PERFORMANCE REPORT – FINAL

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PROJECT: STATE-WIDE ASSESSMENT OF UNIONID DIVERSITY IN TEXAS

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ABSTRACT

Field surveys of freshwater mussels (family Unionidae) were conducted in 2011-2012 at 126 sites in 11 rivers (Colorado, Frio, Guadalupe, Llano, Medina, Neches, Nueces, San Marcos, San Saba, Rio Grande, and Trinity), and in Brady Creek. The surveys concentrated on several rare Texas endemic species Texas pimpleback, Texas fatmucket, Golden orb, smooth pimpleback, Texas fawnsfoot, and regional endemic Mexican fawnsfoot. We estimated the size of the Texas pimpleback, Texas fatmucket, Texas fawnsfoot, and smooth pimpleback populations in the San Saba River and found that some of the Texas endemic species (e.g., Texas fatmucket, Texas and Mexican fawnsfoot) are presently in dangerously low numbers. Considering the critical state of the Rio Grande River, and a number of Central Texas rivers suffering from drought and dewatering, appropriate conservation measures to save the remnant populations and preserve their habitat should be designed and carried out as soon as possible. We located sites on the Neches and Trinity rivers that are among the richest in the state in diversity and abundance of unionid bivalves, and additional sites for Texas endemic golden orb, smooth pimpleback and Texas pimpleback in the San Saba, Nueces, San Marcos and Guadalupe rivers. We recommend these sites with abundant and diverse unionid assemblages for future monitoring and conservation. In addition, we analyzed historic and current distributional data for Texas pigtoe (*Fusconaia askewi*), Triangle pigtoe (*F. lananensis*), and Louisiana pigtoe (*Pleurobema riddellii*) collected during our 2003-2011 state-wide surveys and tested the genetic affinities of *Fusconaia* and similar species. Our study suggested that Triangle pigtoe is not a valid species, and it is likely that there is only one *Fusconaia* species (Texas pigtoe) currently present in Texas, thus simplifying conservation efforts. We found that the distribution range of both Texas pigtoe and Louisiana pigtoe has been reduced in the last 80 years. The present survey provided data required for successful management and conservation of freshwater molluscs (family Unionidae) in Texas.

PERFORMANCE REPORT

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Grant Title: Wildlife Research

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Project No. and Title: Survey of threatened freshwater mussels (Bivalvia: Unionidae) in Texas

Report Period: March 4, 2011 through August 31, 2012

I. Objectives:

1) Survey sites to clarify the status of endemic Texas fatmucket, Texas pimpleback, and Mexican fawnsfoot. Locate existing populations in need of protection, assess their current status, determine species' habitat requirements, and establish sites for future monitoring and conservation.
2) Survey historical sites to clarify if the critically endangered false spike still exists in Texas.

II. Background:

Texas Comprehensive Wildlife Conservation Strategy for 2005-2010 highlights the importance of monitoring of aquatic non-game species, and especially the species that are of immediate interest. Freshwater mussels (Unionidae), one of the most imperiled group of animals in North America, are the species of immediate interest. Due to habitat destruction, habitat alteration and pollution, over 70% of unionid species in North America are threatened, endangered or of special concern (Williams et al., 1993). Our long-term state-wide study of Texas mussels revealed that 65% of all species are rare, including all state and regional endemics, and most endemic species are very rare (Burlakova et al., 2011a). Fifteen rare freshwater mussel species were recently added to the state's list of threatened species (Texas Register 35, 2010), and 11 of those are currently under consideration for federal listing by the U. S. Fish and Wildlife Service (74 FR 66261; 74 FR 66866). In order to target TWAP priorities, we defined the current conservation status of four extremely rare and threatened freshwater mussel species.

