



Molecular phylogeny, biogeography, and conservation status of the Texas-endemic freshwater mussel *Lampsilis bracteata* (Bivalvia, Unionidae)

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Abstract

Lampsilis bracteata (Gould), the Texas Fatmucket, is a regional endemic species in the central Texas biogeographic province which is a candidate to be listed as threatened or endangered under the Endangered Species Act of 1973. *Lampsilis bracteata* is morphologically similar to the common species *L. hydiana* (Lea). Here, we examine the molecular taxonomic identification of *L. bracteata*, and compare its historical range with its current geographic distribution. Tests of genetic affinities based on two mitochondrial genes typically used for DNA barcoding (cytochrome oxidase subunit 1, COI and *NADH* dehydrogenase subunit 1, ND1) support recognition of *L. bracteata* as a full species. An unexpected spin-off result was that ND1 sequences of *L. satura* (Lea), a threatened species in Texas, formed a highly supported cluster within putative *L. cardium* Rafinesque. As an endemic species, the distribution of *L. bracteata* has been historically restricted; however, poor land and water management practices have further reduced its distribution from eighteen to just eight streams in the Colorado River drainage and to one stream in the Guadalupe River drainage. For *L. bracteata*, as for many other imperiled freshwater mussel species, effective conservation measures rely on correct species identification, definition of its geographic range and assessment of its changes in the recent past.

Key words: barcoding, species validation, geographic range contraction

Introduction

Traditionally, the identification of freshwater pearl mussels or unionid species (Order Unionoida) is based on conchological characteristics which is overall reliable for most species. However, these features vary geographically and with the environment which has led to species taxonomy ambiguities and conservation challenges (Williams & Mulvey 1997; Lydeard & Roe 1998; Shea *et al.* 2011). DNA barcoding has proven to be an effective alternative tool for both classification (Hebert *et al.* 2003a; Hebert *et al.* 2003b; Hebert & Gregory 2005) as well as species delineation (Hubert & Hanner 2015) but see also Will & Rubinoff (2004), Ebach & Holdrege (2005) and Will *et al.* (2005).

Unionids are a highly diverse and ecologically important group. Nevertheless, this taxon has experienced among the highest rates of decline and extinction in North America (Bogan 1993; Williams *et al.* 1993; Master *et al.* 2000; Lydeard *et al.* 2004; Haag & Williams 2014) as a result of human impacts including overharvesting, pollution, impoundment of rivers and the introduction of exotic species such as dreissenid mussels (Bogan 1993; Williams *et al.* 1993; Vaughn & Taylor 1999; Haag 2012). The greatest diversity of unionids in North America is found in the southeastern United States (Lydeard & Mayden 1995; Neves *et al.* 1997). In the state of Texas, there are approximately fifty described species (Howells *et al.* 1996; Winemiller *et al.* 2010); twenty-two of them are considered rare and eight are very rare, including state and regional endemics (Burlakova *et al.* 2011b).

The genus *Lampsilis* Rafinesque, 1820, is represented by four species in Texas: *L. teres* (Rafinesque, 1820) (Yellow Sandshell), *L. hydiana* (Lea, 1838) (Louisiana Fatmucket), *L. bracteata* (Gould, 1855) (Texas Fatmucket), and *L. satura* (Lea, 1852) (Sandbank Pocketbook). A fifth species, *L. cardium* Rafinesque, 1820 (Plain Pocketbook),

was listed in Howells *et al.* (1996); however, its distribution in Texas is not clear due to its morphological resemblance to *L. satura*.

Lampsilis bracteata and *L. satura* were both classified as rare (Burlakova *et al.* 2011b), based on their restricted distribution and relative low densities, and they were listed as threatened by the Texas Parks and Wildlife Department (TPWD) along with another thirteen species in Texas (TPWD 2010). *Lampsilis bracteata* is also a candidate to be listed as threatened or endangered under the Endangered Species Act of 1973 (ESA 1973).

Lampsilis bracteata has been recognized as a valid species (Turgeon *et al.* 1998; Williams *et al.* 2017). However, it is morphologically very similar to the common species *L. hydiana* (Fig. 1) which tends to be somewhat larger, heavier-shelled and more inflated (Howells *et al.* 1996). The status of these species has not been evaluated with a molecular approach and molecular delineation of this species is therefore needed (Howells 2010).

Given current environmental threats, mussel populations in Texas are expected to continue to decline (Burlakova *et al.* 2011a) and warrant conservation. However, effective conservation measures rely on correct identifications of the threatened species and determination of their geographic ranges. The aims of this study are 1) to reevaluate the status of *L. bracteata* with molecular techniques and, 2) to compare its historical and current geographic range in Texas. Two mitochondrial genes often used for genetic barcoding (cytochrome oxidase subunit 1, COI and *NADH* dehydrogenase subunit 1, ND1) were used to determine the status of *L. bracteata*. Past and current distributional data were obtained from an extensive survey across the state of Texas conducted in 2004–2012 (Burlakova *et al.* 2011a, b) and the literature.

