Review of the Blanco Blind Salamander (*Eurycea robusta*)

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Executive Summary

The Blanco blind salamander (*Eurycea robusta*) was petitioned for listing under the U.S. Endangered Species Act (Act) in a multi-species petition submitted to the U.S. Fish and Wildlife Service (Service) on June 25, 2007. The Service published a partial 90-day finding on December 16, 2009, that determined the petition presented substantial information that listing may be warranted. The species is scheduled for a 12-month finding in Fiscal Year 2022.

The Blanco blind salamander is known only from one specimen collected in 1951 from an excavated fissure in the dry bed of the Blanco River northeast of the City of San Marcos, Hays County, Texas. This salamander is a stygobite, a species adapted to life in water-filled subterranean voids. Conditions in this environment include complete darkness, low oxygen, stable temperatures, and reduced food availability. Like many other stygobites, the Blanco blind salamander exhibits morphological adaptations to these conditions including reduced eyes and absence of pigmentation.

There is compelling evidence indicating that the Blanco blind salamander does not exist as a current taxonomic entity. When the Blanco blind salamander was formally described in 1981, the description was based on a single specimen. Morphological measurements from the individual were very similar to those of the Texas blind salamander (*E. rathbuni*), a congeneric species known from several caves and springs in the City of San Marcos, very close to the Blanco blind salamander type locality. Characters cited as distinguishing the Blanco blind salamander from the Texas blind salamander are the former's robust body form (i.e., torso body tissue), longer trunk, shorter limbs, broad and rounded tail, and rounded skull.

The species description for the Blanco blind salamander did not account for natural morphological variation and relies on characters that may have been influenced by chemical fixation and preservation. Similar morphology and close geographic proximity suggest that these two species are not separate taxa. The Blanco blind salamander's published description does not fully address the possibility that the specimen is instead an aberrant, variant, or altered individual of the nearby Texas blind salamander. For the species' description, a small series of Texas blind salamanders (i.e., 10 individuals) was measured for comparison to the Blanco blind salamander. Reliance on a small number of individuals limited consideration of the potential range of morphological variation inherent to the Texas blind salamander.

Along with natural variation, the effects of standard herpetological chemical fixation and preservation methods can alter the morphology of preserved amphibians. Several studies have documented the effects of common preservation methods on specimen morphology. Fixation and/or preservation of amphibians can result in changes to soft tissue and bony morphological traits, skewing assessment of actual traits exhibited by living individuals. The preservation method first applied to the Blanco blind salamander specimen is unknown. Chemical preservative has potentially made genetic assessment of this individual impossible as DNA has yet to be isolated. The specimen had been preserved for over 10 years before it was described and 30 years when redescribed. The distinctiveness of the Blanco blind salamander is largely premised on soft tissue traits (e.g., torso body tissue and rounded tail) that may have been altered by fixative and/or preservatives.

The collection locality for the Blanco blind salamander is 3 kilometers (km) (2 miles [mi]) northeast of the nearest occurrence of the Texas blind salamander. A string of Texas blind salamander sites occurs in caves and springs roughly on a line to the southwest. The Texas blind salamander is found along southwest to northeast trending faults, most notably the San Marcos Springs Fault. Groundwater connections exist among several of those subterranean karst features, the San Marcos Springs Fault, and the Edwards Aquifer. Cretaceous-age, highly permeable Edwards Group formations are the primary water-bearing strata of the Edwards Aquifer. Less permeable strata, like the Austin Chalk, overlay and confine the Edwards Group in this area.

Alluvium and Austin Chalk were excavated to enlarge the fissure in the Blanco River where the Blanco blind salamander was collected. It is likely that this fissure communicated with subterranean, water-filled voids in the underlying Edwards Group. This site is along the northeastern extent of the San Marcos Springs Fault. Given documented groundwater connectivity in the area, it is likely that connections exist in the cavernous Edwards Group rocks that extend to and underlie the Blanco River.

The type specimen on which the Blanco blind salamander's description was based either represents a historical occurrence of the Texas blind salamander or it represents a unique species that is no longer extant. No stygobitic *Eurycea* are known to occur sympatrically with other stygobitic members of the genus, and given the potential for groundwater connectivity, it is unlikely that a group of salamanders would be isolated from the larger population of Texas blind salamanders less than 3 km (2 mi) to the southwest. It is plausible that the type locality of the Blanco blind salamander was the most northeastern occurrence of the Texas blind salamander.

Many surveys to locate additional specimens have been conducted. Since 1951, no stygobitic *Eurycea* have been collected from the Blanco River or areas to the north of that river in Hays County. Texas blind salamanders have been regularly collected and observed from multiple sites south of the Blanco River in the City of San Marcos. Efforts to relocate the Blanco Blind salamander in 2006 and 2020-2021 from nearby springs have not resulted in collection of any similar species. If the Blanco blind salamander was a valid taxon, we have no evidence that the species remains extant.

1.0 Introduction

This report is a summary of information assembled and reviewed by the U.S. Fish and Wildlife Service (Service) regarding the taxonomic status of the petitioned Blanco blind salamander (*Eurycea robusta*). The Blanco blind salamander is known only from a 70-year-old specimen collected from a fissure in the Blanco River near the City of San Marcos, Hays County, Texas in 1951 (Potter and Sweet 1981, pp. 70-73). It is a member of a diverse radiation of groundwater-dependent salamanders that occur across the state's Edwards Plateau (Devitt et al. 2019, pp. 2626-2630). Due to uncertainty in taxonomic status, the Service incorporated the best scientific and commercial data available in reviewing the status of this salamander species.

1.1 Federal Actions

On August 21, 1995, the Service received a petition to list the robust blind salamander (referred to hereafter as the Blanco blind salamander) as endangered under the U.S. Endangered Species Act (Act). The petition was submitted by Walter R. Courtney, Ph.D., on behalf of the American Society of Ichthyologists and Herpetologists. The Service published a 90-day finding on September 9, 1998, that stated uncertainties existed regarding the taxonomic validity and distribution of the Blanco blind salamander and the Service found that the petition did not present substantial information indicating that listing may be warranted (63 FR 48166).

On June 25, 2007, the Service received a petition from Forest Guardians (now WildEarth Guardians) requesting that the Service consider 475 species in the southwest Region be listed under the Act as endangered or threatened with critical habitat (Forest Guardians 2007, p. 2). The Blanco blind salamander was included among the list of petitioned species (Forest Guardians 2007, p. 35).

On March 19, 2008, WildEarth Guardians filed a complaint indicating that the Service failed to comply with its mandatory duty to make a preliminary 90–day finding on the petitioned species. The Service subsequently published an initial 90–day finding for 270 of the 475 petitioned species on January 6, 2009, concluding that the petition did not present substantial information that listing of those species may be warranted (74 FR 419). The Blanco blind salamander was not addressed in this initial 90-day finding.

The Service and WildEarth Guardians filed a stipulated settlement agreement on March 13, 2009, agreeing that the Service would submit a 90–day finding to the Federal Register for the remaining species by December 9, 2009. The Service published a partial 90-day finding on December 16, 2009, that determined the petition presented substantial information that listing may be warranted for 67 of the remaining species, including the Blanco blind salamander (74 FR 66866). The finding stated that the petition presented substantial information to indicate that listing the Blanco blind salamander may be warranted due to the present or threatened destruction, modification, or curtailment of its habitat or range resulting from water pollutants and water withdrawal (74 FR 66866).

This report represents the Service's review of the Blanco blind salamander and will be used to inform the 12-month finding to address whether the petitioned action (listing) is warranted.

1.2 *Eurycea* Salamanders and the Edwards-Trinity Aquifer System

An aquifer is a rock unit capable of storing and transmitting water (Ford and Williams 2007, p. 103; White 2012, p. 383). The term "karst" refers to a type of terrain and subsurface structure (e.g., caves) that is formed by the slow dissolution of calcium carbonate from surface and subsurface limestone, and other soluble rock types (e.g., carbonates and evaporites), by mildly acidic groundwater (Holsinger 1988, p. 148; Culver and Pipan 2009, pp. 5-15; Jones and White 2012, pp. 431-438). Flow of groundwater through pores, fractures, and conduits leads to the formation of an interconnected system of subterranean voids that become larger as bedrock is dissolved (Culver and Pipan 2009, pp. 5-8; Veni 2012, pp. 603-608; White 2012, pp. 383-386). Karst aquifers are typified by networks of conduits that can carry water quickly through the aquifer (Bonacci et al. 2009, p. 893; White 2012, pp. 385-387).

The Edwards-Trinity Aquifer system of central Texas' Edwards Plateau is among the most biodiverse groundwater ecosystems in the world (Culver et al. 2000, pp. 389-392; Culver et al. 2003, pp. 445, 449; Gibert et al. 2009, p. 54). The three major karst aquifers that comprise this system, the Edwards-Trinity, Trinity, and Edwards Aquifers (George et al. 2011, pp. 27-33, 35-41, 67-72), host a wide range of endemic aquatic animal species (Hutchins 2017, p. 490; Devitt et al. 2019, p. 2630; Krejca and Reddell 2019, pp. 160-165). Subterranean aquatic habitats consist of fractures, conduits, and water-filled voids of varying sizes (e.g., mesocaverns to macrocaverns) that facilitate groundwater flow throughout the aquifer (Howarth 1983, pp. 370-371; Danielpol et al. 2003, pp. 3-4; Bonacci et al. 2009, pp. 893-894). Surface aquatic habitats are sites where groundwater emerges from underlying aquifers in the form of springs and spring-fed streams (Springer and Stevens 2008, pp. 1-9; Kløve et al. 2011, pp. 774-775).

The Edwards Plateau is a center of diversity for the Plethodontid salamander genus *Eurycea* (Chippindale et al. 2000, pp. 1-4, 9-16; Bendik et al. 2013, pp. 3, 6-12; Devitt et al. 2019, pp. 2626-2630). At present, 12 described and three undescribed *Eurycea* species are known from the region (Devitt et al. 2019, p. 2630). Most populations of these *Eurycea* species are fully aquatic, do not metamorphose (i.e., paedomorphic: maintain larval form throughout life), and are closely associated with the karstic Edwards-Trinity Aquifer system (Gorički et al. 2012, pp. 667-670; Stafford et al. 2014, p. 54). The Valdina Farms salamander (*E. troglodytes*) is the only species currently known to contain some populations in which individuals metamorphose from larval to adult form (Sweet, 1977, pp. 366-372). No other instances of metamorphosis have been observed in other central Texas *Eurcyea*.

In this review, the Edwards Aquifer is of chief concern regarding the Blanco blind and another closely related *Eurycea* species, the Texas blind salamander, which has been federally listed as endangered since 1967 (32 FR 4001). This aquifer is the eastern most of the Edwards-Trinity Aquifer system. The Edwards Aquifer is comprised of three distinct zones referred to as the contributing/drainage, recharge (outcrop), and artesian zones (subcrop; see Figure 1) (Schindel 2019, pp. 12-13). Each of these zones display unique hydrogeological characteristics (Hunt et al. 2019, pp. 76, 80-81).

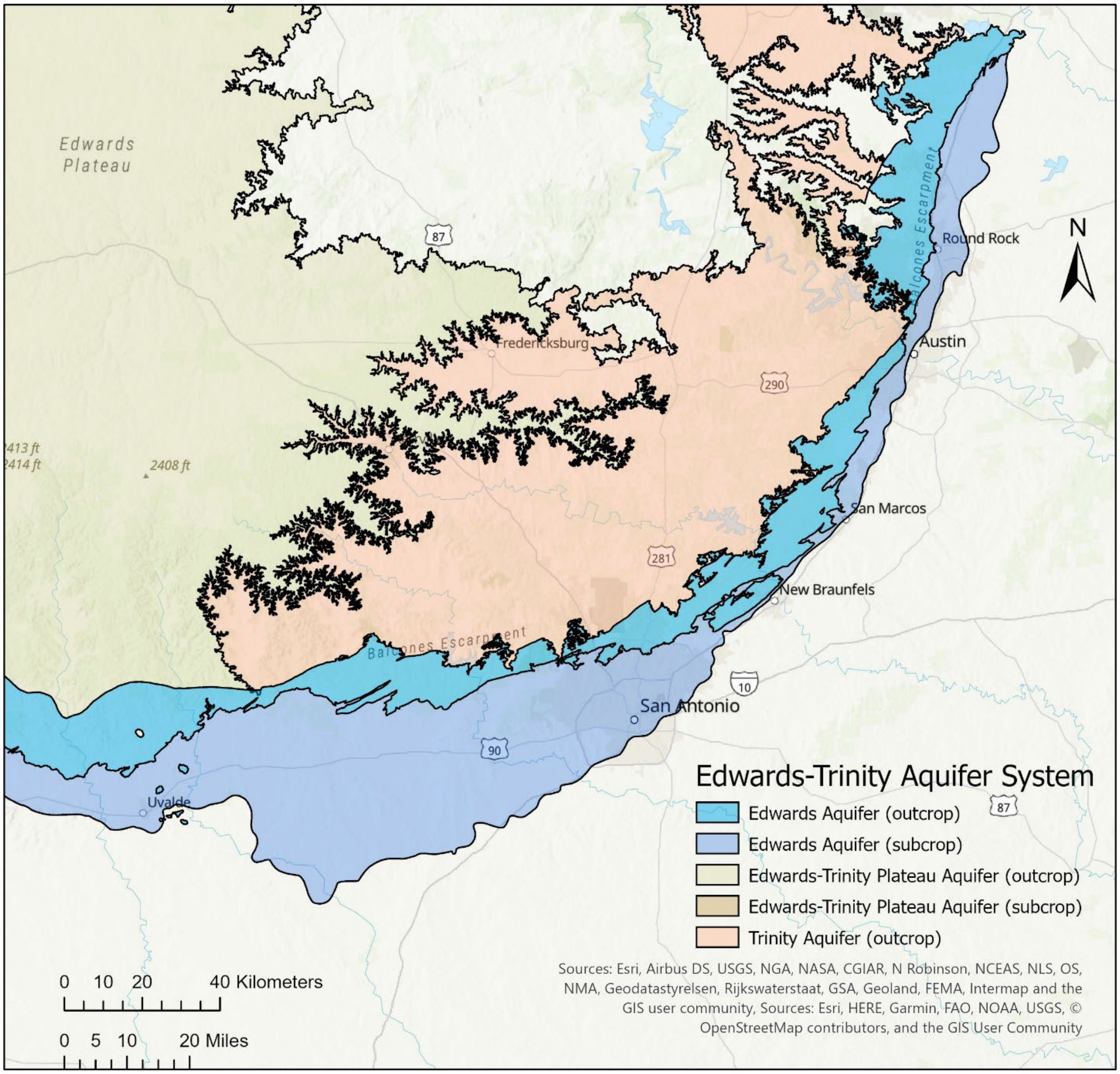

Figure 1. Edwards-Trinity Aquifer System, Texas. The term outcrop refers to recharge area of aquifers while subcrop generally corresponds to an aquifer's artesian zone.

Precipitation falling across the contributing zone flows downstream to the recharge zone where it can enter the aquifer through recharge features (e.g., caves, faults, fractures, and sinkholes) or by infiltrating soils and rock strata that overlie the aquifer (Lindgren et al. 2004, pp. 31-35). Many creeks, streams, and rivers lose significant volumes and sometimes all of their baseflow to recharge features as they cross the recharge zone (Schindel 2019, p. 10). The artesian zone is composed of less permeable geologic layers that confine the inflowing waters from the recharge zone (Schindel 2019, pp. 12-13). The hydraulic pressure of the confined water within the artesian zone's cavities, faults, and fissures forces it to the surface where it escapes through springs and seeps (Lindgren et al. 2004, pp. 35, 39-40). The Blanco blind salamander type locality and most sites occupied by the Texas blind salamander are located in the artesian zone of the Edwards Aquifer.

1.3 Adaptation to Groundwater Ecosystems

Figure 2. Texas blind salamander. Stygobitic morphological characters include vestigial eyes, reduced pigmentation, and elongated limbs. ©2019 Tom Devitt.

