

THE CONSERVATION STATUS OF
TEXAS GROUNDWATER OSTRACODA**Benjamin T. Hutchins^{1,*}, Benjamin F. Schwartz^{1,2} and Okan Külköylüoğlu³**¹ *Edwards Aquifer Research and Data Center, Texas State University,
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Abstract.— Groundwater-obligate organisms are nearly always small range endemic species with a high-degree of imperilment because of a combination of anthropogenic threats and inherent ecological traits such as low reproductive potential. Accurate assessments of conservation status are foundational for development of species-specific research and conservation priorities. Fourteen species of endemic ostracods are restricted to groundwater habitats in Texas. We used NatureServe methodology to evaluate the conservation status of all 14 species. All evaluated taxa were identified as warranting G1S1 (critically imperiled) or G2S2 (imperiled) designation, primarily due to small-range endemism (particularly small area of occupancy and few known occurrences). Most species are recorded from five or fewer locations and occupy 4-km² grid cells. Threat levels varied by site type, with all springs and one surface water site facing the most severe threats and highest number of threats. Deeper, phreatic groundwater sites were typically more secure. Most ostracod sites harbor only a single groundwater species, but two sites, Comal Springs and the San Marcos Artesian Well, harbor six and seven species, respectively. Based on this status assessment, we recommend that all 14 ostracod species be designated as species of greatest conservation need (SGCN). Additionally, we propose that the top priorities for these taxa are 1) identification of existing lots in museum holdings, 2) molecular analyses of populations to better delimit species boundaries, 3) additional field work to fully delineate range sizes and area of occupancy, and 4) long-term sampling at diverse sites to track population trends.

Supplemental material is available for this article online.

Keywords: aquifer, stygobiont, NatureServe, subterranean

Groundwater-obligate organisms (called stygobites or stygobionts) feature prominently among state and national lists of imperiled species because of a combination of external threats (e.g., groundwater

extraction and contamination) and intrinsic vulnerability (e.g., small-range endemism and stenothermy) (Mammola et al. 2019). However, even among this narrowly defined ecological group with strong convergence on similar morphological, physiological, and behavioral characteristics (Culver & Pipan 2019), species differ in conservation status due to differences in range size, rarity, magnitude and severity of threats, and ecological tolerance. Consequently, resource managers and conservation practitioners must rely on consistent methodologies to evaluate the conservation status of groundwater organisms when allocating limited resources (*sensu* Game et al. 2013). Informed conservation of groundwater organisms, however, is hindered by several knowledge shortfalls (Mammola et al. 2019 and references therein), including incomplete knowledge of species distributions (i.e., the Wallacean shortfall) and incomplete knowledge of diversity and species identities (i.e., the Linnaean shortfall). This is particularly true for organisms for which current taxonomic expertise has been lacking.

The groundwater fauna of Texas, and the Edwards Aquifer in particular, has long been recognized as uniquely diverse and a global hotspot of biodiversity (Longley 1981; Hutchins et al. 2021). Until recently, however, the groundwater-obligate ostracod fauna was limited to a single, poorly known species (Hart 1978). Given the high species-richness in other subterranean groups such as amphipods (Holsinger & Longley 1980) and snails (Hershler & Longley 1986), the paucity of ostracod species was improbable. Indeed, ostracods are an important component of groundwater communities globally. In Western Europe, ostracods make up about 4.5% of described groundwater species (Deharveng et al. 2009). In the Pilbara region of Western Australia, and emerging hotspot of groundwater biodiversity, an extraordinary radiation of Candonid ostracods (Karanovic 2007) constitute over a quarter of described groundwater species (Halse 2018a). In two recent special issues on ‘hotspots of subterranean biodiversity’ in the journal *Diversity*, ostracods constituted 0–26% of the groundwater-obligate fauna in sites with ≥ 20 species (Table 1).

In 2011, investigation of Texas’ groundwater ostracod fauna began in earnest, with the description of *Bicornucandona fineganensis* Kulköylüoğlu, Gibson, Diaz & Colin 2011. Hutchins (2018) evaluated

Table 1: Ostracod species richness, total stygobiont species richness, and proportional contribution of ostracods in diverse (≥ 20 spp.) groundwater sites (data compiled from “Hotspots of Subterranean Biodiversity” Vols 1 and 2, special issue of the journal Diversity (ISSN 1424-2818), years 2021–2024).

