# A tale of two catfishes: Yaqui (*Ictalurus pricei*) and Chihuahua (*I*. sp.) – and a bunch of people....

- Dean A. Hendrickson, Biodiversity Center, Department of Integrative Biology, University of Texas at Austin
  - with help from Dave Propst, Jim Brooks, Buddy Jensen, Alejandro Varela, Tyler Pilger, Lex Snyder, Doug Nelson, Adam Cohen, John P. Sullivan, John Lundberg, Julian M. Humphries, Robert Rush Miller and many more....

Presented at 49<sup>th</sup> annual meeting of the Desert Fishes Council, November 2017 in San Luis Río Colorado, Sonora, México

#### Chihuahua Catfish



From East Fork of the Gila River, New Mexico

From near the type locality, Nonoava (Rio Conchos basin), Chihuahua, Mexico



The formal description of the Chihuahua Catfish has a long history of diligent work that culminated in a near final manuscript co-authored by the preeminent and highly esteemed ichthyologist, Dr. Robert Rush Miller, who had a long history of study of fishes of Mexico and the desert southwest of the US. The first author was one of his doctoral students, Julian Humphries.

With their strictly morphology-based research, it appears that the obstacles preventing submission for publication were initially analytical issues likely related to hybridization of the new species with close relatives, and later the illness of Dr. Miller and divergence of the career of Dr. Humphries from ichthyology.

TEXAS AND NORTHEASTERN MEXICO, WITH REMARKS ON
THE STATUS OF ICTALURUS LUPUS

A second

Julian Humphries\* and Robert Rush Miller\*\*

ABSTRACT.— A new species of catfish in the genus Ictalurus is described from the Chihuahuan desert region of the American Southwest. Ictalurus chihuahua has previously been confused with I. lupus, with which it overlaps considerably in geographic range. It is distinguished from that species by a reduced number of anal rays (22-25) and vertebrae (38-42), a weakly forked caudal fin, short and weak pectoral and dorsal spines, broad mouth, deep caudal peduncle, expanded vomer, and unique shape of the mandible, pectoral girdle and pectoral spine. Archeological material dating from 1100 AD has been recovered from a site in the Pecos River basin in New Mexico. Because of confusion between the names Ictalurus lupus and I. vulpes for the species now known as the former, we discuss the taxonomic history of that species and, as first revisors, designate I. lupus as the valid name for the headwater catfish.

#### Methods section of manuscript

- Purely anatomical diagnosis
- Explains they excluded Gila basin specimens from analysis because that population was introduced
  - but elsewhere they noted they were confident the Gila population was mostly pure and that it would likely become the most important for conservation actions
  - The manuscript states they had no clue how/when the species may have been introduced to the Gila basin, and gives no hint that they ever considered the possibility that it may have been a cryptic native of the Gila basin.
- Insights to issues that delayed work are obvious in this section's ending with:

"We have had difficulty in our analyses with specimens that are possible hybrids between *I. chihuahua* and *I. lupus* and between both these species and *I. punctatus*. Based on conventional morphological or meristic evidence alone it is not always possible to be confident whether outlying counts or measurements are due to hybridization or simple variation. We have been cautious in including outliers in our description of *I. chihuahua*, and in comparisons with other species."

#### Robert R. Miller (RRM)

- Bob collected what was to become the type collection for *I. chihuahua* in 1978, but he was apparently aware of the existence of the species before then.
- Wife (Frances Hubbs Miller) died in 1987, and following that Bob suffered from bouts of severe depression until his death in 2003.
- Last large projects were Fishes of Mexico book (published posthumously) and description of Chihuahua Catfish with Humphries.....

## Julian M. Humphries (JMH)

- Doctoral student of Bob's in late 1970s
- 1982 report with Miller to FWS on status of *I. lupus* (probable sister of *I. chihuahua*)
- Post-doc in late 1980s-early 1990s (Univ. New Orleans)
- Museum collection management software developer (Muse) in 1990s
- IT / staff biologist in CT lab at UT Austin late 1990s early 2000s
- Professional photographer by 2000
- Retired and moved from Austin 2015 and "lost" specimens surfaced in his garage

## Mining Miller's files at University of Michigan Museum of Zoology (UMMZ)

- In Dec. 2006 Alejandro Varela (University of Sonora) and I spent nearly a week working in the UMMZ fish collection. He was looking at *I. pricei* complex specimens for his dissertation, and I hoped to find the data set on which the *I. chihuahua* manuscript was based, and other materials likely to be useful for finishing the species description.
- It was clear from correspondence between JMH and RRM found there that the manuscripts were out of sync with the data –specimens (even type) had been added to the ms by RRM but were not included in the data set, analyses or figures and tables being developed by JMH for the manuscript
- No complete data set was ever provided or found. We did scan one hard copy of a data matrix dated 1991 that required considerable cleaning and remains with some ambiguous codes needed to link to localities/catalog numbers. Unfortunately, it turned out to be from the earlier *I. lupus* project, though it did include some specimens later determined to be *chihuahua*.
- We scanned and photographed a massive set of notes documenting RRM's work on the species, figures, labels, x-rays, photographed many specimens, etc.
- THEY WERE CLEARLY VERY CLOSE TO PUBLICATION WHEN WORK ON THE CHIHUAHUA MANUSCRIPT EFFECTIVELY STOPPED
- WHAT WE OBTAINED FROM THE FILES WAS MOSTLY FRAGMENTARY AND DIFFICULT TO MANAGE, BUT OBVIOUSLY POTENTIALLY VERY USEFUL

