis-identified specimens of *N. oxyrhynchus* that extend the record of presence of the species in the Colorado basin much further back in time than previous authors realized, and conclude that the species was almost certainly native there. However, lack of the species in any of the many collections made in the basin over the last half century indicates a low probability that it still persists there.

INTRODUCTION

Our independently funded Fishes of Texas Project (FoTX - Hendrickson and Cohen, 2012; http://www.fishesoftexas.org), which compiled and normalized all known museum specimen-based fish occurrence records from Texas from 46 different institutions, set the stage for this project by bringing all known museum-based data for the state into a single database, georeferencing it, normalizing most data fields and verifying identifications of many distributional outliers, or "suspect" specimens (see detailed methodology in http://www.fishesoftexas.org/documentation/). That work on data cleaning and verification revealed a relatively high specimen identification error rate, especially for species of the family Cyprinidae. This high error rate, and the fact that we had been able to verify identifications for only a very small proportion of these vast collections, prompted us to think that more records of a species of particular conservation concern, *Notropis oxyrhynchus* (Sharpnose Shiner), might be hidden in the museum record, misidentified as other species.

Of particular interest for this project was the origin of the Sharpnose Shiner in the Colorado River basin of Texas. Only six museum specimen-based records of this species from this basin were known at the start of this project, all collected between 1940 and 2008. The literature indicated that the species was not native to the basin and that these occurrences were the result of introduction (Gilbert 1980; Conner and Suttkus 1986; Wang 2004; Hubbs et al 2008), however, evidence supporting that conclusion was not explicitly documented in those publications. Careful review of the complete museum specimen-based collecting record for Texas compiled and normalized in the Fishes of Texas Project database (Hendrickson and Cohen 2012) indicated that historical sampling in the Colorado basin was inadequate to rule out the possibility that the species was native and simply undetected prior to its first collection in 1940. This project therefore attempted to rigorously verify the identifications of the vast majority of older specimens of all cyprinids collected from the Colorado River basin in hopes that such work would allow us to better assess whether the species was native there or not.

METHODS AND RESULTS

This project relied exclusively on preserved museum specimens and the databases associated with them. In a standard ichthyological museum specimen-based research collection, a specimen "lot" consists of all specimens of a single species that were collected and preserved from a single point in time and space. A "lot" thus represents a single occurrence record in time and space for a species, and produces (usually) a single jar on a collection's specimen shelves and a single record in a collection's database. Many lots contain up to hundreds or even (rarely) thousands of specimens, and our experience with examination of large numbers of specimens during the development of our Fishes of Texas Project revealed that most large lots, and especially those identified as cyprinid species, have high probability of containing "sleeper" specimens of species other than what is recorded on jar labels and in museum catalogs. The separate species occurrence records that those "sleeper" specimens would create thus often remain "lost" in collections until astute researchers with expertise in the taxon have cause to carefully examine the total contents of museum lots and discover them. We therefore methodically searched for "sleeper" cyprinids in older lots of cyprinids from the Colorado River basin, anticipating that some previously "lost" historic specimens of Sharpnose Shiner might be found.

Our work started with a query of the Fishes of Texas database (Hendrickson and Cohen, 2012), which (in its combined Track 1 and Track 2 data sets on 01 June 2012) contained 3,175 Colorado River basin cyprinid lots that we had not previously examined. Realizing that resources for this

project were not adequate to enable us to inspect all of these, we prioritized our efforts to focus first on the earliest samples (from the mid-1900's), since we considered that older specimens would be less likely than more recent ones to be the result of introductions. We eventually examined 67% (758) of the 1,126 potentially available pre-1980 lots, while only 34% of the 2,049 post-1979 lots were examined. Our own collection, Texas Natural History Collection (TNHC) at University of Texas at Austin, held more of the total target specimen lots than did any other collection, so we began our work in-house. However, at the same time, we also made arrangements to visit the next largest holdings of target specimens for this project, the Mayborn Museum Complex at Baylor University and Texas A&M's Biodiversity Research and Teaching Collections, and we requested loans of other lots from 18 institutions (seven of which eventually sent the requested lots). Instead of loaning specimens, Oklahoma State University offered to have their own recognized authority, Dr. Anthony Echelle, verify all specimens we had requested, and we accepted his identifications. In all, the determinations reported here were performed by five individuals (see Appendix 1), but 1,243 (85%) were done in our lab by Dr. Floyd Douglas Martin, an employee of the Fishes of Texas Project. Dr. Martin has extensive experience working with all Texas freshwater fishes, and our protocol included consultation with other Fishes of Texas staff regarding any specimens for which he had any doubt about identification, and his overall work was systematically spot-checked by other Fishes of Texas Project staff. We used all available resources for our determinations, including identification keys published in the FoTX and the primary literature, including original species descriptions and diverse state and regional "Fishes of" books. Notes documenting the basis of determinations are included in the data provided as part of this report (Appendix 1) and those notes will be incorporated into the FoTX database.

By the end of this project we had examined 1,445 lots (roughly half of the total target lots) from 10 institutions, resulting in detection and correction of 195 mis-identifications (an error rate of 13.5%). Through detection of multiple species in many lots we increased the number of total cyprinid lots from the Colorado basin to 3,191 (an increase of 16 lots). The final complete data set of all specimens of all cyprinids known to have been collected from the Colorado River basin, indicating which lots were and were not examined by this project, is provided in Appendix 1. Eleven of the examined lots contained more than one species, and one lot containing five species was found. Our use here of "misidentification", however, has to be carefully interpreted - many of the total "misidentifications" are not true errors in identification but simply the result of failure of data-providing institutions to keep up with current taxonomic changes. If, for example, we remove from the analysis all species of the genera *Dionda* and *Macrhybopsis*, both of which have had recent taxonomic revisions, the overall "misidentification rate" lowers to 3%.