Site	Ostracods	Total stygobionts	Proportion	Citation
Robe Valley, Australia	15	58	0.26	Clark et al. (2021)
Coume Ouarnède System, France	0	22	0.00	Faille & Deharveng (2021)
Walsingham Caves, Bermuda	13	62	0.21	Ilfie & Calderón- Gutiérrez (2021)
Postojna-Planina Cave, Slovenia	2	71	0.03	Zagmajster et al. (2021)
San Marcos Artesian Well, USA	11	55	0.20	Hutchins et al. (2021)
Križna Jama, Slovenia	1	32	0.03	Polak & Pipan (2021)
Ojo Guareña, Spain	8	46	0.17	Camacho & Puch (2021)
Vjetrenica Cave, Bosnia & Herzegovina	3	48	0.06	Delić et al. (2023)
Cent Fonts Aquifer, France	7	43	0.16	Prié et al. (2024)

the conservation status of the state’s invertebrate groundwater fauna, concluding that 55% of groundwater taxa described at that time were imperiled or critically imperiled, primarily because of extreme small-range endemism. Four ostracod species had been described at the time of that assessment, but the available distribution data were deemed insufficient for ranking those and eight non-ostracod taxa. Since then, ten additional groundwater-obligate ostracods have been described (Table 2), bringing the total number in Texas up to 14 species. This taxonomic effort has resulted, in part, from more extensive ostracod collecting throughout the state. Indeed, surface and groundwater-ostracods have been reported from over 250 sites throughout the state by the authors of the present study.

Considering these recent advances, and the prevalent threats to and inherent vulnerability of groundwater taxa in general, we felt that a conservation status assessment of the state’s groundwater-obligate ostracods was due. Here, we present a comprehensive status assessment

Table 2. Conservation status ranks, rarity measures, and threat impact for Texas endemic, groundwater-obligate ostracods. Area of occupancy is the number of 4-km² cells occupied by the species. * denotes single-site endemics.

Species	Habitat	Assigned Rank	Range Extent (km ²)	Area of Occupancy	Number of occurrences	Occurrences with good viability	Threat Impact
<i>Bicrnicandona fineganensis</i>	large karst springs, hyporheic zone	G2S2	250 - 1,000	6-25	6-20	4-12	high
<i>Cabralcandona mixoni*</i>	Edwards Aquifer phreatic zone	G1S1	< 100	1	1-5	1-3	low
<i>Comalcandona gibsoni*</i>	large karst springs	G1S1	< 100	1	1-5	1-3	medium
<i>Comalcandona tressleri</i>	large karst springs, small karst springs, hyporheic zone, Edwards Aquifer phreatic zone	G2S2	250 - 1,000	6-25	6-20	1-3	high
<i>Cypria lacrima</i>	large karst springs, Edwards Aquifer phreatic zone	G1S1	< 100	2	1-5	1-3	medium
<i>Hobbsiella moria</i>	caves (ectoparasite)	G1S1	< 100	2	1-5	1-3	medium
<i>Indocandona rusti*</i>	hyporheic zone	G1S1	< 100	2	1-5	1-3	medium
<i>Lacrimacandona wisei*</i>	Edwards Aquifer phreatic zone	G1S1	< 100	1	1-5	1-3	low
<i>Namiothocypria hayensis*</i>	Edwards Aquifer phreatic zone	G1S1	< 100	1	1-5	1-3	low
<i>Pseudocandona lordi</i>	large karst springs, hyporheic zone	G1S1	5,000-20,000	3-5	1-5	1-3	high
<i>Rugosuscandona scharfi</i>	large karst springs, hyporheic zone, Edwards Aquifer phreatic zone	G2S2	100-250	3-5	1-5	1-3	medium
<i>Schornikovdona bellensis</i>	caves, small karst springs, large karst springs	G2S2	1,000-5,000	6-25	6-20	1-3	high
<i>Tuberona leonidasi*</i>	small karst springs	G1S1	< 100	1	1-5	0	high
<i>Ufocandona hamaleae</i>	large karst springs, Edwards Aquifer phreatic zone	G1S1	< 100	2	1-5	1-3	medium

of the state's described groundwater-obligate and groundwater-dependent ostracod fauna using the NatureServe methodology and our recommendations for updated conservation status ranks. Additionally, we discuss research and conservation needs to better understand and manage this important component of our subterranean biodiversity.

