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NOTEWORTHY RECORDS FOR SIX SPECIES OF BATS FROM 13 TEXAS COUNTIES AND THE FIRST VOUCHER SPECIMENS FROM SITES WITH *PSEUDOGYMNOASCUS DESTRUCTANS*

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ABSTRACT

Diseases that result in a regional loss of both species richness and genetic diversity highlight the importance of managing and building upon natural history collections. Occurrence data and specimen vouchers can supplement information regarding distribution and genetic diversity prior to the potential expansion into Texas of the emerging disease known as white-nose syndrome. Herein, six species of bats from 13 counties in Texas are reported. A total of 10 new county records were documented for four species (*Myotis velifer*, *Perimyotis subflavus*, *Eptesicus fuscus*, and *Corynorhinus townsendii*). Noteworthy winter observations were recorded for two additional species (*Myotis austroriparius* and *C. rafinesquii*). In addition to documenting occurrence records of bat species in the state of Texas, the first voucher specimens are provided from three sites positive for *Pseudogymnoascus destructans*, the causative agent of white-nose syndrome.

Key words: bats, county records, *Pseudogymnoascus destructans*, Texas, winter surveys

INTRODUCTION

The documentation of *Pseudogymnoascus destructans*, and the development of white-nose syndrome (WNS) in 2006 within New York State, has been followed by the persistent spread of both the fungus and the disease across the eastern United States (Blehert et al. 2009). This emerging disease affects hibernating bats and has resulted in mass fatalities and precipitous declines in population abundance (Blehert et al. 2009; Frick et al. 2010). The 2016–2017 winter documentation of the fungus, *P. destructans*, in Texas (Meierhofer et al. in prep) emphasizes the importance of maintaining and collecting chronological records in the form of voucher specimens (and their tissues). To our knowledge, no diagnostic signs of WNS have been

observed in any Texas bat (Meierhofer et al. in prep). Therefore, these records will be essential in understanding the potential impact of the fungus to biodiversity, genetic variation, and species distributions should the disease emerge and spread within the state of Texas. This point-in-time sampling will provide valuable information for studying direct and indirect agents of local extinction and will provide material needed to investigate potential genetic coping mechanisms of the bats of Texas. Herein, new county records and noteworthy vouchers are reported for species of bats obtained throughout Texas, from both natural and human-made structures, as well as the first voucher specimens from cave systems positive for *P. destructans*.

METHODS

A total of 207 roost site surveys were conducted across eight ecoregions (Central Great Plains, Cross Timbers, East Central Texas Plains, Edwards Plateau, High Plains, South Central Plains, Southwestern Tablelands, and Texas Blackland Prairies; Omernik 1987) in Texas during fall and winter survey efforts between 23 November 2016 and 5 March 2017. Species identities were confirmed by comparing morphological measurements to the key characteristics of bat species in Ammerman et al. (2012) and specimens that were either new county records or supporting evidence for existing literature or Department of State Health Services (DSHS) records were collected.

To determine new county records, all county level bat observations were compared to distribution maps in *Bats of Texas* (Ammerman et al. 2012) and *The Mammals of Texas* (Schmidly and Bradley 2016). The Natural Science Research Laboratory (NSRL) Vertebrate Database, Texas A&M Biodiversity Research and Teaching Collection (BRTC; previously Texas Cooperative Wildlife Collection (TCWC)), the website listing bat specimens examined for *Bats of Texas* (Ammerman et al. 2012; www.batsoftexas.com), and the recent county records presented by Demere et al. (2012) and Garcia et al. (2016) also were reviewed. Several counties designated by Schmidly (1991, 2004), Ammerman et al. (2012), and Schmidly and Bradley (2016) as having either a literature record or a DSHS database record in the distribution maps, have no voucher specimen because bats submitted to DSHS were, in some years, incinerated after being recorded in a database (Demere et al. 2012). Therefore, specimens were considered as new county records if no museum voucher specimen was known for that county.

All bats were collected following the standardized methods set forth by the guidelines of the American Society of Mammalogists (Sikes et al. 2016) and the animal handling methods of a Texas A&M University Animal Care and Use Protocol (IACUC 2015-0296). Specific locality data are presented for all specimens collected on public land. However, to remain in compliance with Texas Parks and Wildlife Code § 12.103 (1995) and the limitations established in the Landowner Permission for Wildlife Research Form, only directional locality data instead of specific latitude and

longitude coordinates are presented for all specimens collected on private land.