The *Eurycea* species of central Texas vary in their use of groundwater-dependent habitats. Some species, like the Jollyville Plateau (*E. tonkawae*) and San Marcos salamanders (*E. nana*), occur in springs and headwater streams as well as subterranean habitats (Bendik and Gluesenkamp 2012, pp. 4-5; Diaz et al. 2015, pp. 308-309, 315-319; Bendik et al. 2016, pp. 15-17). A few *Eurycea* species, like the Texas blind salamander (Figure 2; *E. rathbuni*), occur only in groundwater-filled caves and conduits and exhibit striking morphological adaptions (e.g. vestigial eyes, elongated legs, depressed skull, and absence of pigmentation) to subterranean conditions (Gorički et al. 2012, pp. 665, 668-669).

Organisms that are obligate inhabitants of groundwater habitats are termed stygobites and often exhibit adaptations to life in complete darkness (Humphreys 2006, pp. 116-117; Culver and Pipan 2009, p. 3; Christiansen 2012, pp. 520-523). These systems are generally thought to be nutrient poor with limited oxygen (Hancock et al. 2005, pp. 104-105; Humphreys 2006, pp. 112-113; Hervant and Malard 2012, pp. 651-652; Hüppop 2012, pp. 1-2, 4; Simon 2012 pp. 100-103) and species may display physiological adaptations to harsh subterranean conditions including delayed maturity, longer lifespans, and reduced numbers of eggs (Gorički et al. 2012, p. 665; Hüppop 2012, pp. 6-8). Studies of the Edwards Aquifer contradict the generic notion that the system is nutrient poor. Along with receiving input of organic matter from the surface, the system also receives nutrient input through chemolithautotrophy (i.e., microbial oxidation of inorganic compounds to produce nutrients; Hutchins et al. 2016, p. 1535). A complex food web seemingly exists in the Edwards Aquifer along a gradient from surface-dominated food webs near recharge features to chemolithoautotrophic-dominated food webs in deeper reaches of the aquifer (Hutchins et al. 2016, pp. 1536, 1539). The Blanco blind and Texas blind salamanders represent examples of stygobites.

Stygophiles are organisms that move between aquatic surface and subsurface habitats (Humphreys 2006, pp. 116-117). The Jollyville Plateau salamander, along with the Georgetown (*E. naufragia*) and Salado (*E. chisholmensis*) salamanders, are examples of stygophilic *Eurycea* species. The morphology of stygobitic *Eurycea* are often markedly different from congeneric species that occupy surface habitats. Surface-dwelling *Eurycea* species generally possess dark pigmentation, developed eyes, and robust limbs (Hillis et al. 2001, p. 269; Gorički et al. 2012, p. 669; Bendik et al. 2013, p. 3). However, some surface species, like the Cascade Caverns salamander (*E. latitans*), contain populations that inhabit subterranean systems and display stygobitic characters (Figure 3; Gorički et al. 2012, pp. 669-670; Bendik et al. 2013, pp. 8-9, 11; Devitt et al. 2019, p. 2625). This intraspecific variability has confounded understanding of *Eurycea* species boundaries based on morphometric and limited genetic (i.e., allozyme allele frequency) analyses (Chippindale et al. 2000, p. 2; Wiens et al. 2003, p. 511).

Recent work by Devitt et al. (2019, pp. 2629-2630), using genome-wide DNA sequence data, has revealed that some species considered distinct instead are populations of other *Eurycea* species. Specifically, the Comal blind (*E. tridentifera*) and Comal Springs (*E*. sp. 8) salamanders were found to be populations of the Cascade Caverns and Fern Bank (*E. pterophila*) salamanders, respectively (Devitt et al. 2019, p 2629.). Devitt et al. (2019, p. 2629) also identified two new *Eurycea* species, previously considered populations of the Valdina Farms salamander (*E. troglodytes*).

Figure 3. Surface (right) and subterranean forms (left; note reduced eyes, reduced pigmentation, and elongated limbs) of the Cascade Caverns salamander (*E. latitans*). ©2019 Tom Devitt.

1.4 Conservation Issues

Several species of *Eurycea* of the Edwards Plateau are of conservation concern. The rapidly growing Austin-Round Rock-San Marcos and San Antonio-New Braunfels metropolitan areas overlay portions of the Edwards-Trinity Aquifer system (Frey 2012, p. 4; U.S. Census Bureau 2012, p. 9; U.S. Census Bureau 2020). Seven *Eurycea* species across this region have been federally listed as endangered or threatened due to threats from groundwater pumping and conversion of natural habitats to exurban, suburban, and urban development (Krejca and Reddell 2019, p. 165, 167-168). Three additional Texas salamander species have been petitioned for listing under the Act due to those same threats (Forest Guardians 2007, pp. 28-29, 35-36; Center for Biological Diversity 2012, pp. 313, 353-357). Texas Parks and Wildlife Department has listed 11 *Eurcyea* species as endangered or threatened at the state-level (Texas Parks and Wildlife Department 2020). Among those is the Blanco blind salamander which the agency listed as threatened.

2.0 History of Discovery

The Blanco blind salamander is known only from a single individual collected and preserved in 1951 (Potter 1963, pp. 1-2; Potter and Sweet 1981, p. 70). The events that lead to the collection of this specimen were detailed by Potter (1963, pp. 1-3) and Russell (1976, pp. 14, 30-31) with some differing details provided by Longley (1978, p. 18). During most of the 1950s, Texas experienced one of the most prolonged and intense droughts of the 20th century (Stahle and Cleaveland 1988, p. 66; Woodhouse and Overpeck 1988, pp. 2697, 2699; Smith and Hunt 2010, p. 615; Nielsen-Gammon 2012, p. 91; Heim 2017, pp. 2586, 2588). Drought conditions lead to substantial declines in flow for many streams across the Edwards Plateau (Winters 2013, pp. 11-12, 23). The type locality for the Blanco blind salamander was an excavated fissure in the then-dry bed of the Blanco River west of Interstate 35 and just northeast of the City of San Marcos in Hays County, Texas (Figures 4 and 5; Potter and Sweet 1981, p. 70). The site is along the very southeastern edge of the Edwards Aquifer in the artesian zone.

The following narrative is summarized from Potter (1963, pp. 1-3) and Russell (1976, pp. 14, 30-31). In the summer of 1951, drought-induced streamflow decline in the Blanco River prompted the Hays County Gravel Company, which had been pumping water from the river for operations, to identify an alternative water source. Employees with the company located a "small spring" flowing from a narrow crack or fissure in the Blanco River's dry bed. At the time, the site was approximately 6.4-8 kilometers (km) (5 miles [mi]) north of the City of San Marcos. The spring reportedly flowed "about five gallons per minute." A trench, 6.1 meters (m) (20 feet [ft]) long and 2.4 m (8 ft) wide, was excavated following the fissure to a depth of approximately 6.1 m (20 ft). Longley (1978, p. 18) reports that "heavy equipment" was used to excavate the trench. A pump removed water during the digging effort, though not all water could be removed during digging, and water pooled at the bottom of the trench.

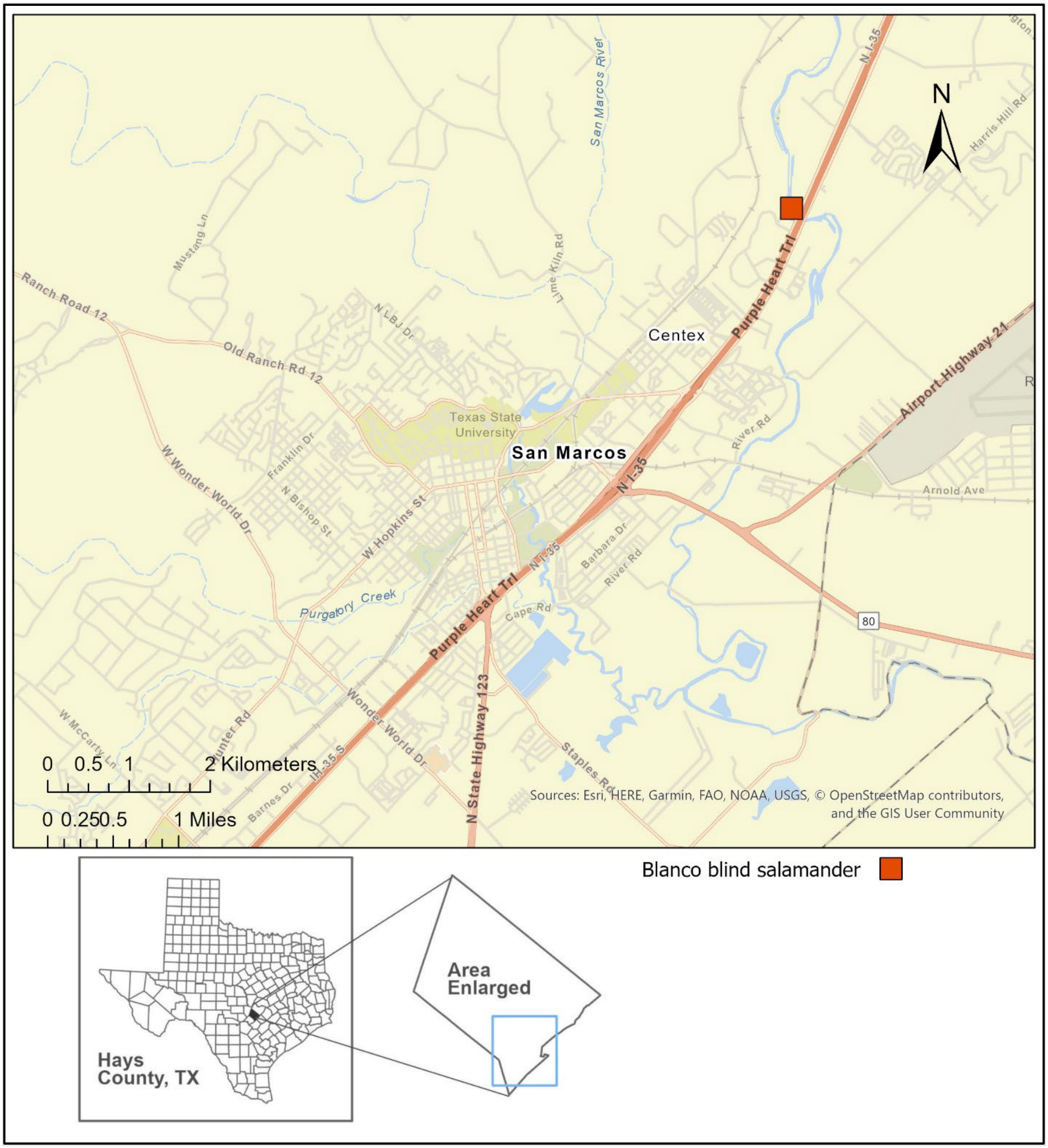

Figure 4. Type locality of the Blanco blind salamander in the Blanco River, City of San Marcos, Hays County, Texas.

Russell (1976, p. 30) states "on several occasions white salamanders were seen in these pools." A gravel company employee collected "three or four of the salamanders" and provided them to a biology faculty member, C.S. Smith, Ph.D. at Southwest Texas State Teachers College, now Texas State University (Figure 6). Potter (1963, p. 1) provides a specific date for the collection event, July 23, 1951, but writes that only "two salamanders" were taken from the excavation and provided to Smith. No other salamanders appeared in the trench, and the gravel company abandoned further digging. Water pooled into the abandoned excavation and was later filled and covered with sediment and rocky material as flow returned to the Blanco River.

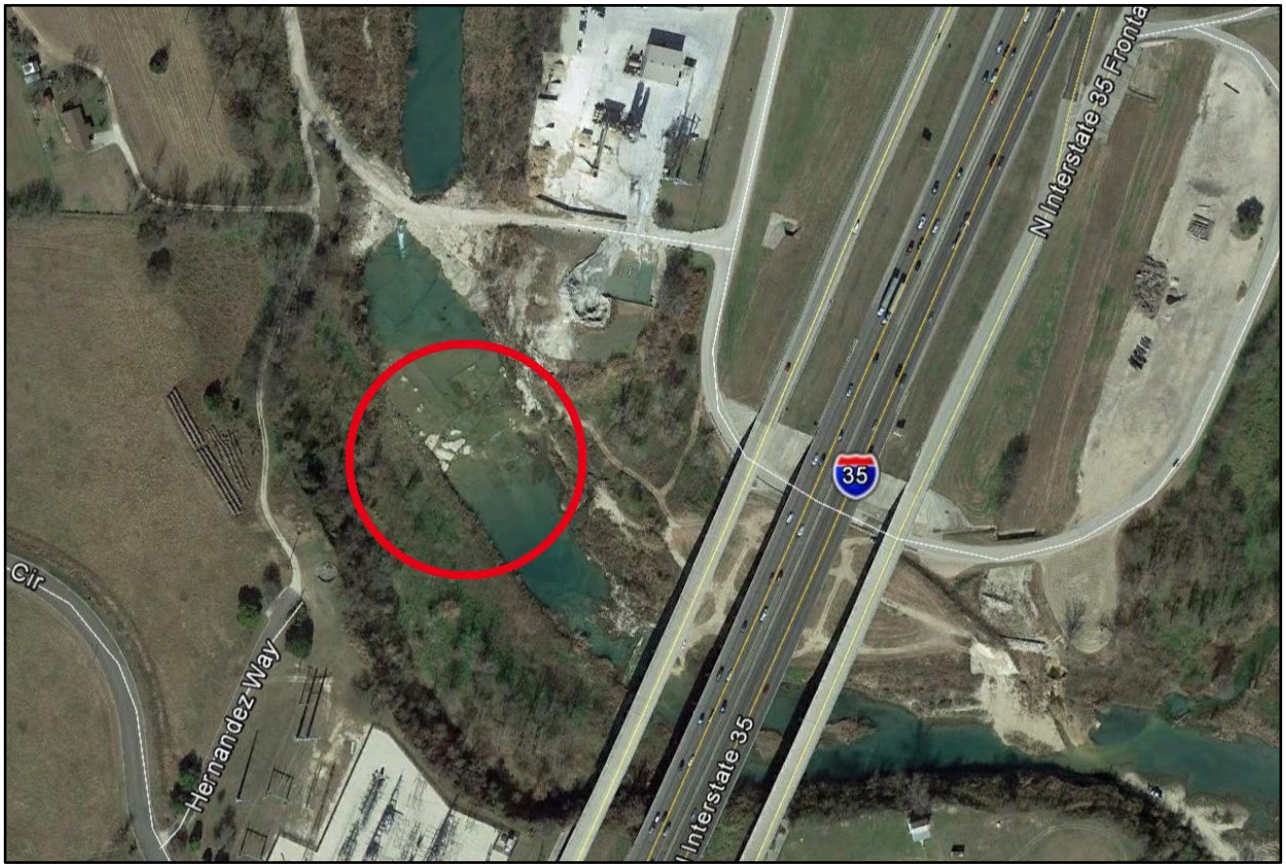

Figure 5. General vicinity (red circle) of Blanco blind salamander collection site in the Blanco River, Hays County, Texas.

3.0 Species Description

By 1952, only one preserved specimen of the salamanders collected from the Blanco River remained at Southwest Texas State Teachers College (Potter 1963, p. 2; Longley 1978, p. 18; Potter and Sweet 1981, p. 70). That specimen was provided to Floyd E. Potter, Jr., in 1961 (Potter 1963, p. 1; Potter and Sweet 1981, p. 70). Potter (1963, pp. 23-24) described this salamander as a new species, *Typhlomolge robusta*, in an unpublished master's thesis at the University of Texas at Austin. As that description appeared in an unpublished document, it was not valid under the International Commission on Zoological Nomenclature (Potter and Sweet 1981, p. 70).

Potter and Sweet (1981, pp. 70-73) published a redescription of *T. robusta* in the journal *Copeia*. The holotype is a mature female that displays distinct morphological adaptations to life underground including lack of skin pigmentation and much reduced eyes (Figure 6; Potter and Sweet 1981, p. 70). The specimen is 100.8 millimeters (mm) (3.99 inches [in]) in total length and described as heavy-bodied with robust limbs and a thick tail with moderately high fins (Potter and Sweet 1981, p. 70). Additional morphological data are provided in Potter and Sweet (1981, pp. 70-73). The Biodiversity Center at the University of Texas at Austin currently houses the holotype as specimen number 20255.