Survey of Threatened Freshwater Mussels. Our state-wide surveys conducted in 2004-2009, funded by SWG, stressed the lack of status data for several rare species (Burlakova et al., 2011a). Thus, the status of False spike (*Quincuncina mitchelli*) is unclear, as this species is either extinct or on the edge of extinction. Our surveys will add to the knowledge of presence of false spike, thus allowing limited resources to be directed toward existing species in critical need of protection (Burlakova et al., 2011a). Only a few live individuals

of Texas fatmucket (*Lampsilis bracteata*), and Mexican fawnsfoot (*Truncilla cognata*) have been found over the last few decades. Based on the fact that during our study in 2004-2009 not a single significant population was discovered for any of these species that could be protected, we recommended to change their current status to critically endangered, and suggested that extensive surveys are of the highest priority for this group (Burlakova et al. 2011a). Another endemic Texas pimpleback (*Quadrula petrina*), according to our surveys, has most likely only one remaining population left in the Concho River. This apparently not reproducing population is currently under direct threat due to restrained water release from several upstream reservoirs, recent drought, and urban and agriculture run-off (Burlakova et al., 2011a). More efforts should be directed toward a detailed survey of the historical habitat to find existing populations of these extremely rare endemic species for further protection, or declare them extirpated (e.g., false spike). All these freshwater mussel species are SGCN and listed as high priority in the 2005 TWAP. Our surveys of high priority SGCN helped to clarify their current distribution and define habitat requirements for the species that lack such information critical for their conservation, filled data gaps related to species status, provided information essential for the development of their recovery and management plans, and suggested sites to monitor these populations in the future.

III. Procedures:

Survey Methods (Objectives 1-2). We used standard freshwater mussel survey techniques long utilized by TPWD and employed during our SWG-funded mussel surveys in 2004-2009. Methods included random mussel collection by diving, snorkeling and wading, as well as area and time searches, that have been proved to detect effectively mussel diversity and presence of rare species in variety of Texas habitats (Burlakova et al., 2011a). Number and size-frequency distribution of mussels were recorded for each species; location (GPS coordinates), and habitat information (depth, dominant substrate, type of substrate where live mussels were found) were recorded for each sampling site. Buffalo State College (BSC) staff identified unionids collected in the field, and returned mussels back to the substrate from which they were collected. Dead shells were collected as voucher specimens and deposited into the BSC Great Lakes Center' Invertebrate Collection along with some tissue specimens preserved for future molecular identification. Mussel assemblage and habitat information are currently being entered into Excel spreadsheets.

To estimate *population densities* we used quadrats and strip transect sampling with random starts (Serber, 1982; Smith et al., 2003; Smith, 2006). This method was used to estimate the densities and the size of populations of endemic Texas pimpleback, smooth pimpleback, and Texas fawnsfoot in the lower San Saba River. The river was surveyed at five locations: at CR 208, north-east of San Saba; at San Saba River Golf Course; at San Saba Road and China Creek Rd (CR 200); at CR 126, and at CR 340. Each location was surveyed using consecutive 50-m long non-overlapping strata, and from 8 to 17 strata were sampled in each location. In each stratum we sampled from 2 to 3 transects that run from one shore to another (perpendicular to the shores) to ensure unbiased sampling and considering that mussels beds often located in riffles across the whole breadth of the river. The location of the first transect in each stratum was chosen randomly, and the other transect(s) run 2 m apart from the first one (systematic strip transect sampling with random

start, Serber, 1982). To ensure full mussel recovery, we searched substrate (up to 10-15 cm deep whenever possible) in quadrats along sampling transects. Depending on the width of the river, from 5 to 20 quadrats were used along each of the sampled transects. A total of 86 transects were searched at 42 strata. We recorded the number of each species in each quadrat. That data was used to calculate the population size at the sampled locations using formulae from Serber (1982). To estimate the total population size of each endemic species, we used the total area of the lower San Saba river where there was enough water to support mussel populations (from the confluence of Brady Creek to the mouth of the river, total length 50 km, and the average width of the river calculated using data from our sampling sites, 18 m). Then we used the calculated population size of each species in surveyed area and the proportion of the surveyed area from the total area of the lower San Saba River that supported the populations.

Survey Locations (Objectives 1-2). Surveys of false spike concentrated on tributaries of Colorado River (San Saba River), and lower Guadalupe River, on sites for which we have historical records and other areas within the known historical range. Presence of Texas fatmucket and Texas pimpleback were examined at historical and other locations not surveyed recently including sites in Llano, San Saba, San Marcos, Frio, Medina, Nueces, and Guadalupe rivers. Additionally, we surveyed several sites on Neches and Trinity rivers for rare East Texas unionid species. Surveys of Mexican fawnsfoot took place in the Rio Grande River.

