A quick overview of knowledge relevant to conservation of the North American blind catfishes of the Edwards Aquifer

Dean A. Hendrickson
Curator of Ichthyology
Biodiversity Center, Texas Natural History Collections (TNHCi)
University of Texas at Austin
Austin, Texas 78758 USA
deanhend@austin.utexas.edu

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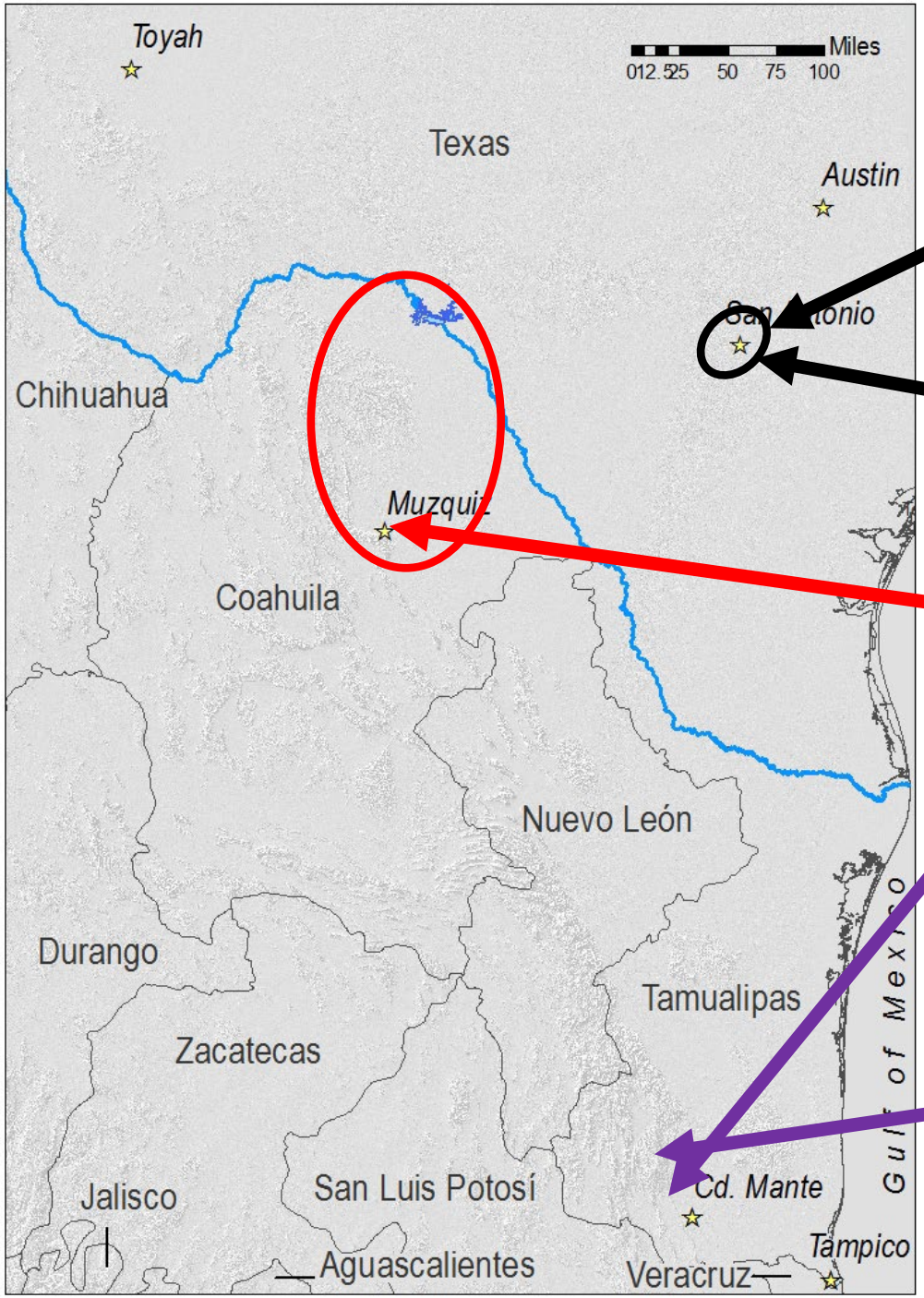


Blind Ictalurids Discovery History

1919 – *Trogloglanis pattersoni* - Eigenmann, C. H. *Trogloglanis pattersoni* a new blind fish from San Antonio, Texas. *Proceedings of the American Philosophical Society*, *58*(6), 397–400. https://doi.org/10/gjcr6n

1931 – *Satan eurystomus* - Hubbs, C. L., & Bailey, R. M. Blind catfishes from artesian waters of Texas. *Occasional Papers of the Museum of Zoology, University of Michigan, Ann Arbor, Michigan*, *499*, 1–17.

1954 – *Prietella phreatophila* - Carranza, J. Descripción del primer bagre anoftalmo y depigmentado encontrado en aguas Mexicanas. *Ciéncia, México*, *14*(7–8), 129–136.

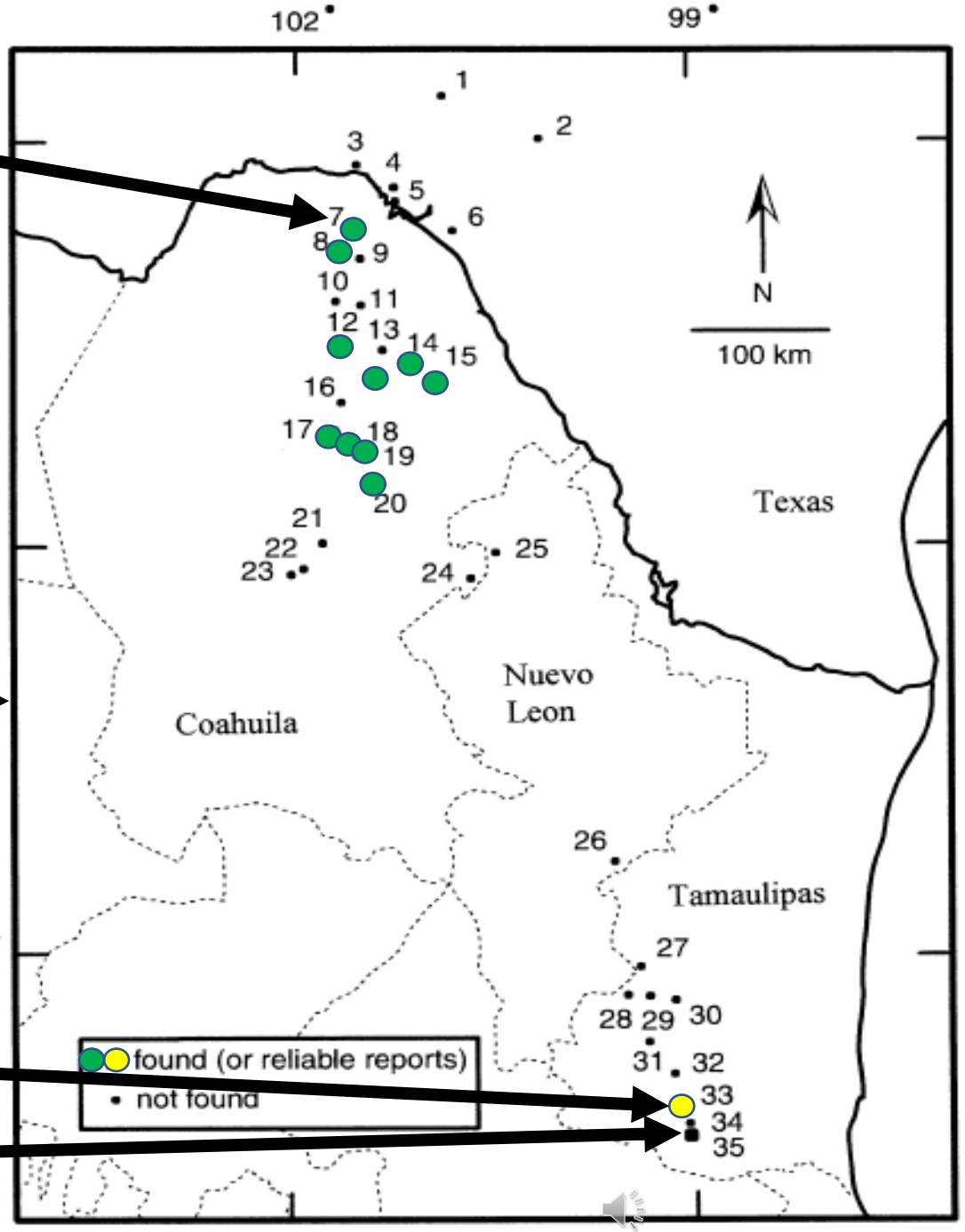
1989 – *Prietella lundbergi* - Walsh, S. J., & Gilbert, C. R. (1995). New Species of Troglobitic Catfish of the Genus Prietella (Siluriformes: Ictaluridae) from Northeastern México. Copeia, 1995(4), 850–861.