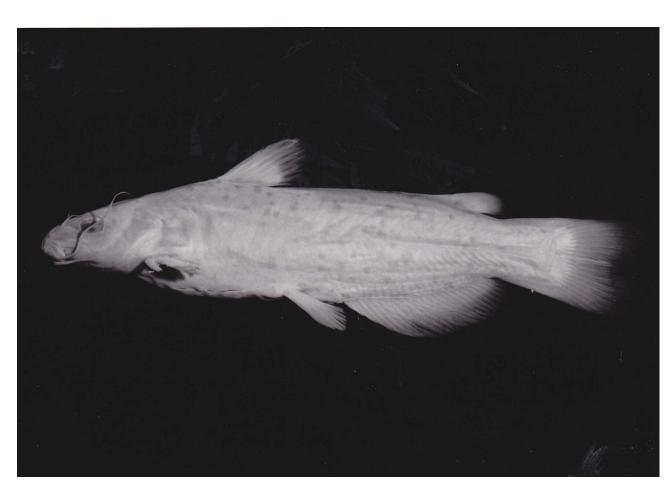
Table 1. Morphometrics of Ictalurus chihuahua. Data are mean percentage SL, standard deviation of the mean, minimum and maximum of the same ratio, actual mm lengths of the holotype. and multivariate allometric coefficient.

						Allometric
Character	Mean	SD	Min.	Max.	Holotype	Coefficient
SL (mm)	103.25	41.27	59.4	256	177.3	0.97
Dorsal-fin oanal fin b.	54.95	1.19	52.78	57.73	95.8	1.02
Dorsal-fin oadipose	50.2	1.56	47.57	53.34	89.6	1.03
Dorsal-fin oanal-fin o.	33.77	1.17	31.93	36.95	59.5	1.04
Pelvic-fin oadipose	39.48	1.29	36.95	41.79	69.6	1.07
Predorsal length	37.16	1.05	35.02	39.1	68.3	0.96
Anal base length	26.22	1.09	24.26	28.7	43.6	1.01
Body Depth	19.46	1.44	16.84	24.06	35.7	1.29
Head length	26.24	0.93	24.5	27.91	47.3	0.97
Head width	20.27	0.95	18.11	22.94	37.2	1.04
Pectoral-fin opelvic-fin o.	25.54	1.14	23.32	28.52	43	1.02
Pectoral-fin odorsal-fin o.	21.5	0.95	19.7	23.64	38.6	1.03
Pectoral-fin oorbit	11.06	0.57	9.85	12.3	20.8	1.03
Pectoral spine length	11.44	0.99	8.52	13.5	18	0.68
Dorsal spine length	11.32	1.38	7.62	14.18	15	0.66
Cleithral process length	9.76	0.82	7.55	11.67	15.2	0.78
Snout length	11.42	0.63	10.38	13.06	20.1	1.05
Fleshly interorbital width	11.52	1.11	9.93	14.39	24.2	1.5
Mouth width	10.87	0.93	9.07	13.8	21.9	1.26
Anal-fin badipose	11.69	0.72	10.25	13.18	21.9	1.2
Caudal peduncle depth	10.89	0.68	8.95	12.42	18.8	1.03
Caudal peduncle length	14.15	0.95	11.65	16.22	25.5	1
Caudal-fin length	14.24	1.22	10.51	17.85	23.5	0.68
Orbit diameter	5.66	0.82	3.4	6.87	8.2	0.34
Premaxilla tooth-plate	6.87	0.76	5.72	8.88	14.6	1.35

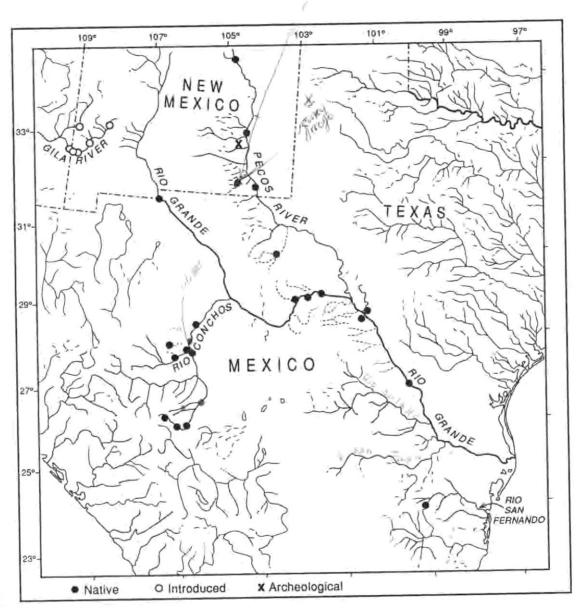
Table 2. Comparison of four species of Ictalurus from Gulf Character	-slope drainages, USA I. chihuahua	and Mexico. Propo	rtions are given as r	mean + S.D.  I. punctatus
Anal-fin rays, range (mean) Typical values	22-26 (23.6) 23-24	23-27 (24.9) 24-26	20-24 (21.7) 21-22	<b>26-32 (29.7)</b> 28-30
Vertebrae range (mean) Typical values	38-43 (40.0) 39-41	39-46 (40.8) 40-42	38-40(39.1)	42-46 (43.9) 9 43-45
Pectoral-fin spine posterior serration	few, non- recurved, short, blunt	many fine, recurved, sharp1	very few, blunt or tiny points	many large, recurved
Caudal forking	shallow	moderate	very shallow	deep
Extent of posterior cleithral process	even with ascending process	slightly past ascending process	even with ascending process	posterior to ascending process
Pectoral-fin spine/mouth width	1.06 + 0.14	1.33 + 0.20	1.04 + 0.11	1.71 + 0.24
Pectoral-fin spine/caudal peduncle depth	1.05 + 0.11	1.30 + 0.11	1.10 + 0.06	1.76 +0.20
Dorsal-fin spine/mouth width	1.05 + 0.18	1.32 + 0.25	1.18 + 0.29	1.77 + 0.19
Dorsal-fin spine/caudal peduncle depth	1.04 + 0.13	1.29 + 0.16	1.23 + 0.21	1.82 +0.18

