SURVEY AND DISTRIBUTION OF INVERTEBRATES FROM SELECTED SPRINGS OF THE EDWARDS AQUIFER IN COMAL AND HAYS COUNTIES, TEXAS

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ABSTRACT—We surveyed four spring sites of the Edwards Aquifer to determine current distribution and abundance of spring and cave-adapted invertebrates, including three that are federally listed as endangered. We found 18 species from 12 families of endangered and stygobiontic fauna, similar to other surveys completed 10 years prior, and recorded new localities for *Tethysbaena texana, Cirolanides texensis, Lirceolus hardeni, Lirceolus smithii, Stygobromus longipes, Stygobromus flagellatus,* and *Artesia subterranea.* We also report discovery of new species of Bogidiellidae (near *Parabogidiella*), *Ingolfiella,* and *Almuerzothyas.*

RESUMEN—Examinamos cuatro sitios de nacimento de aguas del acuífero Edwards para determinar las distribuciones y abundancias de invertebrados adaptados a manantiales y cuevas, incluyendo 3 especies que están en peligro de extinción a nivel federal. Encontramos 18 especies de 12 familias de fauna estigobiónticas parecidas a muestreos realizados hace 10 años. Reportamos nuevas localidades para *Tethysbaena texana, Cirolanides texensis, Lirceolus hardeni, Lirceolus smithii, Stygobromus longipes, Stygobromus flagellatus* y *Artesia subterranea.* Tambien reportamos el descubrimento de nuevas especias de Bogidiellidae (similar a *Parabogidiella*), *Ingofiella* y *Almuerzothyas.*

The limestone aquifers of the Edwards Plateau support about one-half of all springs in Texas, including some of the largest (Brune, 1981). Bowles and Arsuffi (1993) identified 91 endemic species in the permanent aquatic habitats of the Edwards Plateau with >40 described species of stygobionts (Hershler and Longley, 1986*b*; Barr and Spangler, 1992). This aquifer is the major source of water for the rapidly growing city of San Antonio. It also is important for agriculture, fisheries, and tourism. Water flowing down the Guadalupe River supplied by the aquifer provides important coastal habitat for estuarine-reliant species such as economically important shrimp, crabs, and fishes (Bowles and Arsuffi, 1993), as well as waterfowl that feed on these species. Thus, it is important to maintain the quality and a sufficient quantity of water in the Edwards Aquifer. Periodic surveys of the stygobiontic communities can be useful to detect changes within the aquifer that might affect those that rely on this resource. Three aquatic invertebrates associated with springs of the Edwards Aquifer are listed as endangered by the United States Fish and Wildlife Service (1997)—Comal Springs riffle beetle *Heterelmis comalensis,* Peck's cave amphipod *Stygobromus pecki,* and Comal Springs dryopid beetle *Stygoparnus comalensis.* These aquifer-dependent species are subject to population reduction, or possible extinction, if water quality or quantity is decreased due to drought or over-pumping. They only occur in Comal and Hueco springs in Comal County and San Marcos and Fern Bank springs in Hays County, Texas (Fig. 1).

Comal Springs, located in the city of New Braunfels, is the largest spring system (mean discharge = 8,200 during 1927–2005; United States Geological Survey, http://nwis.waterdata.usgs.gov/tx/nwis/discharge/, to 9,900 L/s during1882-1926; Brune, 1981) in Texas and the southwestern United States. Comal Springs is a spring complex with numerous discharge locations occurring along the 1,300-m reach forming Landa Lake. Tracer testing performed by the Edwards Aquifer Authority (EAA) in 2002