- 1 specimen – formalin preserved. Many dives seeking more were unsuccessful

1997 – *New genus new species* – 4 specimens from Cueva Nacimiento del Río Frio (CNRF)

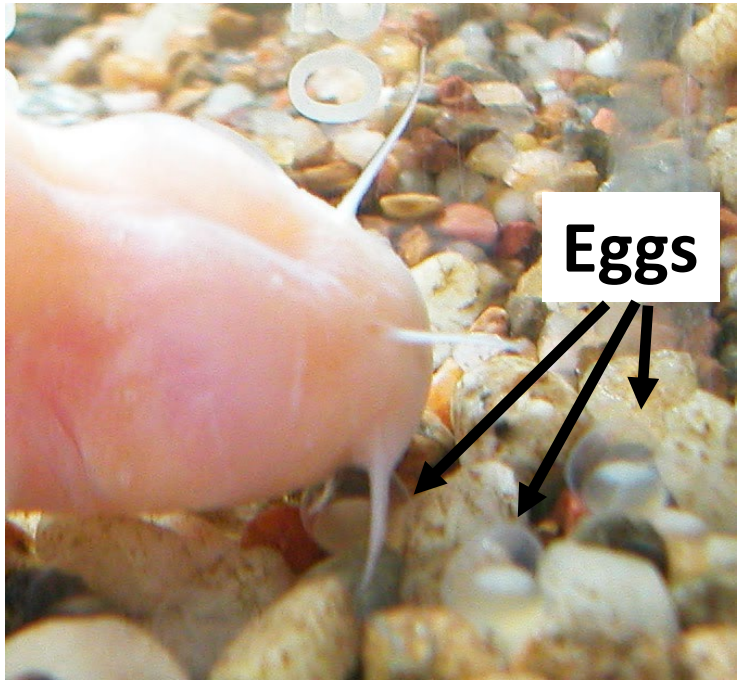
Hendrickson's blindcat addiction:

- **1992 - 6 live specimens ("entry drug") from here were left at my office door**
- **1993, 1994, 1996-8 - fieldwork in México**
- **1992-2016 - Captive stock *P. phreatophila***
- **2001** Hendrickson, D. A., Krejca, J. K., & Rodríguez Martínez, J. M. (2001). Mexican blindcats, genus Prietella (Ictaluridae): An overview of recent explorations. Environmental Biology of Fishes, 62(1–3), 315–337 https://doi.org/10.1023/A:1011808805094
- **2004** Wilcox, T. P., García de León, F. J., Hendrickson, D. A., & Hillis, D. M. (2004). Convergence among cave catfishes: Long-branch attraction and a Bayesian relative rates test. *Molecular Phylogenetics and Evolution*, *31*(3), 1101–1113. https://doi.org/10.1016/j.ympev.2003.11.006:
  - Cueva Nacimiento del Río Frio specimens are ***NOT*** *Prietella*
  - *Prietella lundbergi* (only 1 formalin specimen)



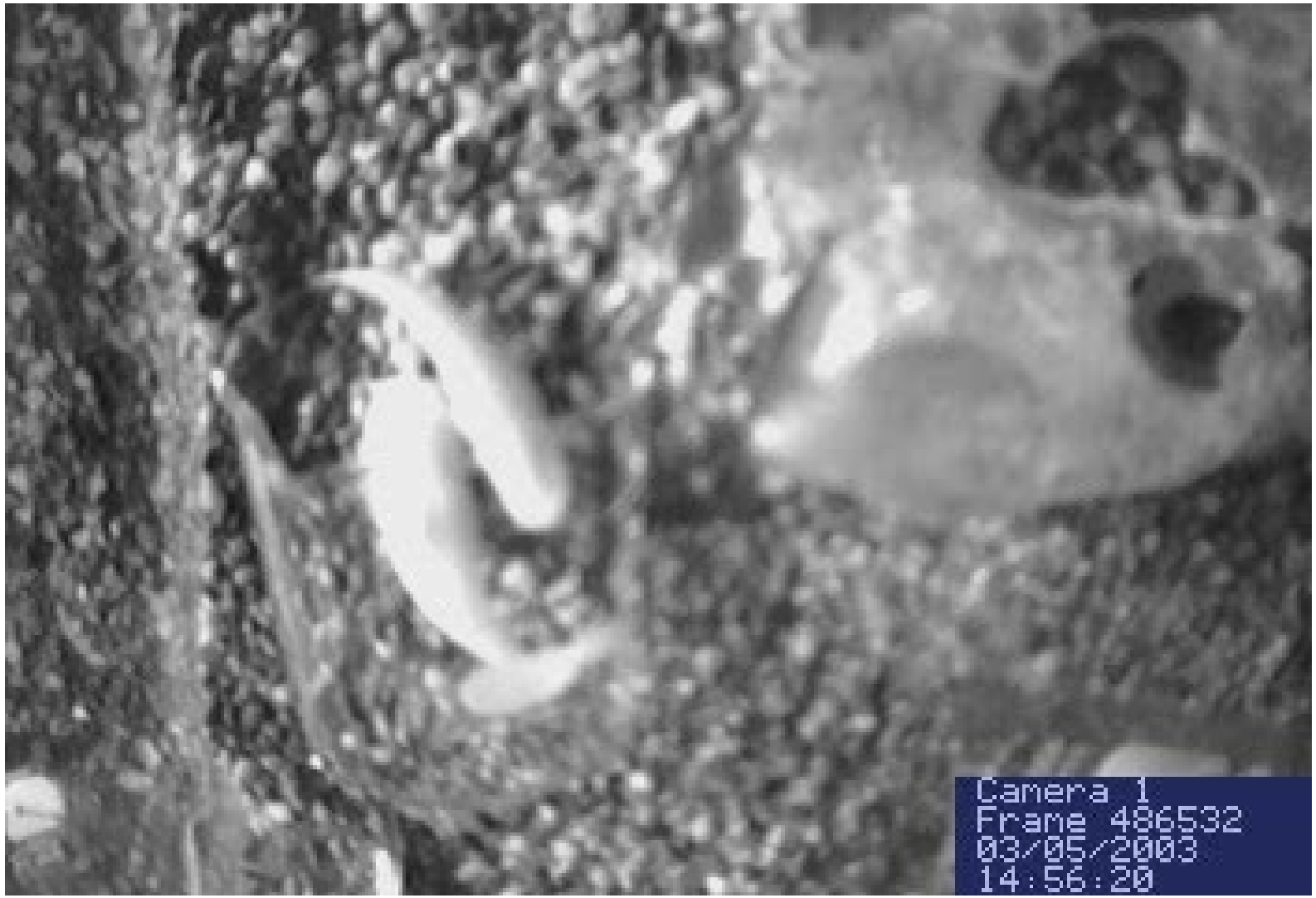
Observations from 25 years hanging out with *P. phreatophila*

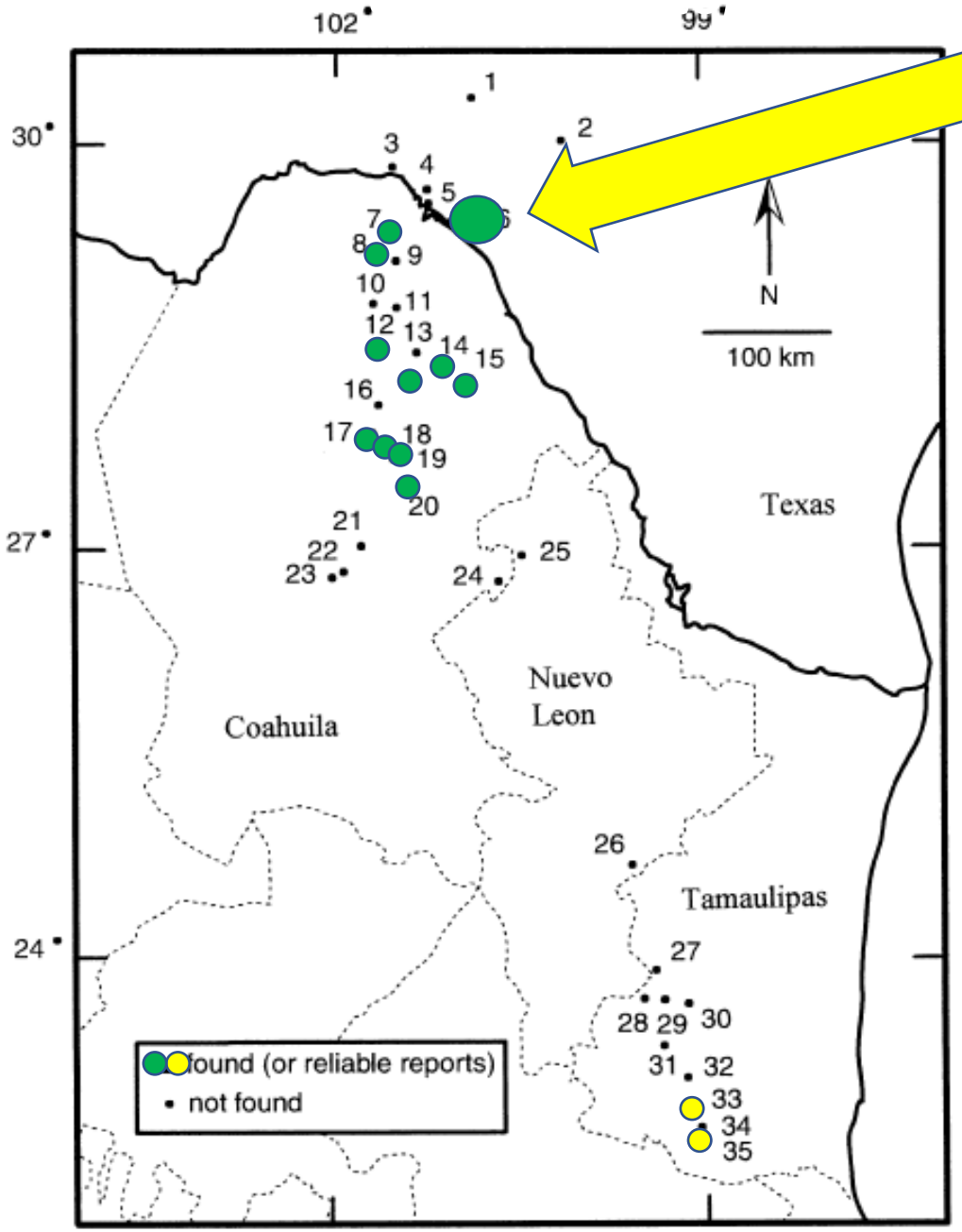
- Male dominance contests?
- Courtship
- Spawning, guarding, no hatching
- Starvation tolerance (44 months)
- Landscape memory mapping
- No reaction to visible light
- Long-lived (> 30 yrs in lab)



Taking a break (this one 16 minutes)