Upon collection, each bat was placed in an individually labeled specimen bag to limit the potential spread of any *P. destructans* spores. Specimens were only removed from their individual bags and handled in a controlled lab setting. Each time an individual specimen was handled, new latex gloves were worn and all contact surfaces and instruments were decontaminated using Clorox® Disinfecting Wipes in accordance with the manufacturer standards. Epidermal swab samples of each voucher specimen were collected to document presence or absence of *P. destructans* by dipping a cotton tipped swab (Puritan®) into a micro-centrifuge tube filled with sterile water, and then rolling the moistened swab across the muzzle and forearm of the individual bat. The swab tip was then placed into a micro-centrifuge tube filled with Invitrogen™ RNAlater® stabilization solution and the tube was stored on ice for shipping.

Samples were analyzed by the Foster Lab in the Department of Molecular, Cellular, and Biomedical Sciences at the University of New Hampshire. DNA was extracted from each sample and analyzed in duplicate runs by real-time qPCR using methods developed by Muller et al. (2013). A cycle threshold (C_T) cutoff value of 40 was used to classify a positive detection of *P. destructans* (Muller et al. 2013). Due to the premiere detection of *P. destructans* in Texas, any samples that produced a positive detection, in either one or both of the duplicates, were run a third time.

After obtaining analytical results from the Foster Lab (University of New Hampshire), each voucher was prepared as a museum specimen, assigned a museum catalog number and a tissue number (ASK), and all materials were deposited in the Angelo State Natural History Collection (ASNHC) for long term storage. In addition to decontaminating all surfaces, all instruments used for specimen preparation were placed in 10% bleach for five minutes, instruments were rinsed with clean water, and instruments were allowed to dry before being used on subsequent specimens. Age, sex, and standard external measurements (total length, tail length, hind foot length, ear length, tragus length, and

length of the forearm) were documented for each specimen. Tissue samples (heart, liver, and kidney) were collected and frozen at -80°C .

To limit the possibility of transmitting fungal spores from *P. destructans* positive sites to museum collections, all specimens collected from sites in the Rolling and High Plains of Texas were preserved in 10% formalin and then stored as a fluid voucher specimen in 70% ethanol. Specimens that were collected from regions other than the Rolling and High Plains,

and that did not have a positive amplification of *P. destructans*, were prepared as standard skin-and-skull museum specimens. Due to the susceptibility of *Perimyotis subflavus* to WNS (Turner et al. 2011), all specimens of this species were preserved as fluid vouchers. Taxonomy and order of authority within the manuscript follow those presented in *Mammal Species of the World* (Wilson and Reeder 2005) and “Revised Checklist of North American Mammals North of Mexico, 2014” (Bradley et al. 2014), with all common names following those specified by Bradley et al. (2014).

RESULTS

Ten new county records and seven noteworthy observations were documented for six species of bats (*Myotis austroriparius*, *Myotis velifer*, *Perimyotis subflavus*, *Eptesicus fuscus*, *Corynorhinus rafinesquii*, and *Corynorhinus townsendii*) from 13 counties in Texas (Fig. 1). Seven of the 14 specimens collected represent the first voucher specimens taken from *P. destructans* positive sites. The collection of *E. fuscus* and *C. townsendii* in Scurry County represent a slight southern expansion of the known distribution of these species within the Rolling Plains.

Family Vespertilionidae

Corynorhinus rafinesquii (Lesson, 1827)

Rafinesque’s Big-eared Bat

An inhabitant of tree hollows, caves, and man-made structures throughout its distributional range in the southeastern United States, Rafinesque’s Big-eared Bat reaches its westernmost distributional boundary in the South Central Plains region of eastern Texas. Although captures of this species have been recorded from May through December (Schmidly and Bradley 2016), only two winter roosts have been documented (Mirowsky et al. 2004). Due to its state-listed threatened status, and the documentation of *C. rafinesquii* as a bat species upon which *P. destructans* has been detected (www.whitenosesyndrome.org), we suggest that efforts should be undertaken to document winter distribution records in order to help future monitoring efforts and contribute to understanding the potential for *C. rafinesquii* to spread the fungus.