Chippindale et al. (2000, pp 23-24) later placed the genus *Typhlomolge* as a junior synonym of the genus *Eurycea*. As a result, the Blanco blind salamander is now referable to the genus *Eurycea*, along with other salamanders originally described as *Typhlomolge* species.

Figure 6. Blanco blind salamander, lateral and dorsal views. ©2019 Travis LaDuc. Biodiversity Center, University of Texas.

4.0 Stygobitic *Eurycea*

At the time of the Blanco blind salamander's redescription in 1981, two other stygobitic salamanders had been described from the eastern margin of the Edwards Plateau. The Texas blind salamander was described in 1896 from specimens taken from an artesian well drilled in the City of San Marcos (Stejneger 1896, pp. 619-621). Additional localities of this salamander were later discovered in that municipality, with one occupied cave (i.e., Rattlesnake Cave) located 3 km (2 mi) to the southwest of the type locality of the Blanco blind salamander (Figure 7; Longley 1978, p. 13).

The Comal blind salamander (*E. tridentifera*), thought to be a solely stygobitic species at the time, was described in 1964 from specimens collected from Honey Creek Cave in Comal County, approximately 56 km (35 mi) to the southwest of the Blanco blind salamander locality (Mitchell and Reddell 1965, p. 14; Sweet 1977, p. 199.1). An additional stygobitic species, the Austin blind salamander (*E. waterlooensis*), was described in 2001 from the Barton Springs system in the City of Austin (Hillis et al. 2001, pp. 269-271). That site is 40 km (25 mi) to the northeast of the type locality of the Blanco blind salamander.

Because of morphological incongruities (e.g., intraspecific sympatry of surface and subterranean forms) among several *Eurycea* populations (Wiens et al. 2003, pp. 503-504), genetic analyses have proven vital in refining the taxonomic relationships of central Texas *Eurycea* species (Chippindale et al. 2000, pp. 9-22; Bendik et al. 2013, pp. 6-15; Devitt et al. 2019, pp. 2626-2628). For example, sympatric, surface-dwelling forms (e.g., dark pigmentation and developed eyes) of the Comal blind salamander are now known (Sweet 1984, pp. 428-431; Bendik et al. 2013, pp. 8-9, 13-15), suggesting that species is not completely stygobitic. Devitt et al. (2019, p. 2629) found that the Comal blind salamander was not genetically distinct from the Cascade Caverns salamander (*E. latitans*) and recommended it be synonymized with the latter species.

Currently the Austin blind, Blanco blind, and Texas blind salamanders represent the only formally described stygobitic salamanders in Texas. An unnamed stygobitic *Eurycea* is known from New Braunfels, Comal County (*E.* sp. New Braunfels; Corbin 2020, pp. 17-19, 21, 24-26, 34, 38). Genetic studies have indicated that the Austin blind and Texas blind salamanders are distinct taxa (Wiens et al. 2003, pp. 507-508; Chippindale and Hillis 1994, p. 12; Chippindale et al. 2000, pp. 9-13, 18, 23-24; Hillis et al. 2001, pp. 274; Devitt et al. 2019, pp. 2627-2628). However, researchers have not conducted genetic analyses of the Blanco blind salamander, although several have tried. DNA has never been successfully isolated from the existing specimen (Chippindale and Hillis 1994, p. 4; Hillis et al. 2001, p. 267; Wiens et al. 2003, p. 504; Chippindale et al. 2000, p. 5; Devitt et al. 2019, p. 2631) purportedly due to the method of chemical preservation (Chippindale 2009, p. 4). Since no additional specimens have been discovered, only morphological analyses can be conducted, and those analyses are limited by the potential chemical alteration of the type specimen.

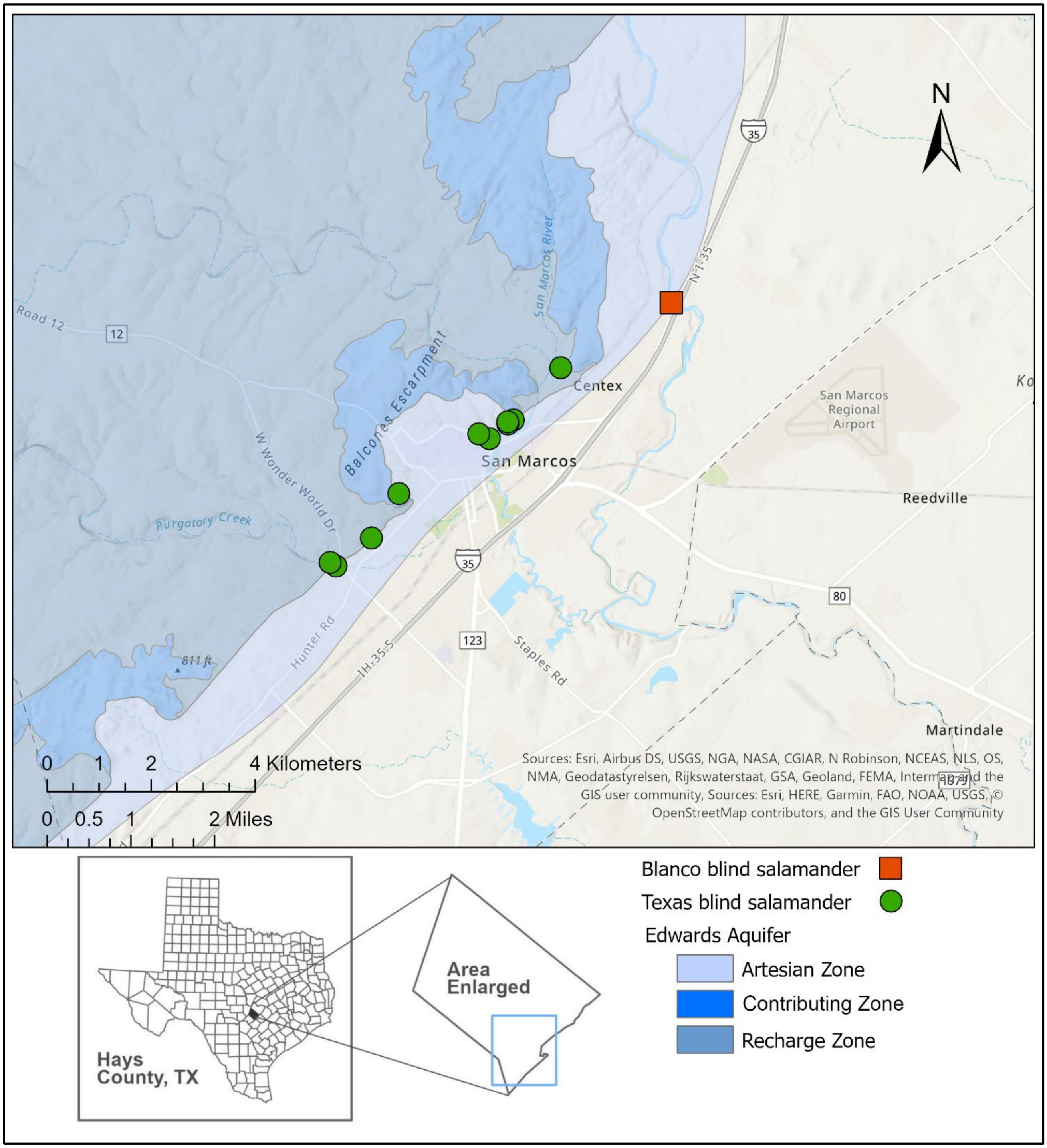

Figure 7. Distribution of the Blanco blind and Texas blind salamanders, along the eastern extent of the Edwards Aquifer, in the City of San Marcos, Hays County, Texas.

5.0 Blanco Blind versus Texas Blind Salamander

5.1 Morphological Comparison

Researchers have noted the morphological similarities that exist between Blanco blind and Texas blind salamander and consider the two sister taxa (Reddell 1967, p. 187; Chippindale et al. 2000, pp. 23-24; Wiens et al. 2003, p. 504). Adaptation to subterranean conditions can result in convergent evolution of stygomorphic traits in otherwise unrelated taxa (Christiansen 2012, pp. 518-520). For example, reduced eyes, depigmentation, elongated appendages, among other characters, often appear in stygomorphic arthropods, fish, and several salamander genera including *Eurycea*, *Gyrinophilus*, and *Proteus* (Wiens et al. 2003, p. 509; Gorički et al. 2012, pp. 666-674).

There are clearly shared traits among the Austin blind, Blanco blind, and Texas blind salamanders that are the result of adaptation to aquatic subterranean habitats (Chippindale et al. 2000, p. 2; Hillis et al. 2001, pp. 269-270). These traits can vary in their magnitude and proportions, which can assist in species delineations. The description of the Austin blind salamander by Hillis et al. (2001, pp. 269-271) provides detailed morphological comparisons with the Blanco blind and Texas blind salamanders. In particular, those authors noted the much shorter limbs, weakly developed tail fin, higher degree of pigmentation, distinct row of lateral iridophores, and smaller gills of the Austin blind salamander in comparison to the other two species (Hillis et al. 2001, p. 270).

In their description of the Blanco blind salamander, Potter and Sweet (1981, pp. 70-73) compared morphological characters from the holotype with a series of 10 Texas blind salamanders (Figure 8 and Table 1). The Blanco blind and Texas blind salamanders overlap in standard and total lengths. The number of costal grooves, vertebrae, and teeth are each identical between the two species (Potter and Sweet 1981, p. 72). Snout to gular fold (i.e., throat skin), snout to third gill ramus, distance between eyes, and tail length are also similar. Potter and Sweet (1981, p. 70) noted the two species shared similarities in skull structure including the "disproportionate lateral expansion of the anterior cranial elements, the absence of ossified orbitospenoids, and an arcuate lateral outline of the mandible." Figure 9 depicts the skulls of the Blanco blind and a Texas blind salamander.

Potter and Sweet (1981, p. 71) defined the Blanco blind salamander as distinct from the Texas blind salamander on the basis of the former's more "robust" body form, longer trunk, shorter limbs, broad, rounded tail, and rounded skull. Regarding the robust body of the Blanco blind salamander, the authors remark that the "musculature of the holotype is strongly developed." In terms of skull structure, Potter and Sweet (1981, p. 72) state that the rounded skull, in dorsal view, of the Blanco blind salamander, versus the oval skull of the Texas blind salamander, "showed no evidence of pathological deformity."

Potter and Sweet (1981, p. 70) indicated their examination of the Blanco blind salamander convinced them it was not aberrant individual. Their position regarding distinctiveness is largely premised on soft tissue differences (e.g., torso musculature and rounded tail) and skull shape. Those authors do apply a caveat to their redescription (Potter and Sweet 1981, p. 72), directly questioning the distinction of the Blanco blind salamander from the Texas salamander in stating:

“It is evident that *T. robusta* is referable to the genus *Typhlomolge* and supports the distinction previously drawn between that genus and *Eurycea*, if *T. robusta* is in fact specifically distinct from *T. rathbuni*.”

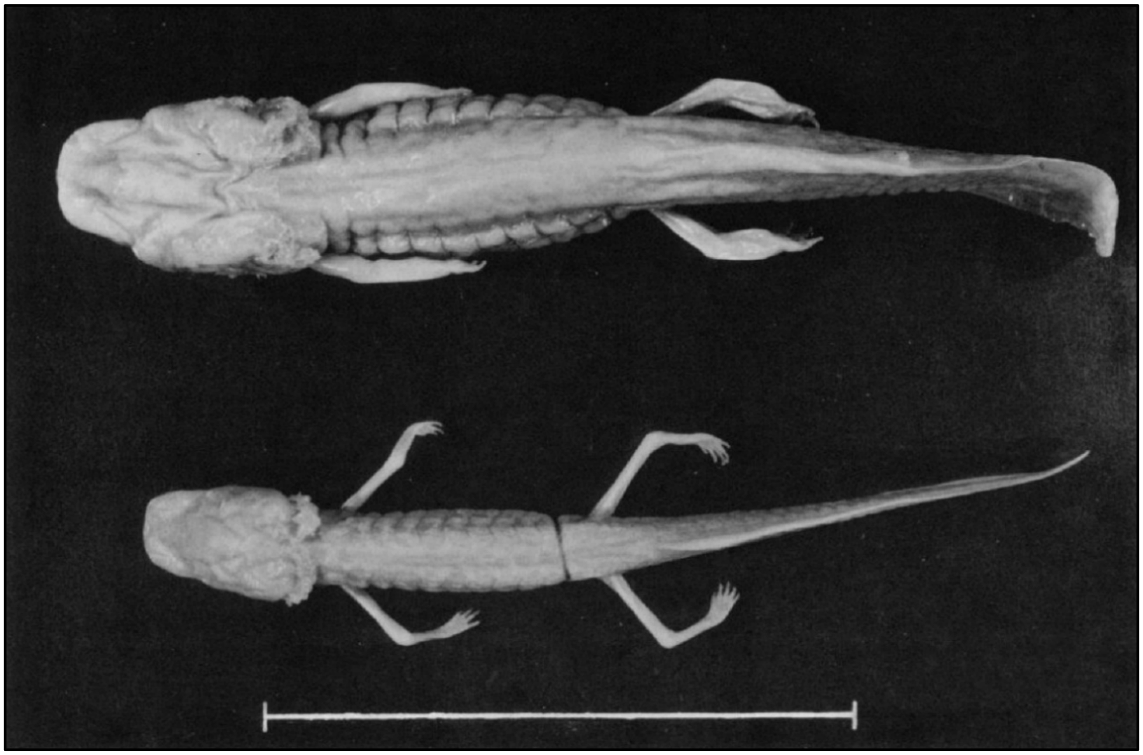

Figure 8. Dorsal view of Blanco blind salamander (top) and Texas blind salamander (bottom). Adapted from Potter and Sweet (1981, p. 70).

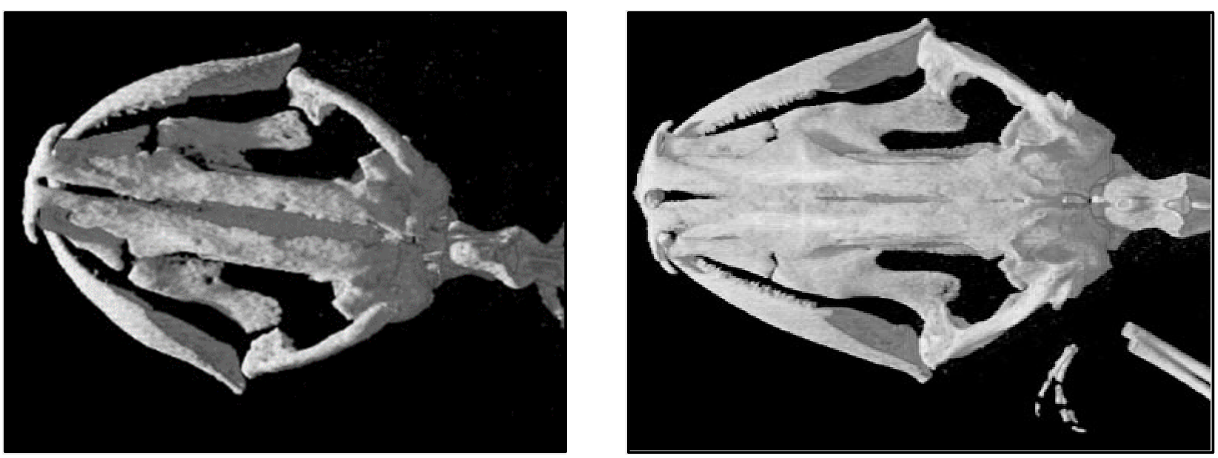

Figure 9. Skulls (dorsal view, not to scale) of the Blanco blind (left) and Texas blind (right) salamanders. Courtesy of Texas Memorial Museum.