IV. Results:

Field surveys of freshwater mussels (family Unionidae) were conducted at 126 sites in 11 rivers (Colorado, Frio, Guadalupe, Llano, Medina, Neches, Nueces, San Marcos, San Saba, Rio Grande, and Trinity), and in Brady Creek. Assessment of populations of rare and endemic species was performed at 52 sites in the San Saba, and Rio Grande rivers where detailed quantitative studies were conducted.

Mexican fawnsfoot *(Truncilla cognata)*

This species is endemic to the Rio Grande drainage that was described from the Devils River, Texas, and Rio Salado, Nuevo Leon, Mexico (Lea, 1857; Johnson, 1999). This species is considered endangered by the American Fisheries Society, and has been recently added to the state's list of threatened species (Texas Register 35, 2010). *Truncilla cognata* is currently under consideration for federal listing by the U.S. Fish and Wildlife Service (USFWS) (Federal Register 74, 2009). In the U.S., *T. cognata* was reported only from few sites in Texas with no living or dead specimens collected since 1972 (Howells *et al.*, 1997; Howells, 2001). In total, only 19 live Mexican fawnsfoot were found from 2001 to 2011 in the Rio Grande River in Laredo, Webb County (Karatayev et al., 2012).

In April 2012 we surveyed the Rio Grande River at 33 sites in Webb County, over 70 river kilometers above and app. 50 river km below La Bota (Laredo). The survey was done using an airboat (owner Mr. S. Barkley), the only type of motorboats capable to efficiently navigate the river during low water levels (Photo 1).

Photo 1. Airboat survey of freshwater mussels in the Rio Grande River. Lower picture, left to right: S. Barclay, T. Vaughn, T. Miller, D. Barclay, and A. Karatayev

Our collaborator Tom Miller (Laredo Community College) assisted us in sampling, and obtained permission to sample on private land, and Mr. Don Barkley and Dr. Thomas Vaughn (Texas International A&M University in Laredo) also participated in the survey. This survey was designed to estimate the population of Texas hornshell (*Popenaias popeii*), however we specifically checked potential Mexican fawnsfoot habitat, and recorded all other species found. We used time searches (at all sites), and area and quadrat searches at 10 sites.

We found live Mexican fawnsfoot at 2 sites: in northern Laredo, above 2.5 miles from Phelps Rd. (6 live molluscs 19.2 – 37 mm shell length in 40 quadrats and 3 mh time search, Photo 2), and near old town of Darwin (1 live, 38 mm). We found mussels in a mixture of sand and gravel, or in soft sediments (e.g., sand, sand and clay) in shallow protected nearshore areas often adjacent to riffles. Sometimes molluscs were found burrow in gravel-cobble substrates. Because of the small size, it is difficult to distinguish Mexican fawnsfoot from gravel, adding to the hardship of detecting this cryptic species. Although, our 2012 survey confirmed the presence of Mexican fawnsfoot in Rio Grande, in Laredo and north of the city, the estimations of population size were not possible due to the low density.

Photo 2. Mexican fawnsfoot (*Truncilla cognata*) (left) and southern mapleleaf (right) found in the Rio Grande River in northern Laredo.

All these Mexican fawnsfoot in 2001-2012 were found in and above Laredo. No live mussels of any species were found in the 50 km stretch of river below Laredo and Nuevo Laredo sewage treatment plants inspite of abundance of suitable substrates. The reasons for the lack of mussels could be the contamination of water or sediments that prevent mollusc reintroduction from upstream sites. Special studies are needed to understand why native

molluscs are no longer found below Laredo (e.g. testing of water and sediment quality, degree of organic enrichment, presence of host fish) and to determine the feasibility of reintroduction.