Numbers of both examined and total available lots are graphically presented by year of collection in Figure 1. Overall, the specimens examined were originally identified as 37 cyprinid taxa, with the most common species being *Cyprinella venusta* (359), *C. lutrensis* (311), and *Campostoma anomalum* (160) (Table 1). As anticipated from the outset, not all potentially available specimens were examined for several reasons: 1) we did not receive specimen loans in time; 2) we did not request loans for institutions where very large numbers of specimens were held, knowing in advance that those institutions did not have adequate resources to process and ship such large loans; and 3) some lots could not be found at the donor institutions, where they may be permanently lost, on loan, or mis-shelved.

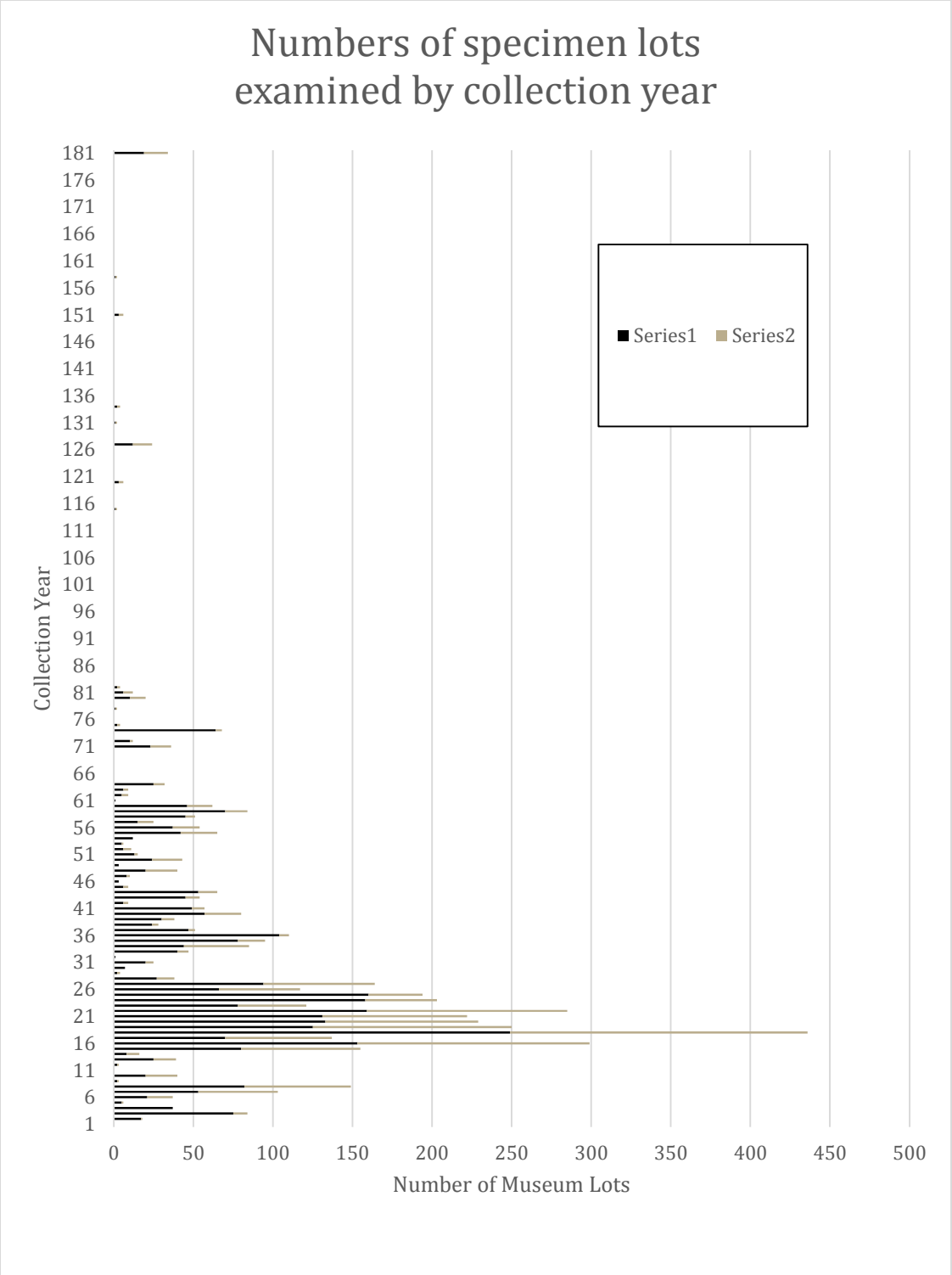

Figure 1. Numbers of Colorado River basin cyprinid specimen lots in the Fishes of Texas (FoTX) database (Track 1 + 2) by year of collection. Collection dates in FoTX are defined by begin and end dates representing a date range (see detailed methodology in http://www.fishesoftexas.org/documentation/). This graph is based on begin dates. The black (left) portion of each bar is the number of lots examined by this project and gray the number of lots not examined.

Table 1. List of all Colorado River basin cyprinid lots in FoTX database (Tracks 1 + 2) flagged initially for examination (any examined by FoTX staff prior to this project were excluded) and summary of outcomes of specimen examinations done by this project. "ID not changed" = N for which the donor institutions' determinations were found to be correct, "ID changed" = N lots that were found to be mis-identified (donor's determination changed), and "Not examined" = N lots not examined by this project.

Original species ID	ID not changed	ID changed	Not examined	All lots available
Cyprinidae (sum)	1260	195	1734	3191
Campostoma anomalum	154	6	195	355
Carassius auratus	2		1	3
***Cyprinella* hybrid**			11	11
Cyprinella lutrensis	300	11	300	611
***Cyprinella* sp.**			1	1
Cyprinella venusta	359		308	667
Cyprinus carpio	5		25	30
Dionda episcopa		2		2
Dionda flavipinnis	1	3	4	8
Dionda nigrotaeniata		136	49	185
***Dionda* sp.**			1	1
***Dionda* sp. 3**		1	3	4
Hybognathus placitus	7	3	2	12
Hybopsis amnis			11	11
Lythrurus fumeus	13	1	11	25
Macrhybopsis aestivalis	5	1	7	13
Macrhybopsis hyostoma			1	1
Macrhybopsis marconis			2	2
***Macrhybopsis* sp.**		4	2	6
Notemigonus crysoleucas	32	1	36	69
Notropis amabilis	50	4	94	148
Notropis braytoni			1	1
Notropis buchanani	3	6	33	42
Notropis oxyrhynchus	1		3	4
Notropis potteri	1			1
Notropis rubricroceus			1	1
Notropis shumardi	8	3	4	15
***Notropis* sp.**			4	4
Notropis stramineus	31		29	60

Notropis texanus	39	3	18	60
Notropis volucellus	66	3	40	109
Opsopoeodus emiliae	18	4	7	29
Phenacobius mirabilis	2		7	9
Pimephales notatus			1	1
Pimephales promelas	25	1	160	186
Pimephales vigilax	138	1	362	501
Unknown sp.		3		3

DISCUSSION

Table 2 summarizes the results for all specimens mentioned below, and photographs of those same specimens are provided in Figures 3 - 7.