Marion County.—On 26 January 2017, a specimen of unknown sex was observed at a Hwy 49 culvert (32.78912°N , 94.25073°W) 8.1 km southwest of Smithland, Texas. Due to the state-listed status of this species, this specimen was not collected. However, a photograph was taken for species verification (Fig. 2). This observation provides support for an existing voucher specimen (Stephen F. Austin State University #2933) that was overlooked in Ammerman et al. (2012) and is the first winter documentation of *C. rafinesquii* in the county. Marion County is the northernmost documentation of Rafinesque’s Big-eared Bat in Texas.

Corynorhinus townsendii (Cooper, 1837)

Townsend’s Big-eared Bat

Townsend’s Big-eared Bat has a distributional range that extends throughout the entire western United States and has two disjunct subspecies populations in the Ozark and Appalachian Mountains. *Corynorhinus townsendii* is a year-round resident of Texas and has been documented from six ecoregions (Central Great Plains, Southwestern Tablelands, High Plains, Edwards Plateau, Chihuahuan Desert, and Arizona/New Mexico Mountains). Although Piaggio and Perkins (2005) indicated that *C. t. australis* was the only subspecies in Texas, this conclusion was based on limited sampling in Texas (one site). More recently, two subspecies (*C. t. pallascens* and *C. t. australis*) have been confirmed in the state (Smith et al. 2008; Tipps 2012). Although not found in Texas, the endangered Virginia Big-eared Bat (*Corynorhinus townsendii virginianus*) has been documented as a carrier of *P. destructans* (www).

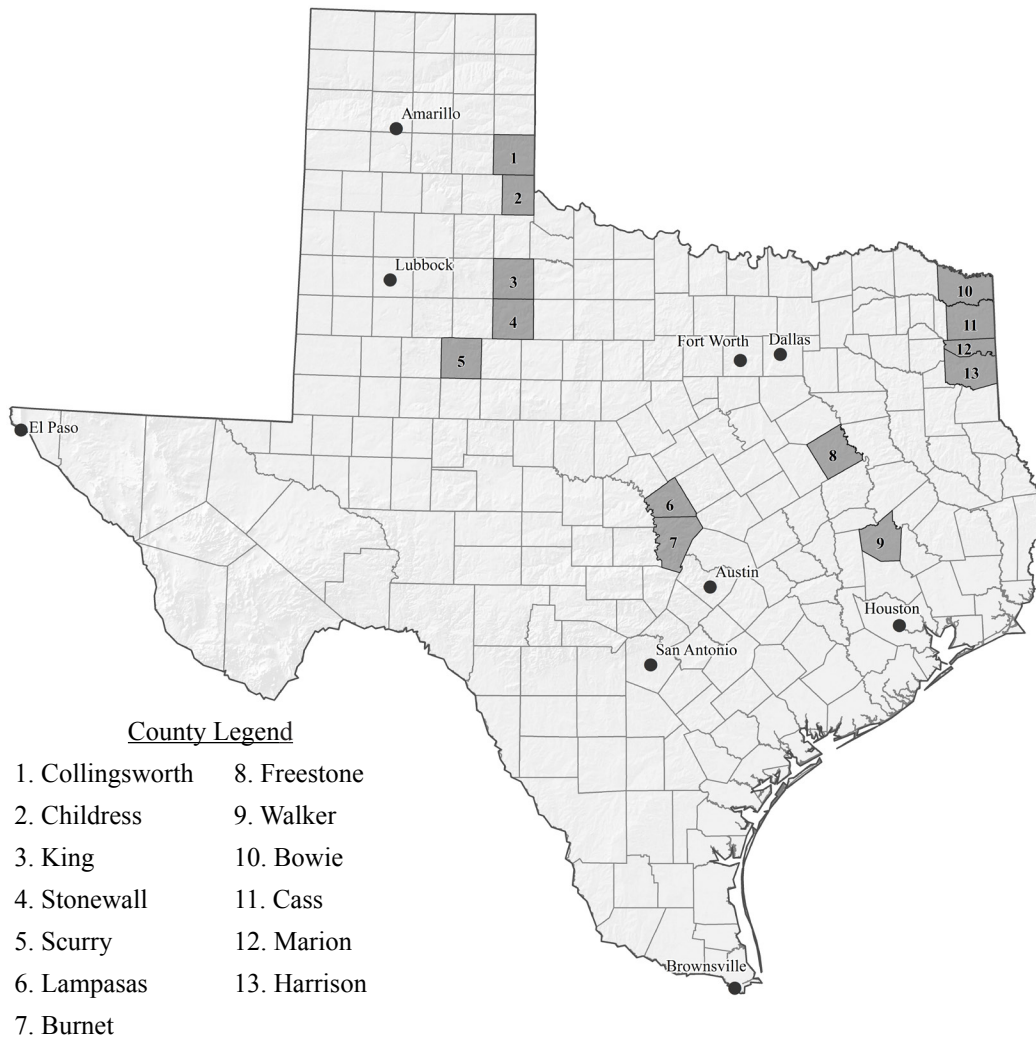


Figure 1. Map depicting 13 counties in Texas where bats were observed or collected during roost site surveys between November 2016 and March 2017.