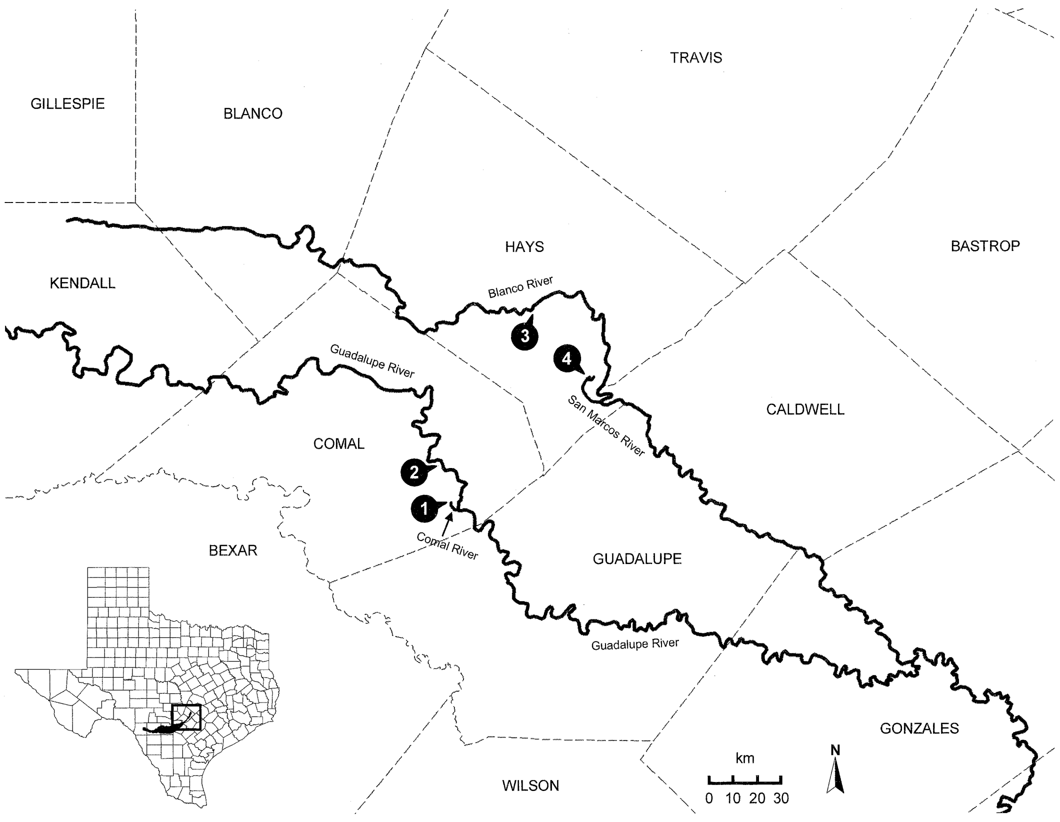

FIG. 1—Sites in south-central Texas surveyed for invertebrates by drift netting and hand sampling at spring openings at (1) Comal Springs, (2) Hueco Springs, and (3) Fern Bank Springs during spring and summer 2003. San Marcos Springs (4) was sampled using cotton-cloth lures during spring 2004 and 2006. In small map of Texas, rectangle indicates expanded region of named counties and dark polygon represents the artesian zone of the Edwards Aquifer.

indicates convergent, conduit-dominated flow paths in the Comal Springs area. Groundwater discharging from Comal Springs originates in regions as far as 225 km to the west. Discharged groundwater ranges in age from hours to many years. The spring complex has had continuous flow with an exception in 1956, when flow ceased for about 6 months after 7 years of drought (Brune, 1975, 1981).

Hueco Springs, located 5.1 km N Comal Springs, is a much smaller spring system (mean discharge = 1,100 during 1924–1978; Brune, 1981, to 1,800 L/s during 2002–2006; United States Geological Survey , http://nwis.waterdata.usgs.gov/tx/nwis/discharge/) issuing from alluvial gravel about 90–150 m west of the Guadalupe River. Hueco Springs, like Comal Springs, has a local and regional component; tracer tests by Ogden et al. (1986) showed some local contribution to the springs and naphthalene detected at Hueco and Comal springs from a diesel spill in January 2000 about 6.5 km SW New Braunfels suggest regional contribution (G. Schindel, pers. comm.). The occurrence of some of the same species at both Comal and Hueco springs also suggests a regional component of flow.

San Marcos Springs, located in the City of San Marcos, is the second largest spring system (mean discharge = 4,100 during 1916–1955; Brune, 1981, to 4,900 L/s during 1956–2005; United States Geological Survey, http://nwis.waterdata.usgs.gov/tx/nwis/discharge/) in Texas. San Marcos Springs is a complex of many artesian springs upwelling into Spring Lake, forming the head of the San Marcos River (Brune, 1981). Records indicate this spring complex has not ceased flowing. Water discharging from San Marcos Springs has multiple sources; southern flow from the San Antonio area in Bexar County that bypasses Comal Springs and flow from the northern area of the aquifer, south of the Barton Springs groundwater divide near Kyle, Hays County. Groundwater from the southern flow paths discharges from the southern portion of Spring Lake while water from the northern flow paths discharges from the northern portion of the lake (G. Schindel, pers. comm.).

Fern Bank Springs is located 8 km E Wimberley and 13 km NW San Marcos. A small cave stream empties into a pool before diffusely flowing over a bluff and cascading into the Blanco River. Many seeps emerge near the base of this cliff. These springs are influenced by local recharge and may be perennial with recorded discharge ranging from 140 L/s on 31 May 1975 to 9 L/s on 1 May 1978 (Brune, 1981; C. B. Barr, in litt.).