Our initial query of the FoTX Project database contained six occurrences of *N. oxyrhynchus* in the Colorado River drainage. The specimens underlying two (Texas Natural History Collections #9322 and University of Michigan #170083) occurrences had been previously verified by specimen examination by FoTX staff outside of this project and so were not re-examined. The specimen (Texas Natural History Collections #2500) representing the third occurrence had been loaned to a researcher and, for approximately 20 years, was thought to be lost. This project sparked a concerted and eventually successful effort to re-locate it at the borrower's location, allowing us to then confirm the borrower's re-determination as *N. oxyrhynchus* (following an original determination as *N. jemezanus*). Similarly, the fourth occurrence in the initial query, University of Michigan's lot #170305, was not found when we visited that collection in 2011, it too was located as a result of this project, examined and confirmed as *N. oxyrhynchus*. The fifth lot (University of Louisiana at Monroe #8711) was also mis-shelved or lost at the time of our 2011 visit to that collection and unfortunately, inquiries to that collection now indicate that the entire collection has been boxed for storage and is essentially inaccessible for the foreseeable future (Dr. John Carr, pers. comm.). Examination of the sixth lot (Texas Natural History Collections #41918) for this project determined it to be *N. amabilis* rather than *N. oxyrhynchus*. Finally, a Mayborn Museum Complex specimen (Catalog# 589) originally identified as *N. oxyrhynchus,* was examined and determined to be *Hybognathus placitus*. Furthermore, though the locality, "3 miles east of Menard, Texas: San Saba River," recorded in the Mayborn's database is in the Colorado basin (thus our interest in it), our examination of the original jar labels clearly indicated it was not from that locality, but from the Brazos drainage. Assignment of this collection to the Colorado basin was apparently based on a simple databasing error on the part of institution.

This project, however, resulted in addition of a "new" verified record of *N. oxyrhynchus* from the Colorado River drainage (row 7 of Table 2). United States National Museum #36581 was collected at Austin in 1884 and cataloged there as *N. atherinoides.* Though we examined this specimen in 2011 (prior to this project) and agreed with that original determination, our examination for this project of large numbers of specimens of diverse cyprinids led us to realize that *N. atherinoides* and *N. oxyrhynchus* are morphologically very similar and thus very prone to be confused. Consequently, we decided to re-examine a small subset of specimens that we had previously examined, including this one, and we now conclude, on the basis of several characters, that it is actually *N. oxyrhynchus*, and thus by far the oldest of all records of this species from the Colorado basin.

Table 2. All Colorado River basin cyprinid records from the Fishes of Texas database indicated at the onset of this project to be N. oxyrhynchus, and one record (USNM 36581) previously incorrectly identified but re-determined by FoTX staff as N. oxyrhynchus.

Institution	Catalog number	Location	Collection year	Notes	Conclusion	Figure No.
University of Michigan	170083	Colorado River at Colorado City	1940	Species determination verified in 2010 by FoTX project staff.	*N. oxyrhynchus*	3
Texas Natural History Collections	9322	Colorado River at SH 208	1955	Species determination verified by FoTX project staff.	*N. oxyrhynchus*	4
Texas Natural History Collections	2500	Colorado River, at ford upstream of San Saba River confluence	1952	Specimen found after having been lost for nearly 20 years. Species determination verified by FoTX staff.	*N. oxyrhynchus*	5
United States National Museum	36581	Barton Creek, at mouth and vicinity	1884	Originally determined as *N. atherinoides* and FoTX staff in 2010 agreed, but re-examined for this project.	*N. oxyrhynchus*	6
University of Michigan	170305	Colorado River at Wharton	1940	Species determination verified in 2014 by FoTX staff.	*N. oxyrhynchus*	7
University of Louisiana at Monroe	8711	San Saba River near Fort McKavett	1963	Not found during 2011 visit. Headwater stream location atypical for species. Probably a mis-identification.	not examined	n/a
Texas Natural History Collections	41918	Flat Creek at US 290	2008	Determined by FoTX staff to be *Notropis amabilis.*	**not** *N. oxyrhynchus*	n/a