MATERIALS & METHODS

Conservation status of the 14 species listed in Table 2 was assessed using the NatureServe Rank Calculator v3.2 (NatureServe 2020) following the methods of Hutchins (2018), modified as described below. Briefly, conservation status assessment was evaluated based on range extent, area of occupancy (defined as the number of 4-km² grid cells occupied), number of occurrences, number of occurrences with good viability, and overall threat impact. Occurrence records (Supplement 1, https://doi.org/10.32011/txjsci_77_1_Article02.S01) were collected from the literature and unpublished records solicited from the authors and collaborators (see acknowledgements). Voucher specimens representing unpublished records are housed at the Aquifer Biodiversity Collection, Edwards Aquifer Research and Data Center. Separation distance was set at 1.5 km (Hutchins 2018) and range size of taxa recorded from one or two locations was set at 4 km² or the linear distance between the two sites x 1 km, respectively. State ranks were calculated, but because all the evaluated species are Texas endemics, state and global ranks are equivalent.

Unlike Hutchins (2018), who evaluated threats on an aquifer-by-aquifer basis, we evaluated threats on a site-by-site basis. Nine threats in eight NatureServe threat categories were evaluated (Table 3). Threat severity was evaluated subjectively, based on the authors' site knowledge, hydrogeologic context, or consultation with other biologists (see Acknowledgements) because population trend data are not available for any species at any site. The severity of water management/use and drought was based on aquifer recharge and storage properties and the existence of sufficient regulatory/conservation mechanisms protecting water quantity. Invasive non-native/alien

Table 3: Number of sites, number of ostracod records, and percentage of sites with good/excellent viability by site type.

Site type	Number of sites	Number of ostracod records	Number of ostracod species	Good viability (%)
cave	3	3	2	100
Edwards Aquifer phreatic zone	2	8	8	100
hyporheic zone	12	12	5	75
large karst spring	19	26	8	58
small karst spring	5	6	3	20
surface waters	1	1	1	0

species refers specifically to habitat degradation by feral hogs. Temperature extremes were considered slight for shallow groundwater habitats (hyporheic zones and some springs), and negligible for deeper groundwater habitats (phreatic zone and some springs). Populations were classified as having good viability/ecological integrity when the calculated overall threat impact for a site was moderate or better. Site-by-site threats were aggregated for each species. The scope of threats was calculated based on the proportion of sites for which a given threat was identified. If the severity of a threat varied across sites for a given species, the highest severity was assigned for the species. All ranks and supporting information were provided to Texas Parks and Wildlife Department: the state agency responsible for providing state-level conservation status updates to NatureServe via periodic data exchanges.

RESULTS

The conservation status of 14 groundwater-dependent ostracods was assessed using the NatureServe Rank Calculator V3.2. Species records were gathered from 43 distinct sampling sites (Supplement 1). Ten of the 14 species were designated as critically imperiled at the state and global level, and the remaining four species were designated as imperiled (Table 2). Rarity was the primary factor driving the conservation status of taxa: 11 of the 14 taxa (79%) are currently known from five or fewer locations and occupy five or fewer 4-km² grid cells (Fig. 1, Table 2). Ten of the 14 species (71%) have a range of 100 km²

or less, and only one species, *Pseudocandona lordi* Kulköylüoğlu, Hutchins, Yavuzatmaca & Schwartz 2021, has a range greater than 5,000 km² (Fig. 1, Table 2). Five species are single-site endemics (Fig. 1, Table 2). Species occur at few sites with good or excellent viability. *Tuberona leonidasi* Kulköylüoğlu, Ataman, Gibson & Diaz 2023 (see Kulköylüoğlu & Meisch (2023) for replacement name) does not occur at any sites with good or excellent viability, and only one species, *Bicornucandona fineganensis* Kulköylüoğlu, Gibson, Diaz & Colin 2011 is known from four or more sites with good or excellent viability (Table 2). Nine new occurrence records are reported for *B. fineganensis* (vouchers: ABC-003566, ABC-002986, ABC-004455–004456, ABC-004463, ABC-004460–004462). Threat impact was variable among species. Most species have a medium or high threat impact, three species have a low threat impact, and one species has a very high threat impact.