Courtship



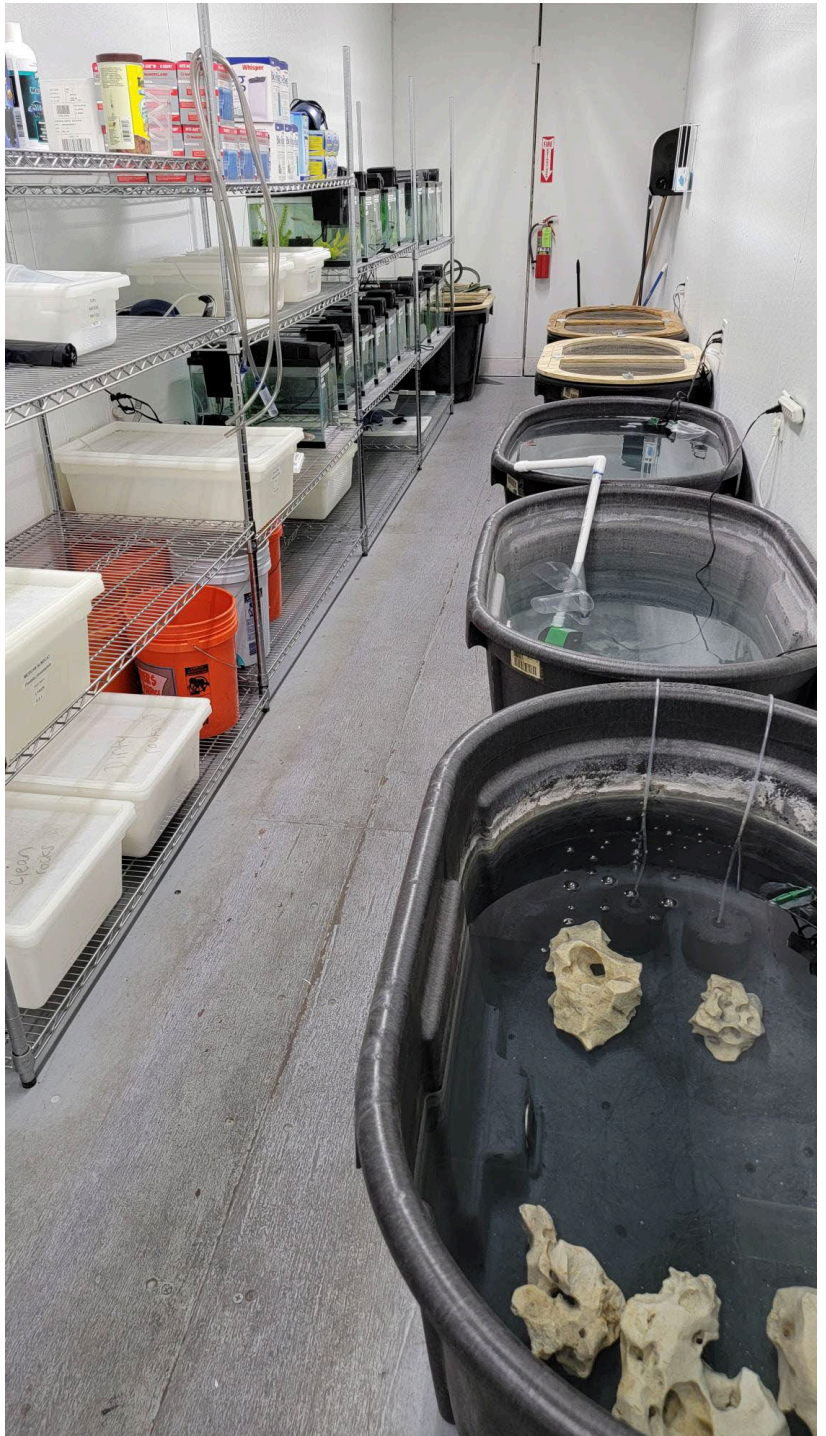


2015-2016 - new Texas locality

- **May 11, 2016 - 3 specimens collected in cave of original sighting**
- **Live specimens to UT Austin Fish Collection (TNHC)**

It immediately had full protection as Endangered in U.S.

Morphology & Cyt b sequence validated conspecificity of Texas and Coahuila specimens (1% divergent)



Summer 2016 – 2 remaining live Texas specimens transferred on loan to San Antonio Zoo Center for Conservation & Research, followed by both remaining live 20+ year-old specimens from Coahuila, then later by more Texas specimens

Summer 2022 – the Zoo's new dedicated blindcat lab opened

Center for Conservation & Research

Satan eurystomus
(Widemouth Blindcat)

20 known specimens

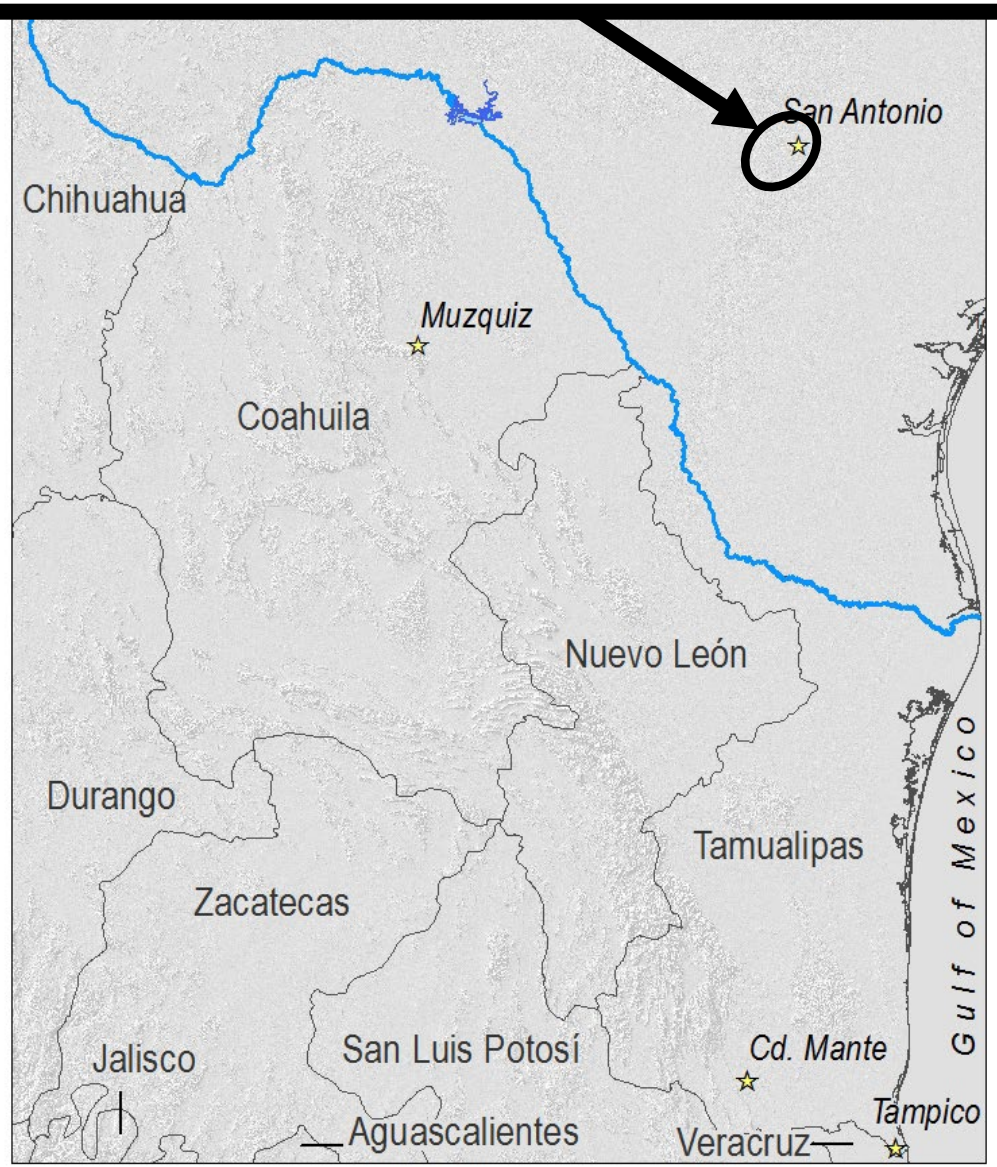
Max TL = 140 mm
Predator

Trogloglanis pattersoni
(Toothless Blindcat)

43 known specimens

Max TL = 104 mm
bacterial/fungal grazer

Satan eurystomus Hubbs and Bailey 1947 - Widemouth Blindcat
Trogloglanis pattersoni Eigenmann 1919 - Toothless Blindcat



Known only from wells 700 to >1400 ft deep under San Antonio, Texas

Late 1800's – San Antonio River spring-fed but water well drilling was catching on quickly and artesian flows diminishing

By 1911 deep gushers in/around SA were spewing blind fishes

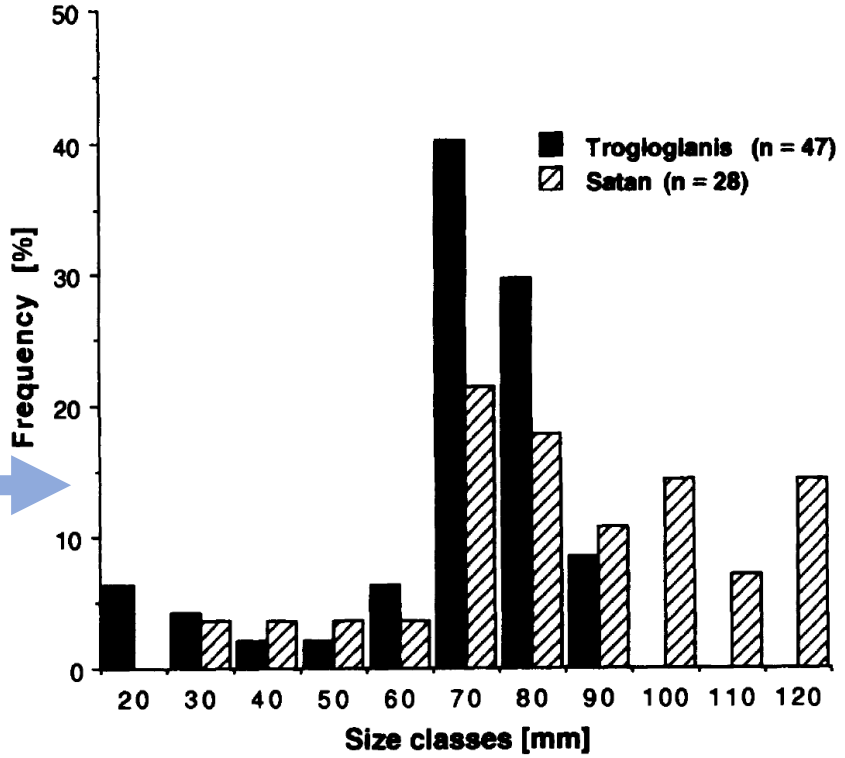
By 1930s SA was (and still is) mostly dependent on groundwater (SA springs now flow very rarely)