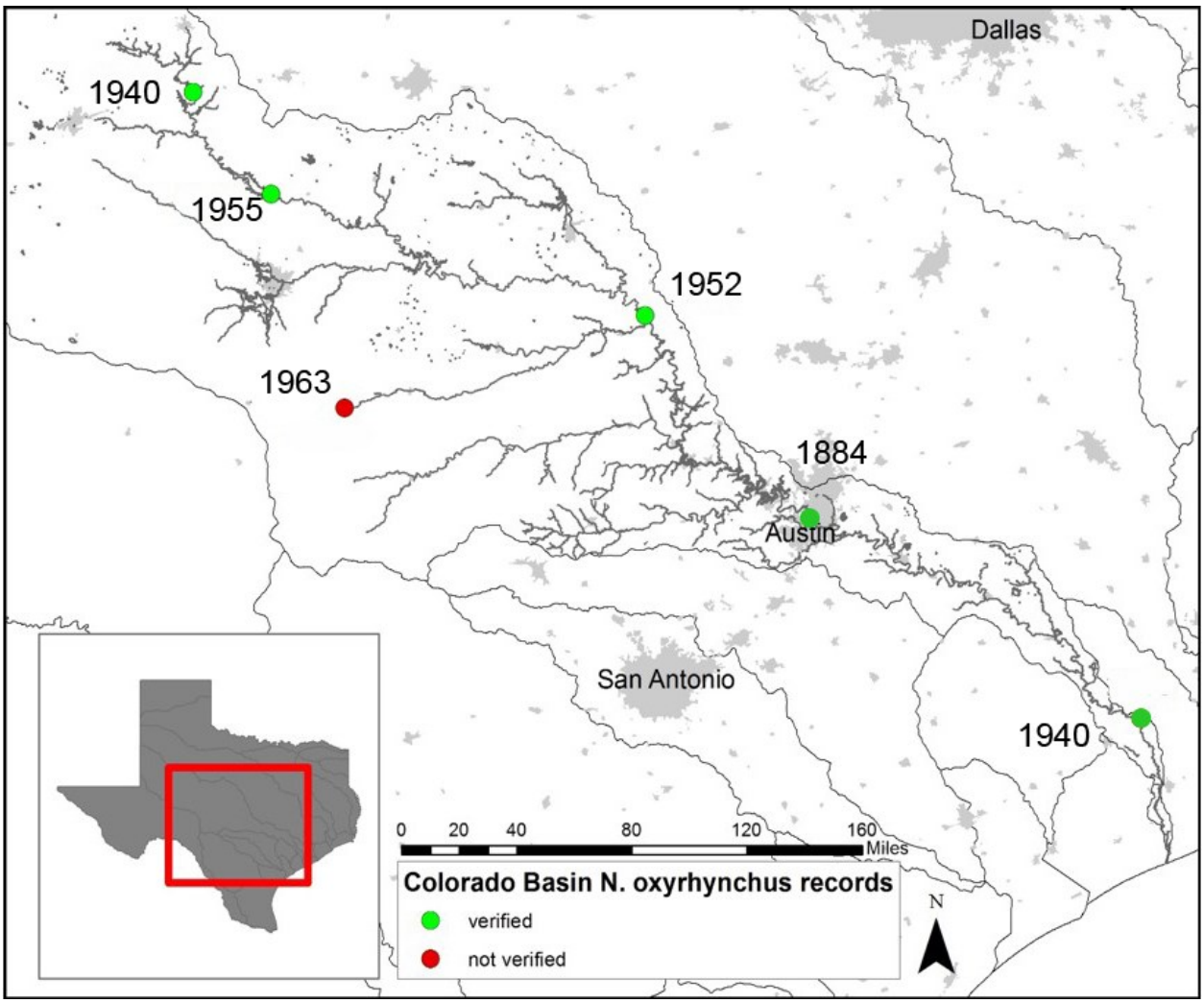

Figure 2. Locations of the five verified N. oxyrhynchus lots in the Colorado River drainage and one dubious record (ULM 8711) not examined but identified at the donor institution as being N. oxyrhynchus.

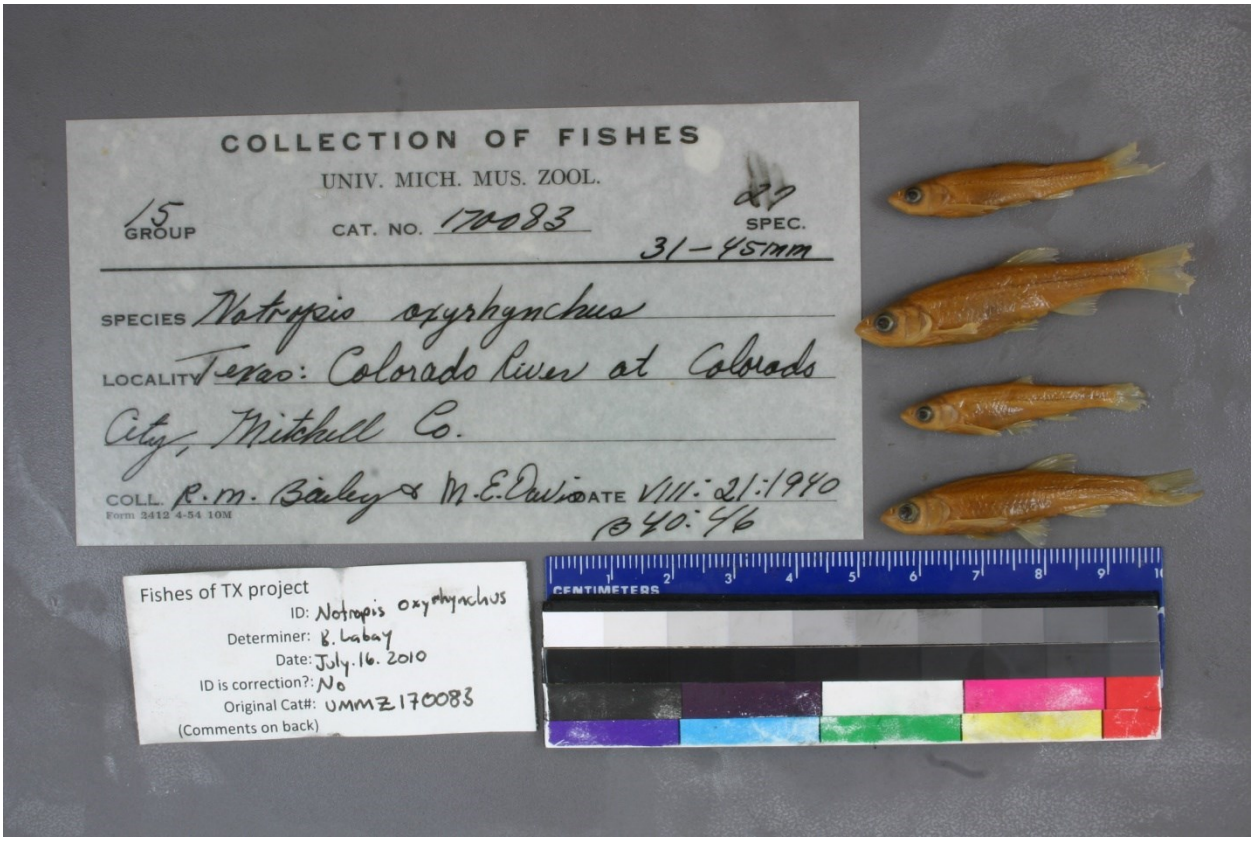

Figure 3. Selected specimens of University of Michigan Catalog# 170083. Verified occurrence of N. oxyrhynchus from Colorado River.