Table 1. Morphological measurements for Blanco blind salamander holotype and averaged measurements of ten Texas blind salamanders (with exceptions where noted) from 1981 (Potter and Sweet 1981, pp. 70-71), measurements for the Texas blind salamander type specimen (Stejneger 1896, p. 621), and measurements for the Blanco blind salamander holotype and adult female Texas blind salamander from 2021. Bolded text denotes measurements cited by Potter and Sweet (1981, pp. 70-71) as distinguishing the Blanco blind salamander from the Texas blind salamander. All characters reported in mm (in) except where otherwise noted.

Character	**Blanco blind salamander type specimen (1981)[a]**	**Texas blind salamander series (n=10; 1981)[b]**	**Texas blind salamander type specimen (1896)[c]**	**Blanco blind salamander type specimen (2021)[d]**	**Texas blind salamander adult female (2021)[e]**
Standard length	57.1 (2.25)	54.0-60.6 (2.12-2.38)	53 (2.09)	56.06 (2.21)	64.35 (2.53)
Total length	100.8 (3.97)	94.0-103.2 (3.70-4.06)	102 (4.01)	94.42 (3.72)	138.06 (5.43)
Number of trunk vertebrae	13	13	NA	NA	NA
Snout to gular fold	14.3 (0.56)	14.2 (0.56)	16 (0.63)	14.21 (0.56)	16.47 (0.65)
Snout to third gill ramus	20.7 (0.81)	19.4 (0.76)	22 (0.87)	20.2 (0.79)	22.69 (0.89)
Width at jaw articulation	**15.1 (0.59)**	**10.7 (0.42)**	**NA**	**12.37 (0.49)**	**15.32 (0.60)**
Distance between eyes	5.2 (0.20)	4.9 (0.19)	6 (0.24)	NA	NA
Trunk width	**13.7 (0.54)**	**9.1 (0.34)**	**NA**	**12.21 (0.48)**	**10.24 (0.40)**
Axilla-groin length	**29.7 (1.17)**	**26.4 (1.04)**	**25 (0.98)**	**28.84 (1.13)**	**47.76 (1.88)**
Number of costal grooves	12	12	NA	12	12
Forelimb length	**17.3 (0.68)**	**19.1 (0.75)**	**20 (0.79)**	**16.6 (0.65)**	**18.98 (0.75)**
Upper forelimb width	3.3 (0.13)	1.6 (0.06)	NA	2.65 (0.10)	2.58 (0.10)
Hind limb length	**19.6 (0.77)**	**21.6 (0.85)**	**20 (0.79)**	**17.01 (0.67)**	**21.4 (0.84)**
Upper hind limb width	3.8 (0.15)	1.8 (0.07)	NA	3.20 (0.13)	3.06 (0.12)
Tail length	43.7 (1.72)	44.8 (1.76)	NA	38.36 (1.51)	73.53 (2.89)

[a] Blanco bline salamander type specimen (Potter and Sweet 1981)
[b] Series of 10 individuals; single values are means (Potter and Sweet 1981)
[c] Texas blind salamander type specimen (Stejneger 1896, p. 621)
[d] Blanco blind salamander type specimen (Measurements taken on June 21, 2021, Michael Warriner, U.S. Fish and Wildlife Service)
[e] San Marcos specimen (Measurements taken on June 21, 2021, Michael Warriner, U.S. Fish and Wildlife Service)

Stejneger (1896, p. 621) provides the original measurements for the type specimen of the Texas blind salamander (Table 1). That type specimen falls within the range of total lengths for the Texas blind salamanders measured by Potter and Sweet (1981, p.71), but falls slightly outside of the lower limits for standard length. The head of the type specimen is also particularly large. The head length is longer, the head width is greater, and the distance between the eyes is greater for the type specimen when compared to both the Blanco blind and Texas blind salamanders measured by Potter and Sweet (1981, p. 71). Several specimens of the Texas blind salamanders, with morphological characters larger than the specimens described in Potter and Sweet (1981, pp. 70-71), are known. For example, a Texas blind salamander collected from a well at Wonder World Cave (formerly Beaver Cave) in Hays County was found to have a total length of 120 mm (4.72 in) (Uhlenhuth 1921, p. 90). This length falls well outside the upper range (103.2 mm [3.70-4.06 in]) for Potter and Sweet's (1981, p. 71) Texas blind salamander series.

On June 21, 2021, Service personnel obtained measurements from the Blanco blind salamander type specimen and from a large Texas blind salamander specimen to provide additional insight into the range of morphological variability inherent to the Texas blind salamander (Table 1) and the potential effects of preservative on amphibian morphology (see Section 5.3 Effects of Preservation on Museum Specimens). The Texas blind salamander specimen (hereon referred to as the San Marcos specimen) was preserved on March 3, 2020, by the Service's San Marcos Aquatic Resource Center. The San Marcos specimen was originally collected from the wild and lived in captivity for over 20 years before its death, so its actual age is unknown.

The San Marcos specimen was among the largest female Texas blind salamander specimens held at that facility to date. Compared to the Blanco blind salamander, the San Marcos specimen has a much longer body (i.e., tail and trunk), a longer and wider head, and longer fore- and hind-limbs (Figure 10). The widths of fore- and hind-limbs, while not as wide as those of the Blanco blind salamander, are of a greater width than those reported for the Texas blind salamander series in Potter and Sweet (1981, pp. 70-71). The San Marcos specimen depicted in Figure 10 is also substantially larger and longer for most morphological characters when compared to the limited sample of Texas blind salamanders referenced by Potter and Sweet (1981, pp. 70-71). The average values for fore- and hind-limb length for the San Marcos specimen are somewhat shorter and wider than values reported for the Texas blind salamander series in Potter and Sweet (1981, pp. 70-71).

What these contemporary measurements suggest is that the range of morphological variability for the Texas blind salamander is much greater than that considered by Potter and Sweet (1981, entire). Potter (1963, p. 22) conceded as much in the initial description of the Blanco blind salamander, specifically that the Texas blind salamander "could have a much higher range of morphological variability than is current recognized." Older and larger individuals of that species may display morphological values that overlap or exceed those noted for the Blanco blind salamander as distinctive characters (i.e., longer trunk and shorter, wider limbs). Very little has been published to date on the range of morphological variation inherent to mature, female Texas blind salamanders. The limited morphological data on that species, coupled with the close proximity of the Blanco blind salamander, brings into question the validity of the latter species.

Figure 10. Blanco blind salamander type specimen (A) and Texas blind salamander (B) from the San Marcos Aquatic Resources Center, San Marcos, Texas. Images taken June 21, 2021.

5.2 Morphological Variation

In general, there are several limitations with designating species based on a single individual through morphological traits alone. Thessen et al. (2012, p. 1) proposes that such individuals may not actually be new species but rather an aberrant, damaged, or extreme variant of an existing species. A single individual for assessment limits consideration of the range of natural intraspecific variation (Dayrat 2005, p. 411; Thessen et al. 2012, p. 22).

Since only a single individual of the Blanco blind salamander exists, its range of morphological variation is unknown. Dayrat (2005, pp. 408-409) describes species delineated through morphology alone as "hypotheses that should be tested through different data and methodologies." This is especially relevant to the taxonomy of central Texas *Eurycea* where morphological variations have not consistently aligned with genetic assessments of species validity (Chippindale et al. 2000, pp. 9-22; Bendik et al. 2013, pp. 6-15; Devitt et al. 2019, pp. 2626-2628). Availability of a larger series of specimens would potentially have revealed more morphological variation than was currently known for the Texas blind salamander; variation that may have been consistent with character values from the Blanco blind salamander holotype.

An example of this potential variation was recently noted in the Berry Cave salamander (*Gyrinophilus gulolineatus*), a stygobitic, paedomorphic species from Tennessee (Gorički et al. 2012, pp. 673-674; Gladstone et al. 2018, pp. 31-32). Prior to 2018, most individuals are described as ranging between 80-105 mm (3.15-4.13 in) snout-vent lengths, with the largest known individual measuring 136 mm (5.35 in) (Brandon 1965, p. 349). Gladstone et al. (2018, pp. 32-33) discovered an even larger individual of that species that measured 145 mm (5.71 in) snout-vent length. Gladstone et al. (2018, p. 35) postulates that paedomorphic salamanders may be able to grow unimpeded in permanently aquatic habitats. Salamanders are generally typified by indeterminate growth (Hariharan et al. 2016, pp. 8-9). Indeterminate growth can be defined as the absence of developmental mechanisms that arrest growth (Hariharan et al. 2016, pp. 2-3).

Continued growth throughout life highlights the potential for morphological traits outside documented ranges of variation. Huxley (1950, p. 469) suggests that there is no fixed form for animals with indeterminate growth and that precise proportions are subject to constant change. Little information exists regarding the potential lifespans of stygobitic Plethodontid salamanders. In captivity, Texas blind salamanders have been recorded as living 20 years (Vieira et al. 2020, p. 7). Another stygobitic *Gyrinphilus* species, the Big Mouth Cave salamander (*G. palleceus necturoides*), has reached an age of 18 years in captivity (Snider and Bowler 1992, pp. 5-6). It is conceivable that both salamanders may be capable of living much longer, with growth continuing indefinitely but at a very slow rate (Niemiller and Poulson 2012, pp. 232-233). The morphological traits exhibited by very old stygobitic Texas *Eurycea* have not been assessed but could be an explanation for Blanco blind salamander being a morphologic variant of the Texas blind salamander.

5.3 Effects of Preservation on Museum Specimens

Potter and Sweet (1981, p. 72) touch on the potential for the morphology (i.e., robust body or well-developed musculature) of the Blanco blind salamander to "result from edema or storage in hypotonic preservative." They state that this phenomenon was not observed in preserved specimens of the Comal blind salamander. The Blanco blind salamander specimen was maintained at Southwest Texas State Teachers College for 10 years before transfer to Floyd E. Potter, Jr., in 1961 for his eventual description of the Blanco blind salamander (Potter and Sweet 1981, p. 70).

Standard processing for amphibians and reptile specimens involves chemical fixation and preservation with formalin, cleansing with water, and long-term storage in ethyl or isopropyl alcohol (Simmons 2002, pp. 41-45; Simmons 2019, pp. 492-496, 503-504). Both formalin and alcohol have the potential to distort preserved specimens resulting in morphological changes (e.g., shrinkage or swelling; Simmons 2019, p. 504) not seen in living or unpreserved, dead individuals. The details are unknown as to how the holotype was euthanized, if it was injured or expired during collection or transport, what chemicals were applied for fixation and preservation, how preservative levels were maintained, and the specimen's prior storage conditions.

Chemical fixation and preservation techniques have been noted to alter morphology of preserved specimens from a number of taxa including amphibians, fishes, and reptiles (Klauber 1943, p.

20; Lazell 1972, pp. 11, 18-19, 63; Andriguetto and Haimovici 1988, pp. 214-215; Vervust et al. 2009, pp. 323-328; Vajargah and Hedayati 2014, pp. 106-108; Haubrock et al. 2018, pp. 82-83; Sotola et al. 2019, pp. 5-12). Lee (1982, pp. 267-268) compared recently euthanized cane toads (*Bufo marinus*) to specimens preserved (i.e., fixed in formalin and preserved in alcohol) for six and 14 months. Morphological changes appeared six months post-preservation in some characters, including bony material and soft tissues (e.g., head width and snout-vent length) increasing in size when compared to unpreserved individuals (Lee 1982, pp. 268-269, 271, 279). Another contingent of morphological characters were significantly smaller after six months in preservative (Lee 1982, pp. 268-269). Lee (1982, p. 280) states that "the uncritical use of morphological data secured from preserved specimens could lead to spurious conclusions."

Bernal and Clavijo (2009, pp. 33, 28, 42), in a similar study with three frog species, also noted increases and decreases in morphological characters for preserved specimens after five months when compared to unpreserved individuals. Shu et al. (2017, pp. 5, 10) found that long-term preservation (i.e., up to 10 years) of 13 frog species resulted in significant decreases in body mass and length of preserved specimens. Those authors state, "preserved specimens are unlikely to accurately reflect the morphologies of live specimens, which may lead to different biological interpretations and result in false conclusions." Pierson et al. (2020, pp. 138-139) compared the effects of five fixative and preservation methods on Bay Springs salamanders (*Plethodon ainsworthi*) over 18 months. Those authors noted that direct preservation of the Bay Springs salamander in ethyl alcohol, rather than initial fixation in formalin, may result in inflated values for some morphological traits (i.e., snout-vent length: head width ratios).

Whether certain morphological traits exhibited by the Blanco blind salamander are an artifact of fixation and preservation is not known. However, much emphasis is placed on the trunk length (i.e., axilla-groin length) and width (i.e., well-developed musculature), shorter limbs, and rounded tail of the Blanco blind salamander holotype as distinguishing features from the Texas blind salamander (Table 1; Potter and Sweet 1981, p. 72). Substantial research indicates that a number of common herpetological fixation and preservation techniques can alter a specimen's soft and bony material. Values for such morphological characters have the potential to decrease or increase because of fixation and/or preservation. Dependence upon preserved specimens, especially for species descriptions, may not reflect the actual characteristics of living individuals. In this case, defining morphological characters of the Blanco blind salamander have the potential to have been altered by preservation methods and not represent actual traits of living salamanders. The measurements collected from the individual used as the type specimen may not represent actual traits of the salamander when it was alive.

As referenced above, the Blanco blind salamander type specimen was measured by Service staff on June 21, 2021. Measurement of the same characters 40 years after those cited by Potter and Sweet (1981, pp. 70-71) produced smaller values than reported by those researchers (Table 1). Specifically, standard and total lengths, width at jaw articulation, trunk width, axilla-groin length, and hind- and fore-limb lengths were all smaller than values from Potter and Sweet (1981, pp. 70-71). While there can be variability in measured values generated by different individuals, it does appear that the Blanco blind salamander specimen has experienced some degree of shrinkage, potentially due to chemical preservation. The specimen is currently stored in 70% ethanol but we do not know its full history of exposure to different chemical preservatives.

6.0 Hydrogeological Connectivity

The type locality of the Blanco blind salamander and sites occupied by the Texas blind salamander are located along the southeastern extent of the recharge or artesian zones of the San Antonio segment of the Edwards Aquifer. The San Antonio segment, along with local recharge, supports spring flow at San Marcos Springs and several other springs in Hays County (Smith et al. 2012, pp. 61-62; Smith et al. 2015, pp. 158-159; Green et al. 2019, pp. 44-45; Schindel 2019, p. 16). This segment of the Edwards Aquifer is defined from the Barton Springs segment to the north by a groundwater divide (Figure 11; i.e., boundary between two adjacent groundwater basins, which is represented by a high point in the water table).

The groundwater divide between the San Antonio and Barton Springs segments exists in Hays County between San Marcos Springs and the Blanco River to the south and Onion Creek to the north (Johnson et al. 2012, p. 4; Smith et al. 2012, pp. 62-64, 67). This divide shifts in relation to rainfall and recharge to the Edwards Aquifer, with groundwater moving north or south depending upon dry or wet conditions (Smith et al. 2012, pp. 58-59). With wet conditions, some amount of groundwater flows southward from Onion Creek towards the Blanco River and San Marcos Springs (Smith et al. 2012, pp. 62-63; Smith et al. 2015, pp. 158-159). Conversely, during dry periods groundwater generally flows from San Marcos Springs and the Blanco River to the northeast towards the Barton Springs segment of the Edwards Aquifer (Land et al. 2011, pp. 48-52; Johnson et al. 2012, pp. 82-84; Smith et al. 2012, pp. 62-63, 67; Smith et al. 2015, pp. 159-160).