Heterelmis comalensis was described by Bosse et al. (1988) and was known only from Comal Springs until C. B. Barr (in litt.) collected one adult from San Marcos Springs. *Stygoparnus comalensis* and *Stygobromus pecki* were described and originally known only from Comal Springs (Holsinger, 1967; Barr and Spangler, 1992), but both are stygobiontic and difficult to collect. T. L. Arsuffi (in litt.) recommended use of drift nets for sampling these species because of poor results (only a few *S. pecki* from Comal Springs and no *S. comalensis*) with a Hess sampler and handpicking. C. B. Barr (in litt.) collected many *S. pecki* from the headwaters of the Comal River by placing drift nets at spring orifices and recorded a range extension by capturing a single specimen at Hueco Springs. In September 2002, J. Krejca (unpublished data) collected *S. pecki* (verified by SJH) from Panther Canyon Well (18-m deep), about 110 m from Comal Springs. C. B. Barr (in litt.) also caught a few *S. comalensis* by drift netting at Comal Springs and at Fern Bank Springs (31.5 km NE Comal Springs, range extension). Neither *S. comalensis* nor *S. pecki* have been found from other spring orifices or wells, suggesting that these species are restricted to the known locations and not distributed throughout the aquifer (United States Fish and Wildlife Service, 1997).

To determine current distribution and abundance of endangered and other spring and cave-adapted invertebrates in the Edwards Aquifer region, we surveyed several spring sites in central Texas. We also compared our results with those of C. B. Barr (in litt.).

Materials and Methods—We collected invertebrates using drift nets (0.45 by 0.30-m rectangular opening, 250 μm mesh) at Comal, Fern Bank, and Hueco springs (Fig. 1). From 2 May to 16 August 2003, we sampled each site with five drift nets twice in both spring and summer except Fern Bank Springs, which was sampled with two nets once in spring and twice in summer, totaling 46 samples. We deployed nets for about 24 h and checked them every 4–6 h. Because so few specimens were found at Fern Bank Springs during the first sampling period, nets were left out for 67–74 h and checked once daily during the last two sampling periods. Specimens of *H. comalensis* and *S. pecki* large enough to identify in the field were returned to the area where they were collected and all other invertebrates from nets were preserved in 70% ethanol for later study. We calculated combined drift time (sum of hours nets were placed at a site) for each spring system and drift rate (number collected/day) of each species.

During March 2004 and March and April 2006, we used cotton-cloth lures in San Marcos Springs to see if *H. comalensis* was still present after C. B. Barr (in litt.) collected the only known specimen from this location in 1992. This method was adapted from the mop head and also the gauze technique (Hershler and Longley, 1986*a*) for collecting cave snails and used by G. Cole for collecting isopods (Holsinger and Minckley, 1971). We lodge cotton cloths, about handkerchief-sized, inside spring orifices. Over 3–5 weeks, microorganisms (primarily fungi) grow on the cotton substrate, serving as a probable food source for some invertebrates. After we remove the cloths and place them in a tray filled with water, invertebrates are easily picked off unharmed. We recommend this passive collecting technique for the endangered beetles, *S. comalensis* and *H. comalensis*, because it is less labor intensive, less damaging to the animals, and non-destructive to the habitat than methods previously employed.

Additionally, we reviewed the literature and collection records of the stygobiontic species found at Comal, Hueco, and Fern Bank springs. We determined the current distribution of these species using this and specimens collected or identified by the authors. We also enlisted taxonomic expertise of D. Bowles, J. Holsinger, and I. Smith.

Results and Discussion—We collected 18 stygobiontic and listed species (Table 1), representing 12 families in 5 orders. Total numbers and drift rates of listed and stygobiontic species collected at each locality during the 2003 drift survey are in Table 2. Species currently listed as endangered were present at the localities sampled by C. B. Barr (in litt.).

Endangered Species—We collected *S. comalensis* at Comal Springs and Fern Bank Springs. During a combined drift time of 451.6 h at Comal Springs, we collected two adults and three larvae, which is similar to C. B. Barr (in litt.) who collected one adult and four larvae during a 397.5-h combined drift time. Additionally, we collected a single larva by hand sampling and an adult on a cotton-cloth lure placed in a small spring opening. Subsequently, we collected three more adults on cloth lures placed in seeps of spring runs and along the western shoreline of Landa Lake in 2004. In 2005, we observed three adults on pieces of rotting wood resting on upwellings on the bottom of Landa Lake. At Fern Bank Springs, we collected a single larva from a spring at the base of a hillside during a 304.8-h combined drift time of two drift nets compared to C. B. Barr (in litt.) who collected one adult and 12 larvae from a single small hillside spring during a 131.5-h combined drift time. We placed one net in a small cave where the largest spring (depth = 0.15 m; mean current velocity = 0.36 m/s) emerged and collected stygobionts other than *Stygoparnus*. However, the diffuse streams issuing from the spring pool did not produce any stygobionts. We placed the second drift net over a small spring that flowed out of the base of the bluff directly into the Blanco River. Here, we collected stygobionts, including a single larva of *S. comalensis* during a 140-h total drift time. A variety of trees and shrubs were growing on top of this travertine bluff. The face of this bluff was covered with bryophytes and ferns and produced stalactites that dripped ground water directly into the river. Water percolates through this bluff, potentially supplying aquatic areas for the adult dryopids and damp terrestrial areas for the larvae. Also, microbial growth, on roots and organic debris found within the bluff, provides a potential food source. During this study, too few *S. comalensis* were collected to compare relative abundance among samples and with previous studies in which more aggressive techniques were used. While specific habitat requirements of *S. comalensis* are unknown, it is possible that the preferred habitat is difficult to sample or that it comprises a small area, or both, which results in this beetle rarely being collected.