Sites were classified as one of six habitat types (Table 3). Most sites are large springs or hyporheic sites, and most ostracod records come from karst springs. Most sites only have a single recorded, groundwater-obligate ostracod species, though two sites, Comal Springs and San Marcos Artesian Well, have six and seven described species, respectively. Cave and Edwards Aquifer phreatic zone sites have good viability (low to medium threat impact), hyporheic sites and large karst springs had intermediate viability (75% and 58% of sites, respectively), and small karst springs and a single surface water site had poor viability (20% and 0% of sites, respectively).

Groundwater ostracod sites were evaluated for impact from nine different threats (Table 4). Relative to deeper habitats (caves, large karst springs, and the Edwards Aquifer phreatic zone), shallow sites (hyporheic, springs, and a single surface water site) faced a greater number of threats and the threat impact was more severe. Water management /use (extraction for human use) and climate change effects (drought and temperature extremes) were the most common threats,

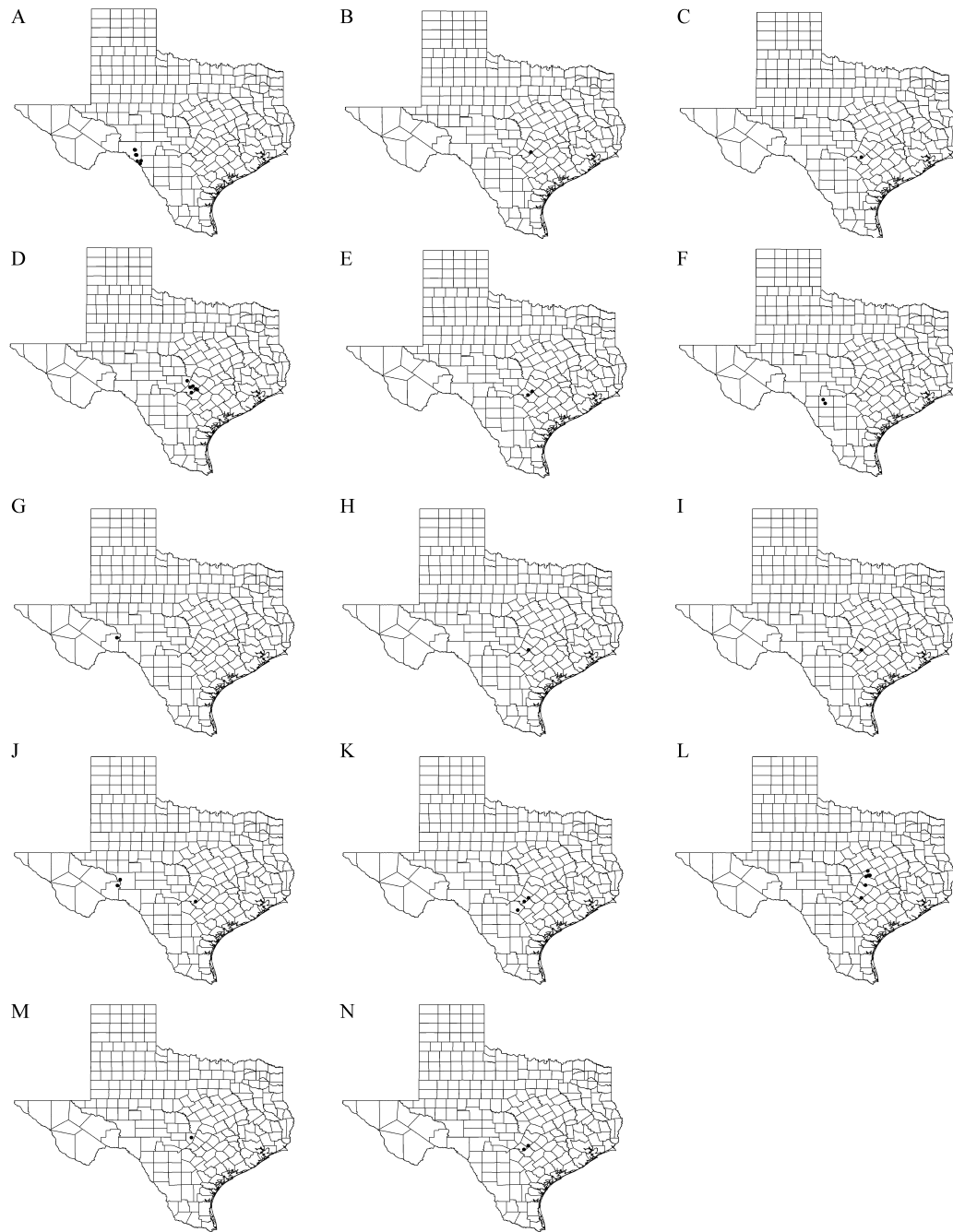


Figure 1: Distributions of groundwater-obligate ostracods in Texas. A: *B. fineganensis*, B: *C. mixoni*, C: *C. gibsoni*, D: *C. tressleri*, E: *C. lacrima*, F: *H. moria*, G: *I. rusti*, H: *L. wisei*, I: *N. haysensis*, J: *P. lordi*, K: *R. scharfi*, L: *S. bellensis*, M: *T. leonidasi*, N: *U. hannaleeae*.

Table 4: Severity of threats and percentage of sites, by site type, affected by threats (number in parentheses = total number of sites).

Threat	Severity	Cave (3)	Edwards Aquifer phreatic zone (2)	Hyporheic zone (12)	Large karst spring (19)	Small karst spring (5)	Surface waters (1)	All Sites (42)
Housing & Urban areas	Slight	0	0	25	0	20	100	13
Livestock & Farming	Slight	33	0	17	32	40	0	31
Oil & Gas drilling	Slight	0	0	25	5	0	0	5
Recreational activities	Slight	0	0	17	5	0	0	4
Water management/ use	Slight	33	100	0	21	20	0	20
	Moderate	67	0	100	79	80	100	80
Invasive non- native species	Slight	0	0	0	58	60	100	46
Domestic and urban waste water	Slight	0	100	33	32	40	100	38