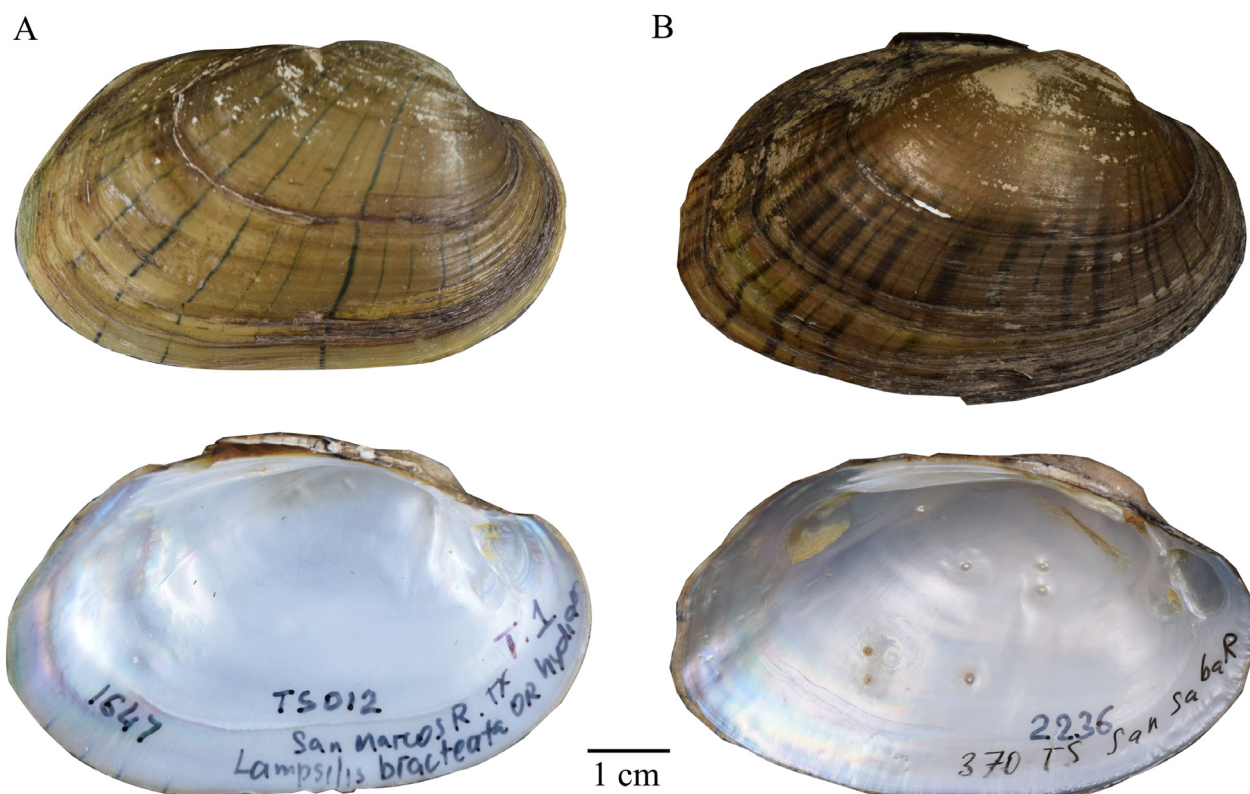


FIGURE 1. (a) *Lampsilis hydiana* voucher no. 1647 (TS012) and (b) *Lampsilis bracteata* voucher no. 2236 (TS370) from Texas. Specimens from the Great Lakes Center Invertebrate Collection, Buffalo State College, Buffalo, NY.

Material

In order to test for phylogenetic relationships, mantle tissue from *Lampsilis bracteata*, *L. hydiana*, *L. teres*, *L. satura*, *L. cardium*, *Obovaria jacksoniana* (Frierson, 1912) and *Villosa lienosa* (Conrad, 1834) was obtained from preserved individuals in the Buffalo State Great Lakes Center Invertebrate Collection (BSGLC) (SUNY Buffalo State College, Buffalo, NY) (Table 1). Given the endangered status of *L. bracteata*, sampling was limited to the

Llano and San Saba rivers both from the Colorado River drainage. Additional *L. satura* tissue was obtained from the BSGLC collection, Neil Ford (University of Texas). One complete individual of *Lampsilis radiata* (Gmelin, 1791) was collected by Denise Mayer (New York State Museum) from the Hudson River, NY and *Lampsilis siliquoidea* mantle tissue was obtained by Porto-Hannes and Burlakova from Honeoye Creek, NY, but these specimens were not incorporated in any collection; there are no voucher numbers, therefore. A list of all other examined specimens with voucher numbers and GenBank accession numbers is given in Table 1.

TABLE 1. Analyzed specimens from three genera that successfully amplified for mitochondrial genes, cytochrome oxidase subunit 1 (COI) or first subunit of the *NADH* dehydrogenase subunit 1, ND1. Voucher no. = collection number at Great Lakes Center Invertebrate Collection, Buffalo State College, Buffalo, NY. Accession number = GenBank accession number for both genes. NA = not available.

* No voucher specimen available, sample is a mantle clip taken by Porto-Hannes, I.

Species	Voucher no.	Locality	River drainage	County	State	Accession number	
						COI	ND1
<i>Lampsilis bracteata</i>	279TS	Llano River	Colorado	Kimble	TX	MK226680	MK226713
	367TS	San Saba River	Colorado	Menard	TX	MK226681	MK226714
	368TS					MK226682	MK226715
	369TS					MK226683	MK226716
	370TS					MK226684	
<i>L. hydiana</i>	012TS	San Marcos River	Guadalupe River	Gonzales	TX	MK226685	MK226709
	013TS					MK226686	
	016TS	Guadalupe River	Guadalupe River	Gonzales	TX	MK226687	MK226704
	138TS	Sandy Creek	Neches River	Shelby	TX	MK226688	MK226708
	159TS	Angelina River	Neches River	Nacogdoches	TX	MK226689	MK226706
	200TS	Village Creek	Neches River	Hardin	TX	MK226690	MK226707
	214TS					MK226691	MK226705
<i>L. teres</i>	047TS	San Marcos River	Guadalupe River	Gonzales	TX	MK226694	MK226711
	048TS	San Antonio				MK226695	
	113TS	Colorado River	Colorado River	Colorado	TX	MK226696	MK226712
	165TS	Angelina River	Neches River	Nacogdoches	TX	MK226697	
<i>L. satura</i>	198TS	Village Creek	Neches River	Hardin	TX		MK226717
	505TS	Neches River	Neches River	Anderson	TX		MK226718
	506TS						MK226719
	507TS						MK226720
	508TS						MK226721
	509TS						MK226722
<i>L. cardium</i>	299TS	Honeoye Creek	Genesee River	Livingston	NY		MK226723
<i>L. siliquoidea</i>	NA*	Honeoye Creek	Genesee River	Livingston	NY	MK226693	MK226710
<i>L. radiata</i>	NA	Hudson River	Hudson River	Saratoga	NY	MK226692	
<i>Obovaria jacksoniana</i>	214TS	Village Creek	Neches River	Hardin	TX	MK226698	MK226726
<i>Villosa lienosa</i>	123TS	Sandy Creek	Neches River	Shelby	TX	MK226699	MK226724
	125TS					MK226700	MK226725
	255TS	Neches River	Neches River	Anderson	TX	MK226701	
	388TS					MK226702	
	389TS					MK226703	