Similar to C. B. Barr (in litt.), we found *S. pecki* to be abundant in our drift-net samples from Comal Springs. We collected 173 specimens during a 451.6-h combined drift effort (9.2/day) and C. B. Barr (in litt.) collected 154 specimens during a 384-h combined drift effort (9.6/day). At Hueco Springs, we collected 23 *S. pecki* during a 455.6-h combined drift effort (1.2/day). C. B. Barr (in litt.) collected only one specimen during a 96-h combined drift effort (0.2/day).

We found *S. pecki* in gravel, rocks, and associated debris (leaves, roots, and wood) directly in or near springs, seeps, and upwellings of Comal Springs and Landa Lake, but we did not observe them in surrounding habitats of the spring runs and lake. Many *S. pecki* collected at Comal Springs were entirely bright orange. All other stygobiontic amphipods appeared white or slightly yellow, but their gut would sometimes appear yellow, orange, tan, or dark brown. All the stygobiontic fauna of Hueco Springs, including *S. pecki*, were white. Color of some amphipods, and crustaceans in general, often is dependent on diet, which usually includes carotenoids produced by plants, algae, and probably some bacteria (Negre-Sadargues et al., 2000; Fraser and Bramley, 2004; Gaillard et al., 2004). It is possible that the orange coloration found within the Comal Springs population is derived from food sources (e.g., leaf litter) that were scarce where *S. pecki* inhabits Hueco Springs.

Overall, we retrieved two *H. comalensis* daily from each spring sampled with drift nets at Comal Springs. Prior to our survey, Norris (2002) collected invertebrates at Comal Springs using the same drift nets we used, placed in the center of the spring runs >100 m downstream from the spring heads, and found *H. comalensis* (mostly larvae) to be much higher in the drift (as much as 18 beetles/day). It is possible that drift of *H. comalensis* was higher in Norris' survey because he potentially captured beetles drifting from all areas of spring runs upstream of the drift net, which encompassed many springs along the margins as well as numerous upwellings throughout the streambeds. These springs and upwellings range from large, high-flow, cave-like orifices to small, low-flow, gravel, sand, or mud seepages. We found *H. comalensis* primarily in or near spring outlets, but microhabitat preferences still are not known. Bowles et al. (2003) collected *H. comalensis* by hand sampling the streambed within spring runs and found the beetles to be randomly dispersed throughout runs; however, the sampling resolution was 1 m^2 and microhabitat preferences might not have been detectable at that level.

We used the cotton-cloth-lure method to collect >50 *Heterelmis* sp. (adults and larvae combined) from upper reaches of San Marcos

TABLE 1—Distribution of stygobiontic and listed species found at Comal, Hueco, and Fern Bank springs, Texas. All records without citations were specimens collected or identified by authors. Counties are in uppercase and all located in Texas. Superscripts are as follows: a = new county record; b = new locality record; and c = first known record.

Taxa	Distribution
Almuerzothyas n. sp.	COMAL: Comal Springs[c]
Artesia subterranea	COMAL: Comal Springs[a] HAYS: TSU artesian well (Holsinger and Longley, 1980), Ezell's Cave[b] (collected by J. Krejca)
Cirolanides texensis	COMAL: Comal and Hueco springs[b]; Panther Canyon Well (Krejca, 2005) southern Edwards Plateau region in Texas and northern Mexico (Krejca, 2005)
Comaldessus stygius	COMAL: Comal Springs HAYS: Fern Bank Springs[a]
Haideoporus texanus	COMAL: Comal Springs HAYS: TSU artesian well (Young and Longley, 1976)
Heterelmis comalensis	COMAL: Comal Springs HAYS: San Marcos Springs
Ingolfiella n. sp.	COMAL: Comal Springs[c]
Lirceolus hardeni	BLANCO, COMAL, HAYS, KENDALL, AND TRAVIS (Lewis and Bowman, 1996; Krejca, 2005)
Lirceolus pilus	BANDERA: Lost Maples State Park (Lewis and Bowman, 1996) COMAL: Comal and Hueco springs[b], Preserve Cave (Krejca, 2005) HAYS: San Marcos Springs[a] MEDINA: Valdina Farms Sinkhole (Lewis and Bowman, 1996)
Mexiweckelia hardeni	BEXAR: San Antonio River alluvium[a] COMAL: Comal and Hueco springs[a] MEDINA: Hondo Creek alluvium (Holsinger, 1992)
Parabogidiella?	BEXAR: Verstraeten well no. 1 (Holsinger and Longley, 1980) COMAL: Comal Springs[a] HAYS: TSU artesian well (Holsinger and Longley, 1980)
Seborgia relicta	COMAL: Comal and Hueco springs[a] HAYS: TSU artesian well (Holsinger and Longley, 1980); Ezell's Cave[b] (collected by J. Krejca) MEDINA: Hondo Creek alluvium (Holsinger, 1992)
Stygobromus flagellatus	COMAL: Comal Springs[a] HAYS: TSU artesian well; San Marcos Springs; Ezell's and Rattlesnake caves (Holsinger, 1966, 1967; Holsinger and Longley, 1980) TRAVIS: Barton Springs[a] (collected by D. Chamberlain)
Stygobromus longipes	HAYS: San Marcos Springs[a] KENDALL: Cave without-a-name (Holsinger, 1966) KENDALL/COMAL: Honey Creek Cave (Reddell, 1985)
Stygobromus pecki	COMAL: Comal and Hueco springs; Panther Canyon Well (collected by J. Krejca)
Stygobromus russelli	widespread in central Texas (Holsinger and Longley, 1980)
Stygoparnus comalensis	COMAL: Comal Springs HAYS: Fern Bank Springs
Tethysbaena texana	COMAL: Hueco springs[b] wells in BEXAR, HAYS, and UVALDE (Stock and Longley, 1981)