1950s – ***many*** new wells started going in

1977 - USFWS funded well biodiversity survey using nets over well outlets, producing majority of existing specimens (Longley and Karnei 1978 reports)

1977-78 collections produced basic biological insights available in:

- Karnei Jr., H. S. (1978). A survey of the subterranean aquatic fauna of Bexar County,Texas [M.S., Texas State University, San Marcos]. https://catalog.library.txstate.edu/record=b1036939~S1a
- Longley, G., & Karnei Jr., H. S. (1978a). Status of *Satan eurystomus Hubbs and Bailey*, the Widemouth Blindcat (contract #14-16-0002-77-035; p. 55). U.S. Fish and Wildlife Service. http://www.edwardsaquifer.net/pdf/Widemouth_Blindcat_by_Karnei_and%20Longley_1978.pdf
- Longley, G., & Karnei Jr., H. S. (1978b). Status of *Trogloglanis pattersoni Eigenmann,* the Toothless Blindcat (contract #14-16-0002-77-035; p. 61). U.S. Fish and Wildlife Service. http://www.edwardsaquifer.net/pdf/Toothless_Blindcat_by_Karnei_and_Longley_1978.pdf
- Langecker, T. G., & Longley, G. (1993). Morphological Adaptations of the Texas Blind Catfishes *Trogloglanis pattersoni* and *Satan eurystomus* (Siluriformes: Ictaluridae) to Their Underground Environment. *Copeia*, *1993*(4), 976–986. https://doi.org/10.2307/1447075



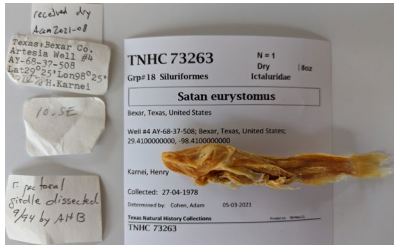
1977-78 specimens still comprise the majority of all specimens, but

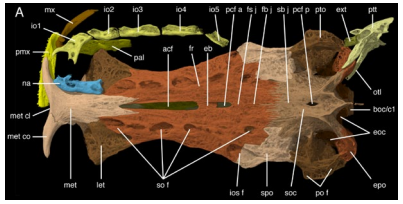
- Unfortunately, specimens from the 1978-contracted USFWS survey were stored permanently in formalin (de-ossifies), extensively dissected, and/or used in destructive analyses and extracted parts (e.g. ovaries, guts) were lost. Thus, most specimens were severely degraded and/or lost by 2022, when they were deposited at TNHCi.
- We continue working on cleaning data for all specimens (and anecdotal occurrences) across all collections, and our collections data and images in GBIF are updated weekly.
- Subsequent specimens (e.g. Zara 2010 and 2014, and USFWS until 2023) were also deposited at TNHCi.
- TNHCi specimens are available on loan to qualified researchers following UT policies.

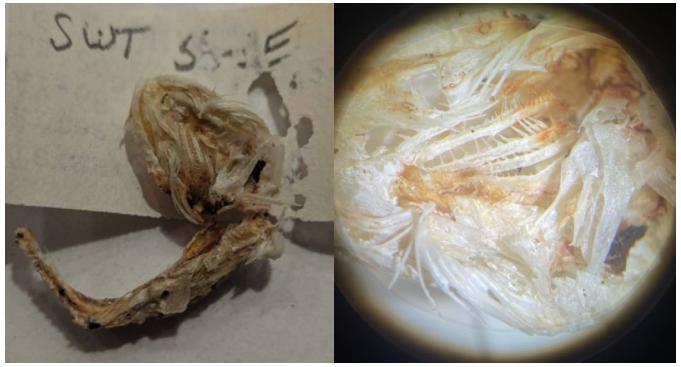
TNHCi specimens in GBIF

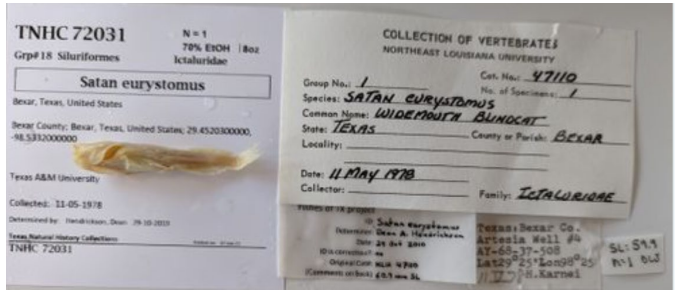
Satan:

https://www.gbif.org/occurrence/gallery?taxon_key=2341042

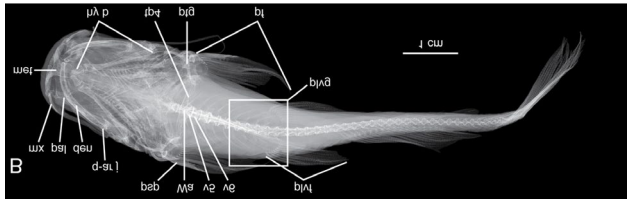






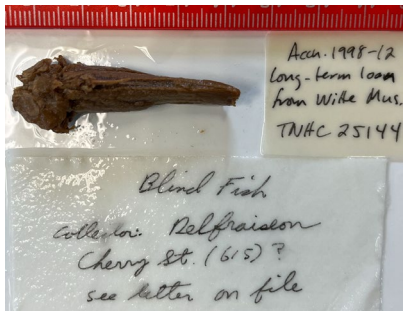


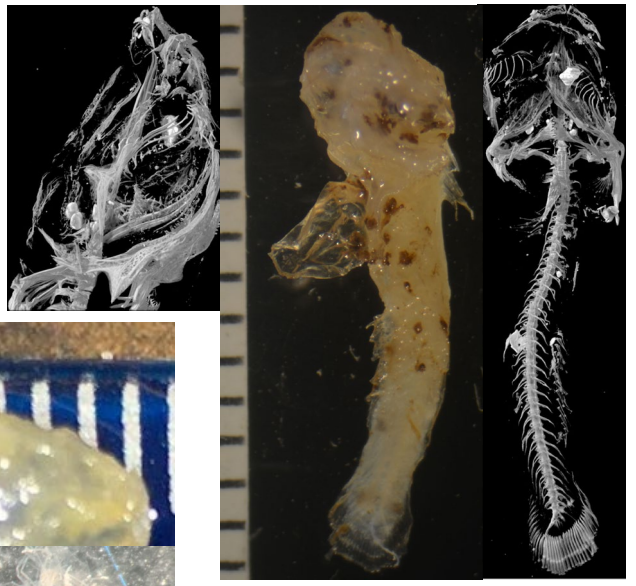
Title: Satan eurystomus RAD119285-001
Creator: Division of Fishes
Publisher: Smithsonian Institution, NMNH, Fishes
Source: Division of Fishes NMNH Smithsonian Institution

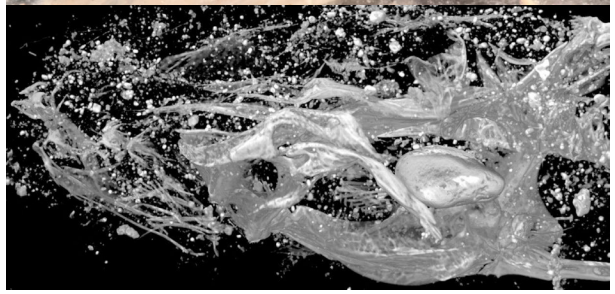


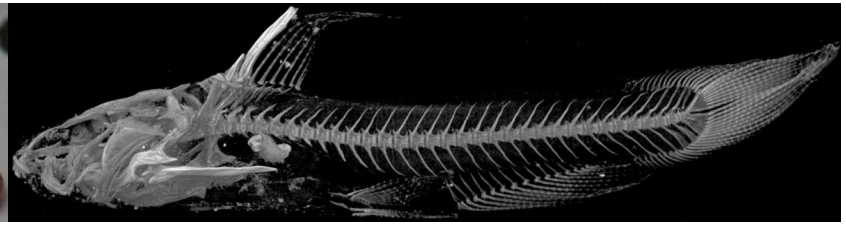
Trogloglanis:

https://www.gbif.org/occurrence/gallery?taxon_key=2341042









Satan & Trogloglanis specimen data curation

- 6 institutions hold specimens. All from the 1978 survey were transferred to UT Austin and catalogued in TNHCi

	Year	1944	1947	1953	1960	1977	1978	1980	1983	1984	2008	2009	2010	2011	2012	2013	2020	2021	????	TOTALS
CAS	*Trogloglanis*																		1	1
SIO	*Trogloglanis*	1																	1	2
TCWC	*Satan*									3										3
TCWC	*Trogloglanis*									2										2
TNHC	*Satan*		1	1		1	7												1	11
TNHC	*Trogloglanis*					1	7	1	3		1	1	6	5	3	1	1	2	3	35
UMMZ	*Satan*																		1	1
UMMZ	*Trogloglanis*																		3	3
USNM	*Satan*				2														2	4
	Totals																			
	Satan	-	1	1	2	1	7	-	-	3	-	-	-	-	-	-	-	-	4	**19**
	Trogloglanis	1	-	-	-	1	7	1	3	2	1	1	6	5	3	1	1	2	8	**43**