Molecular identification. Genomic DNA was extracted using a modified protocol described by Wilson (1997). Genetic differentiation was determined using two regions from the mitochondrial DNA genome (mtDNA): female cytochrome c oxidase subunit 1 gene (COI) (Folmer *et al.* 1994) and first subunit of the *NADH* dehydrogenase sub-

unit 1, (ND1) (Buhay *et al.* 2002; Serb & Lydeard 2003). Each mitochondrial gene was amplified via a polymerase chain reaction (PCR) in a 25 µl reaction containing the following concentrations: 2.0 ng/ul of extracted genomic DNA 0.3 mM dNTPs, 10 mM Tris-HCl buffer (pH 8.3), 50 mM KCl, 2.5 mM MgCl₂, 0.2 µM each primer and 1U Taq polymerase.

The amplification conditions for both genes were as follows: initial heating to 94°C for 2 min; 5 cycles of, 94°C for 40 s, annealing at 50°C for 40 s, and a 90 s extension time at 72°C; 25 cycles of, 94°C for 40 s; annealing at 40°C for 40 s, and a 90 s extension time at 72°C; a final extension of 10 min at 72°C. All PCR products were screened on 2% agarose gel to confirm amplification and targeted sequence size. Successfully amplified samples were sent to TACGen (Richmond, CA) for sequencing.

Chromatograph files of COI (610 bp) and ND1 (743 bp) sequences were edited and aligned using Geneious v10 (Kearse *et al.* 2012). Sequences were translated using the mitochondrial invertebrate genetic code to ensure the absence of stop codons. All phylogenetic analyses were performed using the nucleotide sequences. Additional COI and ND1 nucleotide sequences were obtained from other studies (Table 2). *Amblema plicata* (Say, 1817) was used as an outgroup; it belongs to the subfamily Ambleminae along with *Lampsilis*, *Villosa* Frierson, 1927, and *Obovaria* Rafinesque, 1819 (tribe Lampsilini) but belongs to the putative sister tribe Amblemini (Lopes-Lima *et al.* 2017).

TABLE 2. Cytochrome c oxidase subunit 1 gene (COI) and first subunit of the *NADH* dehydrogenase subunit 1, ND1 sequences used here from other studies, with GenBank accession numbers.

Species	GenBank no.		Reference
	COI	ND1	
<i>Lampsilis hydiana</i>	EF033270		Chapman <i>et al.</i> 2008a
<i>L. teres</i>	AF406803	AY655102	Hoeh <i>et al.</i> 2002; Campbell <i>et al.</i> 2005
<i>L. ornata</i>	AF385112	AY158748	Roe <i>et al.</i> 2001b; Serb & Harris 2003
<i>L. cardium</i> 1	AF120653		
<i>L. cardium</i> 2	AF156519		Combosch <i>et al.</i> 2017
<i>L. cardium</i> 3	KX713472		
<i>L. cardium</i> 1		FJ601356	Szumowski <i>et al.</i> 2012
<i>L. cardium</i> 2		FJ601350	
<i>L. cardium</i> 3		FJ601357	
<i>L. ovata</i>	EF033262	AY613797	Campbell <i>et al.</i> 2005; Chapman <i>et al.</i> 2008b
<i>L. radiata</i>		EF446098	Kneeland & Rhymer 2007
<i>L. virescens</i>	JF326433		Campbell & Lydeard 2012
<i>Obovaria olivaria</i>	KF035241	KF035386	Inoue <i>et al.</i> 2013
<i>O. subrotunda</i>	KF035263	KF035406	Inoue <i>et al.</i> 2013
<i>O. jacksoniana</i> 1	KF035209	KF035357	Inoue <i>et al.</i> 2013
<i>O. jacksoniana</i> 2	KF035206	KF035354	Inoue <i>et al.</i> 2013
<i>Villosa iris</i>	AF156523	HM849364	Graf & O’Foighil 2000; Breton <i>et al.</i> 2010
<i>V. lienosa</i>	KT285660	DQ445208	Pfeiffer <i>et al.</i> 2016
<i>Actinonaias ligamentina</i>	AF231730	HM852921	Bogan & Hoeh 2000
<i>Amblema plicata</i> (outgroup)	AF156512	AY158796	Graf & O’Foighil 2000; Serb <i>et al.</i> 2003
<i>Obliquaria reflexa</i> (outgroup)	AY655008	AY655108	Campbell <i>et al.</i> 2005