Figure 2. *Corynorhinus rafinesquii* observed roosting in a box culvert in Marion County, Texas, on 26 January 2017. Photograph by Krysta D. Demere.

whitenosesyndrome.org). We recently reported the first documentation of *C. t. pallescens* as a subspecies on which the fungus has been detected, but no diagnostic signs of WNS have been observed (Meierhofer et al. in prep).

King County.—On 2 December 2016, two *C. townsendii* were observed roosting in a cave system 26.1 km southeast of Paducah, Texas. An adult female *C. townsendii* (ASNHC 18118, ASK 12675) was collected as a voucher specimen. This specimen represents a new county record for the species, and tested negative for the DNA of *P. destructans*. Although separate, the cave system in which these individuals were observed is located within 50 m of a cave housing *M. velifer* that have been reported as positive carriers of the *P. destructans* fungal spores. Townsend's Big-eared Bats were observed lying between these two cave sites.

Scurry County.—An adult male *C. townsendii* (ASNHC 18119, ASK 12676) was collected on 5 December 2017 from a gypsum cave system 27.7 km northeast of Snyder, Texas. In total, 57 *C. townsendii* were observed during survey efforts in Scurry County. Although this voucher specimen was *P. destructans* negative, two other *C. townsendii* that were swabbed at the site were positive for *P. destructans*. A public observation (Chris Taylor, pers. comm.) in 2000 served as the first historic mention of *C. townsendii* in Scurry County, but was not officially documented. Therefore, this voucher specimen is considered to be the first verified county record for *C. townsendii* in Scurry County.

***Eptesicus fuscus* (Palisot de Beauvois, 1796)**

Big Brown Bat

Eptesicus fuscus is primarily recorded from the eastern, northern, and western parts of Texas. A disjunct distribution separates the two known subspecies (*E. f. fuscus* and *E. f. pallidus*) in Texas (Ammerman et al. 2012). This permanent resident is one of seven species that has been identified with diagnostic signs of WNS in the eastern United States (www.whitenosesyndrome.org). Although the Big Brown Bat has not been confirmed as a positive carrier for *P. destructans* in Texas, *E. fuscus* were observed roosting at locations in Childress and Scurry counties where either *C. townsendii* or *M. velifer* were documented to carry the fungus.

Cass County.—An adult female *E. fuscus* (ASNHC 18125, ASK 12683) was collected from a Hwy 77 culvert (33.19392°N, 94.43376°W) 7.5 km west of Douglassville, Texas, on 27 January 2017. An additional observation for the county was made at a Hwy 59 bridge (33.05429°N, 94.29038°W) over Frazier creek 1.0 km northeast of New Colony, Texas. The collected voucher specimen represents a new county record for Cass County and tested negative for *P. destructans*.

Scurry County.—An *E. fuscus* was observed roosting in a horizontal crevice located in a cave system 27.7 km northeast of Snyder, Texas. This observation of *E. fuscus* in Scurry County represents a new record and extends the southern distribution for this species in the Texas panhandle (Ammerman et al. 2012). Unfortunately, due to the dimensions of the crevice, the specimen could not be reached for collection. A photograph was taken for species verification (Fig. 3). This individual was not tested for *P. destructans*, but was found in a cave system where sampling efforts yielded positive results for the fungal spores on *C. townsendii*.

Stonewall County.—On 2 December 2016, an adult male *E. fuscus* (ASNHC 18117, ASK 12674) was collected from a bridge (33.205273°N, 100.249774°W) located 8.2 km north of Aspermont on Hwy 83. This is the same location from which a *M. velifer* voucher for Stonewall County was collected (mentioned below). This specimen represents a new record for Stonewall County and was one of 25 *E. fuscus* observed on this date. This species appears to maintain a presence in the county throughout the winter because individuals also were observed at this location on 9 October 2016 and 5 February 2017.