1919 and 1931

Now known to be 1931...1947

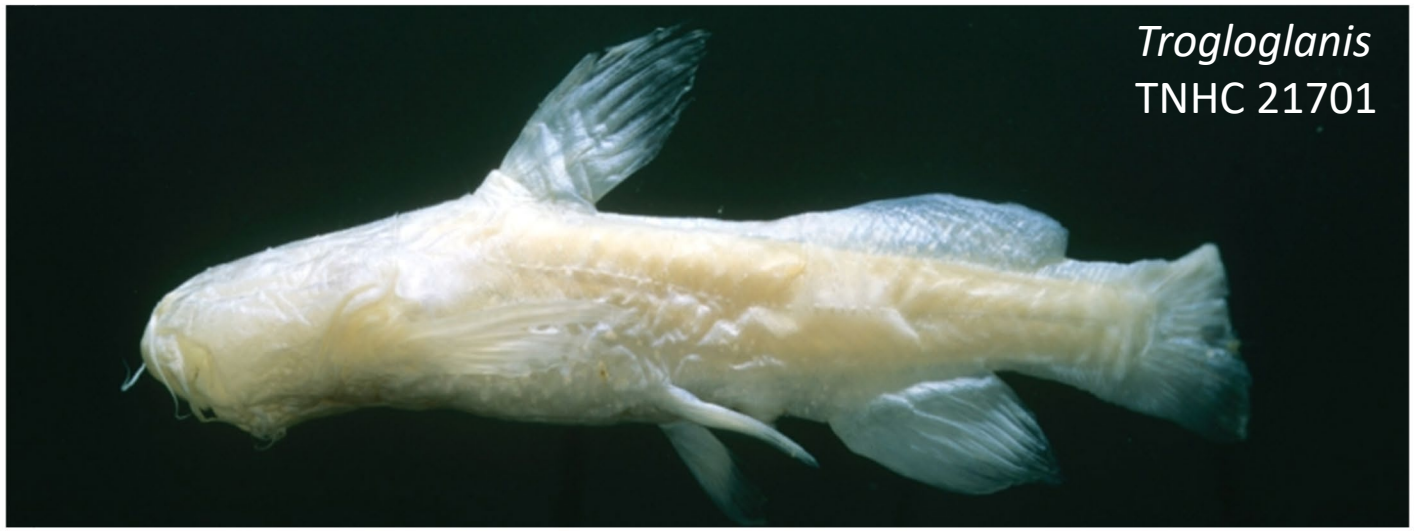
Longley says 11 S & 22 T

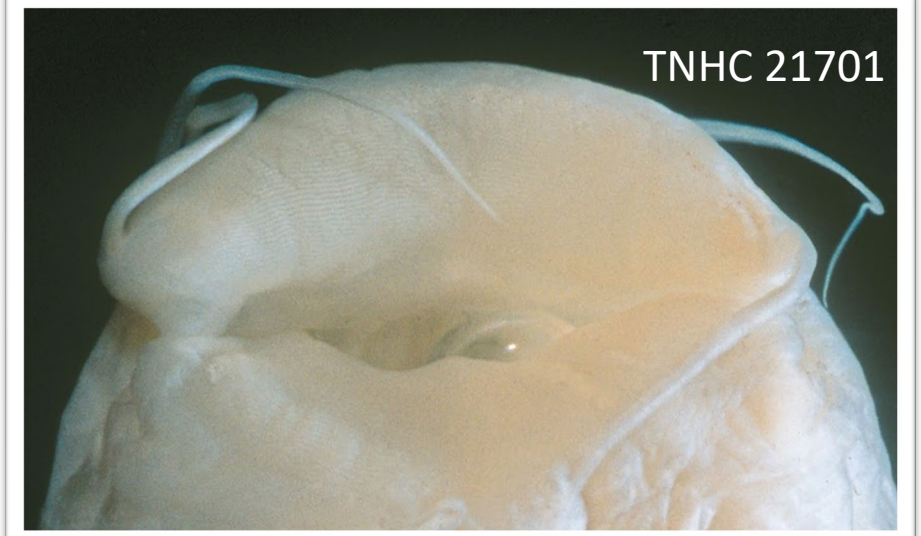
1986

GBIF.org (04 March 2022) GBIF Occurrence Download https://doi.org/10.15468/dl.ud74xm

Key specimen-based biology insights

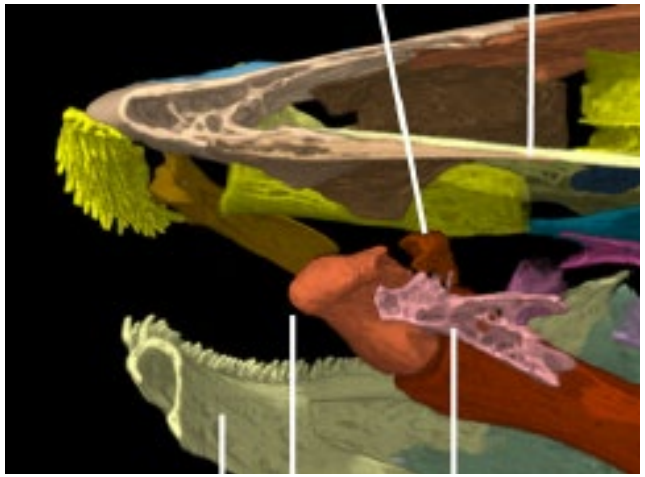
- **mostly > 300 m deep in 27-28C water.** One specimen survived > 100 days at ±24C
- **All *Trogloglanis* were near badwater line (= chemolithoautotrophic zone)**
  - **likely grazes bacterial/fungal mats, i.e. primary consumer (supported by unpublished preliminary stable isotope data)**
  - **Some had *~ 200 eggs in various stages of development***





Key specimen-based biology insights

- ***Satan* gut analysis demonstrates it is an invertebrate predator**
  - **No direct evidence that it preyed on *Trogloglanis*, but <u>preliminary stable isotope data</u> support it as top predator, and oral jaw dentition and sensory systems are clearly designed for efficient predation on large-bodied prey.**

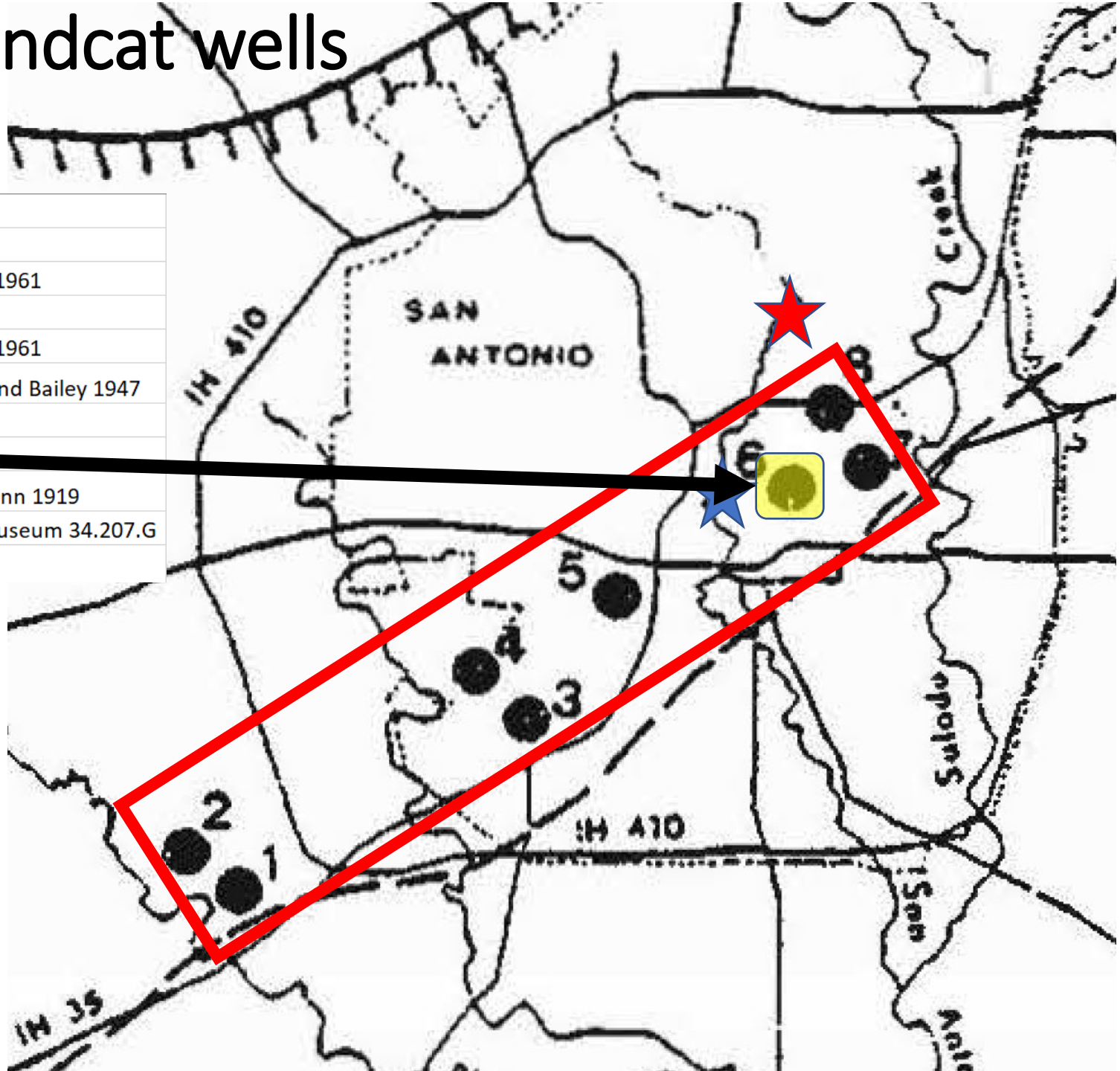


  - ***Trogloglanis* surely would not have spines unless it had a gape limited predator (sister *P. phreatophila* lost its spines in absence of predation)**
  - **Six *Satan* dissected by Langecker and Longley had <u>*no evidence of sexual maturity*</u>**

Karnei's 1978 map of blindcat wells

15-mile-long known range (both species)

		Karnei & Longley		Earlier		
		Satan	*Troglo*	*Satan*	*Troglo*	
1	O.R. Mitchell	3	3	1	1	Suttkus 1961
2	Verstraeten Well No. 1		1			
3	El Patio Foods Well			1		Suttkus 1961
4	William Kempin Well			1		Hubbs and Bailey 1947
5	BMD Well	1				
6	Artesia Pump Station Well #4	11	22			
7	G.W. Brackenridge Well				1	Eigenmann 1919
8	Josef Boeke Well				1	Witte Museum 34.207.G
	OVERALL TOTALS 1979	18	29			