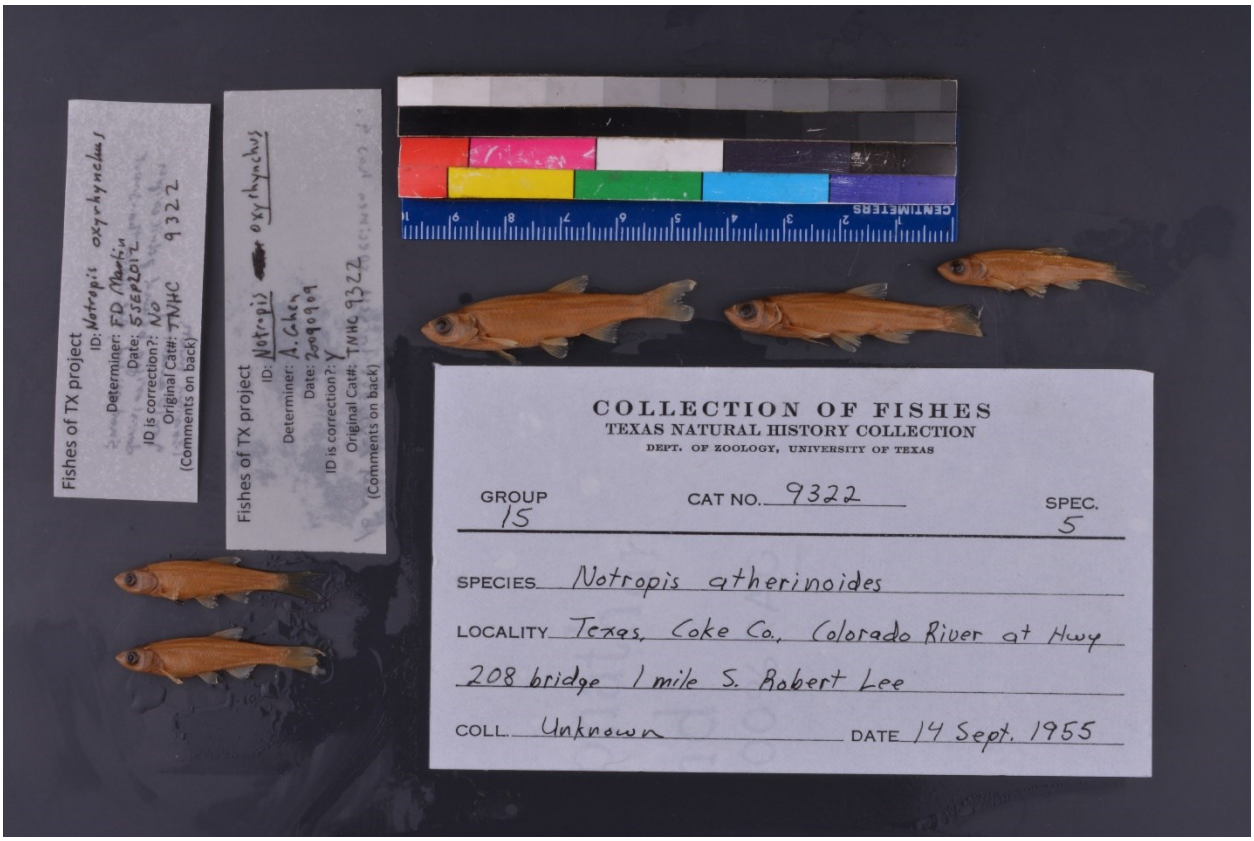

Figure 4. All specimens of Texas Natural History Collections catalog #9322. Verified occurrence of N. oxyrhynchus from Colorado River.