1 Very large specimens have reduced to worn serrae

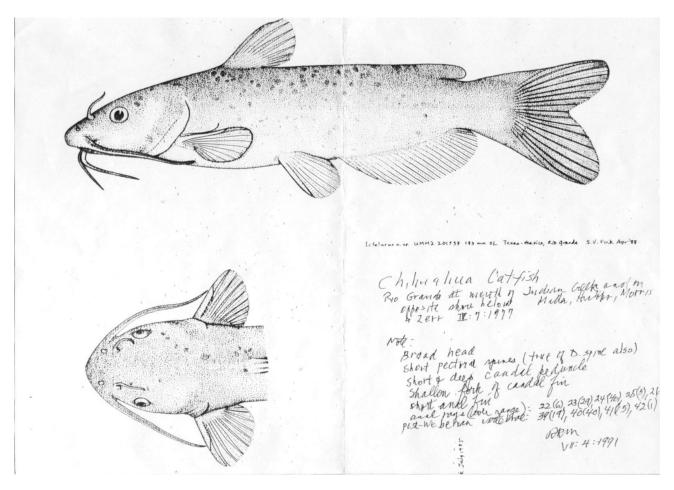




Chihuahua Catfish 133.5 mm SL MSB 376 New Mexico: Gila River, 1 mi E of Arizona border VIII: 23: 1949 Koster & Coburn



Some examples of notes found in RRM's files at UMMZ follow – they are a potentially rich, but not always easily deciphered, source of insights into his knowledge of these catfishes:



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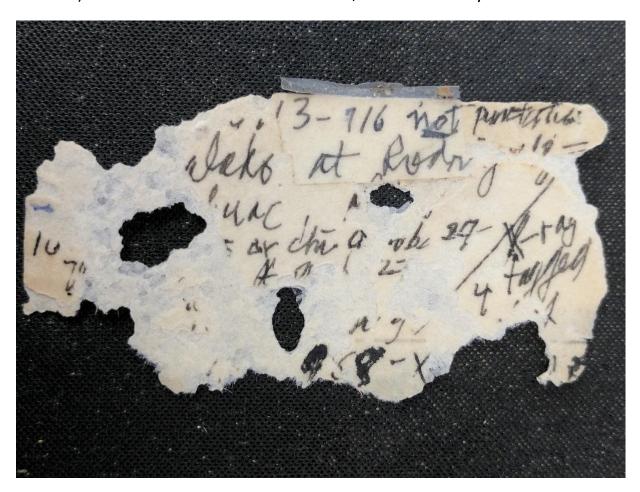
longest rays

Vertebral:

PAM IV:4:88

Some years after we photographed RRM's catfish project notes, the entire file at UMMZ disappeared. So, it's lucky we scanned them when we did.

For many specimens, jar labels are often the only source of data from RRM, and often they are not readily legible. This label was found Dec 2016 in the bottom of a box of specimens retrieved months earlier from JMHs' house just before he moved from Austin. It must have once been taped on (but almost surely was once inside) a jar of *Ictalurus* and fell off. It's clearly RRM's writing, and it's clear he determined it <u>NOT</u> to be *I. punctatus*. It's also clear there's an x-ray of it somewhere and that field (or other) number contains **13-716**, and locality was XXX "at Rodriguez".



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## Between manuscripts and files we now have a list of Chihuahua Catfish types as of 2002, and scattered comments about other specimens