Genetic divergence (i.e., number of net nucleotide substitutions per site between species) among species was calculated in DnaSP v6 (Rozas *et al.* 2017) using the *analysis of DNA divergence between population* command. The two genes were analyzed separately and then concatenated to provide a larger number of informative characters. Phylogenetic trees were estimated using Maximum likelihood (ML) and Bayesian inference (BI). Sequences were analyzed under a GTR+G+I nucleotide substitution model for the ML and BI analysis. The ML tree was calculated using PhyML (Guindon & Gascuel 2003) in Seaview v4.3 (Gouy *et al.* 2010). Support values were generated using the Shimodaira-Hasegawa likelihood ratio test, SH-LRT (Shimodaira & Hasegawa 1999). Bayesian inference was as implemented in MrBayes v3.2.6 (Ronquist & Huelsenbeck, 2003) using Markov chain Monte Carlo simulations. Searches were conducted for 700,000 generations (until the mean SD of the split frequencies fell below 0.01) and

each run consisted of four chains. The molecular data were partitioned by gene and one tree was saved every 1000 generations in each analysis. To allow each gene to evolve independently at its own rate, the option `prset ratepr = variable` was employed and `shape`, `pinvar`, `statefreq`, and `revmat` were all unlinked. Other parameters were set to default values. A consensus tree was obtained by including all the post burn-in sampled trees.

Distribution data. We here use distributional data from a statewide survey conducted between 2004 and 2012. The aim of that survey was to clarify the status of endemic mussels by locating existing populations in need of protection, assess their current status, determine species' habitat requirements, and establish sites for future monitoring and conservation. See Burlakova *et al.* (2011a,b) for the complete survey. Locations where live or recently dead specimens of *L. bracteata* had been found are summarized in Table 3. Additional distributional data were obtained from Strecker (1931), Horne & McIntosh (1979), Howells *et al.* (1996, 1997, 2003) and Howells (2010), from unpublished Texas Parks and Wildlife Department (TPWD) surveys summarized in Howells (2010) and from other reports (Johnson & Groce 2011; Wilkins *et al.* 2011; Randklev *et al.* 2013; Sowards *et al.* 2013; Morton *et al.* 2016).

We digitized the distributional data from the 2004–2012 survey and the literature and created a shape file using ArcGIS v10.1. In the present study historical distributional data are defined as records that were reported by waterbody and county and were collected prior to the 2004–2012 survey. We maintained that format to facilitate comparisons among different data sets and we present current distributional data as GPS coordinate points.

TABLE 3. Locations where live or recently dead specimens of *Lampsilis bracteata* were found during the 2004–2012 freshwater mussel (Family Unionidae) survey in Texas conducted by Burlakova *et al.* (2012).

Species	Waterbody	Drainage	County	Live	Recently dead	Time search, (man-hour)
<i>L. bracteata</i>	Guadalupe River	Guadalupe River	Kerr	6	1	4
	Live Oak Creek	Pedernales/ Colorado River	Gillespie	2	2	5
	San Saba	Colorado River	Menard	12	0	3.75
	San Saba		Menard	1	0.5	3.33
	San Saba		Menard	0	65	Area Search
	San Saba		McCulloch	0	7	1.67
	Elm Creek	Colorado River	Runnels	1	1	10.5
	Llano River	Colorado River	Kimble	1	2	3
	Llano River		Llano	5	0	9
	Llano River		Llano	3		2.5
	Llano River		Mason	2	0	1.75

Results

Molecular identification of *Lampsilis bracteata*. After sequence alignment and editing, we obtained a 743 bp fragment of the ND1 gene. A total of four sequences of *L. bracteata*, one of *L. cardium*, six of *L. hydiana*, two of *L. teres*, six of *L. satura*, two of *V. lienosa* and one of *O. jacksoniana* were successfully amplified (Table 1).

A 610 bp fragment of the COI gene was obtained for analysis in all sampled individuals after sequences were aligned and edited. A total of five sequences of *L. bracteata*, four sequences of *L. teres*, seven sequences of *L. hydiana*, five sequences of *V. lienosa* and one of *O. jacksoniana* (Table 1) were successfully sequenced. *Lampsilis satura* COI was not successfully amplified. *Villosa lienosa* and *O. jacksoniana* from the Neches River drainage were included in the analysis because of their morphological resemblance to *L. hydiana*.

Maximum likelihood and Bayesian analyses of both genes separately (data not shown) and concatenated (Figs. 2, 3) confirm that *L. bracteata* is a distinct species as it forms a monophyletic group with strong support (Shimodaira-Hasegawa-like likelihood ratio test and posterior probability = 1). All *L. bracteata* individuals sequenced in this study share the same haplotype and the genetic differentiation (i.e., nucleotide differences) between *L. bracteata* and *L. hydiana* is 8% for COI and 11% for ND1. Furthermore, the phylogenetic analyses reveal that *L. bracteata* is more closely related to *Lampsilis satura*, *L. ornata*, *L. cardium* and *L. ovata* than to *L. hydiana*. Three individuals identified as *L. hydiana* based on shell morphology collected from the San Marcos and Guadalupe Rivers (Figs. 2, 3) grouped with other *L. hydiana* specimens.

Phylogenetic relationships. Our Maximum likelihood and Bayesian analyses show that ND1 sequences (re-

ported here for the first time) of *L. satura* from Village Creek and from the Neches River in Texas form a cluster with high support (>0.86; Figs. 2, 3). However, this cluster is nested within putative *L. cardium* sequences. Furthermore, nucleotide differences between these two species is only 0.8%.