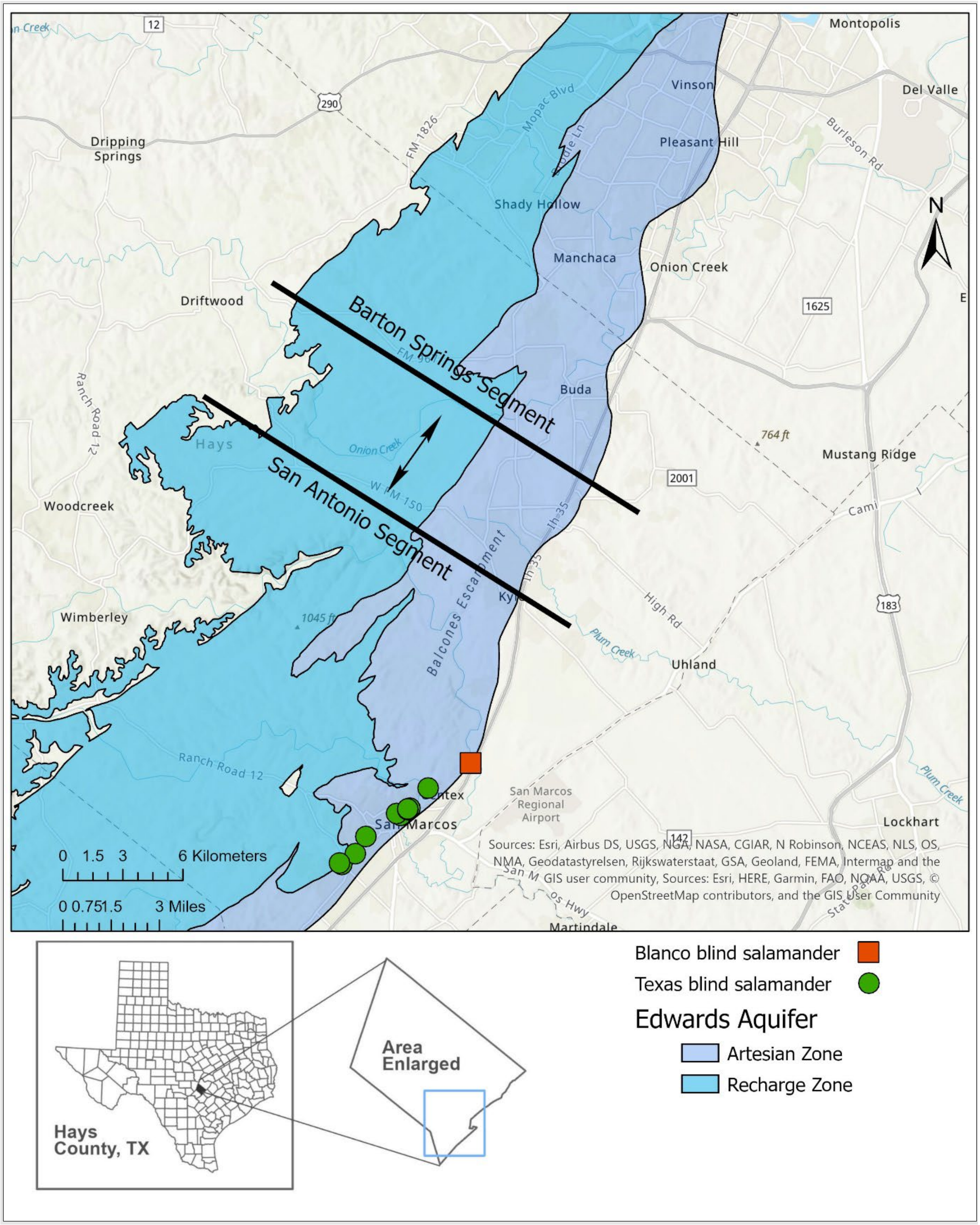

Figure 11. Northern and southern extents of the San Antonio and Barton Springs segments of the Edwards Aquifer (Johnson et al. 2012, p. 4). Arrow represents approximate location of groundwater divide between aquifer segments and generalized groundwater flow during dry (north) and wet periods (south).

Cretaceous-age Edwards Group formations (i.e., Kainer and Person) are the primary water-bearing strata of the Edwards Aquifer (DeCook 1963, p. 27; DeCook 1963, pp. 11-12, 30-31; MacLay 1995, p. 13; Clark et al. 2018, p. 4). Karstification of Edwards Group dolomite and limestone has created a highly permeable network of conduits, fissures, and fractures that facilitate groundwater flow (DeCook 1963, pp. 34-36; Hanson and Small 1995, pp. 4, 8-9; Ferrill et al. 2004, pp. 407, 409; Lindgren et al. 2004, pp. 13, 15-16; Ferrill et al. 2019, pp. 180-181). In the artesian zone, the Edwards Group is confined, bottom to top, by younger, less permeable formations including Del Rio Clay, Buda Limestone, Eagle Ford Shale, and Austin and Pecan Gap Chalks (DeCook 1963, pp. 37-38, 45; Grimshaw and Collins 1986, p. 72; Hanson and Small 1995, pp. 5-7; Clark et al. 2018, p. 4).

The San Marcos Springs Fault represents the southeastern boundary of the Edwards Group in Hays County (Figure 12; DeCook 1963, p. 45; Russell 1976, p. 8; Musgrove and Crow 2012, p. 5). The San Marcos Springs Complex issues from this fault (Hanson and Small 1995, p. 9; Musgrove and Crow 2012, pp. 6-8). Near San Marcos Springs, the Edwards Group is in contact with Pecan and/or Austin Chalks (DeCook 1963, pp. 39, 45; Grimshaw and Woodruff 1986, p. 74). The San Marcos Springs Fault strikes from the southwest to the northeast, separating into two northeastern trending branches (Grimshaw and Collins 1986, pp. 72, 74; Blome et al. 2005; Grimshaw 2013). One diverging branch of the San Marcos Springs Fault extends under the Blanco River, just west of Interstate 35, in close proximity to the Blanco blind salamander collection site.

Faults, along with bedding planes and joints, can serve as groundwater flow conduits, with permeability increasing laterally and vertically parallel to these planar breaks (Senger and Kreitler 1984, pp. 7-8; Faunt 1997, pp. 30-32; Ferrill et al. 2004, pp. 407, 409, 415; Bense et al. 2013, pp. 182, 185). Groundwater flow along faults can increase the size of these features, resulting in the development of larger subterranean voids (e.g. caverns; Ford and Williams 2007, pp. 31-35, 104, 112-114; Stafford et al. 2014, p. 13). Dissolution of the Edwards Group has resulted in the formation of conduits and water-filled caves in southeastern Hays County, several inhabited by the Texas salamander including, from south to north, Primer's Fissure and Ezell's, Wonder World, and Rattlesnake Caves (Figure 13; Uhlenhuth 1921, pp. 74-96; DeCook 1963, pp. 31-32; Russell 1976, pp. 1-2, 11; Longley 1978, pp. 17-18, 28-29, 38-40; Wermund et al. 1978, pp. 10, 12). These caves are located along or adjacent to the northeastern trending San Marcos Springs Fault, and a network of smaller, intersecting faults, in the City of San Marcos (DeCook 1963, p. 31).

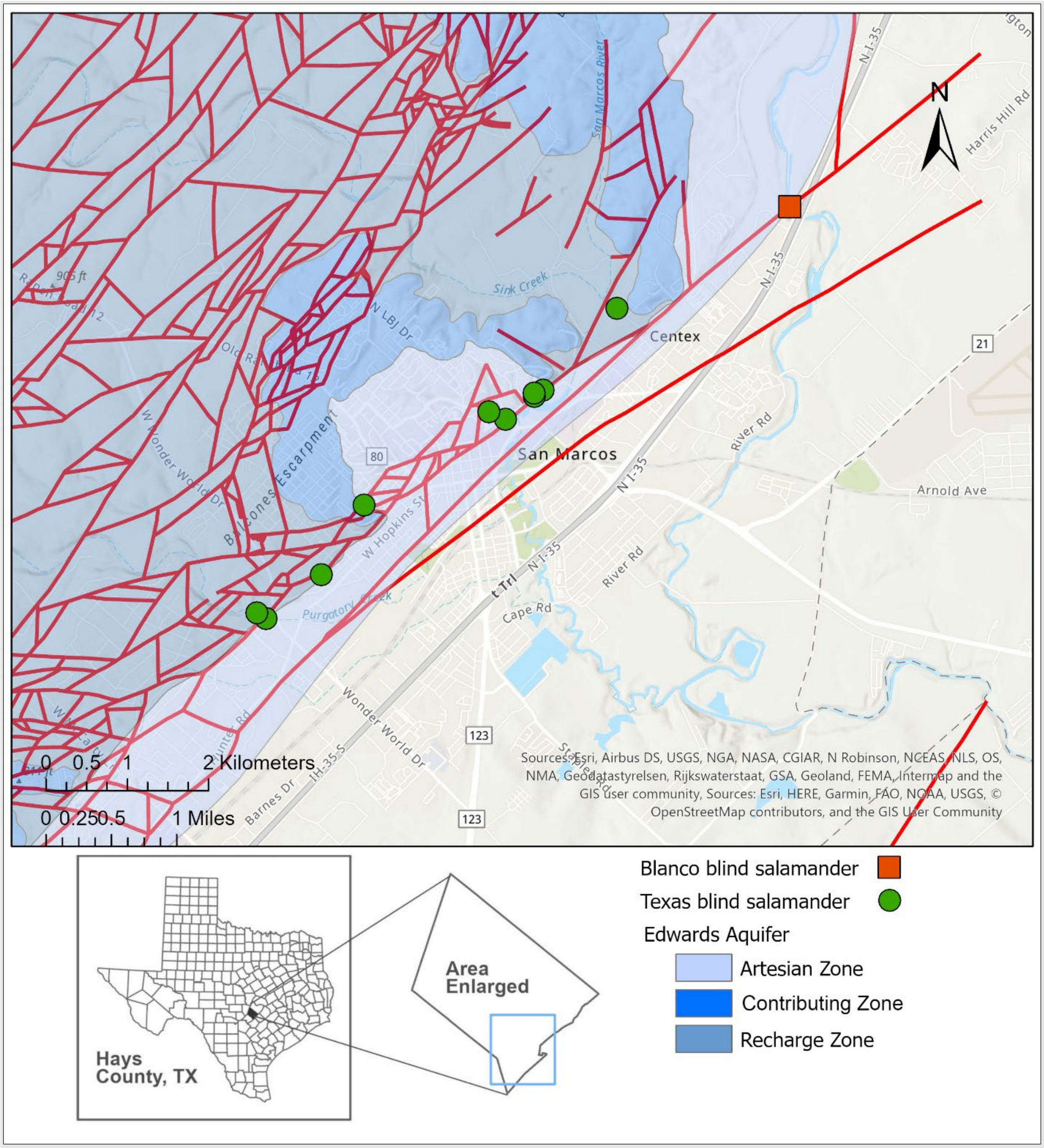

Figure 12. Distribution of Blanco blind salamander Texas blind salamander in relation to faults in the City of San Marcos, Hays County, Texas. Red lines depict observed, concealed, and inferred faults from Grimshaw (2013).

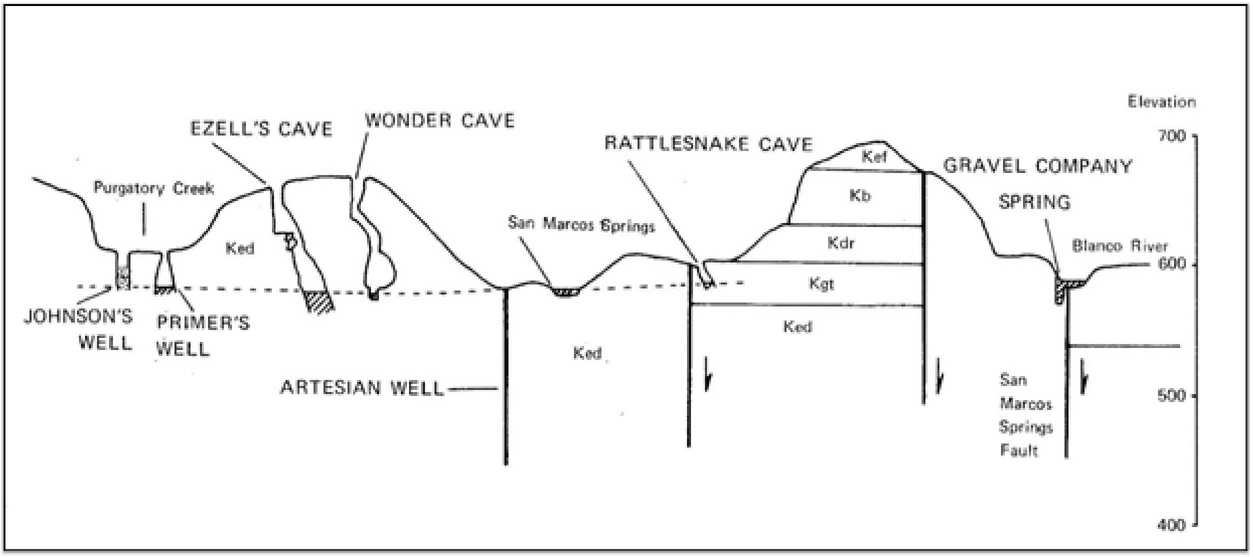

Figure 13. Geologic cross section of sites occupied by the Texas blind salamander, roughly parallel to the San Marcos Springs Fault. The location labeled Gravel Company Spring is type locality of the Blanco blind salamander. The dashed line is groundwater level. Abbreviations - Kef: Eagle Ford; Kb: Buda Limestone; Kdr: Del Rio Clay; Kgt: Georgetown Limestone; and Ked: Edwards Limestone. Adapted from Russell (1976, p. 11).

Uhlenhuth (1921, pp. 79, 85-86, 88, 90, 92-95, 98, 101) postulated that water in Texas blind salamander caves was interconnected and from the same source as San Marcos Spring. Dye-tracing studies have since confirmed groundwater connectivity among many of these water-filled caves, San Marcos Springs, and smaller springs in the area (Figure 14; Ogden et al. 1986, pp. 117-118; Johnson et al. 2012, pp. 8-10, 22-87; Johnson et al. 2019, pp. 286, 288, 291). These studies have delineated a complex network of conduits that facilitate groundwater flow in and around the City of San Marcos (Johnson et al. 2012, pp. 9, 24, 26, 33, 40, 52, 67, 69, 75, 83).

This groundwater flow system plausibly serves as dispersal corridors for the Texas blind salamander. Research to evaluate movement patterns (i.e., mark-recapture) of that species have been limited given the relative inaccessibility of its habitat, however (Krejca and Gluesenkamp 2007, entire). Preliminary evaluation of Texas blind salamander genetic population structure suggests that sampled localities for this species are not reproductively isolated and interbreed (Chippindale 2009, pp. 8-9; Corbin 2020, p. 75), indicative of connectivity among cave and spring sites.

Russell (1976, p. 31) described the small spring, discovered in the dry bed of the Blanco River, as occurring in an exposure of Austin Chalk. Austin Chalk along the Blanco River consists of beds of chalky limestone interbedded with calcareous shale (DeCook 1963, pp. 37-38). This formation has some capacity to transmit water through solution cavities, with cavernous formations including some of the largest subterranean voids in central Texas (i.e., Robber Baron Cave; Livingston et al. 1936, p. 69; Arnow 1963, pp. 17-18; Stein 1995, p. 29; Cokendolpher 2004, pp. 37-38, 42, 53; Banta and Clark 2012, pp. 2-3). Compact layers of chalk, marl, and shale can limit the Austin Chalk's relative permeability, however (Livingston et al. 1936, p. 69; DeCook 1963, p. 38).