***Myotis austroriparius* (Rhodes, 1897)**

Southeastern Myotis

The Southeastern Myotis has been widely documented throughout the southeastern United States and is known from three ecoregions in Texas; South Central Plains, East Central Plains, and Western Gulf Coastal Plains (Ammerman et al. 2012). It is in eastern Texas that the species reaches the westernmost part of its North American range. This year-round resident of the state is a species on which *P. destructans* has been detected, but no diagnostic signs of WNS have been observed (www.whitenosesyndrome.org).



Figure 3. *Eptesicus fuscus* observed roosting in a gypsum cave system in Scurry County, Texas, on 5 February 2017. Photograph by Melissa B. Meierhofer.

Bowie County.—On 27 January 2017, a Southeastern Myotis of unknown sex was observed roosting in a culvert along I-30 near Hook, Texas (33.46593°N, 94.23925°W). This individual was located approximately 30 m from the entrance of the culvert at a height of 2.3 m and was alert and responsive. Due to the roosting height of the bat, this individual was not swabbed nor tested for signs of *P. destructans* and was not collected as a voucher. Although not a county record for the species (Schmidly and Bradley 2016), this documentation of *M. austroriparius* represents the first winter observation of the Southeastern Myotis for Bowie County.

Walker County.—On 20 January 2017, an adult male *M. austroriparius* (ASNHC 18126, ASK 12682) was collected from a culvert 12.8 km south of Hunts-

ville, Texas (30.62102°N, 95.50771°W) on I-45. This individual was swabbed, tested, and reported negative for *P. destructans*. Walker et al. (1996) previously reported a single captured specimen of *M. austroriparius* (TCWC 52871). Therefore, this voucher specimen provides additional verification of the occurrence of *M. austroriparius* in Walker County.

***Myotis velifer* (J. A. Allen, 1890)**

Cave Myotis

A year-round resident of Texas, the Cave Myotis has been documented in every ecoregion of the state with the exception of the South Central Plains (Ammerman et al. 2012). This species exhibits seasonal variation in its distribution and is thought to be restricted to the central and north-central parts of the state during

the winter months (Ammerman et al. 2012). Although *P. destructans* was recently documented on *M. velifer* in Texas (K. Gilles, pers. comm; Meierhofer et al. in prep), no diagnostic signs of WNS were observed.

Childress County.—On 12 January 2017, three dead female *M. velifer* (ASNHC 18121, ASK 12678; ASNHC 18122, ASK 12679; ASNHC 18123, ASK 12680) were found during a survey of a gypsum cave system located 31.0 km northeast of Childress, Texas. These bats were located at the base of a substantial rock fall and had visual signs of physical trauma including abrasions and gashes. As a precautionary measure, the specimens were collected and tested for the presence of *P. destructans*. The initial on-site swab results for all three of these specimens were reported as negative for *P. destructans*. However, the specimens were swabbed again in a lab setting and one (ASNHC 18123) produced positive results. The specimen did not display diagnostic symptoms of WNS, was in good physical condition, and was documented at a weight of 15 g with fat deposits present. The presence of the fungus also was documented on two additional *M. velifer* in this cave system. Although not a county record (Schmidly and Bradley 2016), these specimens represent the first voucher specimens of the Cave Myotis collected from a *P. destructans* positive site in Childress County. In total, 340 *M. velifer* were observed at this site.

Lampasas County.—An adult male specimen (ASNHC 18124, ASK 12681) was collected from a culvert (31.11936°N, 98.51707°W) 2.3 km north of Bend, Texas, on 23 November 2016. The bat was located in a swallow nest approximately 1.6 m from the portal and at a height of 1.4 m. Lampasas County was previously listed as a DSHS record (Ammerman et al. 2012). However, to our knowledge the only specimen representing this record was discarded. Therefore, this voucher specimen is considered as a county record. This individual tested negative for *P. destructans*.

Scurry County.—On 5 February 2017, a male *M. velifer* (ASNHC 12677, ASK 18120) was collected approximately 27.7 km northeast of Snyder, Texas. This bat was roosting in a gypsum cave system approximately 19.7 m from the cave entrance at a height of 1.4 m; a total of approximately 150 *M. velifer* were observed at this site. Although this record is within the previously known distribution of the species, Scurry

County is located at the proposed contact zone of the northern and southern subspecies *M. v. magnamolaris* and *M. v. incautus* (Dalquest and Stangl 1984; Parlos 2008; Ammerman et al. 2012). Without further investigation, it is not possible to determine which of the two subspecies (*M. v. magnamolaris* or *M. v. incautus*) was collected in Scurry County. Analysis of the collected swab reported this individual to be negative for *P. destructans*. However, two Townsend's Big-eared Bats from this site tested positive for *P. destructans*. Therefore, this specimen represents a new county record and the first *M. velifer* to be collected from a site positive for *P. destructans* in Scurry County.