TABLE 2—Total numbers and drift rates (number/day) of stygobionts and listed species collected at Comal, Hueco, and Fern Bank springs, Texas, 2 May–16 August 2003. *Stygobromus* spp. were immature and probably were *S. pecki* and *S. russelli.*

	Comal Springs		Hueco Springs		Fern Bank Springs	
Taxa	No.	No./day	No.	No./day	No.	No./day
Crustaceans						
Amphipoda						
Crangonyctidae						
Stygobromus pecki	173	9.2	23	1.2		
Stygobromus russelli	371	19.7	91	4.8	14	1.1
Stygobromus spp.	120	6.4	42	2.2	14	1.1
Hadziidae						
Mexiweckelia hardeni	54	2.9				
Sebidae						
Seborgia relicta	13	0.7	7	0.4		
Bogidiellidae						
Artesia subterranea	6	0.3				
Isopoda						
Asellidae						
Lirceolus hardeni	14	0.7	439	23.1	9	0.7
Lirceolus near *pilus*	144	7.7	102	5.4		
Cirolanidae						
Cirolanides texensis	2	0.1	4	0.2		
Thermosbaenacea						
Monodellidae						
Tethysbaena (=*Monodella*) *texana*			3	0.2		
Insects						
Coleoptera						
Dytiscidae						
Comaldessus stygius	10	0.5			1	0.1
Haideoporus texanus	3	0.2				
Dryopidae						
Stygoparnus comalensis	5	0.3			1	0.1
Elmidae						
Heterelmis comalensis	37	2.0				

Springs (Spring Lake) in the area where C. B. Barr (in litt.) had collected a single adult, 10 years prior. Of these specimens, 15 were preserved and identified as *H. comalensis* by D. Bowles. We collected 28 adults and larvae of *Heterelmis* in only three springs in this same area during the subsequent survey of 29 upwellings throughout Spring Lake. It appears that *H. comalensis* primarily occurs in the headwaters of Spring Lake. Additionally, we found two other species of elmid (*Microcylloepus pusillus, Stenelmis sexlineata*) in eight springs sampled from the headwaters to 180-m downstream.

Other Stygobionts—All other stygobiontic fauna collected by C. B. Barr (in litt.) also were collected during our survey (Table 2). Additionally, we obtained several new records and made discoveries at each locality, with taxa including beetles, crustaceans, and a mite (Table 1).

At Comal Springs, we collected two species of stygobiontic beetles other than *S. comalensis*; both were dytiscids and rare in our samples. These included *Haideoporus texanus* (three larvae), known only from the artesian well in San Marcos (Young and Longley, 1976; Longley and Spangler, 1977) and spring runs of Comal Springs (Bowles and Stanford, 1997), and *Comaldessus stygius* (10 adults), known only from Comal Springs (Spangler and Barr, 1995). We also found a single adult of *C. stygius* in a drift-net sample from Fern Bank Springs, a range extension for this rare species. However, to confirm this record, additional collecting should be performed. As is characteristic of the family, both of these species likely are predators as larvae. Larvae of *C. stygius* have yet to be collected and described. Longley and Spangler (1977) presumed that larvae of *H. texanus* pupate along the margins of their aquatic habitat and in trapped air spaces within the aquifer because dytiscids typically pupate in damp areas out of water. Adult dytiscids typically frequent the surface to replenish their air supply carried in a subelytral chamber. Because these beetles are subterranean, they may locate air spaces within the aquifer for this purpose. Near springs, these air spaces may be maintained due to the relative stability groundwater level, possibly providing habitat for all life stages of these beetles. Other than in Australia, stygobiontic beetles are rare throughout the world, and the presence of three taxa (in two families and three genera) of such beetles in these spring systems is remarkable.