Droughts	Slight	33	100	0	0	0	0	5
	Moderate	67	0	100	100	100	100	95
Temperature extremes	Slight	33	0	100	5	80	100	56

affecting all sites. Additionally, water management/use and climate change effects were the only threats with more than slight severity at some sites, although these threats were less severe in deeper sites (some caves and the phreatic zone of the Edwards Aquifer). Oil and gas drilling and recreational activities, followed by housing and urban areas, were the most restricted threats, each impacting less than 10% of evaluated sites. Impact from invasive species (feral hogs) only impacted some springs and surface waters.

DISCUSSION

As in some other groundwater communities, ostracods constitute an important component of the Texas groundwater fauna. These taxa represent unique components to the biodiversity of the state. Three examples are provided here. 1) The subfamily *Cabralcandoninae* (Külköylüoğlu et al. 2019) is known only from Texas (Meisch et al. 2024). It contains eight genera (*Bicornucandona*, *Cabralcandona*, *Comalcandona*, *Lacrimacandona*, *Rugosuscandona*, *Schornikovdona*, *Tuberona*, *Ufocandona*) reported from the San Marcos Artesian Well and/or springs associated with the Edwards or Trinity Aquifers. It appears that members of the tribe show unique adaptations to hypogean habitats (e.g., smaller body size, anophthalmy, reduced setation, etc., Külköylüoğlu et al. (2019) and references therein). 2) *Indocandona rusti* was collected from the hyporheic zone of Independence Creek. It is the third species of the genus, and the first reported outside of India. The genus may represent a pronounced example of convergent evolution rather than monophyly (Külköylüoğlu et al. 2021). 3) The genus *Hobbsiella* Danielopol & Hart 1985 belongs to the subfamily Sphaeromicolinae in the family Entocytheridae. The family includes about 220 living species, representing the third most diverse nonmarine ostracod family. The genus includes three ectosymbiont ostracods living commensally on other crustaceans. *Hobbsiella moria* (Hart, 1978) is the only ectosymbiont stygobiont known from Texas.