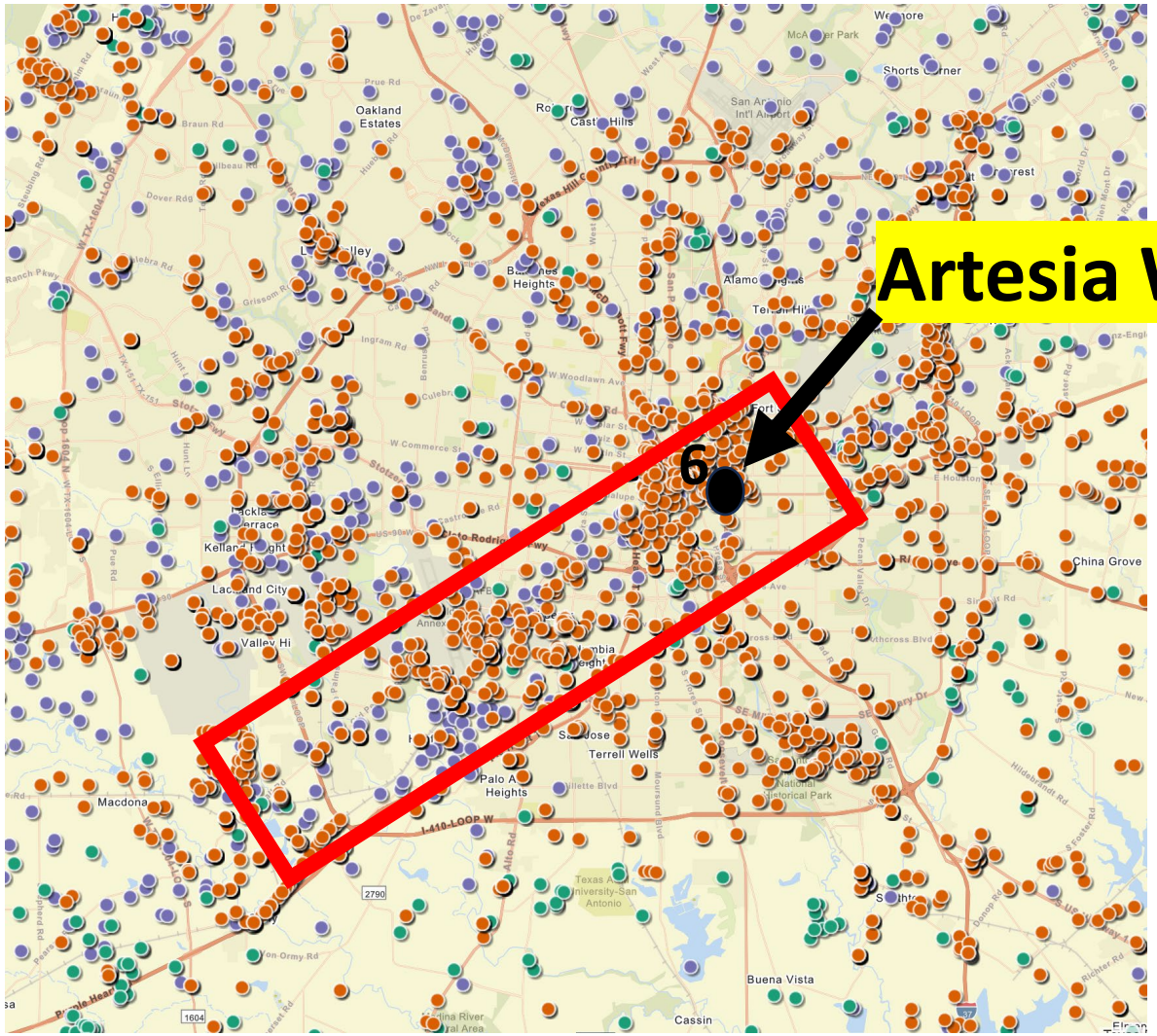
San Antonio Zoo

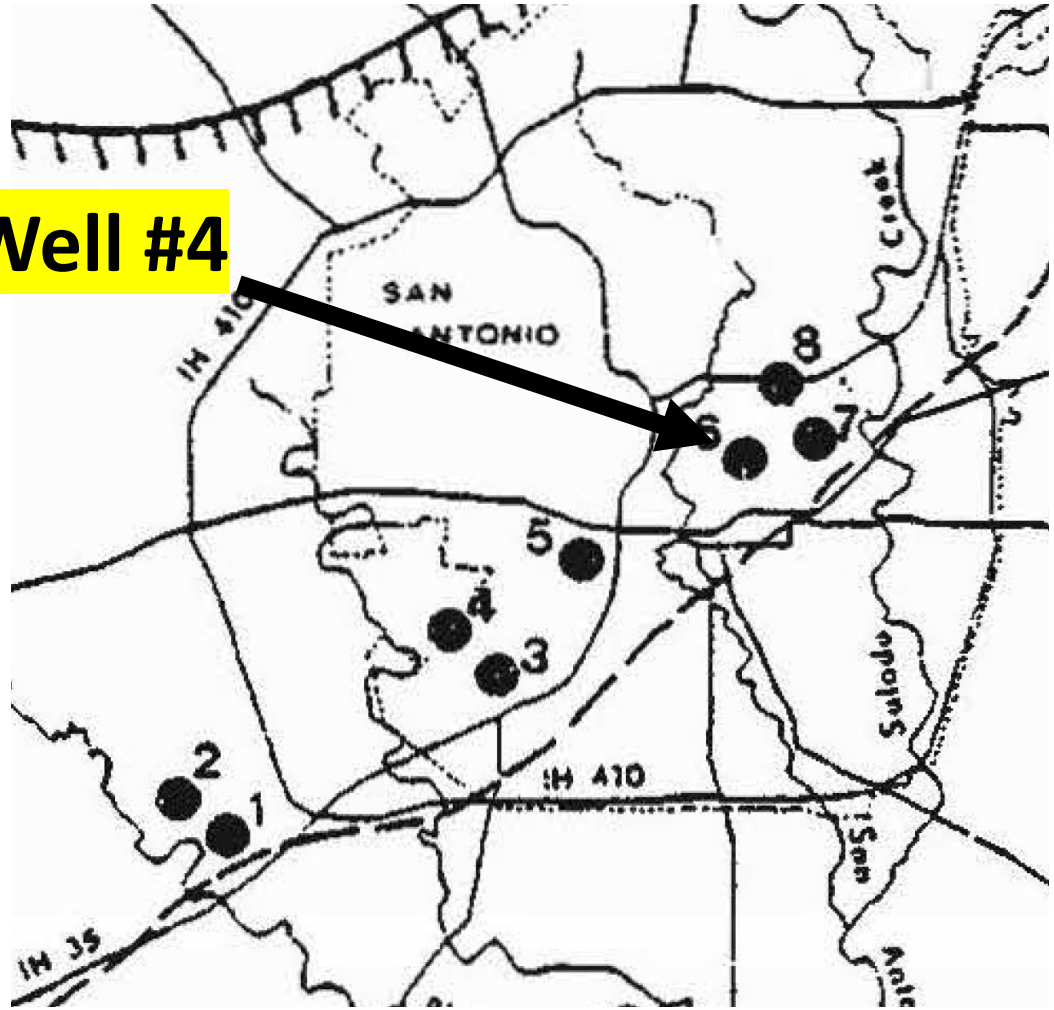
Alamo

Balcones Escarpment (recharge)

Badwater Line / chemolithotrophic zone (fish on N side)

There are LOTS of unsampled wells (most drilled long ago; plugged ones not shown) throughout the presumed range of blindcats





Wells at this station were drilled to similar depths in 1950s, have mostly been operational since, and are currently being renovated.

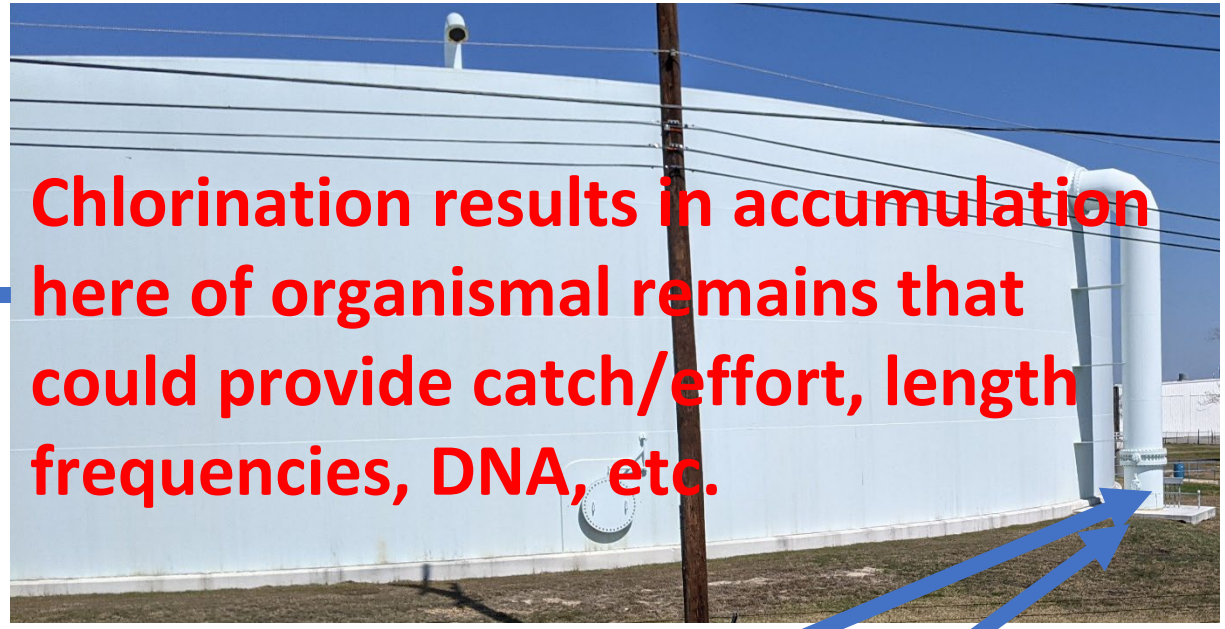
Only #4 was sampled by Longley, producing ~60% of all known *Satan* and *Trogloglanis* specimens in 68 days of netting 21,000 m^3/day, resulting in

- **<u>Annual</u> catch/effort-based mortality estimates via #4 alone:**
  - **2,473 *Trogloglanis* and around half as many *Satan***

Actual mortality could be relatively easily and precisely determined at via public water supply wells