Thermosbaenaceans are in a primitive crustacean order found in groundwater and marine habitats primarily in the Mediterranean and Caribbean regions. However, *Tethysbaena* (=*Monodella*) *texana* occurs in caves and wells of Hays, Bexar, and Uvalde counties (Stock and Longley, 1981). We collected three specimens at Hueco Springs; the first record of this species in Comal County.

We collected *Cirolanides texensis* at Comal and Hueco springs. These are the first records for this species at both locations, but their occurrence there is not surprising because Krejca (2005) collected it in Panther Canyon Well adjacent to Comal Springs prior to our survey. This marine-relict isopod is believed to have descended from ancestors in the Gulf of Mexico (Bowman, 1964) and has been found in caves and phreatic groundwaters throughout the southern Edwards Plateau region of Texas. Recently, Botosaneanu and Iliffe (2002) recorded a subspecies in caves of northern Mexico. Krejca (2005) used genetic analysis to show *C. texensis* had a pattern of evolution that followed subterranean hydrological connections compared to the freshwater-derived *Lirceolus* isopods that appear to have followed surface river drainages. The genus *Lirceolus* occurs in caves, springs, and riverbed alluvium in Texas and Mexico. *Lirceolus hardeni* has been found in cave streams and springs in Blanco, Comal, Hays, Kendall, and Travis counties of central Texas (Lewis and Bowman, 1996; Krejca, 2005). We collected them at all sites (Table 2) and report the first records for this species at San Marcos and Fern Bank springs. *Lirceolus pilus* has been recorded from a spring system in Bandera County and a sinkhole in Medina Co., Texas (Lewis and Bowman, 1996). Krejca (2005) collected isopods of the genus *Lirceolus* in Preserve Cave, Comal County, and found them to be genetically similar to typical *L. pilus*. We found other populations in Comal County resembling this species, *Lirceolus* near *pilus*, which was the dominant isopod at Comal Springs (92%), but it was less common at Hueco Springs (19%). At San Marcos Springs, we collected *L. hardeni* on cotton-cloth lures, and found *L. pilus*, and *Lirceolus smithii* in drift samples collected by personnel of the Edwards Aquifer Research and Data Center and Aquarena Center. *Lirceolus smithii* previously was recorded only from the artesian well at Texas State University in San Marcos (Bowman and Longley, 1976).

Most *Stygobromus* caught in this study were small (estimated as <5 mm long). This may be because they were dislodged easier than larger ones, they were drifting for dispersal, or the population near spring openings was comprised mostly of small individuals. We recorded those that were too small to readily identify to species as *Stygobromus* spp., although they most likely consisted of both *S. russelli* and *S. pecki*. The most common amphipod collected at all sites was *S. russelli* (Table 2). This species is the most widespread stygobiontic amphipod in Texas (Holsinger and Longley, 1980). It is found in karst habitats and gravel stream beds, and likely is more common in shallow aquifer habitats than deep cave systems (Holsinger, 1967). At Hueco Springs, the largest numbers of *S. russelli* (10/day) were from shallow seeps and less (2/day) were from large upwellings. Also, more *S. pecki* (1.2/day) than *S. russelli* (0.2/day) were collected from the artesian well. This suggests that *S. pecki* inhabits deeper portions of the Hueco Spring system than does *S. russelli*. Holsinger (1967) distinguished the *flagellatus* group of *Stygobromus*, which includes *S. pecki*, as having insular patterns of speciation being greatly restricted in ranges, and occupying deeper groundwater niches as compared to the more common *tenuis* group, which includes *S. russelli*.

Because *S. pecki* has been collected only near or within spring systems, it is likely a phreatic species and does not disperse well in downstream gravel beds where *S. russelli* occurs. Similarly, SJH assisting J. Krejca and P. Sprouse (unpublished data) found *S. pecki* near the upwellings and not further downstream in the gravel at Hueco Springs during summer 2003. However, *S. russelli* was in both areas. Because of the phreatic nature of *S. pecki* and the hydrological connection demonstrated by the aforementioned diesel spill, we suspect this species inhabits deep groundwater niches in the area around Comal and Hueco springs and follows hydrological connections between these locations.