FMNH	100478	1	Rio Camacho at Linares, Tamaulipas	synonym
			Rio San Pedro, trib. to Rio Conchas, at road crossing 7	
			km S of Satev6, Chihuahua, 27°53'N, 106°07'W, elev. 1350 m., 23 May, 1978, R. R. Miller, E. Marsh and M. L.	
UMMZ	214590	1	Smith.	Holotype
UMMZ	203009	28	collected with holotype	Paratopotypes
MSB	3180	1	Pecos R. below Eddy, Loving Co.	Paratypes
UMMZ	66188	1	Middle Berendo Cr. N. of Roswell, Chavez Co.	Paratypes
TNHC	4240	2	Rio Grande, El Paso	Paratypes
TNHC	2037	1	San Felipe Cr., Del Rio, Val Verde Co	Paratypes
UMMZ	201537	1	Rio Grande, mouth Indian Creek, Terrell Co.	Paratypes
UANL	1892	6		
			Rio Florida, Jimenez, Chihuahua	Paratypes
UANL	7121	10	Rio Florida, Saucillo, Chihuahua	Paratypes
UMMZ	203240	3	Rio de Allende, Allende, Chihuahua	Paratypes
UANL	2153	7	Rio de Allende, Allende, Chihuahua	Paratypes
			Rio Florida between El Cristo and Villa Ocampo,	
UMMZ	161739	1	Durango	Paratypes
	64-0859	1	Rio San Pedro Meoqui, Chihuahua	Paratypes
UMMZ	196733	1	Rio San Pedro Meoqui, Chihuahua	Paratypes
UANL	5661	2	Julimes, Chihuahua	Paratypes
FMNH	100478	1	Linares, Nuevo Leon	Paratypes
			Gila drainage: Although we do not formally list these	
			specimens as types because their origin is uncertain	
			there is no question that most of those from the Gila	
			River are I. chihuahua. How they got into that drainage	
MSB	unspecified		is a puzzle discussed below	Nontype also examined
			Pecos drainage, Pecos. R. below Summer Reservoir,	
MSB	7778	1	DeBaca Co. 1981	Nontype also examined

RRM early on developed a still useful key to Ictalurus that included *I.* chihuahua, though that species was omitted from the key in Fishes of Mexico book

Fo	OR	PERSONAL USE ONLY - NOT TO BE REPRODUCED	
		PERSONAL USE ONLY - NOT TO BE REPODICED  BONGELLE  X:3:1989	
		X:3:1989	
		Artificial Key to Mexican Species of <u>Ictalurus</u>	
1	la.	Premaxillary teeth with lateral backward projection	
		Balsas catfish, <u>I</u> . <u>balsanus</u> (Fig. 00)	
1	b.	Premaxillary teeth without lateral backward projection2	
2	a.	Distal margin of anal fin straight; swim bladder	
		3-chambered; anal rays 28-38	
		Blue catfish, <u>I</u> . <u>furcatus</u>	
2	b.	Distal margin of anal fin rounded; swim bladder 2-chambered;	
		anal rays 20-323	
3	a.	Basal length of anal fin subequal to or greater than head	
		length; anal rays 26-32; post-Weberian vertebrae 43-47;	
		supraoccipital spine in firm contact with supraneural	
		Channel catfish, <u>I</u> . punctatus	
3	b.	Basal length of anal fin less than head length; anal rays	
		20-27; post-Weberian vertebrae 33-44; supraoccipital spine	All Mexican <i>Ictalurus</i>
		and supraneural not in contact4	
4	a.	Length of dorsal spine greater than fleshy interorbital	
		width; pectoral spine of juvenile with few and weak serrae	
		(2-3), these lacking or with only vestigial nubs in adult	
		Panuco catfish, <u>I</u> . <u>mexicanus</u>	
4]	b.	Length of dorsal spine equal to or subequal to fleshy	
		interorbital width; pectoral spine of juvenile to adult	
		with well developed serrae (5-14)*5	
*:	fee	ble in the <u>I</u> . <u>dugesi</u> -type from the upper Río Balsas (E of	
	Cu	autla) USNM 130895.	

5a. Gillrakers 16-24; pectoral fin-rays 11
Yaqui catfish, <u>I</u> . <u>pricei</u>
5b. Gillrakers 11-17; pectoral fin-rays 9 or 10 (rarely 8)6
6a. Caudal peduncle depth 0.9-1.1 in pectoral-spine length. Rio
Grande from near Laredo to El Paso, Pecos River to above
Roswell, and Río Conchos basin
<u>Ictalurus</u> n. sp., Page 00
6b. Caudal peduncle depth 1.2-1.6 in pectoral-spine length7
7a. Gill rakers 13-17, long8
7b. Gill rakers 11-13, short. Pacific slope, rheophilic in the
Río Lerma basin
<u>I</u> . <u>dugesi</u> , Page 00
a. Serrae of pectoral spine weak, vestigial or absent in adult.
Pacific slope, in Lake Chapala and Río Ameca basin
<u>I</u> . <u>ochoterenai</u> , Page 00
b. Serrae of pectoral spine well developed in juvenile to
adult. Atlantic slope in Río Grande basin, below Río
Conchos