Artesia Well #4

SAWS customers

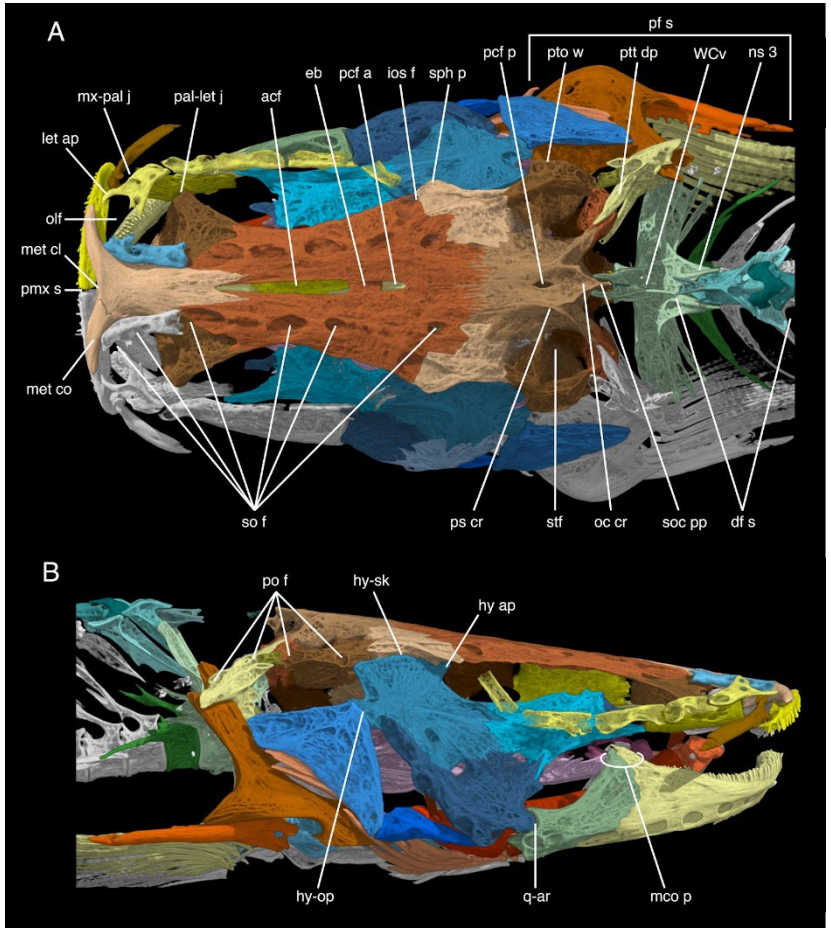


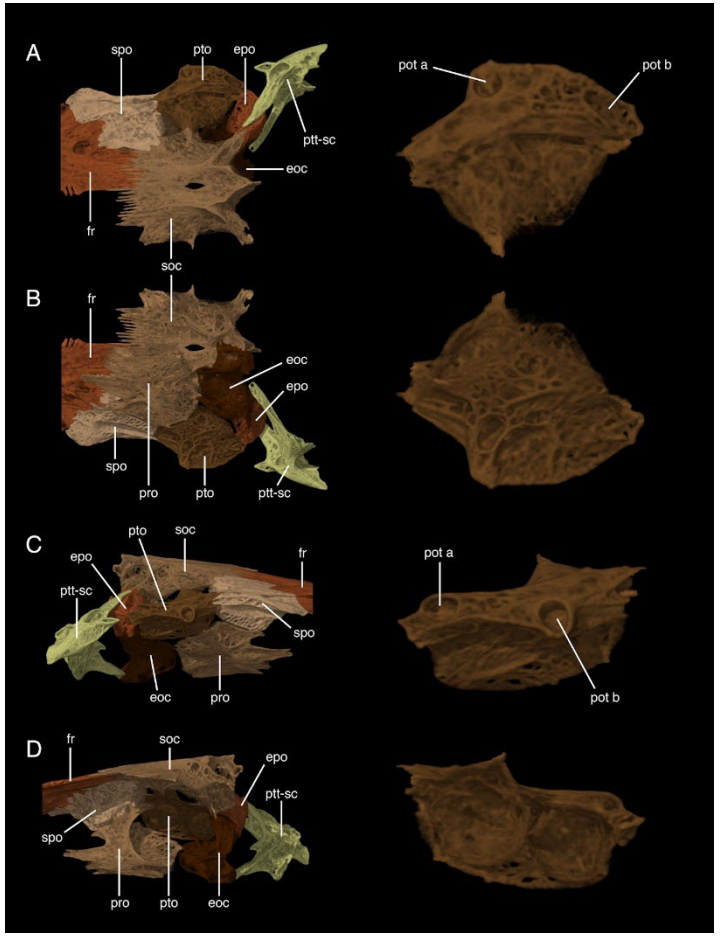
Chlorination results in accumulation here of organismal remains that could provide catch/effort, length frequencies, DNA, etc.

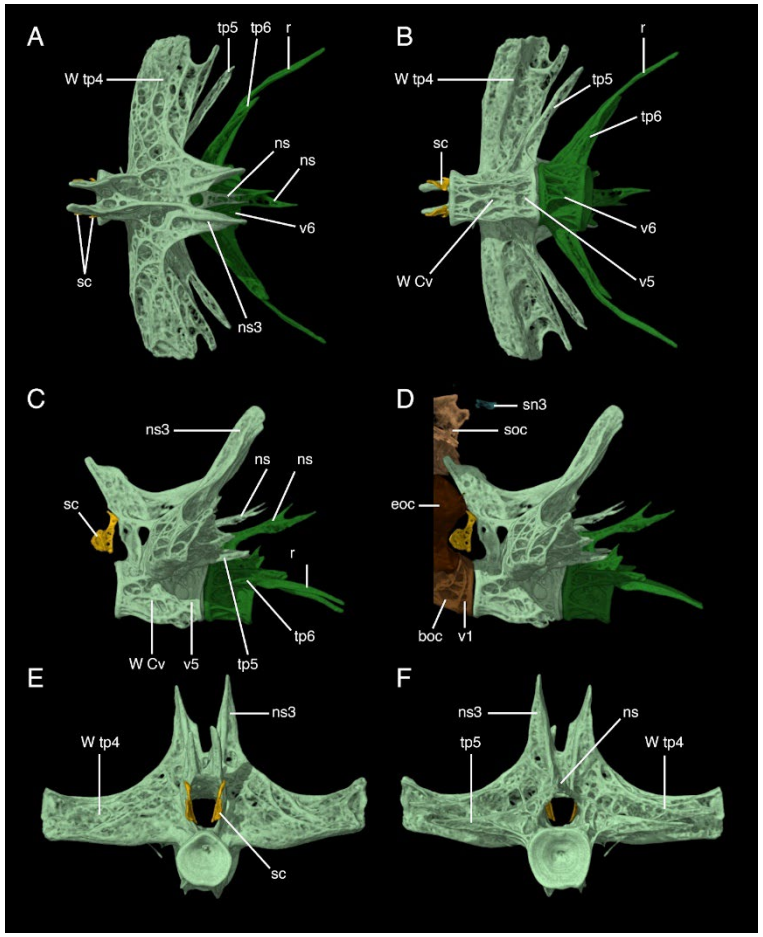
244 liters/sec

Satan's anatomy is now very well illustrated and analyzed

- Lundberg, J. G., Hendrickson, D. A., Luckenbill, K. R., & Mariangeles, Arce H, M. (2017). Satan's skeleton revealed: A tomographic and comparative osteology of *Satan eurystomus*, the subterranean Widemouth Blindcat (Siluriformes, Ictaluridae). *Proceedings of the Academy of Natural Sciences of Philadelphia*, *165*(1), 117–173. https://doi.org/10.1635/053.165.0108







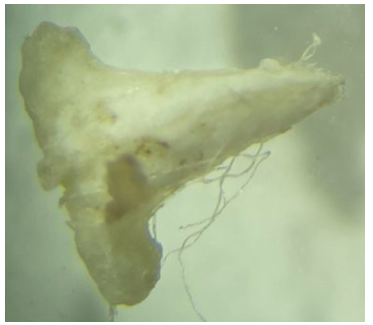
Trogloglanis osteology

- **A bit more difficult** - Lundberg, J. G. (1982). The Comparative Anatomy of the Toothless Blindcat, *Trogloglanis pattersoni* Eigenmann, with a Phylogenetic Analysis of the Ictalurid Catfishes. *Miscellaneous Publications, Museum of Zoology, University of Michigan, Ann Arbor, Michigan*, *163*, 85p. https://deepblue.lib.umich.edu/bitstream/handle/2027.42/56407/MP163.pdf;sequence=1
  - **poor ossification makes osteological comparisons difficult, but our CT scans can help**

- **Long denticulated pectoral and dorsal spines easily diagnose the species and are clear anti-predator adaptions:**
  - No fish predators have ever been seen in habitats of its sister, *Prietella phreatophila*, which totally lacks spines.

DNA?