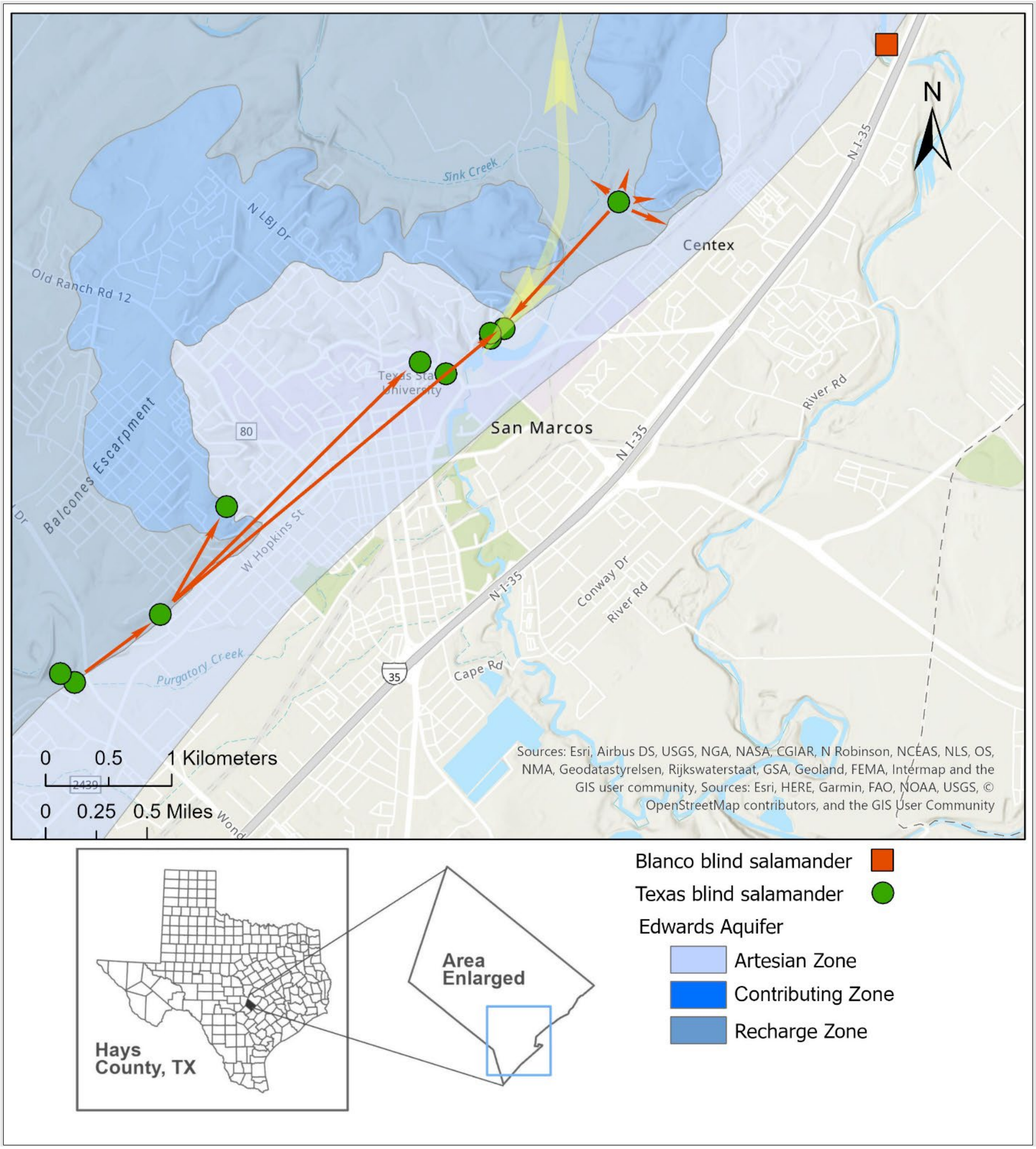

Figure 14. Groundwater connectivity among Texas blind salamander localities (red arrows indicate flow direction) based on dye-trace studies (Ogden et al. 1986, pp. 117-118; Johnson et al. 2012, pp. 8-10, 22-87; Johnson et al. 2019, pp. 286, 288, 291). Yellow arrow indicates generalized groundwater flow between San Antonio and Barton Springs segments of the Edwards Aquifer. Groundwater flows north to Barton Springs during wet periods and to the south to San Antonio segment during dry periods (Land et al. 2011, pp. 41, 48; Johnson et al. 2012, pp. 9, 24, 26, 33, 75, 83; Smith et al. 2012, pp. 63, 65).

Groundwater may be supplied to Austin Chalk by underflow where it is in contact with the underlying Edwards Group (DeCook 1963, p. 51; Clark et al. 2018). Livingston (1936, p. 70) proposed that fissures in the Austin Chalk, where the formation outcrops near faults, facilitate the transport of water to the underlying Edwards Group along fault planes. That situation exists at the Blanco blind salamander type locality. After the type specimen was collected and the excavated fissure was abandoned, water continued to upwell and pool in the excavation (Russell 1976, p. 30). This continued flow of groundwater suggests that some connection probably existed between the Austin Chalk and Edwards Group strata.

Russell (1976, pp. 30-31) suggested the Blanco blind salamander did not originate from the Austin Chalk, given its relative impermeability, but instead "the most obvious choice for the source of the salamander is the subsurface Edwards Limestone." That researcher postulated that a large, stygobitic salamander inhabiting the more interconnected and cavernous Edwards Group was more plausible than the less permeable Austin Chalk; therefore, the type specimen more likely came from the aquifer below the fissure rather than from the fissure itself. Such a stance discounts the actual permeability inherent to the Austin Chalk and the existence of connectivity between that rock layer and the Edwards Group.

Potter (1963, pp. 5-6) partially premised his recognition of the Blanco blind salamander as distinct from the Texas blind salamander on the presumption that the two species were hydrogeologically separated for some time (i.e., since the Eocene, 56 to 34 million years ago). That author provides limited evidence for that assertion. Potter and Sweet (1981, pp. 72-73) largely recapitulate Russell (1976, pp. 30-31) but suggest that the Blanco blind salamander may be restricted to north and east of the Blanco River.

Subterranean networks of water-filled conduits can facilitate stygobite gene flow across a karst system. Buhay and Crandall (2005, pp. 4623-4624, 4629) noted moderate to high levels of genetic diversity and gene flow among populations of two stygobitic crayfish species (i.e., *Orconectes incomptus* and *O. australis*) in the southeastern United States. Research on the population structure of the European stygobitic salamander, the olm (*Proteus anguinus*), noted gene flow between two hydrogeologically connected caves 10 km (6 mi) apart (Zakšek et al. 2017, pp. 243-245). Additional work on that species has found further evidence of *P. anguinus* movement across an interconnected subterranean system (Gorički et al. 2017, p. 7; Vörös et al. 2019, p. 217).

Most localities of the Texas blind salamander occur along an area of high fault density with Rattlesnake Cave as an outlier along a fault to the northeast. The entrance of this cave is formed in the Edwards Group and provides access to the water table (Johnson et al. 2012, p. 73). Flowpaths have been documented from that cave to multiple springs approximately 1.6 km (1 mi) to the southeast in the San Marcos Springs complex (Johnson et al. 2012, pp. 8-10, 73-79). Dye-tracing of springs and wells immediately to the north, northwest, and northeast of Rattlesnake Cave also receive some groundwater flow from that cave (Johnson et al. 2012, pp. 74-77).

Like known Texas blind salamander sites, the type locality of the Blanco blind salamander is situated along the San Marcos Fault. Groundwater movement likely exists along this fault, and associated conduits, that enabled Texas blind salamanders to disperse below the Blanco River.

No evidence exists of a hydrogeological barrier that would reproductively isolate individual salamanders at that site from individuals to the southwest. Based on the high degree of hydrogeological connectivity documented across the range of the Texas blind salamander, it is probable that the salamander specimen retrieved from the Blanco River represents an aberrant or altered (i.e., effect of preservative) Texas blind salamander.

7.0 Survey Efforts and Potential Extirpation

The Edwards Aquifer, in the area of southeastern Hays County, has been and continues to be intensively sampled for its diverse and unique groundwater fauna. Beginning in the late 19th century, caves, springs, and wells in the area have yielded many new species including the Texas blind salamander and a contingent of endemic groundwater invertebrates (Benedict 1896, entire; Ulrich 1992, pp. 85-98; Reddell and Mitchell 1969, pp. 3-6, 8-9, 11, 14; Bowman and Longley 1976, pp. 490-494; Young and Longley 1976, pp. 788-791; Holsinger and Longley 1980, pp. 1-3, 5-50; Külköylüoğlu et al. 2017, pp. 176-182; Schwartz et al. 2019, pp. 501-509; Alvear et al. 2020, pp. 12; Hutchins et al. 2021, pp. 2-3, 6-13).

Significant groundwater connectivity exists across the Edwards Aquifer in Hays County (See 6.0 Hydrogeological Connectivity) and this connectivity is evidenced in documented flow paths among many locations inhabited by the Texas blind salamander (Ogden et al. 1986, pp. 117-118; Johnson et al. 2012, pp. 8-10, 22-87; Johnson et al. 2019, pp. 286, 288, 291). Connections among populations of this salamander are underscored by the apparent lack of reproductive isolation among sites (Chippindale 2009, pp. 8-9; Corbin 2020, p. 75). Similar patterns of occurrence, at many of the same springs and wells inhabited by the Texas blind salamander, have also been documented for several Edwards Aquifer groundwater invertebrates (e.g., *Artesia subterranea*, *Cirolandes wassenichae*, *Palaemonetes antrorum*, and *Seborgia relicta*) (ZARA Environmental LLC 2010, pp. 52, 54-55; Hutchins et al. 2013, p. 15; Schwartz et al. 2019, p. 503).

Gluesenkamp and Krejca (2007, pp. 3, 7, 9) conducted surveys in 2006 to relocate the Blanco blind salamander at the type locality and several groundwater wells north of that site in Hays and Travis counties (Figure 15). Those researchers excavated three crevices in the dry bed of the Blanco River but none of the excavations extended to subterranean voids and no salamanders were observed (Gluesenkamp and Krejca 2007, p. 4). Surveyed wells ranged from 8-25 km (5-15 mi) north of the Blanco River and did not yield stygobitic *Eurycea* (Gluesenkamp and Krejca 2007, pp. 4-6, 8). Recent survey efforts of wells and springs in Hays County in 2020 and 2021 have also not resulted in discovery of specimens of the Blanco blind salamanders or other stygobitic *Eurycea* to date (Tovar 2021, pers. comm.). Conversely, Texas blind salamanders are regularly observed and collected by 10(a)(1)(A) permitted researchers from several localities in the City of San Marcos. Since 1951, no other stygobitic *Eurycea* have been taken from the Blanco River or sites to the north of the river in Hays County. If the Blanco blind salamander was a valid species, we have no evidence it remains extant.

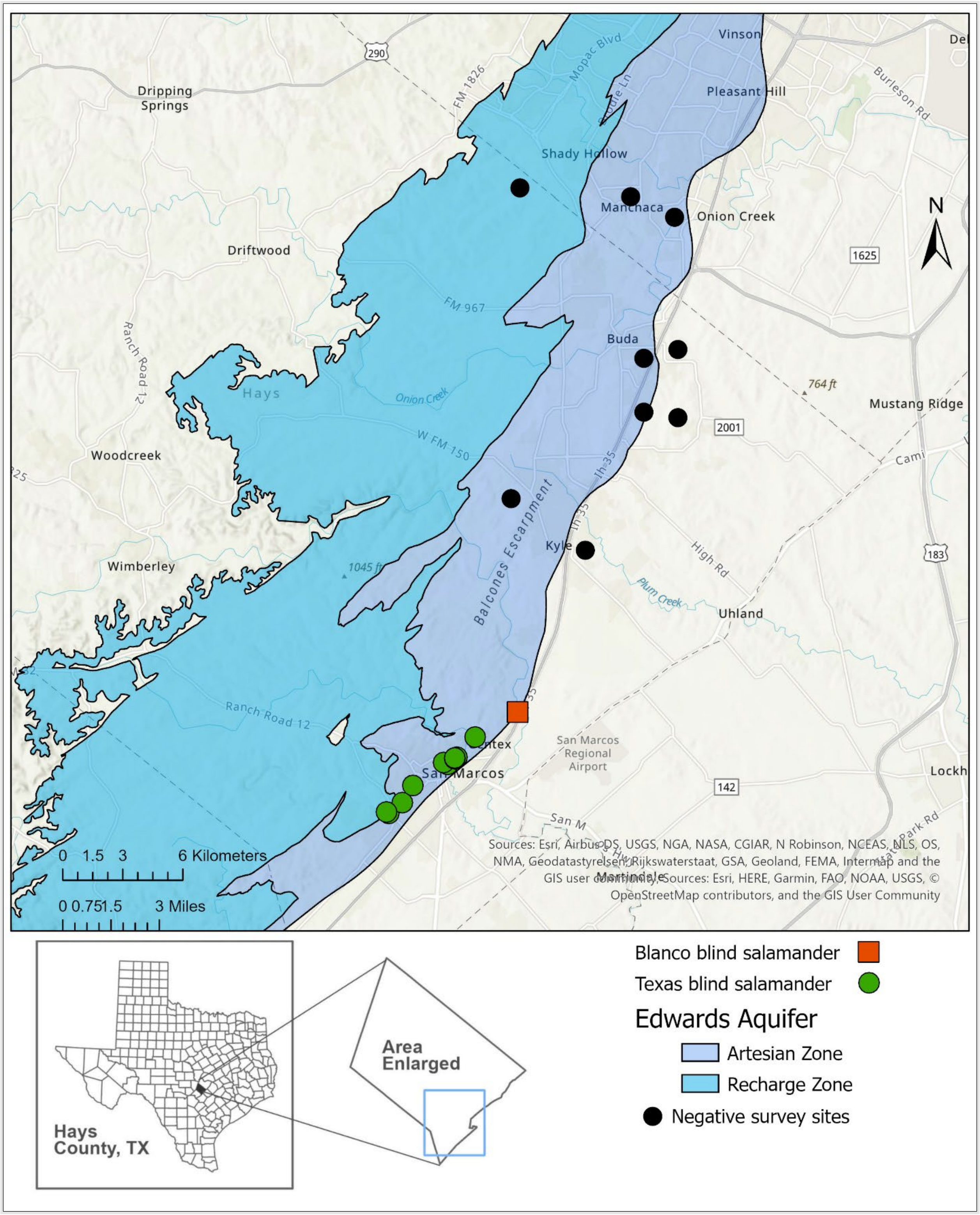

Figure 15. Sites surveyed in 2006 to relocate the Blanco blind salamander (Gluesenkamp and Krejca 2007, pp. 4-8).

8.0 Conclusions

In assessing the best available scientific information for the status of a species, the Service generally relies on information published in peer-reviewed journals or other reports. Particularly related to taxonomic determinations, we defer to the scientific literature and to professional authorities for taxonomical assignments (Service 1992, pp. 1-3). For salamander species, we typically defer to the Society for the Study of Amphibians and Reptiles (Crother 2021, entire). However, when that information is in question, the Service can and should conduct its own analysis and exercise our best scientific judgement.

There are several lines of compelling evidence indicating that the Blanco blind salamander does not exist as a current taxonomic entity. The type specimen on which the species' description was based either represents a historical occurrence of the Texas blind salamander or it represents a unique species that is no longer extant. When it was described, the single specimen was compared to a series of ten Texas blind salamander individuals that did not sufficiently account for natural morphological variation of the species. Several morphological characters of the Blanco blind salamander overlap or are identical to the Texas blind salamander. The Blanco blind salamander species description relies on characters that could have been influenced by chemical fixation and preservation and may not be reflective of living or freshly dead individuals. Genetic analyses of the single specimen have been attempted but were unsuccessful.

In addition, the type locality of the Blanco blind salamander is located along the northeastern reach of the San Marcos Springs Fault. Significant groundwater connectivity has been documented among several springs inhabited by the Texas blind salamander in the City of San Marcos. Hydrogeological connectivity also exists along the San Marcos Springs Fault, among those sites, and the Blanco River. As a result, subterranean dispersal corridors likely existed to facilitate movement of Texas blind salamanders to water-filled voids beneath that river. Nowhere in North America do two species of stygobitic *Euycea* co-exist at a location, and given the potential for groundwater connectivity, it is unlikely that a group of salamanders would be isolated from the larger population of Texas blind salamanders less than 3 km (2 mi) to the southwest. The type locality of the Blanco blind, therefore, could be recognized as the most northeastern occurrence of the Texas blind salamander. Since 1951, no stygobitic *Eurycea* have been collected from the Blanco River or areas to the north of that river in Hays County. Texas blind salamanders are collected on an annual basis from several sites immediately to the south of the Blanco River in the City of San Marcos. Additional surveys at the location of the Blanco blind salamander type specimen did not locate salamander individuals of any species. If the Blanco blind salamander was a valid taxon, we have no evidence that the species remains extant.