Stonewall County.—On 2 December 2016, an adult male *M. velifer* (ASNHC 12673, ASK 18116) was collected from a bridge (33.205273°N, 100.249774°W) located 8.2 km north of Aspermont, Texas, on Hwy 83. This specimen represents the first voucher specimen for Stonewall County and was the only Cave Myotis observed on this date. However, this location was surveyed on three additional occasions, 9 October 2016, 11 January 2017, and 5 February 2017, and species counts of 350, 1, and 5, respectively, were recorded. Therefore, this species might maintain an active presence in the county throughout the winter. Stonewall County was previously listed as a literature record (Hayward 1970), however no voucher specimen was found. Thus, this voucher is considered as the official county record. This individual was not positive for *P. destructans*.

***Perimyotis subflavus* (F. Cuvier, 1832)**
American Perimyotis

The American Perimyotis is a year-round resident of Texas that has been documented from all of the vegetative regions with the exception of the far western reaches of the Trans-Pecos (Schmidly and Bradley 2016). This species is known to hibernate in caves and box culverts throughout the winter within its range (Sandel et al. 2001). Recent records in Lubbock, Presidio, Brewster, Moore, Potter, and Hutchinson counties suggest that the American Perimyotis may be expanding its range westward in the state (Yancey et al. 1995; Schmidly 2004; Ammerman 2005; Demere et al. 2012). *Perimyotis subflavus* is one of seven bat species identified with diagnostic symptoms of WNS (www.whitenosesyndrome.org).

Burnet County.—An adult female *P. subflavus* (ASNHC 12670, ASK 18113) was collected on 5 March 2017 at Longhorn Caverns State Park (Permit # 2017-R3-04), 14.2 km southwest of Burnet, Texas. This voucher specimen represents a new county record for Burnet County. The *P. subflavus* population for the cavern system was estimated at 100 individuals.

Cass County.—On 27 January 2017, two American Perimyotis were observed roosting torpid at two locations in Cass County. The first individual was located in a Hwy 77 culvert (33.19392°N, 94.43376°W) located 7.4 km west of Douglassville, Texas. The second individual was observed roosting under a bridge on State Hwy 59 (33.05429°N, 94.29038°W) over Frazier creek 1.0 km northeast of New Colony, Texas. Although many voucher specimens have been taken from Cass County (Ammerman et al. 2012; www.batsoftexas.com), these observations are noteworthy winter documentations for the county.

Collingsworth County.—An adult male American Perimyotis (ASNHC 18115, ASK 12672) was collected from a gypsum cave located 22.1 km northeast of Wellington, Texas, on 13 January 2017. This individual represents a new county record and is one of the northernmost documentations of *P. subflavus* in Texas. In

total, 30 *P. subflavus* were observed across four sites in Collingsworth County.

Freestone County.—On 23 January 2017, an adult female *P. subflavus* (ASNHC 18114, ASK 12671) was collected from a culvert along IH-45 (31.52346°N, 96.11293°W) 8.4 km northwest of Buffalo, Texas. A total of 712 *P. subflavus* were documented at this location. An additional 969 *P. subflavus* were documented across other sites in Freestone County. Although American Perimyotis has previously been documented in Freestone County (Walker et al. 1996), to our knowledge no voucher specimens exist. Thus, this voucher specimen is considered as a new county record for Freestone County. This individual was negative for spores of *P. destructans*.

Harrison County.—On 26 January 2017, a *P. subflavus* was observed roosting in an IH-20 culvert (32.48579°N, 94.12468°W) 6.2 km west of Waskom, Texas. This bat was roosting at a height of 2.8 m and was not collected as a voucher due to its location. This observation provides additional support for a previous voucher from Harrison County (Demere et al. 2012) and is the first locality-specific documentation of *P. subflavus* in the county.

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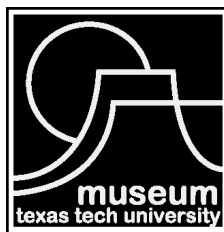
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