Note inclusion of

geography

Yaqui cat

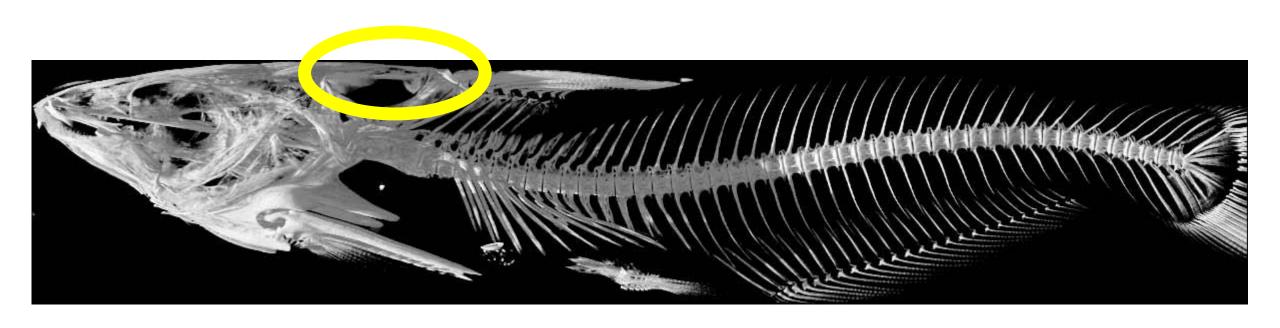
Chihuahua cat

Headwater cat

I. lupus, Page 00

#### Chihuahua Catfish -

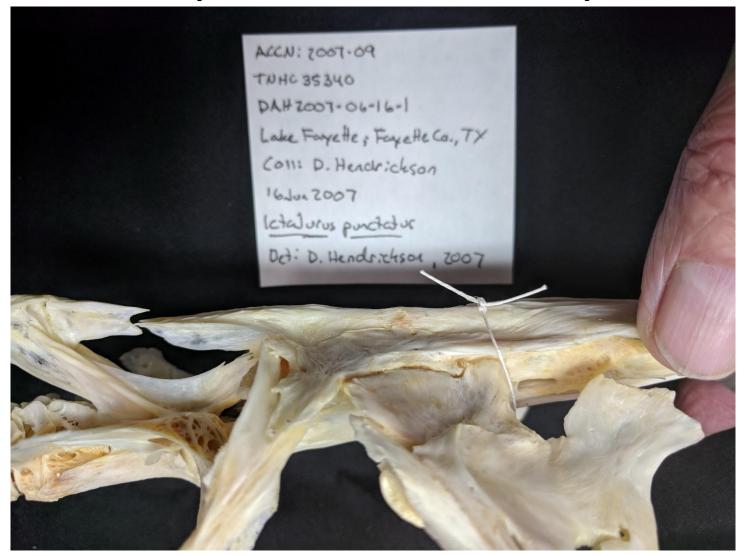
- The 2002 ms does not mention it, but Miller's notes and the older key do – these bones are <u>not in contact in any of the native Mexico</u> <u>species</u>
- X-rays are available of many Chihuahua Cat specimens, but they are clearly not optimal for visualizing such thin sheets of bone, which are much more easily seen in C&S, dry skeletons and CT
- However, the character can be easily evaluated on any specimen by pressing at short intervals between the dorsal spine and skull with the edge of a thumbnail positioned transversely. Gaps, even very small ones, can be sensed this way, even on quite small specimens.



Ictalurus punctatus, hatchery strain, A.E. Woods State Fish Hatchery, San Marcos, Texas (CT scan)



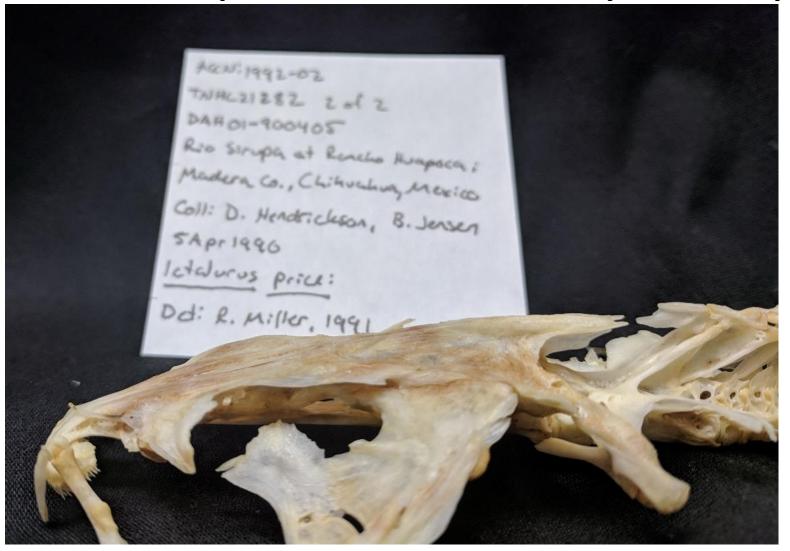
Ictalurus punctatus, hatchery strain, A.E. Woods State Fish Hatchery, San Marcos, Texas



*Ictalurus punctatus,* Lake Fayetteville, Texas



Ictalurus punctatus – Colorado River at Smithville, TX



Ictalurus pricei - Rio Yaqui basin



Ictalurus lupus - TNHC 24963 - Rio Grande near Hot Springs Rapids

#### More recently:

- Jim Brooks in 2006 collected 8 catfish specimens from near Nonoava, not far upstream from the manuscript's type locality, took fin clips, deposited vouchers and tissues in UNISON and Varela later published the resulting Cyt-b sequence of "I. sp." in GenBank.
- Dave Propst provided an unvouchered sequence from the East Fork of the Gila basin

 In 2011 JMH provided DAH hard copies of 4 versions of the manuscript and authorization to proceed with work that would culminate in eventual publication of the species description The 2006 Nonoava specimens seemed to fit the manuscript description quite well, as well as supported additional details gleaned from RRM's notes, such as:



The body of Chihuahua Catfish appears more round in cross-section than I. punctatus, but this is hard to quantify with standard 2-D morphology. 3-D scans could be very helpful for quantifying this and other characters.