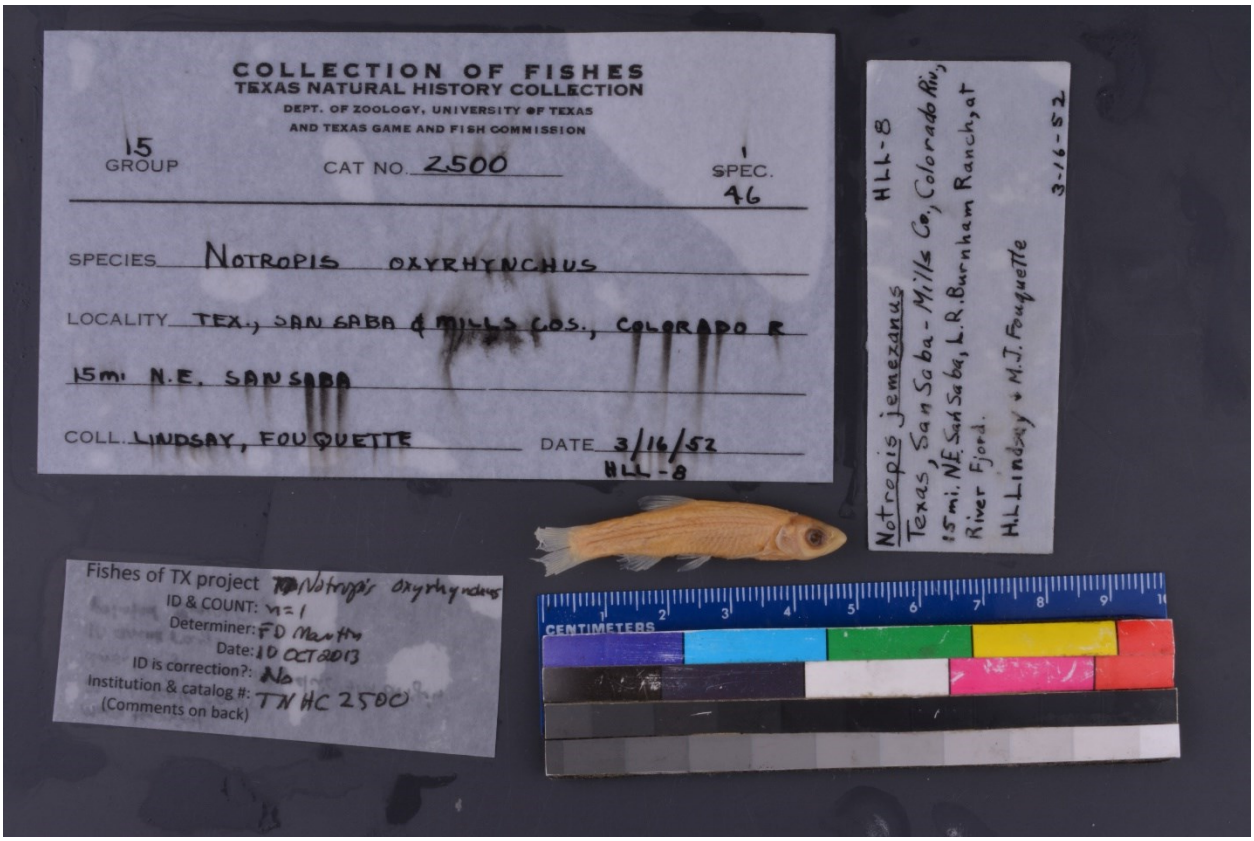

Figure 5. Texas Natural History Collections catalog #2500. Verified occurrence of N. oxyrhynchus from Colorado River.

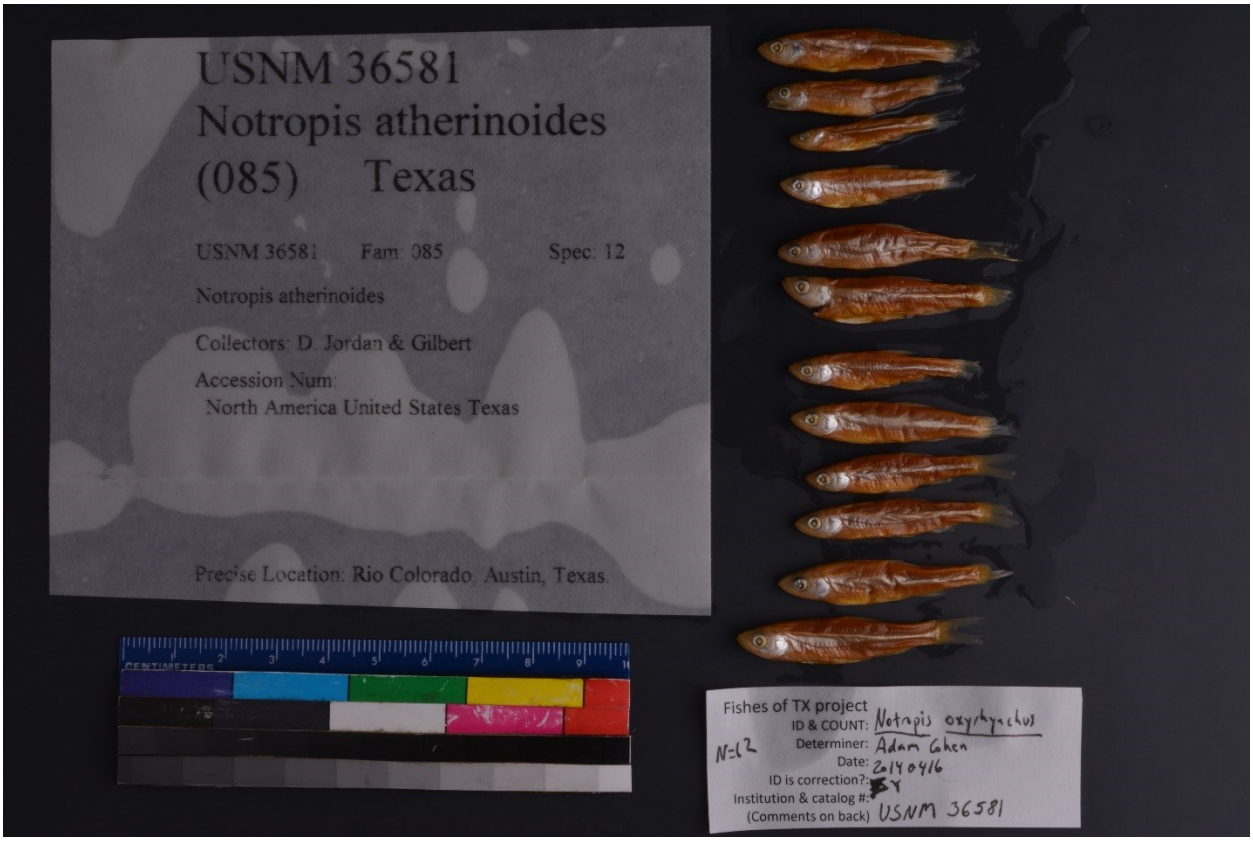

Figure 6. United States National Museum catalog #36581. Previously identified as N. atherinoides, re-examination for this project resulted in re-determination of this lot from the Colorado River in 1884 to N. oxyrhynchus.