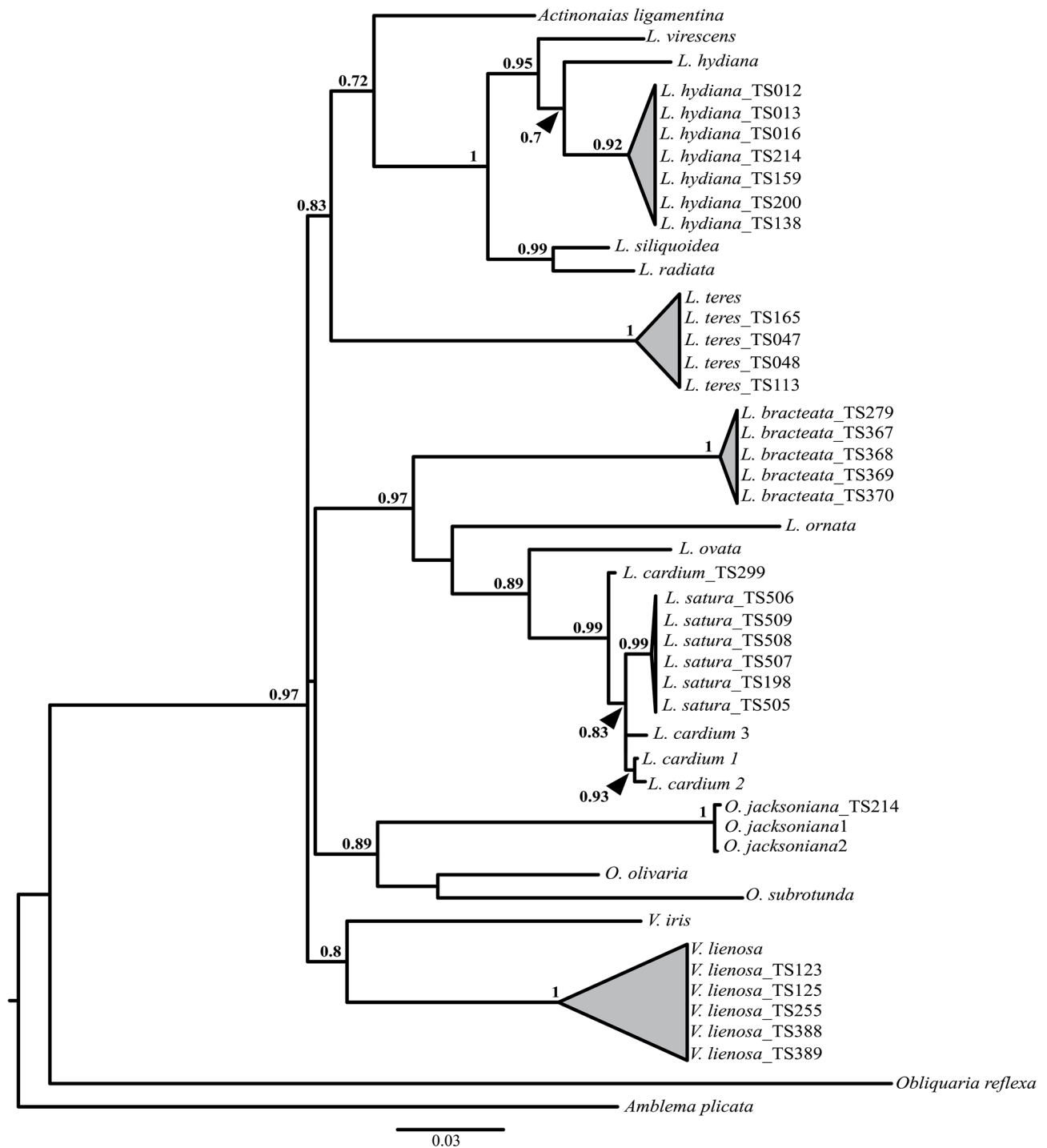


FIGURE 2. A Maximum likelihood tree based on concatenated ND1 and COI nucleotide sequences showing the phylogenetic relationships of *Lamprolaima* and other unionid species from Texas. Numbers after the species names indicate voucher number (Table 1), accession number for the remaining species are in Table 2. Numbers on lines indicate node support values generated using the Shimodaira-Hasegawa-like likelihood ratio test and shown if ≥ 0.70 . The scale bar represents number of nucleotide substitution.

Actinonaias ligamentina nested within a *Lamprolaima* cluster that contains *L. siliquoidea*, *radiata*, *hydiana* and *virescens* with strong support (>0.98). Lastly, our results show that the genus *Lamprolaima* is not monophyletic (Figs. 2, 3). On the Bayesian topology three clusters are formed. Cluster 1 is represented by *L. hydiana*, *virescens*, *sili-*

quoidea, *radiata* and *A. ligamentina*; cluster 2 contains only *L. teres* and cluster 3 is composed of *L. cardium*, *ovata*, *satura* and *ornata*. The Maximum likelihood topology recovered two clusters similar to clusters 1 and 2 from the Bayesian analysis, but cluster 1 includes *L. teres* and cluster 2 is genetically more similar to *Obovaria* than to other *Lampsilis* (<0.70; Fig. 3).

Current biogeographic distribution. The 2004–2012 surveys report a total of thirty-three live specimens of *Lampsilis bracteata* at seventeen locations within five streams (Table 3; Fig. 4); the majority, twenty-seven individuals (Table 3) being from the Colorado River drainage. Six individuals are from one location in the Guadalupe River drainage and none from the San Antonio River drainage (Table 3; Fig. 4). The average density of *L. bracteata* across several sampled locations in San Saba River is very low (0.003 mussels/m², data not shown). The average densities of live mussels found in time searches across all sites is 0.97±0.32 mussels/man-hour (mean + standard error of the mean), and live and recently dead mussels is 1.22±0.25 mussels/man-hour (Table 3).