Ictalurus punctatus (here from Guadalupe drainage, TX) have flatter sides



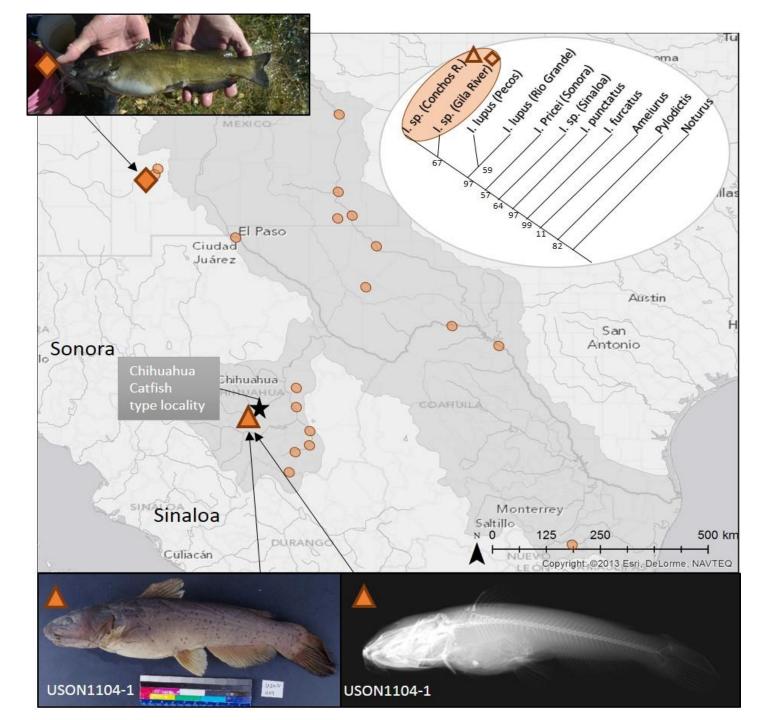
Nonoava Chihuahua Cats (here JEB 2006 collection) are more rounded in cross-section

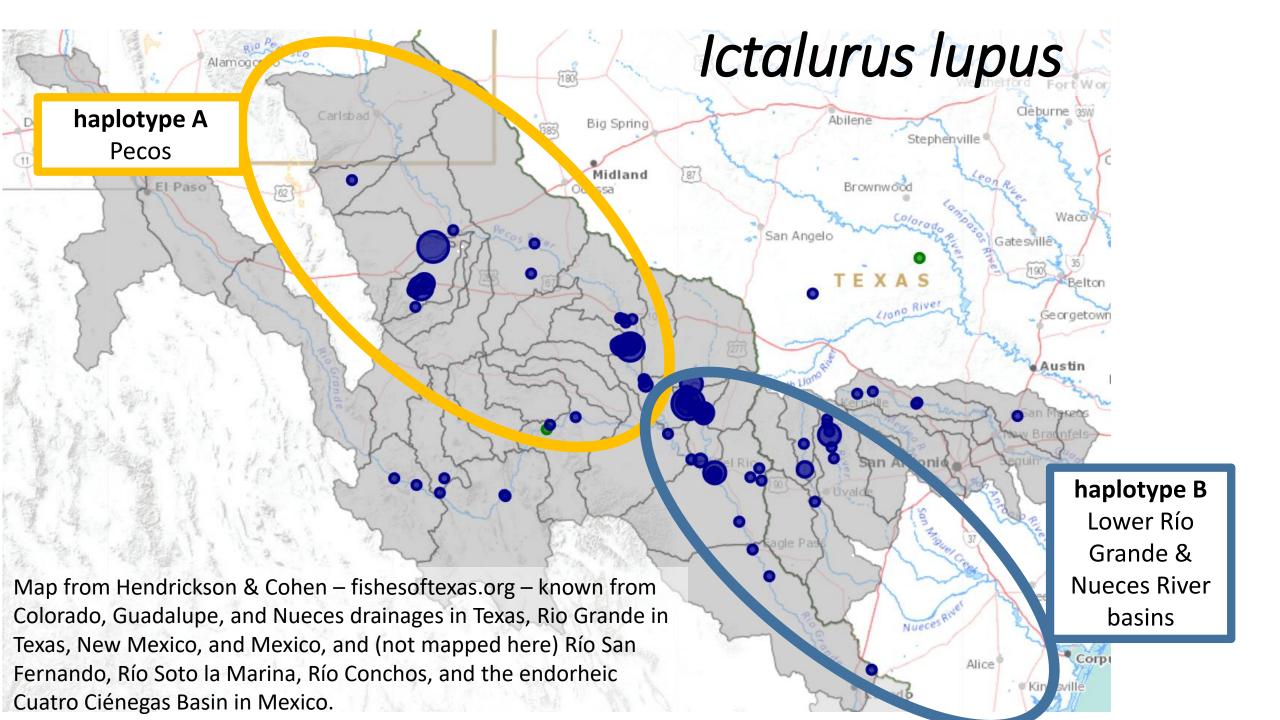
But perhaps more immediately useful, tissues from Nonoava and the Gila basin specimens were sequenced and used to produce a preliminary phylogeny: Solid circles mark occurrences of Chihuahua Catfish, based on specimens examined & identified by RRM and listed in the unpublished manuscript. RRM chose all specimens that he collected in 1979 at the black star (Nonoava, Chihuahua) as the species' type series (holotype and 28 paratypes). Presumed native range of Chihuahua Catfish is shaded grey.