Until recently, Texas groundwater ostracods were too poorly known to draw conclusions about their conservation status. Our knowledge of Texas' groundwater ostracods is still very incomplete, but it is apparent that Texas' groundwater ostracod fauna is globally significant and, like groundwater species around the world, imperiled. All 14 of the taxa evaluated were ranked as G1/S1 or G2/S2 species. In contrast, only 55% of the taxa ranked by Hutchins (2018) had G1/S1 or G2/S2 ranks. Though there were some differences in methodological approaches, several biological and ecological factors more likely contribute to greater imperilment in ostracods relative to other Texas stygobionts. Some of the taxa evaluated in Hutchins (2018) have unusually large ranges (resulting in lower imperilment) for groundwater taxa (e.g., *Sphalloplana mohri* Hyman 1938 and *Phreatodrobia nugax* (Pilsbry &

Ferriss 1906)) and may represent complexes of multiple cryptic taxa. Indeed, *S. mohri* includes four previously described taxa that were synonymized by Kenk (1977). Other taxa evaluated in Hutchins (2018) (e.g. *Stygobromus russelli* (Holsinger 1967) and *Caecidotea reddelli* (Steeves 1968)) are relatively large, easily detected in the field, and abundant in areas that have received a great deal of biological investigation because of the presence of endangered species; factors which may contribute to a greater number of known occurrences across a larger area (resulting in lower imperilment). In contrast, the ostracods evaluated here are not readily hand-collected or noticeable without a microscope and occur in habitats that are historically under-sampled in Texas (e.g. the hyporheic zone). Hutchins (2018) reported that 92% of taxa had low to medium threat levels, and nearly half had low threat levels. Conversely, only 18% of ostracods have low threat levels and over 40% had high or very high threat levels. Differences in how threat level was calculated in Hutchins (2018) versus the current effort may contribute in part to higher threat levels for ostracods, but these differences can also be attributed to the prevalence of ostracods in shallow groundwater habitats which, as shown in this current study, are generally more threatened. Indeed, half of the ostracods examined are known from shallow groundwater habitats (Table 2). Conversely, only 17% of the taxa evaluated in Hutchins (2018) were from shallow hyporheic or hypotelminorheic habitats although small springs and caves were not distinguished from large springs or other aquifer habitats in that study.

The imperiled status of ostracods, however, is not primarily the result of higher threat levels, but rather, pervasive small-range endemism and the small number of sites from which most species are known. Nine of the 14 taxa evaluated occurred in only one or two 4-km² grid cells: a condition that automatically triggers an S1 designation using the NatureServe methodology. Of the five taxa with larger ranges, all would still receive a S1 or S2 designation if threat level was not considered, although a low or medium threat level designation could result in a more secure designation (S1 taxa becoming S2 or S2 taxa becoming S3) for some taxa. Currently, these taxa have medium and high threat designations. The outsized role that small range size and few occurrence records play in determining the conservation status of

groundwater ostracods underscores the need for continued field surveys and examination of museum materials, both of which could result in discovery of additional populations. For example, a recent TPWD-funded survey of groundwater dependent invertebrates in the Trans-Pecos region produced one new record of *Indocandona rusti* Kulköylüoğlu, Hutchins, Yavuzatmaca & Schwartz 2021 and nine new records of *Bicornucandona fineganensis* Kulköylüoğlu, Gibson, Diaz & Colin 2011 (Schwartz et al. 2023). Additionally, major collections of groundwater ostracods (particularly the Aquifer Biodiversity Collection at Texas State University) await additional attention from taxonomic experts: hundreds of lots, containing thousands of individuals remain unidentified. However, given the prevalence of small-range endemism in groundwater faunas (Trontelj et al. 2009; Culver & Pipan 2019), substantial range extensions for all taxa seem unlikely. Culver and Pipan (2019) showed that small range endemism is the norm for the United States, parts of Europe and the Pilbara region of Western Australia, which have received considerable attention. Gladstone et al. (2022) evaluated the conservation status of stygosnails in the United States and Mexico, concluding that 82% of evaluated species were imperiled or critically imperiled, again due to small range endemism, but also due to changes in hydrology and nutrient input and habitat modification: threats that Texas' groundwater fauna also face.