Other locations where this species has been observed since 2004 are the Pedernales River mainstream in Gillespie County (Johnson & Groce 2011; Morton *et al.* 2016), and Rocky Creek, a tributary of the Pedernales River in Blanco County (Sowards *et al.* 2013), Threadgill Creek in Gillespie and Mason Counties (Howells 2005), and Onion Creek in Travis County (Howells 2010; Wilkins *et al.* 2011) (Fig. 4). It is worth mentioning that a survey of fifty-eight sites in the Colorado River drainage (Morton *et al.* 2016) found 136 live individuals of *L. bracteata* at twenty sites. Furthermore, thirty live individuals were found in the San Saba River in 2011 (Randklev 2012).

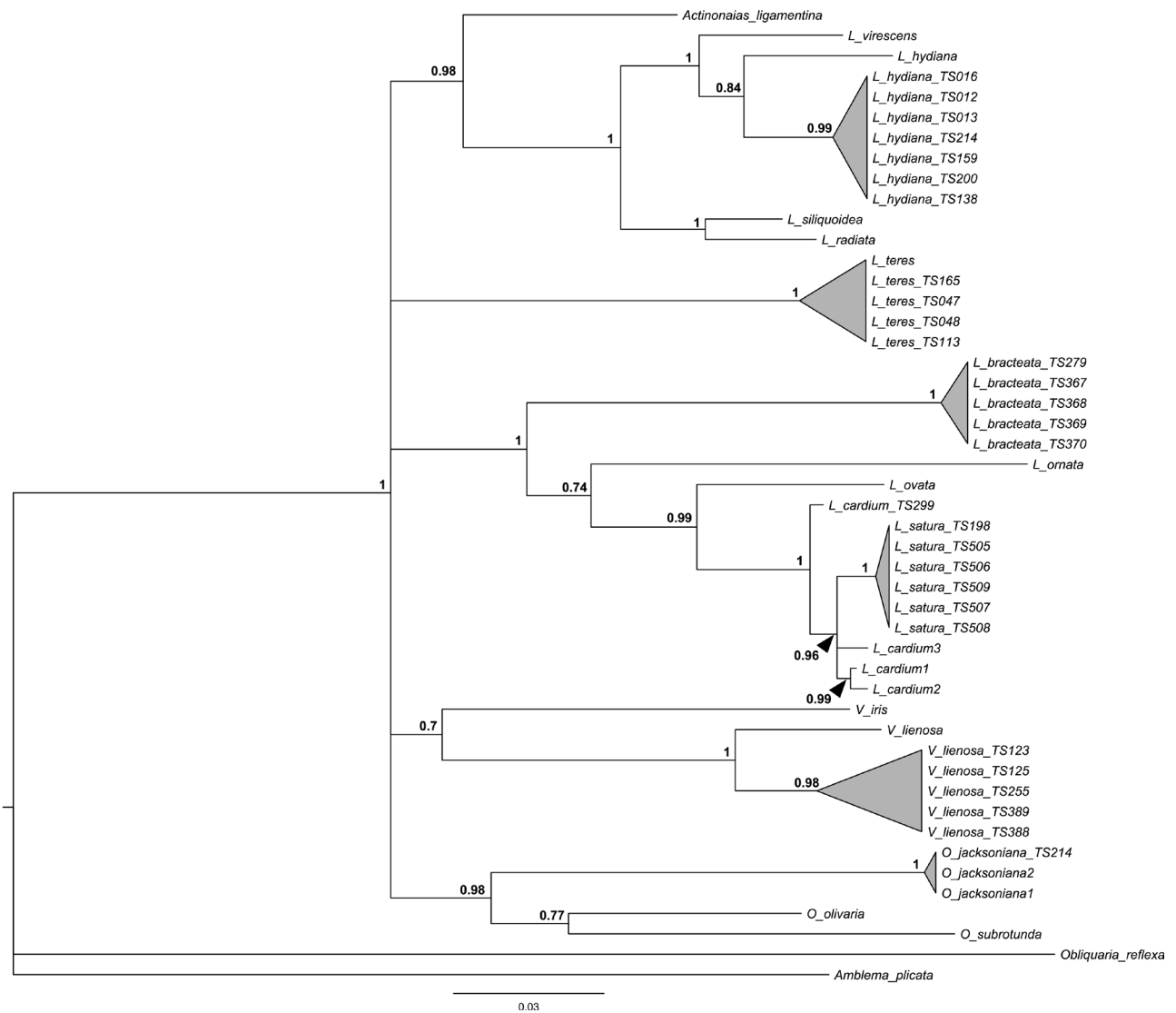


FIGURE 3. A Bayesian likelihood consensus tree based on concatenated ND1 and COI nucleotide sequences showing the phylogenetic relationships of *Lampsilis* and other species from Texas. Numbers after the species names indicate voucher number (Table 1), accession number for the remaining species are in Table 2. Numbers on lines indicate node posterior probabilities (≥ 0.70). The scale bar represents number of nucleotide substitution.