- ***Trogloglanis* - complete mitochondrial genome** from fragments (chopped up by pumps) collected by Zara (2014) that had diagnosable bones: Hendrickson, D. A., Peterson, R., Mandoiu, I., Alqahtani, F., & Pirro, S. (2021b, May 8). The complete mitochondrion sequence of **Trogloglanis pattersoni**—MZ151889.1. NCBI GenBank. https://www.ncbi.nlm.nih.gov/nuccore/MZ151889.1?report=genbank
- ***Prietella phreatophila* - complete mitochondrial genome** - Hendrickson, D. A., Peterson, R., Mandoiu, I., Alqahtani, F., & Pirro, S. (2021a, May 8). The complete mitochondrion sequence of ***Prietella phreatophila*** - MZ151888.1. NCBI GenBank. https://www.ncbi.nlm.nih.gov/nucleotide/MZ151888.1?report=genbank
- ***Satan* – all specimens were preserved in formalin and the majority dessicated/rotted,**
  - ***HOWEVER*** **(THANK YOU BEN HUTCHINS!!!!) last year a vial of fish bones collected in 1986 from a San Antonio well, then lost for nearly 40 years, was re-discovered (photos at https://www.gbif.org/occurrence/4538848777)!!!**

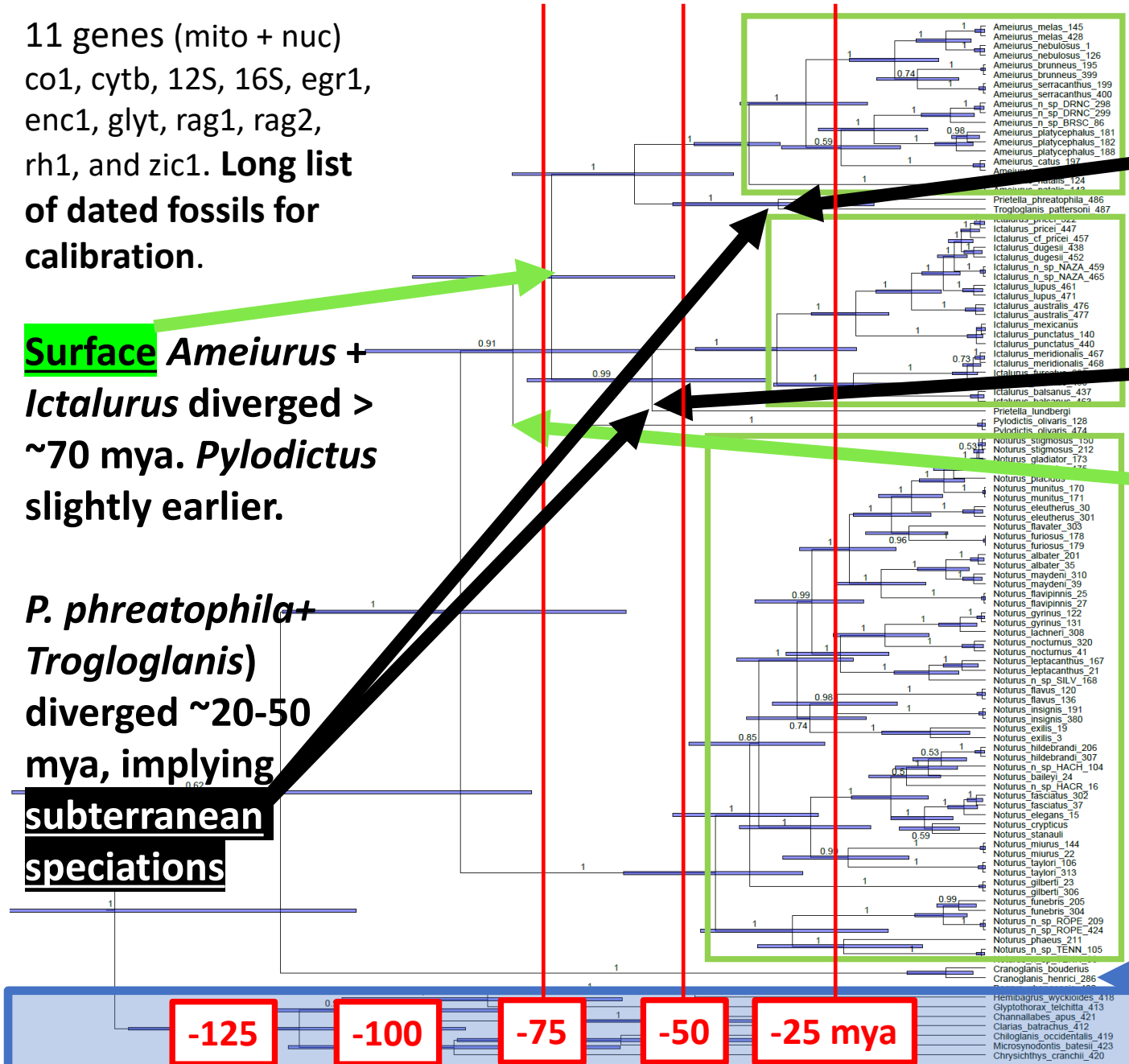

  - **contains unambiguously diagnostic bones of 2 specimens preserved in ethanol, *but***
  - **so far, the only identifiable sequence fragments match *Trogloglanis*.**

FROM: Janzen, F. H. et al. 2023. Phylogenetic relationships of the North American catfishes (Ictaluridae, Siluriformes): Investigating the origins and parallel evolution of the troglobitic species. Molecular Phylogenetics and Evolution, 107746. https://doi.org/10.1016/j.ympev.2023.107746

11 genes (mito + nuc) co1, cytb, 12S, 16S, egr1, enc1, glyt, rag1, rag2, rh1, and zic1. **Long list of dated fossils for calibration.**

Surface *Ameiurus* + *Ictalurus* diverged > ~70 mya. *Pylodictus* slightly earlier.

***P. phreatophila*+ *Trogloglanis*) diverged ~20-50 mya, implying subterranean speciations**



Ameiurus (17 OTUs / 7 described spp.)

***P. phreatophila* + *Trogloglanis* (sisters)**

Ictalurus (20 / 7)

***New genus+species* (*Cueva* Nac. Rio Frio)**

Pylodictis (2 / 1) (+ Satan?? No DNA)

Noturus (55 / 30)

surface stygobites African

Cranoglanidae **(not including sister Lacantuniidae of Chiapas/Guatemala)**

Varied African /Asian

Conservation status / Population dynamics

Longley and Karnei 1978 reports conclude:

- ***Satan*:** "The fish does have a very restricted habitat, but this is apparently the only significant reason for concern with its status. The fish is currently protected under state non-game law, although the need for this protection is highly questionable. The inaccessibility of the habitat of this fish protects it very well."
- ***Trogloglanis:*** "If any danger exists for the survival of *T . pattersoni*, it would probably stem from the large quantities of water being withdrawn from the Edwards Aquifer in the San Antonio area without adequate provision for additional recharge. The high volume of flow from wells may somehow decrease the numbers of fish below the number adequate to sustain a healthy breeding population."

2008-2014 Zara Environmental sampled wells using Karnei and Longley's basic methods (nets over well outlets), recording catch per unit effort (CPUE) by recording water volume filtered

- Zara Environmental. (2010). *Final Report for Deep Aquifer Biota study of the Edwards Aquifer* (p. 109). Edwards Aquifer Authority. http://www.edwardsaquifer.net/pdf/Deep_Aquifer_Biota_by_ZARA_2010.pdf

- Zara Environmental LLC. (2014). *Fauna of Wells Near the Saline Water line of the Edwards Aquifer, Texas* (LocInArchive_content; pp. 1–37). Edwards Aquifer Authority; Archive_content. https://www.edwardsaquifer.org/science_docs/fauna-of-wells-near-the-saline-water-line-of-the-edwards-aquifer-texas/

FAUNA OF WELLS NEAR THE SALINE WATER LINE OF THE EDWARDS AQUIFER, TEXAS

In line barrel sampler on an irrigation well in Bexar County, Texas.

	1977-78 Karnei	2008-14 Zara
N wells sampled	**36**	**41**
N *Trogloglanis*	**29**	**14**
N *Satan*	**18**	**0**

Using water volume filtered as Effort, and CPUE from Karnei 1978, **expectation for Zara's samples was ~ 7 - 14 *Satan*. At 3 Zara wells with *Trogloglanis*, CPUE was > CPUE of Karnei.**

Zara's bottom line… **"suggests lack of *Satan* not due to an ineffective sampling scheme, and *Trogloglanis* is more abundant now than in 1978."**

IS SATAN EXTINCT?????!!!!! Last specimen 1986

Limited sampling for specimens continues

- Zara (2010-2014) obtained a few *Trogloglanis* (mostly fragmentary **and small**) from an agricultural well SW of San Antonio. All preserved in EtOH and deposited at TNHCi.
- USFWS (last 3-4 years) continues sampling there, sporadically obtaining more fragmentary specimens of **mostly *small*** *Trogloglanis*
    - All preserved and stored in 95% EtOH
- Last *Satan* specimen - 1986

Population biology under natural (stable) conditions:

- **Deep Edwards Aquifer seems likely to have been one of the most stable environments on the planet during the long occupancy of it by blindcats.**
- **Assume gape-limited predator-prey relationship fine-tuned over tens of millions of years of co-evolution in extremely stable, low energy deep aquifer**
- **use insights from lab *Prietella* and other cavefishes:**

Life history parameters	*Trogloglanis* (prey)	*Satan* (predator)
Max age	1-2 decades?	many decades – century?
Average life span	1 decade?	many decades
Age at sexual maturity	Relatively low	Relatively great
Frequency of reproduction	frequent	rare
Fecundity / reproductive event	200	8-20 ???

- **Can assume no "pre-adaptation" to avoidance of novel wells given that wells first showed up in their world in last 0.0003% of their 30,000,000 years of evolution in the aquifer.**
  - **Springs (which some might propose are natural well-like outlets) are mostly outside of their known range. No blindcat specimens have ever been collected from SA-area springs.**

Population biology under current conditions:

- ***Satan***
  - **given long time to maturity and low fecundity, and,**
  - **as typical of most predators and feeding also on invertebrates, hypothesize a relatively larger and more open-water home range than that of *Trogloglanis:***
    - **population(s) would be hyper-sensitive to "overfishing" (=wells)**
      - **Abundance would decrease**
      - **Average length would be slow to decline given slow growth, innate low recruitment**
      - **Time to extirpation under "overfishing" pressure would be far less than expected for *Trogloglanis***
- ***Trogloglanis***
  - **given innate higher fecundity and frequent recruitment, and declining predator population**
  - **And, living on bottom scraping surface, assume likely relatively small home range, and likely less susceptible to well extraction (in ceilings of cavities) than *Satan*.**
    - **Abundance would rebound following predator decline/removal**
    - **relative frequency of small individuals of prey would increase**

eDNA (metabarcoding)

- With **DFC support to Hendrickson +** FWS and San Antonio Zoo support - preliminary results from first samples from 4 sites (many more going to the lab soon)
  - successfully detects *Prietella phreatophila* (in the Zoo's outflow)
  - *Trogloglanis* was ***not*** detected in 2 samples from the well that has occasionally produced fragmentary specimens over the last couple of years – sampling continues.
  - Still no eDNA target for *Satan*
- Still working toward contacting SA-area well owners to seek permission to sample new wells for at least eDNA
- Future eDNA sampling will extend further NE in hopes of re-discovering some "lost" blind salamanders

THE SEARCH FOR *SATAN* AND THE BLANCO BLIND SALAMANDER CONTINUES!!!!

The End