References

Abbott, P.L. and C.M. Woodruff, Jr., editors. The Balcones Escarpment Geology, Hydrology, Ecology, and Social Development in Central Texas. Geological Society of America. 200 pp.

Alvear, D., P.H. Diaz, J.R. Gibson, B.T. Hutchins, B. Schwartz, and K.E. Perez. 2020. Expanding the known ranges of the phreatic snails (Mollusca, Gastropoda, Cochliopidae) of Texas, USA. Freshwater Mollusk Biology and Conservation 23: 1-17.

Andriguetto, J.M., Jr. and M. Haimovici. 1988. Effects of fixation and preservation methods on the morphology of a Loliginid squid (Cephalopoda: Myopsida). American Malacological Bulletin, Vol. 6(2): 213-217.

Arnow, T. 1963. Ground-water geology of Bexar County, Texas. Geological Survey Water-supply Paper 1588. 36 pp.

Banta, J.R. and A.K. Clark. 2012. Groundwater levels and water-quality observations pertaining to the Austin Group, Bexar County, Texas, 2009–11. U.S. Geological Survey Scientific Investigations Report 2012–5278. 18 pp.

Bendik, N.F. and A.G. Gluesenkamp. 2012. Body length shrinkage in an endangered amphibian is associated with drought. Journal of Zoology doi:10.1111/jzo.12009.

Bendik, N.F., K.D. McEntire, and B.N. Sissel1. 2016. Movement, demographics, and occupancy dynamics of a federally threatened salamander: evaluating the adequacy of critical habitat. PeerJ 4:e1817; DOI 10.7717/peerj.1817.

Bendik N.F., J.M. Meik, A.G. Gluesenkamp, C.E. Roelke, and P.T. Chippindale. 2013. Biogeography, phylogeny, and morphological evolution of central Texas cave and spring salamanders. BMC Evolutionary Biology 13:201.

Benedict, J.E. 1896. Preliminary descriptions of a new genus and three new species of crustaceans from an anrtesian well at San Marcos, Texas. Proceedings of the United States National Museum 18: 615-617.

Bense, V.F., T. Gleeson, S.E. Loveless, O. Bour, and J. Scibek. 2013. Fault zone hydrogeology. Earth-Science Reviews 127: 171-192.

Bernal, M.H. and J.A. Clavijo. 2009. An essay on precision in morphometric measurements in anurans: inter-individual, intra-individual and temporal comparisons. Zootaxa 2246: 32-44.

Blome, C.D., J.R. Faith, D.E. Pedraza, G.B. Ozuna, J.C. Cole, A.K. Clark, T.A. Small, and R.R.

Bowman, T. and G. Longley. 1976. Redescription and assignment to the new genus *Lirceolous* of the Texas troglobitic water slater, *Asellus smithii* (Ulrich)(Crustacea: Isopoda: Asellidae). Proceedings of the Biological Society of Washington 88(45): 489-496.

Bonacci, O., T. Pipan, and D.C. Culver. 2009. A framework for karst ecohydrology. Environmental Geology 56: 891-900.

Brandon, R.A. 1965. A new race of the neotenic salamander *Gyrinophilus palleucus*. Copeia 1965 (3): 346-352.

Buhay, J.E. and K.A. Crandall. 2004. Subterranean phylogeography of freshwater crayfishes shows extensive gene flow and surprisingly large population sizes. Molecular Ecology 14: 4259-4273.

Center for Biological Diversity. 2012. Petition to list 53 amphibians and reptiles in the United States as threatened ro endangered under the Endangered Species Act. Petition submitted to the Secretary of the Interior. 454 pp.

Chippindale, P. 2009. Population genetic analysis of the Texas blind salamander, *Eurycea rathbuni*. Final Report Grant No. TX E-78-R, Texas Parks and Wildlife Department. 26 pp.

Chippindale, P.T., D.M. Hillis, and A.H. Price. 1994. Relationships, status, and distribution of central Texas hemidactyliine plethodontid salamanders (*Eurycea* and *Typhlomolge*). Final Report Grant No. E-I-4, Texas Parks and Wildlife Department. 24 pp.

Chippindale, P.T., A.H. Price, J.J. Wiens, and D.M. Hillis. 2000. Phylogenetic relationships and systematic revision of central Texas Hemidactyliine Plethodontid salamanders. Herpetological Monographs (14): 1-80.

Christiansen, K. 2012. Morphological adaptations. Pages 517-528 *in* White, W.B. and D.C. Culver, editors. Encyclopedia of Caves, second edition. Elsevier, Inc. 945 pp.

Clark, A.K. D.E. Pedraza, D.E., and R.R. Morris. 2018. Geologic framework and hydrostratigraphy of the Edwards and Trinity aquifers within Hays County, Texas. U.S. Geological Survey Scientific Investigations Map 3418, 1 sheet, scale 1:24,000, pamphlet, https://doi.org/10.3133/sim3418. 11 pp.

Cohen, A.B. 2020. Population genomics and conservation of Texas cave and spring salamanders (Plethodontidae: *Eurycea*). University of Texas at Arlington, Ph.D. Dissertation. 92 pp.

Cokendolpher, J.C. 2004. *Cicurina* spiders from caves in Bexar County, Texas (Araneae: Dictynidae). Texas Memorial Museum, Speleological Monographs, 6:13-58.

Corbin, A.B. 2020. Population genomics and conservation of Texas cave and spring salamanders (Plethodontidae: Eurycea). Ph.D. Dissertation, University of Texas at Arlington. 92 pp.

Culver, D.C. and T. Pipan. 2009. The biology of caves and other subterranean habitats. Oxford University Press. 256 pp.

Crother, B. I. (editor). 2017. Scientific and Standard English Names of Amphibians and Reptiles of North America North of Mexico, with Comments Regarding Confidence in Our Understanding. SSAR Herpetological Circular 43. 102 pp.

Culver, D.C., M.C. Christman, W.R. Elliott, H.H. Hobbs III, and J.R. Reddell. 2003. The North American obligate cave fauna: regional patterns. Biodiversity and Conservation 12: 441-468.

Culver D.C., L.L. Master, M.C. Christman, and H.H. Hobbs. 2000. Obligate cave fauna of the 48 contiguous United States. Conservation Biology 14: 386-401.

Danielpol, D.L., C. Griebler, A. Gunatilaka, and J. Notenboom. 2003. Present state and future prospects for groundwater ecosystems. Environmental Conservation 30(2): 1-27.

Dayrat, B. 2005. Towards integrative taxonomy. Biological Journal of the Linnaean Society 85: 407–415.

DeCook, K.J. 1963. Geology and ground-water resources of Hays County, Texas. Geological Survey Water-Supply Paper 1612. 72 pp.

Devitt, T.J., A.M. Wright, D.C. Cannatella, and D.M. Hillis. 2019. Species delimitation in endangered groundwater salamanders: Implications for aquifer management and biodiversity conservation. PNAS 116 (7): 2624-2633.

Diaz, P.H., J.N. Fries, T.H. Bonner, M.L. Alexander, and W.H. Nowlin. 2015. Mesohabitat associations of the threatened San Marcos salamander (*Eurycea nana*) across its geographic range. Aquatic Conservation: Marine and Freshwater Ecosystems 25: 307-321.

Faunt, C.C. 1997. Effect of faulting on ground-water movement in the Death Valley region, Nevada and California. U.S. Geological Survey, Water-Resources Investigations Report 95-4132. 42 pp.

Ferrill, D.A., A.P. Morris, and R.N. McGinnis. 2019. Geologic structure of the Edwards (Balcones Fault Zone) Aquifer. Pages 171-188 *in* Sharp, J.M., Jr., R.T. Green, and G.M. Schindel, editors. The Edwards Aquifer: The Past, Present, and Future of a Vital Water Resource: Geological Society of America Memoir 215. 312 pp.

Ferrill, D.A., D.W. Sims, D.J. Waiting, A.P. Morris, N.M. Franklin, and A.L. Schultz. 2004. Structural framework of the Edwards Aquifer recharge zone in south-central Texas. GSA Bulletin 116 (3/4): 407–418.

Ford, D. and P. Williams. 2007. Karst Hydrogeology and Geomorphology. John Wiley & Sons Ltd. 576 pp.

Forest Guardians. 2007. A petition to list all critically imperiled or imperiled species in the southwest United States as threatened or endangered Under the Endangered Species Act, 16 U.S.C. §§ 1531 et seq. Petition submitted to the U.S. Fish and Wildlife Service. 55 pp.

Frey, W.H. 2012. Population growth in metro America since 1980: putting the volatile 2000s in perspective. Metropolitan Policy Program, The Brookings Institution, Washington, D.C. 27 pp.

George, P.G., R.E. Mace, and R. Petrossian. 2011. Aquifers of Texas. Texas Water Development Board Report 380. 172 pp.

Gibert, J., D.C. Culver, M. Dole-Olivier, F. Malard, M.C. Christman, and L. Deharveng. 2009. Assessing and conserving groundwater biodiversity: synthesis and perspectives. Freshwater Biology 54: 930-941.

Gladstone, N.S., E.T. Carter, K.D. Kendall Niemiller, L.E. Hayter, and M.L. Niemiller. 2018. A new maximum body size record for the Berry Cave Salamander (*Gyrinophilus gulolineatus*) and genus *Gyrinophilus* (Caudata, Plethodontidae) with a comment on body size in plethodontid salamanders. Subterranean Biology 28: 29-38.

Gluesenkamp, A.G. and J.K. Krejca. 2007. Assessing the status of *Eurycea* salamanders and aquifer invertebrates in northern Hays County, Texas. Final Report Grant No. E – 70-R, Texas Parks and Wildlife Department. 15 pp.

Green, R.T., F.P. Bertteti, B. Fratesi, and G.M. Schindel. 2019. Pages 29-46 *in* Abbott, P.L. and C.M. Woodruff, Jr., editors. The Balcones Escarpment Geology, Hydrology, Ecology and Social Development in Central Texas. Geological Society of America. 200 pp.

Gorički, S., M.L. Niemiller, and D.B. Fenolio. 2012. Pages 665-675 *in* White, W.B. and D.C. Culver, editors. Encyclopedia of Caves, second edition. Elsevier, Inc. 945 pp.

Gorički Š, D. Stanković, A. Snoj, M. Kuntner, W.R. Jeffery, P. Trontelj, M. Pavićević, Z. Grizelj, M. Năpăruş-Aljančič, and G. Aljančič. 2017. Environmental DNA in subterranean biology: range extension and taxonomic implications for *Proteus*. Scientific Report 7:45054

Grimshaw, T.W. 2013. Geologic map of the San Marcos North Quadrangle and adjacent portions of the Mountain City and San Marcos South Quadrangles, Hays, Caldwell and Guadalupe Counties, Texas. Jackson School of Geosciences, The University of Texas at Austin.

Grimshaw, T.W. and C.M. Woodruff, Jr. 1986. Structural style in an en echelon fault system, Balcones Fault Zone, central Texas: Geomorphologic and hydrological implications. Pages 71-75 *in* Abbott, P.L. and C.M. Woodruff, Jr., editors. The Balcones Escarpment Geology, Hydrology, Ecology and Social Development in Central Texas. Geological Society of America. 200 pp.

Hancock, P.J., A.J. Boulton, and W.F. Humphreys. 2005. Aquifers and hyporheic zones: Towards an ecological understanding of groundwater. Hydrogeology Journal 13: 98–111.

Hanson, J.A. and T.A. Small. 1995. Geologic framework and hydrogeologic characteristics of the Edwards Aquifer outcrop, Hays County, Texas. U.S. Geological Survey, Water-Resources Investigations Report 95-4265. 10 pp.

Hariharan, I.K., D.B. Wake, and M.H. Wake. 2016. Indeterminate growth: Could it represent the ancestral condition? Cold Spring Harbor Perspectives in Biology 2016;8:a019174.

Haubrock, P.J., P. Balzani, I. Johović, A.F. Inghilesi, E. Tricarico. 2018. The effects of two different preservation methods on morphological characteristics of the alien channel catfish *Ictalurus punctatus* (Rafinesque, 1818) in freshwater habitats. Croatian Journal of Fisheries 76: 80-84.

Heim, R.R. 2017. A comparison of the early twenty-first century drought in the United States to the 1930s and 1950s drought episodes. Bulletin of the American Meteorological Society 98(12): 2579-2592.

Hervant, F. and F. Malard. 2012. Responses to low oxygen. Pages 651-658 *in* White, W.B. and D.C. Culver, editors. Encyclopedia of Caves, second edition. Elsevier, Inc. 945 pp.

Hervant, F., J. Mathieu, and J.P. Durand. 2000. Metabolism and circadian rhythms of the European blind cave salamander *Proteus anguinus* and a facultative cave dweller, the Pyrenean newt (*Euproctus asper*). Canadian Journal of Zoology 78: 1427-1432.

Hillis, D.M., D.A. Chamberlain, T.P. Wilcox, and P.T. Chippindale. 2001. A new species of subterranean blind salamander (Plethodontidae: Hemidactyliini: *Eurycea*: *Typhlomolge*) from Austin, Texas, and a systematic revision of Central Texas paedomorphic salamanders. Herpetologica 57(3): 266-280.

Holsinger, J.R. 1988. Troglobites: The evolution of cave-dwelling organisms. American Scientist 76: 147-153.

Holsinger, J.R. and G. Longley. 1980. The subterranean amphipod crustacean fauna of an artesian well in Texas. Smithsonian Contributions to Zoology, Number 308. 62 pp.

Howarth, F.G. 1983. Ecology of cave arthropods. Annual Review of Entomology 28: 365-389.

Humphreys, W.F. 2006. Aquifers: the ultimate groundwater-dependent ecosystems. Australian Journal of Botany 54: 115-132.

Hunt B.B., B.A. Smith, and N.M. Hauwert. 2019. Barton Springs segment of the Edwards (Balcones Fault Zone) Aquifer, central Texas. Pages 75-99 *in* Abbott, P.L. and C.M. Woodruff, Jr., editors. The Balcones Escarpment Geology, Hydrology, Ecology, and Social Development in Central Texas. Geological Society of America. 200 pp.

Hüppop, K. 1985. The role of metabolism in the evolution of cave animals. NSS Bulletin 47(2): 136-146.

Hüppop, K. 2012. Adaptation to low food. Pages 1-9 *in* White, W.B. and D.C. Culver, editors. Encyclopedia of Caves, second edition. Elsevier, Inc. 945 pp.

Hutchins, B.T. 2017. The conservation status of Texas groundwater invertebrates. Biodiversity Conservation 27: 475–501.

Hutchins, B.T., R.U. Tovar, and B.F. Schwartz. 2013. New records of stygobionts from the Edwards Aquifer of central Texas. Speleobiology Notes 5: 14-18.

Hutchins, B.T., A.S. Engels, W.H. Nowlin, and B.J. Schwartz. 2016. Chemolithoautotrophy supports macroinvertebrate food webs and affects diversity and stability in groundwater communities. Ecology 97(6): 1530-1542.

Hutchins, B.T., J.R. Gibson, P.H. Diaz, and B.F. Schwartz. 2021. Stygobiont diversity in the San Marcos artesian well and Edwards Aquifer groundwater ecosystem, Texas, USA. Diversity 13, 234.

Huxley, J.S. 1950. Relative growth and form transformation. Proceedings of the Royal Society of London B, Biological Sciences 137(889): 465-469.

Johnson, S., G.M. Schindel, and J.V. Brahana. 2019. Tracer testing in the Edwards Aquifer. Pages 283-292 *in* Abbott, P.L. and C.M. Woodruff, Jr., editors. The Balcones Escarpment Geology, Hydrology, Ecology and Social Development in Central Texas. Geological Society of America. 200 pp.

Johnson, S., G. Schindel, G. Veni, N. Hauwert, B. Hunt, B. Smith, and M. Gary. 2012. Tracing groundwater flowpaths in the vicinity of San Marcos Springs, Texas. Edwards Aquifer Authority Report No. 12-03. 139 pp.