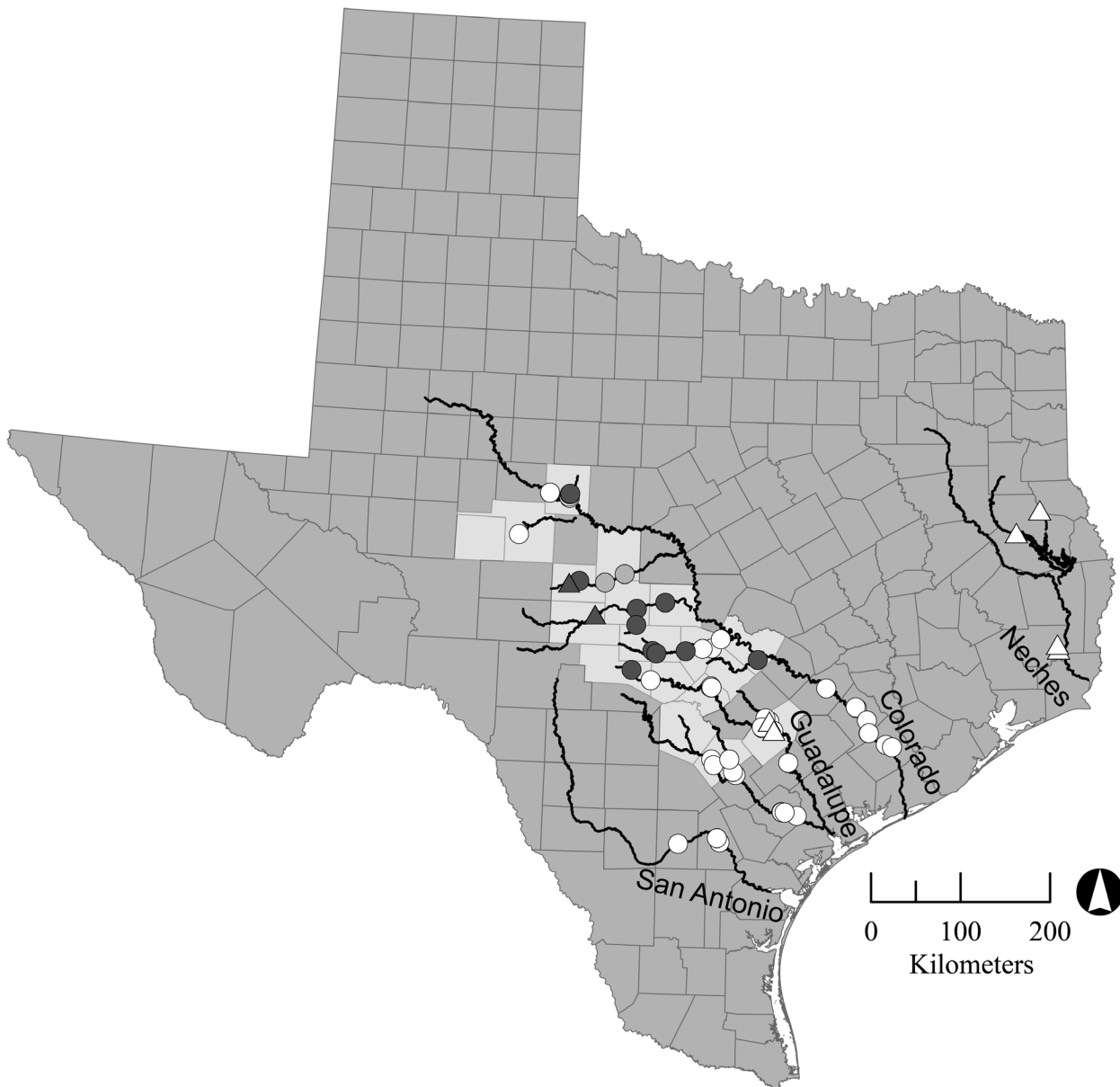


FIGURE 4. Historical and current distribution of *Lampsilis bracteata*. Greyscale codes: **Light grey squares**—historical geographic range by county. **Dark grey circles**—live specimens according to surveys over the past ten years. **Light grey circles**—recently dead specimens in the Colorado and Guadalupe River drainages (Survey 2004–2012; Howells 2005; Johnson & Groce 2011; Wilkins *et al.* 2011; Sowards *et al.* 2013; Morton 2016). **White circles**—locations that were surveyed in 2004–2012 but where *L. bracteata* was not present (Burlakova & Karatayev 2012). **Triangles**—locations where tissue samples for DNA analysis were obtained for *L. bracteata* (**grey**) and *L. hydiana* (**white**).

Map created in ArcGIS v10.1. Data source: Texas Hydrology, TCEQ; Texas counties, US Census Bureau. Projected Coordinates System: WGS_1984_Web_Mercator_Auxiliary_Sphere.

Discussion

Molecular identification of *Lampsilis bracteata*. Genetic barcoding of freshwater mussels supports the distinctiveness of problematic taxa in some cases (Kat 1983; Mulvey *et al.* 1997; Burlakova *et al.* 2012; Roe 2013; Zanatta & Harris 2013; Inoue *et al.* 2014; Lane *et al.* 2016) but not in others (Mulvey *et al.* 1997; Buhay *et al.* 2002; Burlakova *et al.* 2012; Campbell & Lydeard 2012; Inoue *et al.* 2013).

Furthermore, based on molecular analyses, genera have been placed in different subfamilies (Wu *et al.* 2018),

species in different genera (Pfeiffer *et al.* 2016; Perkins *et al.* 2017), cryptic species (Roe & Lydeard 1998; Burlakova *et al.* 2018), lineages within species have been identified (Serb 2006; Zanatta & Murphy 2008; Grobler *et al.* 2011; Doucet-Beaupré *et al.* 2012; Chong *et al.* 2016) and species that were thought to be locally extinct have been rediscovered (Campbell *et al.* 2008). In this study, genetic barcoding supports the distinction between *L. bracteata* and *L. hydiana* but challenges the distinction between *L. satura* and *L. cardium*.