Stygobromus longipes previously was known only from Cave Without-A-Name and Honey Creek Cave in Kendall and Comal counties (Holsinger, 1966, 1967; Reddell, 1985). We collected a single specimen of *S. longipes* on the cotton-cloth lures placed in San Marcos Springs, extending the known range into Hays County. *Stygobromus flagellatus* previously was known only from Ezell's Cave, Rattlesnake Cave, the artesian well at Texas State University, and San Marcos Springs, all in the city of San Marcos (Holsinger, 1966; Holsinger and Longley, 1980). We identified specimens as *S. flagellatus* collected by Dee Ann Chamberlain (unpublished data) in 2003 at Barton Springs and JRG collected a single specimen in a drift survey of Comal Springs extending the range of this species from Hays County, north to Travis and south to Comal counties. In 2005, we found *S. longipes* and *S. flagellatus* in drift samples at San Marcos Springs collected by Edwards Aquifer Research and Data Center and Aquarena Center, indicating these populations are sympatric. Holsinger and Longley (1980) reported *Stygobromus bifurcatus* (*tenuis* group) from San Marcos Springs. Thus, there have been four species of *Stygobromus* collected here, the most recorded together at any locality. Within this genus, co-occurrence is rare and usually involves different species groups, with hybridization being even rarer (Holsinger, 1978). Although *S. flagellatus* is the closest known relative to *S. longipes* (Holsinger, 1967), we found no obvious hybridization (intermediate characters) in >100 specimens examined. We found only a few *S. longipes* in samples that were dominated by hundreds of *S. flagellatus* and *S. russelli*. Presumably, *S. longipes* occupies a different niche and area than *S. flagellatus* in order to co-occur and rarely drifts out into San Marcos Springs. Genetic comparisons of the different populations and species of this genus might be useful to show degree of isolation and connectivity of habitats as well as assist in clarifying taxonomy of the group.

We discovered an apparent groundwater-adapted population of *Crangonyx pseudogracilis* at Hueco Springs. Some other amphipods in the same *gracilis* species group are stygomorphic and restricted to groundwater habitats (Zhang and Holsinger, 2003). The three specimens taken from Hueco Springs have somewhat degenerate eyes and little-to-no pigmentation. The same species was in an interstitial habitat within stream-bank gravels of the Guadalupe River in Kendall County, about 50 km W Hueco Springs (pers. observ., SJH). The specimens from Kendall County resemble those from Hueco Springs in having reduced eyes and pigmentation. Gibson (2000) found *C. pseudogracilis* in sandy springs throughout eastern Texas with the typical pigmentation and well-developed eyes. This species is widespread in the east-central United States and southern Canada, with a few possibly introduced populations in Arizona and Nevada, and likely introduced populations in western Europe (Zhang and Holsinger, 2003). The populations in Comal and Kendall counties may represent the southwestern range limit for the species.

Holsinger and Longley (1980) considered amphipods in the family Crangonyctidae, including *Stygobromus* and *Crangonyx*, to be of freshwater origin. All other stygobiontic amphipods collected during our survey are species that they considered to be derived from relict marine ancestors that might have colonized the region during regression of a Cretaceous sea.

We collected six *Artesia subterranea* at Comal Springs during our survey extending the known range from Hays County to Comal County. Because *A. subterranea* had previously been collected in Ezell's Cave by J. Krejca while diving in the aquifer during September 2006 (verified by JRG) and from the artesian well at Texas State University (59.5 m deep) in San Marcos (Holsinger and Longley, 1980), it is likely that this species primarily inhabits deeper areas of the aquifer. *Artesia welbourni* is the only other known species in this genus and is represented by three specimens collected from open water of a deep phreatic pool within Border Cave, Culberson

Co., Texas (Holsinger, 1992). Another bogidiellid was collected in the drift at Comal Springs in November 2006 (pers. observ., JRG). The two specimens likely are in the genus *Parabogidiella* and may be the same rare species mentioned by Holsinger and Longley (1980) collected in the well at Texas State University, Hays County, and another well in Bexar County. All of these specimens were missing the last pair of legs, making identification inconclusive. *Parabogidiella americana*, the only described species in the genus, has been collected in the well at Texas State University, two wells in Bexar County, and Honey Creek Cave in Comal and Kendall counties (Holsinger and Longley, 1980; Fiers and Iliffe, 2000). These specimens and *A. subterranea* are the only bogidiellids reported north of Mexico.

We collected *Mexiweckelia hardeni* only at Comal Springs during this study. This species was first discovered living in the interstitial alluvial groundwater within the gravel banks (hyporheic zone) of Hondo Creek in Medina County (Holsinger, 1992). It has since been found at Comal Springs (C. B. Barr, in litt.) and alluvial deposits along the San Antonio River, Bexar Co., Texas (pers. observ., SJH). The other two species in this genus occur in northern Mexico (Holsinger and Minckley, 1971; Holsinger, 1973).

We collected *Seborgia relicta* at Comal and Hueco springs, as did C. B. Barr (in litt.). This species was described from the artesian well at Texas State University in San Marcos (Holsinger and Longley, 1980) and later found in the hyporheos of Hondo Creek with *M. hardeni* (Holsinger, 1992). *Seborgia hershleri*, found in a West Texas spring in Val Verde County, is a close relative to *S. relicta* (Holsinger, 1992), and Pesce and Iliffe (2002) collected *S.* near *hershleri* in a cave in Tamaulipas, Mexico. The remaining two members of this genus are brackish water, Old World species—*Seborgia minima* isolated in an island lake in the South Pacific (Bousfield, 1970) and *Serborgia schieckei* on the coast of an island in the Indian Ocean (Ruffo, 1983).