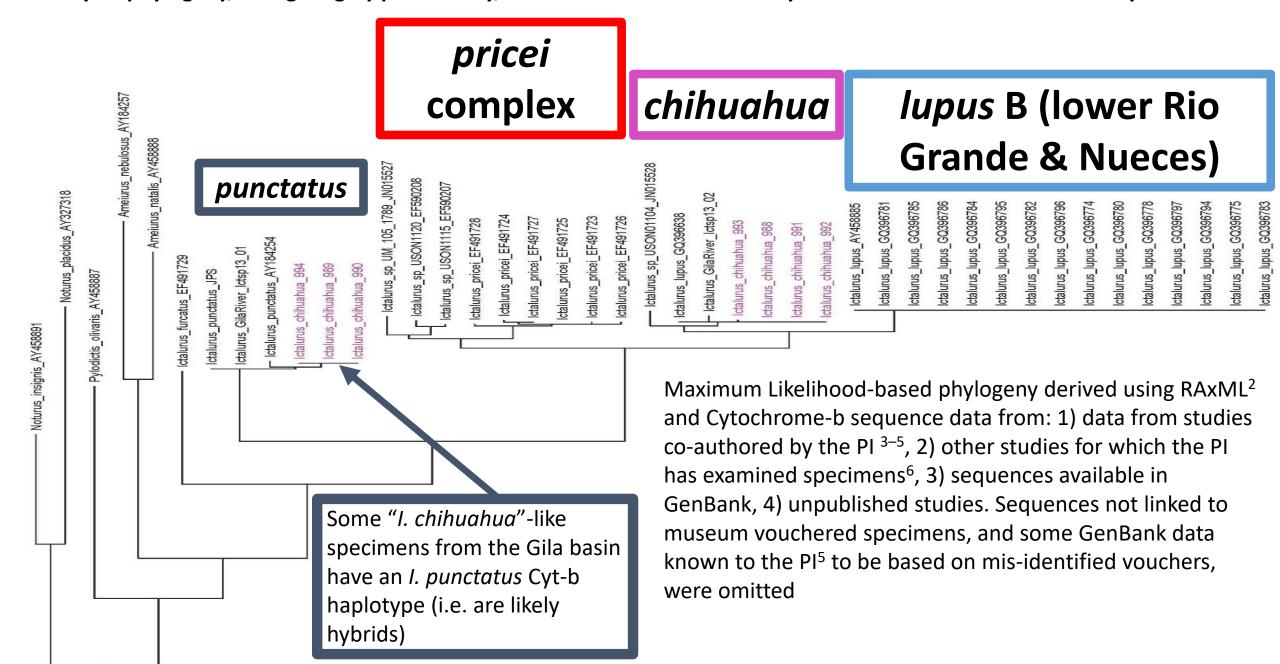
More recent collections for which DNA (Cyt-b) sequences are available are marked by orange symbols: triangle is a 2006 collection (USON 01104 ("I. sp. (Conchos R)" in the phylogeny). About 70 km upstream of the manuscript's type locality, these specimens diagnose morphologically as Chihuahua Catfish. Sequence (GenBank JN015528) is from one of the 9 specimens. Diamond marks location of a fin clip taken in 2013 by Dr. David Propst from an unidentified catfish before releasing it. Tyler Pilger provided unpublished Cyt-b sequence.

The simplified Maximum Likelihood-based phylogeny was derived using RAxML2 & Cyt-b sequence data for specimens mentioned above and from: 1) studies co-authored by DAH, 2) other studies for which DAH has examined specimens, 3) sequences available in GenBank. Sequences not linked to museum voucher specimens (excepting 2013 Gila specimen above), and some GenBank data, known by DAH to be based on incorrectly identified vouchers, were omitted.

Note close relationship of Gila and Nonoava specimens, and together their sister relationship to *Ictalurus lupus*, known from other studies (see below) to be comprised of two clades.







2010. (2015)Propst - vouchers & tissues at TNHC Echelle. OSU vouchers & tissue/DNA vouchers Brooks (2013)Unpublished, provided by Propst TNHC - (Pecos) - McClure Fork Gila, NM E. Fork Gila, tissues on loan @ Dexter NFH. 1104) vouchered - USON chihuahua" 06-021-1)sp." I. Iupus A haplotype. I. lupus B - San Felipe Creek - Rio Grande, TX -Willcox et al. 2004 - voucher TNHC29350-1 lctalurus\_sp\_UM\_105\_1789\_JN015527 lctalurus\_sp\_USON01104\_JN015528 I. lupus B - (Lower Rio Grande + Nueces) - Bean et lctalurus\_sp\_USON1120\_EF590208 al. 2011 - vouchers at Texas A&M, tissues at TX State lctalurus\_GilaRiver\_Ictsp13\_02 lctalurus\_lupus\_GQ396785 ctalurus\_lupus\_GQ396795 lctalurus\_lupus\_GQ396782 lctalurus\_lupus\_GQ396796 lctalurus\_lupus\_GQ396780 lctalurus\_lupus\_GQ396778 lctalurus\_lupus\_GQ396797 lctalurus\_lupus\_GQ396781 lctalurus\_lupus\_GQ39678 ctalurus\_lupus\_GQ39677 lctalurus\_lupus\_GQ396638 lctalurus\_pricei\_EF491724 lctalurus\_pricei\_EF491726 ctalurus\_pricei\_EF491728 Ictalurus\_pricei\_EF491727 lctalurus\_pricei\_EF491723 ctalurus\_pricei\_EF491725 ctalurus\_chihuahua\_993 ctalurus\_chihuahua\_988 ctalurus\_chihuahua\_991 lctalurus\_chihuahua\_992