The uncertainty whether *L. bracteata* and *L. hydiana* are the same species arose from the presence of less massive and more laterally compressed *L. hydiana* in the Guadalupe-San Antonio and Nueces-Frio system (Howells 2010). Our findings support that *L. hydiana* from these systems is not *L. bracteata* and their geographic ranges do not overlap. *Lampsilis bracteata* is only known from the upper Guadalupe River in Comal, Kendall, and Kerr counties while *L. hydiana* is known from the lower Guadalupe in Victoria County (Strecker 1931); *L. bracteata* is not present in the Nueces-Frio system.

The genetic diversity of *L. bracteata* appears to be low but sample size is limited to five individuals restricted to two streams. Howells *et al.* (2010) pointed out that there may be two ecophenotypes of *L. bracteata*; some individuals from some rivers seem to be more elongated than from others; these were previously designated as subspecies “*elongatus*”. However, this classification is no longer recognized. Further testing is needed to describe morphological variation using landmark morphometric as well as genetic diversity and population genetic structure of this species across its distribution.

Phylogenetic relationships. As reported here, *Lampsilis satura* from Texas nest within *L. cardium* specimens using ND1. But, while *L. satura* is found in the upper and lower Sabine, Angelina and Neches rivers and in Village Creek (Howells *et al.* 1996; Bordelon 2003; Ford & Nicholson 2006; Howells 2006; Karatayev & Burlakova 2007; Ford *et al.* 2009; Randklev *et al.* 2009; Ford *et al.* 2014; Ford *et al.* 2016), *Lampsilis cardium* has been reported from the Red River in northeast Texas (Howells *et al.* 1996). The co-occurrence of *L. satura* in the Red River drainage is uncertain (Randklev *et al.* 2013) as the *satura*-like specimens could represent an ecophenotype that has been evolving in isolation from *L. cardium*. This assumption is speculative, however, due to the limited genetic data presently available. Hence, a complete systematic study of *L. satura* as species is needed, especially because it is currently listed as threatened in Texas.

As observed here as well as in previous studies (Williams *et al.* 2017), the placement of eastern North American *Actinonaias* Crosse & Fischer, 1894, with *A. ligamentina* and *A. pectorosa* (Conrad, 1834) and their phylogenetic relationship with other *Actinonaias* species from Mexico (type locality) need to be revised. *Actinonaias ligamentina* was previously placed in *Lampsilis* (Baker 1898; Simpson 1900; Frierson 1927; Haas 1969) but Williams *et al.* (2017) suggested that *A. ligamentina* and *A. pectorosa* should be placed in two different genera. Based on shell morphology, Graf & Cummings (2019) and Watters *et al.* (2009) suggested that eastern North American *Actinonaias* should be placed in *Ortmanniana* Frierson, 1927.

Lastly, *Lampsilis* is not monophyletic (Campbell *et al.* 2005; Zanatta & Murphy 2006; Kuehnl 2009). This genus belongs to the tribe Lampsilini which is monophyletic (Campbell *et al.* 2005; Lopes-Lima *et al.* 2017) but the relationships among genera contained within this tribe such as *Villosa*, *Ligumia* and *Obovaria* (Graf & O’Foighil 2000; Lydeard *et al.* 2000; Roe *et al.* 2001; Campbell *et al.* 2005; Zanatta & Murphy 2006; Kuehnl 2009) are problematic and need thorough investigation. Furthermore, the present study indicates that *Lampsilis* is subdivided in at least two clusters which suggests that the genus may need to be split.

Distribution data and survival prospects. *Lampsilis bracteata* historical range has always been limited to the Texas Hill Country and east-central Edwards Plateau region of central Texas (Howells *et al.* 1996), spanning eighteen rivers from the upper Colorado, Guadalupe and San Antonio drainages (Strecker 1931; Howells *et al.* 1996; Howells 2010). However, during the last decades its range has been reduced putting this species at high risk of extinction.

Surveys conducted over the past decades reveal that *L. bracteata* is mostly found in the Colorado River drainage and in some localities in the San Saba and Llano rivers. Living and dead *L. bracteata* were found in greater densities than reported previously. These results do not necessarily indicate that *L. bracteata* is recovering but probably reflect the greater effort put into the more recent surveys.

Lampsilis bracteata was known to occur in the upper Guadalupe River and tributaries but it has not been observed in Kendall, Comal and northern Gonzales counties in over three decades (Strecker 1931; Horne & McIntosh 1979; Howells *et al.* 1996). Other surveys conducted by the US Fish and Wildlife Service in 2012 and 2013 found a few live and recently dead *L. bracteata* within the reservoirs in Kerr County, which makes it the first official re-

port of this species occurrence in lentic water systems (USFWS 2016). This species was thought to be restricted to moderate-size streams and small rivers in flowing waters (Howells *et al.* 1996; Howells 2010), and previous reports on *L. bracteata* presence in ponds or reservoirs were thought to be *L. hydiana* which is commonly found in lentic systems.

The most likely threats to mussel species in Texas have been land and water management policies that have resulted in impoundments, sedimentation and dewatering, gravel mining and chemical contaminants (Neck 1982; Howells *et al.* 1997; Winemiller *et al.* 2010). Habitat alteration, mainly the construction of dams and reservoirs, has resulted in the loss of rare and endemic species and in the homogenization of unionid communities (Burlakova *et al.* 2011b). Drought and dewatering of waterbodies can have devastating negative effects potentially extirpating entire populations. For example, during the 2004–2012 survey, one location in the middle part of the San Saba River the river bed was completely dry, with a few pools of water left, and sixty-five very recently dead *L. bracteata* were found (Karatayev and Burlakova pers. observ.). Unfortunately, the preferred habitat for *L. bracteata* is streambeds characterized by very low water capacity and high water permeability. Despite the greater number of sites where live *L. bracteata* individuals have been recently found, considering the critical state of Central Texas rivers these populations are highly threatened and are expected to continue declining.

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