Comal Springs is the only locality for an undescribed species of *Ingolfiella*, represented by a single specimen captured in the drift during 2004 (pers. observ., JRG). Valentina Iannilli, S. Ruffo, R. Vonk, and J. Holsinger (pers. comm.) currently are describing this and another species from Bustamante, Mexico, that appear to be closely related. These are the only known ingolfiellids in the subterranean freshwaters of mainland North America and, perhaps like the thermosbaenacean *Tethysbaena texana*, their closest known relatives may occur in the Mediterranean region, appearing to have a Tethys Sea origin. This small and primitive suborder of amphipod (Ingolfiellidea) has been found in interstitial marine, brackish, and fresh groundwater habitats worldwide (Stock, 1977; Ruffo and Vonk, 2001). The apparent rarity of this species may be due to its small size and potential to inhabit tight interstitial spaces that are difficult to sample. It also is possible that this species occupies the interstitial groundwater of streambeds similar to those where *M. hardeni* and *S. relicta* can occur.

Holsinger and Longley (1980), suggested that invasion of adjacent, interstitial habitats could have occurred in the Tertiary and that the Balcones Fault Zone provides a potential mixing zone for karst-groundwater fauna and those from the adjacent interstitial habitat of the Texas coastal plain. This idea is supported by the fact that some of the same taxa (e.g., *M. hardeni* and *S. relicta* mentioned earlier) subsequently have been found by SJH in alluvial biotopes in the coastal plain and areas within the Balcones Fault Zone. Further evidence that marine fauna may have invaded karst habitats through a hyporheic corridor is that all of the larger stygobionts known from the Edwards Aquifer, such as salamanders, fishes, and shrimps, are of ancient freshwater lineages. All of these animals also are mobile, swimming forms. The marine relicts, on the contrary, are all relatively small and benthic.

We collected many nymphal and adult specimens of a new species of hydryphantid water mite from a single spring orifice at Comal Springs. This mite, *Almuerzothyas* n. sp., belongs to a group of genera distributed throughout tropical and warm-temperate parts of the world and its closest known relative is a species living in Costa Rica (Goldschmidt and Gerecke, 2003; I. Smith, pers. comm.).

As apparent by this and other surveys, the Edwards Aquifer has a rich and diverse fauna that reflects its complex geological structure and history. We recommend occasional surveys to monitor subterranean communities for changes that could reflect adverse conditions within the aquifer and to record new faunal localities that hint toward connectivity within the aquifer. Additionally, we suggest use of genetic analysis on the more common taxa that could reflect hydrological connections, degree of isolation of cave and spring fauna, and geological and taxonomic history, which are all important in understanding the complex nature of the aquifer for conservation of the rare and endemic species and associated habitats and management as a major water source.

Special thanks go to C. Thompson for help in all levels including finding funding, correspondence, reviewing, and field work. We thank the Keith and Pfeuffer families for allowing us access to springs on their land. We also thank D. Bowles (National Park Service), J. Holsinger (Old Dominion University), and I. Smith (Agriculture and Agri-Food Canada) for their taxonomic expertise. We appreciate BIO-WEST, Inc., City of Austin Watershed Protection and Development Review Department, the Edwards Aquifer Authority, and Zara Environmental Ltd. for providing us additional information, as well as the staff at Aquarena Center (notably E. Chappell and T. Kunz) and the Edwards Aquifer Research and Data Center for supplying specimens. Thanks go to T. Castro, J. Gracia, B. Grod, J. Hulbert, and J. Thompston for their help in the field. We also thank M. Alexander, T. Arsuffi, D. Bowles, T. Brandt, G. Longley, and J. Fowler-Propst for reviewing drafts. This project was a cooperative effort between the San Marcos National Fish Hatchery and Technology Center and Texas State University. Funding, in part, was provided by a grant from Austin Ecological Services, United States Fish and Wildlife Service. Endangered species collection operated under Texas permit SPR-0390-045 (National Fish Hatchery and Technology Center), federal permit TE676811-9 (National Fish Hatchery and Technology Center), Texas permit SPR-0503-302 (Texas State University), and federal permit TE068895-0 (Texas State University). A representative series of specimens were deposited in the Stephen F. Austin State University Invertebrate Collection. Voucher specimens of *Heterelmis comalensis* from San Marcos Springs are in David Bowles' personal reference collection at the National Park Service and deposited in the insect collection at Texas A&M University. Views presented do not necessarily reflect those of the United States Fish and Wildlife Service.

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Submitted 16 January 2006. Accepted 25 June 2007. Associate Editor was Joseph P. Shannon.