not

at

The chihuahua clade includes both Nonoava and Gila basin specimens that look morphologically like chihuahua, and is clearly distinct from the lupus B clade, but nested in it is the lupus A

ctalurus\_lupus\_

The Cyt B data provide evidence of *chihuahua* Gila River, NM provided by Propst 93904. Mud x punctatus hybridization in the Gila basin River, Kentucky E. Fork Gila River, NM provided by Propst vouchered - INHS vouchers/tissue I. punctatus ctalurus\_sp\_USON1115\_EF590207 Ictalurus\_sp\_USON01104 ctalurus\_lupus\_GQ396638 ctalurus\_pricei\_EF491726 ctalurus\_pricei\_EF491723 Ictalurus\_pricei\_EF491727 ctalurus chihuahua 992 lctalurus\_chihuahua\_991 AY184254 Ictalurus\_chihuahua\_989 ctalurus\_chihuahua\_990

lctalurus\_lupus\_GQ396795 lctalurus\_lupus\_GQ396782 ctalurus\_lupus\_GQ396780 lctalurus\_lupus\_GQ39677 lctalurus\_lupus\_ And perhaps *chihuahua x lupus* hybridization. This is *I. lupus* A (Pecos) of McClure et al 2010, who had specimens from San Solomon Spring, Independence Ck., Delaware River and elsewhere, including some from Rocky Arroyo, NM, which they remarked "were morphologically chihuahua except had pectoral spine dentation of *lupus*". On the basis of the DNA, they chose to call them *lupus* instead of *chihuahua*.









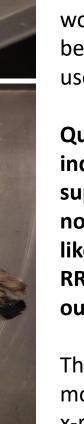
#### Yaqui Catfish

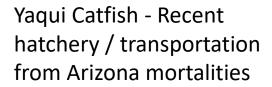
Recent hatchery/transportation mortalities from Arizona:

- large tissue samples taken from all are now archived in liquid nitrogen.
- Otoliths extracted from all and most now aged
- Microchemistry analyses of otoliths proposed
- Most had PIT tags to link to hatchery / refuge monitoring records
- Very heavy parasite loads in many









These were all descendants of the original Dexter broodstock developed with help of Bob Miller, x-rays, morphology, and allozyme work. Only those judged to be "pure" Yaqui Catfish were used in production.

Quick examination of most indicated Supraoccipital – supraneurals consistently do not contact in these. Looks like the old genetics and RRM's expertise helped sort out the hybrids.

They are available for morphological analyses and x-ray/CT/ 3D scanning.







#### Conclusions

- The chihuahua/lupus complex appears to share a lot with its sister, the I. pricei complex
  - Hybridization with *punctatus* (and for *chihuahua*, with *lupus* as well) complicates species diagnosis, as well as conservation actions
  - Genetic distances among taxa within both complexes is comparable
- Chihuahua Cat still exists in the US
  - In East Fork of Gila River, where not common, and somewhat sporadic and apparently restricted to a relatively short reach (Dave Propst, pers. comm), specimens believed to be of this species have been collected relatively regularly in annual sampling. Voucher specimens are relatively few, however.
- Status in US portions of Río Grande basin unclear
  - The JMH/RRM manuscript hypothesized it is likely extirpated here. Last TX specimens were collected in 1980 in a Pecos trbutary, but photos of more recent, released specimens indicate the species may still be present in the Pecos basin of Texas.
- Still very little information on basic ecology of chihuahua/lupus/pricei.
  - Nonoava and upper Gila, where Chihuahua Cat have been recently taken, are not where many of us who collected
    the old occurrences records (all at lower elevations) two or more decades ago would have been likely to go looking
    for these species.
- Their habitats are clearly very different from where historic *I. pricei* refuge populations were attempted.

#### **Future**

- We (Hendrickson lab) now have most specimens of Chihuahua Cat that RRM & JMH examined (and more) on loan
- Seeking student salary to permanently archive and make openly available digital copies of RRM's collection of notes, figures, manuscripts, x-rays, etc. for catfishes and share them via Fishes of TX project (where comments are possible). Could include hatchery records perhaps.
- Reasonable numbers of specimen-vouchered tissues appear to be already available for each chihuahua, lupus and certainly punctatus, for a reasonable genetics analysis (SNP-based) that would be informative regarding hybridization, though some limited additional collecting would likely be helpful.

#### • We hope to:

- CT and/or 3D surface scan select specimens for 3D morphometrics
- Describe species on basis of SNP results and morphology of selected, genotyped specimens
- Investigate use of eDNA could be a big help for detecting remnant populations, but likely not so useful for determining the distribution of pure versus hybrid populations
- Produce Species Distribution Models for genotyped *chihuahua, lupus, pricei, punctatus* and hybrids to help get at habitat questions
- Utilize the now large number of *I. pricei* specimens available in diverse ways (DNA, parasites, otolith microchemistry, further osteological studies)