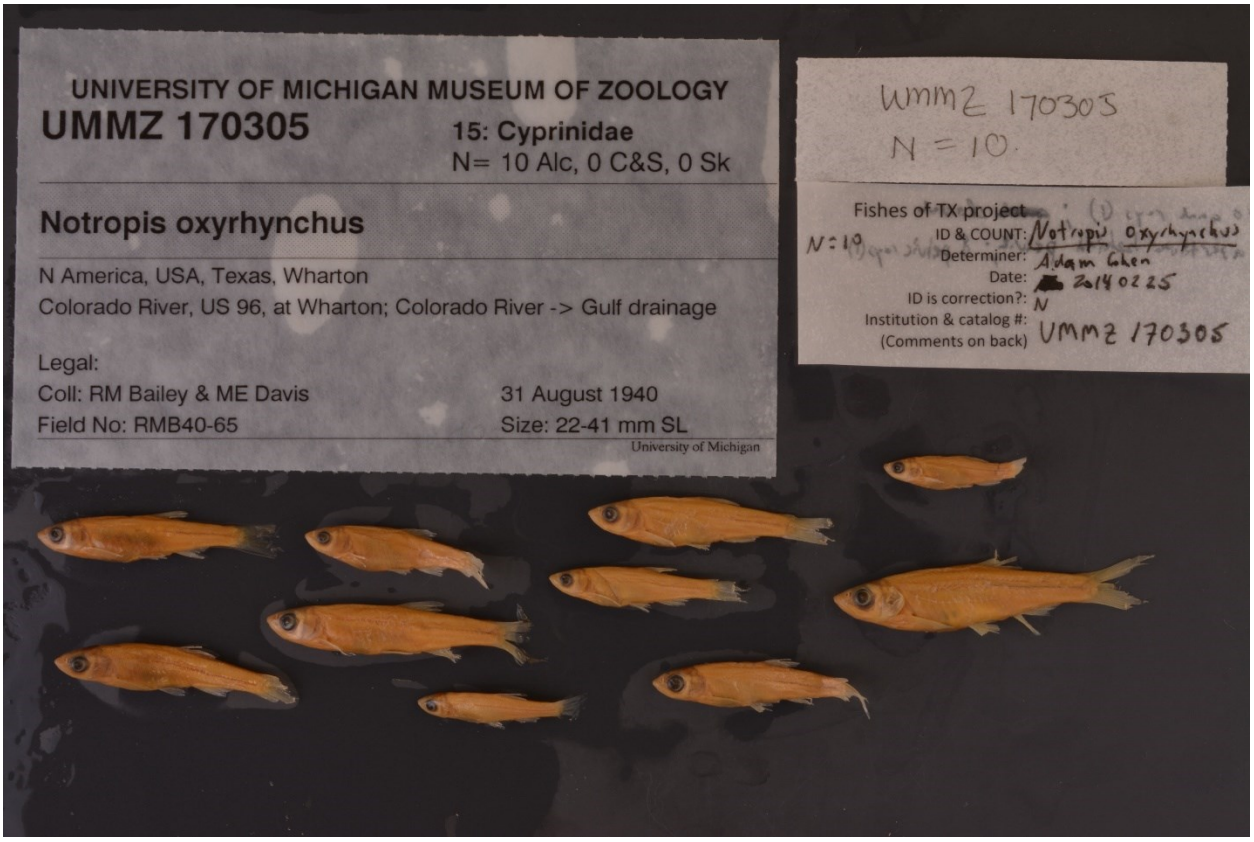

Figure 7. University of Michigan Catalog# 170305. Verified occurrence of N. oxyrhynchus from Colorado River.

CONCLUSIONS

Our review of the museum documents and the specimens themselves revealed that only six records, five confidently verified and one unverified, are available for assessment of nativity and conservation status of *Notropis oxyrhynchus* in the Colorado River basin. The unverified record (Univ. Louisiana at Monroe #8711), from the headwaters of the San Saba River, a Colorado River basin tributary, seems unlikely to be valid because this is primarily a mainstem riverine species. Nearly all other verified records of it in both the Brazos and Colorado basins are from mainstem sites, far from headwaters. This record is also a temporal outlier, occurring eight years after the next most recent verified record for the basin. We anticipate that, if the specimen could be examined, it would prove not to represent this species.

The five verified records of *N. oxyrhynchus*, with identifications confirmed by FoTX staff, are further discussed in detail below.

The two records (Figures 3 and 7) from the University of Michigan's Museum of Zoology (UMMZ) are the upstream- and downstream-most records of the species in the Colorado basin. Both were collected (and later identified) by renowned ichthyologist, Dr. Reeve M. Bailey, 10 days apart on a 1940 expedition. Dr. Bailey was an astute ichthyologist of global repute (Stewart and Smith 2000), well known for his meticulous field notes and attention to details, as well as extensive knowledge of the North American freshwater fish fauna (pers. comm. Douglas Nelson, UMMZ Fish Collection Manager, whose tenure in that position overlapped with Dr. Bailey for decades). The

validity of his records is not commonly questioned, but to increase our confidence in these records and more definitively rule out the possibility of data errors or specimen mix-ups, we reconstructed his itinerary on the basis of collection dates in the UMMZ catalog. Collections in the Brazos (the only other river where the species is known to occur) were not made as part of this expedition, thus lessening the possibility of an error caused by contamination by Brazos specimens. After his first collection of *N. oxyrhynchus* in the upper Colorado, Dr. Bailey proceeded west to collect in the Pecos (where the species has never been documented to occur) before returning eastward through Cameron, TX to make the expedition's second *N. oxyrhynchus* collection from the lower Colorado. From there he proceeded to the Houston area, and though he obviously crossed the Brazos (where the species is extensively documented), nothing in his collection records or notes indicate that he stopped to collect. He instead continued to other areas in east Texas, Louisiana and Arkansas, where this species has never been documented. It seems very unlikely, therefore, that he, or other collection workers processing the specimens, might have somehow attributed specimens of *N. oxyrhynchus* collected elsewhere to his collections from the Colorado.

Texas Natural History Collections (TNHC) #2500 (Figure 5) was collected as part of a 2-day excursion by H.L. Lindsay and M.J. Fouquette through Mills, San Saba and Williamson counties. That trip never took them into the mainstem Brazos. Thus, as for Bailey's collections, contamination by Brazos specimens on the part of these collectors or those later handling the specimens and data, seems similarly unlikely. We also verified collection data against the TNHC's original hand-written ledger for errors of omission or transcription, and none were found.

Texas Natural History Collections #9322 (Figure 4) is the least substantiated record of the species in the Colorado basin since no collectors were recorded for this lot, but we checked these collection data against the original hand-written ledger for possible transcription errors or omissions, and none were found. We also checked the FoTX database and found no specimens collected from the Brazos basin within 4.5 months before and 10 days after the collection date of TNHC #9322, making contamination by Brazos specimens unlikely.