Increasingly, natural resource managers recognize the need for prioritizing conservation and management of subterranean organisms and subterranean ecosystems (Niemiller et al. 2018 and references therein). Not only do subterranean habitats harbor unique lineages that uniquely contribute to regional biodiversity, but these species are inherently vulnerable because of low reproductive rates and limited dispersal (Culver & Pipan 2014). Groundwater taxa may also be particularly sensitive to anthropogenic contaminants, although the existing ecotoxicological research to date is equivocal (Castaño-Sánchez et al. 2020). The current study addressed three science gaps identified in the subterranean fauna conservation road map developed by Wynne et al. (2021): assessing biological diversity, quantifying and delineating ranges, and conducting status assessments (using NatureServe rather than IUCN methodology). This is the first step in strategically identifying evidence-based conservation objectives

(Mammola et al. 2022). Conservation, however, operates within existing legal and managerial frameworks, and Texas laws regarding groundwater use (e.g., the rule of capture and separate management of groundwater vs. surface water) provide particular challenges in a region where groundwater extraction and anthropogenic climate change are the greatest threats to groundwater species. Unlike some municipalities where subterranean species and/or subterranean habitats are protected *de facto* (e.g., Belgium, Slovenia, Croatia, and Western Australia (Halse 2018b; Niemiller et al. 2018), conservation efforts in the United States usually target specific species with state or federal protected status (i.e., via the Endangered Species Act).

Fifty-seven percent of federally protected, subterranean species in the United States are Texas endemics (Niemiller et al. 2018). Although it is premature to consider the ostracods evaluated here as appropriate targets for a federal listing petition, status assessments do suggest a more pro-active course of action: designation as state species of greatest conservation need (SGCN). The available data clearly demonstrate that Texas' groundwater fauna, including ostracods, are uniquely diverse and comprised of small-range endemics that are both inherently vulnerable and restricted to habitats facing growing anthropogenic pressures. Designation as species of greatest conservation need would bring attention to these poorly known taxa and create opportunities for additional research and conservation funding.

We propose that the top priorities for these taxa are 1) identification of existing lots in museum holdings, 2) molecular analyses of populations to better delimit species boundaries, 3) additional field work to fully delineate range sizes and area of occupancy, and 4) long-term sampling at diverse sites to track population trends. Although our knowledge is incomplete, obvious conservation targets are already emerging (i.e., major karst springs). Two sites, Comal Springs and the San Marcos Artesian Well stand out as high diversity sites, with six and seven species, respectively (Supplement 1). Both sites are afforded substantial protection through the Edwards Aquifer Habitat Conservation Plan and have good viability/ecological integrity. Except for two sites, each with two species, the remaining sites evaluated only contained a single groundwater obligate ostracod species, although that

number is expected to increase with additional taxonomic work, particularly at large karst springs like Caroline Springs and the Robertson Spring complex. Indeed, more Texas groundwater ostracod species are recorded from large karst springs than from any other habitat, and Hutchins (2018) previously identified large karst springs as conservation priorities because of high species richness and the ability to conserve many species in a small area. Although shallow groundwater sites were not the most diverse sites identified, they are particularly imperiled because of groundwater quality and quantity issues associated with climate change and over-extraction. They also face threats like habitat modification and non-native species that similarly affect epigean aquatic habitats (Culver & Pipan 2019). However, conservation of small springs may be more straightforward relative to large springs, because of their smaller groundwater basins.

The need for pro-active conservation is more than academic: alarming rates of groundwater extraction, resulting in spring failure and groundwater depletion at the aquifer scale have been documented around the world (Mammola et al. 2019) and recently, an entire groundwater fauna of more than 50 species was declared extirpated on Curaçao (Humphreys 2022). Taxonomic investigations over the past 12 years have shown that ostracods are an important component of Texas' unique and diverse groundwater fauna. Compared to Texas' other groundwater taxa, ostracods are equally or more imperiled on average, and all these taxa are threatened by growing anthropogenic pressures across diverse habitat types.

ACKNOWLEDGMENTS

James Reddell, Randy Gibson (USFWS), Pete Diaz (USFWS) and Jacob Owen (U.S. Air Force, Camp Bullis) provided information on site locations and site-specific threats.

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