Jones, W.K. and W.B. White. 2012. Karst. Pages 430-438 *in* White, W.B. and D.C. Culver, editors. Encyclopedia of Caves, second edition. Elsevier, Inc. 945 pp.

Klauber, L.M. 1943. Tail-length differences in snakes with notes on sexual dimorphism and the coefficient of divergence. Bulletin of the Zoological Society of San Diego, 18: 1-76.

Kløve, B., P. Ala-aho, G. Bertrand , Z. Boukalova, A. Ertürk, N. Goldscheider, J. Ilmonen, N. Karakaya, H. Kupfersberger, J. Kvoerner, A. Lundberg, M. Mileusnić, A. Moszczynska, T. Muotka, E. Preda, P. Rossi, D. Siergieiev, J. Šimek, P. Wachniew, V. Angheluta, and A. Widerlund. 2011. Groundwater dependent ecosystems. Part I: Hydroecological status and trends. Environmental Science & Policy 14: 770-781.

Krejca, J.K. and A.G. Gluesenkamp. 2007. Mark-recapture study of Eurycea rathbuni at two sites in San Marcos, Texas. Final Report Grant No. E-68-R, Texas Parks and Wildlife Department. 22 pp.

Krejca, J. and J. Reddell. 2019. Biology and ecology of the Edwards Aquifer. Pages 159-169 *in* Abbott, P.L. and C.M. Woodruff, Jr., editors. The Balcones Escarpment Geology, Hydrology, Ecology, and Social Development in Central Texas. Geological Society of America. 200 pp.

Külköylüoğlu, O., D. Akdemir, M. Yavuzatmaca, B.F. Schwartz, and B.T. Hutchins. 2017. *Rugosuscandona*, a New Genus of Candonidae Crustacea: Ostracoda) from Groundwater Habitats in Texas, North America. Species Diversity 22: 175-185.

Land, L.E., B.B. Hunt, B.A. Smith, and P.J. Lemonds. 2011. Hydrologic connectivity in the Edwards Aquifer between San Marcos Springs and Barton Springs during 2009 drought conditions. Texas Water Journal 2(1):39-53.

Lazell, J.D., Jr. 1972. The anoles (Sauria, Iguanidae) of the Lesser Antilles. Bulletin of the Museum of Comparative Zoology 143(1): 1-115.

Lee J.C. 1982. Accuracy and precision in anuran morphometrics: artifacts of preservation. Systematic Biology 31: 266-281.

Lindgren, R.J., A.R. Dutton, S.D. Hovorka, S.R.H. Worthington, and S. Painter. 2004. Conceptualization and simulation of the Edwards Aquifer, San Antonio region, Texas. U.S. Geological Survey, Scientific Investigations Report 2004–5277. 143 pp.

Livingston, P., A.N. Sayre, and W.N. White. 1936. Water resources of the Edwards limestone in the San Antonio Area, Texas.

Longley, G. 1978. Status of *Typhlomogle* (=*Eurycea*) *rathbuni*, the Texas blind salamander. Final Report to the U.S. Fish and Wildlife Service, Albuquerque, New Mexico. 45 pp.

MacLay, R.W. 1995. Geology and hydrology of the Edwards Aquifer in the San Antonio area, Texas. U.S. Geological Survey, Water-Resources Investigations Report 95-4186. 64 pp.

Mitchell, R.W. and J.R. Reddell. 1965. *Eurycea tridentifera*, a new species of troglobitic salamander from Texas and a reclassification of *Typhlomolge rathbuni*. Texas Journal of Science 17(1): 12-27. United States Department of the Interior, Geological Survey. Water Supply Paper 773-B: 59-113.

Morris. 2005. Geologic Map of the Edwards Aquifer Recharge Zone, South-Central Texas, Scale 1:200,000, Scientific Investigations Map 2873, Version 1.1

Musgrove, M. and C.L. Crow. 2012. Origin and characteristics of discharge at San Marcos Springs based on hydrologic and geochemical data (2008–10), Bexar, Comal, and Hays counties, Texas. U.S. Geological Survey Scientific Investigations Report 2012–5126. 94 pp.

Nielsen-Gammon, J.W. 2012. The 2011 Texas drought. Texas Water Journal 3(1): 59–95.

Niemiller, M.L. and T.L. Poulson. 2010. Subterranean fishes of North America: Amblyopsidae. Pages 169-280 *in* Trajano E., M.E. Bichuette, and B.G. Kapoor, editors. The Biology of Subterranean Fishes. Science. 516 pp.

Ogden, A.E., R.A. Quick, and S.R. Rothermel. 1986. Hydrochemistry of the Comal, Hueco, and San Marcos Springs, Edwards Aquifer, Texas. Pages 115-130 *in* Abbott, P.L. and C.M. Woodruff, Jr., editors. The Balcones Escarpment Geology, Hydrology, Ecology, and Social Development in Central Texas. Geological Society of America. 200 pp.

Pierson, T.W., T.J. Kieran, A.G. Clause, and N.L. Castleberry. 2020. Preservation-induced morphological change in salamanders and failed DNA extraction from a decades-old museum specimen: implications for *Plethodon ainsworthi.* Journal of Herpetology 54(2): 137-143.

Potter, F.E., Jr. 1963. Gross morphological variation in the genus *Typhlomolge* with a description of a new species. Unpublished Master's Thesis, University of Texas at Austin. 66 pp.

Potter, F.E., Jr., and S.S. Sweet. 1981. Boundaries in Texas cave salamanders, and a redescription of *Typhlomolge robusta* (Amphibia: Plethodontidae). Copeia 1981(1): 64-75.

Poulson, T.L. and W.B. White. 1969. The cave environment. Science 165: 971-981.

Reddell, J.R. 1967. A checklist of the cave fauna of Texas. III. Vertebrata. Texas Journal of Science 19(2): 184-226.

Reddell, J.R. and R.W. Mitchell. 1969. A checklist and annotated bibliography of the subterranean aquatic fauna of Texas. Texas Technological College, Water Resources Center, Special Report 24. 48 pp.

Russell, W.H. 1976. Distribution of troglobitic salamanders in the San Marcos area, Hays County, Texas. BITE Report 7601. 35 pp.

Schindel, G.M. 2019. Genesis of the Edwards (Balcones Fault Zone) Aquifer. Pages 9-18 *in* Sharp, J.M. Jr., T.R. Green, and G.M. Schindel. The Edwards Aquifer: The Past, Present, and Future of a Vital Water Resource. The Geological Society of America Memoir. 312 pp.

Schwartz, B.F., B.T. Hutchins, Z.G. Schwartz, A.J. Hess, and R.M. Bonett. 2019. *Cirolanides wassenichae* sp. nov., a freshwater, subterranean Cirolanidae (Isopoda, Cymothoida) with additional records of other species from Texas, United States. Zootaxa 4543(4): 498-514.

Senger, R.K. and C.W. Kreitler. 1984. Hydrogeology of the Edwards Aquifer, Austin area, central Texas. University of Texas at Austin, Bureau of Economic Geology, Report of Investigations No. 141. 35 pp.

Shu, G., Y. Gong, F. Xie, N.C. Wu, and C. Li. 2017. Effects of long-term preservation on amphibian body conditions: implications for historical morphological research. PeerJ 5:e3805; DOI 10.7717/peerj.3805.

Simmons, J.E. 2002. Herpetological collecting and collection management. Society for the Study of Amphibians and Reptiles, Herpetological Circular 31. 153 pp.

Simmons, J.E. 2019. Storage in fluid preservatives. Pages *in* 161-186 Rose, C.L., C.A. Hawks, and H.H. Genoways, editors. Storage of Natural History Collections: A Preventive Conservation Approach. Society for the Preservation of Natural History Collections, Iowa City, Iowa. 448 pp.

Simon, K.S. 2012. Cave ecosystems. Pages 99-102 *in* White, W.B. and D.C. Culver, editors. Encyclopedia of Caves, second edition. Elsevier, Inc. 945 pp.

Smith, B.A. and B.B. Hunt. 2010. A comparison of the 1950s drought of record and the 2009 drought, Barton Springs Segment of the Edwards Aquifer, central Texas. Gulf Coast Association of Geological Societies Transactions 60: 611-622.

Smith, B.A., B.B. Hunt, and S.B. Johnson. 2012. Revisiting the hydrologic divide between the San Antonio and Barton Springs segments of the Edwards Aquifer: insights from recent studies. GCAGS Journal 1: 55-68.

Smith, B.A., B.B. Hunt, A.G. Andrews, J.A. Watson, M.O. Gary, D.A. Wierman, and A.S. Broun. 2015. Hydrologic influences of the Blanco River on the Trinity and Edwards Aquifers, central Texas, USA. Pages 153-161 *in* Andreo, B., F. Carrasco, J.J. Durán, P. Jiménez, and J. LaMoreaux, editors. Hydrogeological and Environmental Investigations in Karst Systems. Springer International. 663 pp.

Snider, A.T. and J.K. Bowler. 1992. Longevity of Reptiles and Amphibians in North American Collections, second edition. Oxford, Ohio: Society for the Study of Amphibians and Reptiles. 40 pp.

Sotola V.A., C.A, Craig, P.J. Pfaff, J.D. Maikoetter, N.H. Martin, and T.H. Bonner. 2019. Effect of preservation on fish morphology over time: Implications for morphological studies. PLoS ONE 14(3): e0213915. https://doi.org/10.1371/journal. pone.0213915.

Springer, A.E. and L.E. Stevens. 2008. Spheres of discharge of springs. Hydrogeology Journal doi:10.1007/s10040-008-0341-y.

Stafford, K.W., K. Arens, A. Gluesenkamp, O. Knox, J. Mitchell, J. Reddell, A.M. Scott, J. Kennedy, M. Miller, W.H. Russell, P. Sprouse, and G. Veni. 2014. Karst of the Urban Corridor: Bell, Bexar, Comal, Hays, Travis, and Williamson Counties, Texas. Karst Awareness and Education Series, 1: Austin, Texas, Texas Speleological Survey. 110 pp.

Stahle, D.W. and M.K. Cleaveland. 1988. Texas drought history reconstructed and analyzed from 1698 to 1980. Journal of Climate 1(1): 59-74.

Stein, W.G. 1995. Hays County ground-water divide. Pages 23-34 *in* Hauwert, N.M. and J.A. Hanson, coordinators. A Look at the Hydrostratigraphic Members of the Edwards Aquifer in Travis and Hays Counties, Texas. Austin Geological Society Guidebook. 81 pp.

Stejneger, L. 1896. Description of a new genus and species of blind tailed Batrachians from the subterranean waters of Texas. Proceedings of the United States National Museum 18: 619-621.

Streever, W.J. 1996. Energy economy hypothesis and the troglobitic crayfish *Procambarus erythrops* in Sim's Sink Cave, Florida. The American Midland Naturalist 135(2): 357-366.

Sweet, S.S. 1977. Natural Metamorphosis in *Eurycea neotenes*, and the Generic Allocation of the Texas *Eurycea* (Amphibia: Plethodontidae). Herpetologica 33(3): 364-375.

Sweet, S.S. 1984. Secondary contact and hybridization in the Texas cave salamanders *Eurycea neotenes* and *E. tridentifera*. Copeia 1984 (2): 428-441.

Sweet, S.S. 1977. *Eurycea tridentifera*. Catalogue of American Amphibians and Reptiles 199.1-199.2.

Texas Parks and Wildlife Department. 2020. Updated List of State T & E Species in Texas - Effective March 30, 2020. Retrieved on July 1, 2020 from https://tpwd.texas.gov/huntwild/wild/wildlife_diversity/nongame/listed-species/media/fedState-ListedSpeciesComplete-3302020.pdf.

Thessen, A.E., D.J. Patterson, S.A. Murray. 2012. The taxonomic significance of species that have only been observed once: The genus *Gymnodinium* (Dinoflagellata) as an example. PLoS ONE 7(8): e44015. doi:10.1371/journal.pone.0044015.

Tovar, R. Re: Blanco Blind Salamander. Email message to Michael Warriner, February 26, 2021.

Uhlenhuth, E. 1921. Observations on the distribution and habits of the blind Texan cave salamander, *Typhlomolge rathbuni*. Biological Bulletin 40: 73-104.

Ulrich, C.J. 1902. A contribution to the subterranean fauna of Texas. Transactions of the American Microscopical Society 23: 83-101.

U.S. Census Bureau. 2012. 2010 Census of Population and Housing, Population and Housing Unit Counts, CPH-2-45, Texas. U.S. Government Printing Office, Washington, D.C.

U.S. Census Bureau. 2020. Most of the Counties with the Largest Population Gains Since 2010 are in Texas. Retrieved on July 12, 2020 from https://www.census.gov/newsroom/press-releases/2020/pop-estimates-county-metro.html.

U.S. Department of Agriculture (USDA). 2020. Hays USDA Historic Imagery, 1951-02-06. Web. 2020-07-10.

U.S. Fish and Wildlife Service (Service). 1992. Taxonomy and the Endangered Species Act. Internal Service Memorandum, November 25, 1992. 3pp.

Vajargh, M.F. and A. Hedayati. 2014. The effect of preservation in formalin on the morphological characters of spirlin (*Alburnoides eichwaldii*). Journal of Environmental Treatment Techniques 2(3): 106-108.

Veni, G. 2012. Passages. Pages 603-608 *in* White, W.B. and D.C. Culver, editors. Encyclopedia of Caves, second edition. Elsevier, Inc. 945 pp.

Vervust, B., S. Van Dongen, and R. Van Damme. 2009. The effect of preservation on lizard morphometrics – an experimental study. Amphibia-Reptilia: 321-329.

Vieira, W.A., K. Anderson, L.G. Campbell, and C.D. McCusker. 2020. Characterizing the regenerative capacity and growth patterns of the Texas blind salamander (Eurycea rathbuni). Developmental Dynamics Sep 4. doi: 10.1002/dvdy.245. Epub ahead of print. PMID: 32885536.

Voituron, Y., M. de Fraipont, J. Issartel, O. Guillaume, and Jean Clobert. 2011. Extreme lifespan of the human fish (*Proteus anguinus*): a challenge for ageing mechanisms. Biology Letters 7: 105-107.

Vörös, J., S. Ursenbacher, and D. Jelič. 2019. Population genetic analyses using 10 new polymorphic microsatellite loci confirms genetic subdivision within the olm, *Proteus anguinus*. Journal of Hereditary 110(2): 211-218.

Wermund, E.G., J.C. Cepeda, and P.E. Luttrell. 1978. Regional distribution of fractures in the southern Edwards Plateau and their relationship to tectonics and caves. University of Texas at Austin, Bureau of Economic Geology, Geological Circular 78-2.

White, W.B. 2012. Hydrogeology of karst aquifers. Pages 383-391 *in* White, W.B. and D.C. Culver, editors. Encyclopedia of Caves, second edition. Elsevier, Inc. 945 pp.

Wiens, J.J. P.T. Chippindale, and D.M. Hillis. 2003. When are phylogenetic analyses misled by convergence? A case study in Texas cave salamanders. Systematic Biology 52(4): 501-514.

Winters, K.E. 2013. A historical perspective on precipitation, drought severity, and streamflow in Texas during 1951–56 and 2011. U.S. Geological Survey, Scientific Investigations Report 2013–5113. 24 pp.

Woodhouse, C.A. and J.T. Overpeck. 1998. 2000 years of drought variability in the central United States. Bulletin of the American Meteorological Society 79(12): 2693-2714.

Young, F.N. and G. Longley. 1976. A new subterranean aquatic beetle from Texas (Coleoptera: Dytiscidae-Hydroporinae). Annals of the Entomological Society of America 69(5): 787-792.

Zakšek, V., M. Konec, and P. Trontelj. 2017. First microsatellite data on *Proteus anguinus* reveal weak genetic structure between the caves of Postojna and Planina. Aquatic Conservation: Marine and Freshwater Ecosystem 28: 241-246.

ZARA Environmental LLC. 2010. Hays County karst invertebrate distribution and cave development. Final Report Grant no. TX E-105-R., Texas Parks and Wildife Department 56 pp.