Finally, the United States National Museum's (USNM) record #36581 (Figure 6) is described in a publication by David S. Jordan and Charles H. Gilbert (Jordan and Gilbert 1886) that reports on their 1884 expedition. That publication, and other museum specimen records in the FoTX Project database, both indicate that during this expedition Jordan and Gilbert collected in Hays, Comal, Bell and Travis counties and that no collections were made from the Brazos River mainstem, though they did collect from the Lampasas River, a Brazos tributary. According to our Fishes of Texas database, *N. oxyrhynchus* has never been documented to occur in the Lampasas River, and it has very rarely been collected in any small tributaries. Bailey, Jordan and Gilbert obviously crossed the Brazos mainstem to get to and from their collection sites for this expedition, but it appears that they did not collect specimens there, so again, a specimen mix-up seems highly unlikely.

None of those who published claims that *N. oxyrhynchus* is not native in the Colorado (Gilbert 1980; Conner and Suttkus 1986; Wang 2004; Hubbs et al 2008) offer any evidence supporting that hypothesis, and it is clear that they reached this conclusion largely in isolation without rigorous consideration of all possible evidence, or were simply reiterating the conclusions of others. Gilbert's (1980) report of a single record from the Colorado River near San Saba has no reference to the source of the occurrence or its date of collection, nor explanation for the conclusion that it represents an introduced population. Connor and Suttkus (1986) conclude the species to be introduced in the basin, but also without explanation other than paradoxically citing Jurgens (1954), who clearly thought it possible that the species was native to the upper Colorado. Jurgens (1954) reported collecting a Sharpnose Shiner from Lake Travis (a short distance above Austin) at Hurst Creek in 1953, and suspected that he had collected other specimens (presumably from the same location) before 1951,

but confused them with *N. amabilis.* Unfortunately, Jurgens apparently did not deposit any voucher specimens in collections. Wang (2004) cites Gilbert (1980) and offers no other evidence.

On the basis of some of the same evidence described above, prior to completion of the rigorous examination provided by this project, the United States Fish and Wildlife, despite contrary opinions expressed in the literature, concluded that *N. oxyrhynchus* was probably native in the Colorado River (United States Fish and Wildlife Service 2013). Our work further supports that conclusion, providing now more rigorously verified and specimen-based evidence and extending that evidence of occurrence of the species in the Colorado River basin much further back in time to 1884. The likelihood of five independently documented occurrences, held at three institutions and collected by four parties all being erroneous is extremely unlikely. Furthermore, these records mimic the same basic pattern of distribution that the species has in the Brazos, being restricted primarily to large, mainstem river habitats. That the species naturally occupied both rivers should not be surprising given that the Brazos and Colorado basins share many other species and there are well-documented historical, flood-related hydrologic connections between them (Burnett 2008) that would allow such small, mainstem fishes to easily swim across basin divides. The fact that roughly only half of the total available Colorado basin cyprinid specimen lots were verified by this project leaves open the possibility that additional historical occurrences of Sharpnose Shiner may still remain undiscovered. However, 51 years have now passed since the most recent (1963) of the unverified collections of the species, and since no occurrences of the species have been recorded among the 2,655 post-1963 cyprinid occurrences from the Colorado basin in the FoTX Project database, extirpation of this population seems likely.

Since all verified occurrences in the Colorado basin are restricted to the mainstem and larger tributaries, as are occurrences of the species in the Brazos, it is likely that life history requirements of the Colorado population are or were similar to those of the Brazos population (Durham and Wilde 2014), and so it would be susceptible to the same kinds of threats. Dams, which are widely accepted to be the primary threat to the Brazos River population (Moss and Mayes 1993; Durham and Wilde 2009; Perkin and Gido 2011; Labay et al. 2013), were built along the Colorado River to create the Highland Lakes. Lake Buchannan and Inks Lakes were operational in 1938 and are the first two of the five highland lakes (http://waterdatafortexas.org/reservoirs/statewide) on the mainstem Colorado River. The other three lakes were operational by 1951. The timing of the construction of the Highland Lakes, which fragmented the river, and the last observation of *N. oxyrhynchus* in 1955, are probably not coincidental. Highland Lakes construction likely caused reductions in the size of this population, or perhaps its extirpation. However we note that relatively few recent collections have been made in some of the long reaches between reservoirs in the upper Colorado River, where the shifting sandy depositional substrates preferred by the species in the Brazos are prevalent. More sampling is warranted in these habitats to further test the hypothesis that the species has been extirpated from the Colorado.

The *N. oxyrhynchus* specimens that we examined (5 lots = 32 specimens) from the Colorado basin are morphologically more similar to specimens of the species from the Brazos than they are to any other species known from the Colorado (or other Texas basins), and our confidence in our determinations is high. However, we have observed that the Colorado specimens appear to have distinctly shorter snouts than do individuals from the Brazos, including the specimens described in the original description of the species (Hubbs and Bonham 1951). This may have contributed to mis-identifications of this species in the Colorado, since the snout is perhaps this species' most diagnostic character, but the hypothesis that this population represents a unique and undescribed species remains to be tested. We are now performing morphometric analyses that will shed more light on this possibility. Furthermore, unlike all other known specimens from the Colorado, the 1884 lot held by the United States National Museum may have intact DNA since it was almost certainly originally

preserved in alcohol, which was standard practice during the time of that collection (vs. the more modern standard of preserving specimens first in formalin which destroys DNA). If so, it could potentially be used to more rigorously examine the hypothesis of species status for the Colorado population using powerful genetics-based analyses.

These findings illustrate, in many ways, the value of preserved museum specimens for understanding historical biogeography, as well as the value of large-scale data compilation and quality control efforts like the FoTX Project, to the future of specimen-based ichthyological research and biodiversity knowledge and conservation. The ability to easily peruse all available data normalized, georeferenced and easily accessible in one place prompted generation of new hypotheses that could be addressed by methodical re-examination of specimens. Although the vast majority of corrections and verifications done by this project did not produce findings relevant to *N. oxyrhynchus*' status in the Colorado basin, at least one re-determination made as part of this project was critical for supporting the conclusion that *N. oxyrhynchus* is native to the Colorado basin. And, future research on other species benefitted from this project since ID changes and verifications made for many other cyprinids are now reflected in the FoTX Project database and (hopefully one day) donor institution's databases.

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