Among various types of human activities on the Rio Grande drainage most destructive for unionid species, including Mexican fawnsfoot, are impoundments, habitat degradation, salinization, pollution, and water over extraction (Karatayev et al., 2012, Appendix 1). Our analysis of changes in species distribution over the last century has shown that it is likely that the Pecos River population of Mexican fawnsfoot is already extirpated (due to elevated salinity and the construction of Amistad Reservoir that flooded the lover Pecos), and the 27 live specimens that were found in the Rio Grande near Laredo in 2001 – 2012 represent the only known population of this species left in the U.S. (Karatayev et al., 2012) (Figure 1). Creation of Falcon Reservoir most likely decimated the lotic habitat of the bivalves in the lower Rio Grande (Neck and Metcalf, 1988). Any future projects to construct a new dam, or to modify existing low-head dams and associated water diversion structures in the Rio Grande River in Laredo could potentially impact Mexican fawnsfoot. We would advise the U.S. Fish and Wildlife Service, Texas Parks and Wildlife Department, Texas Commission on Environmental Quality, and local authorities to be alerted to possible threats that could extirpate the only known habitat of Mexican fawnsfoot.

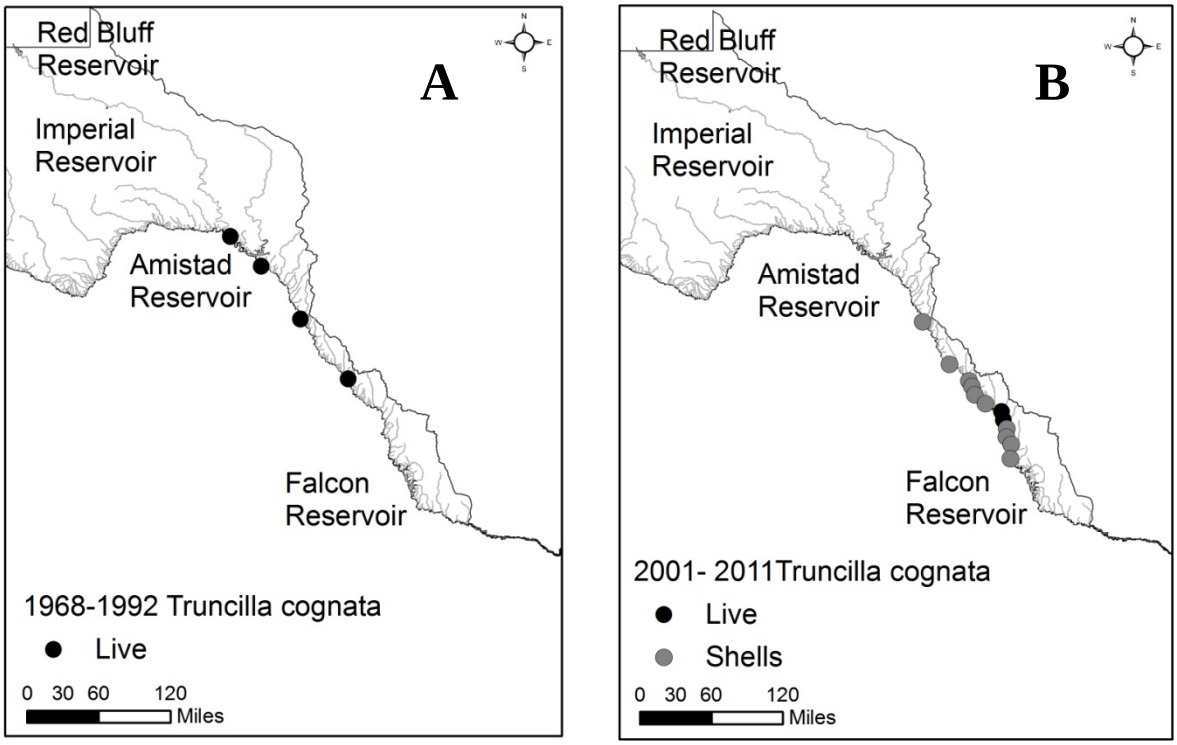

Figure 1. Map of the Rio Grande river basin in Texas with sites where live and/or dead shells of *Truncilla cognata* were found in 1968-1992 (A), and from 2001 to 2011 (B) (Karatayev et al., 2012).

The most important measures to preserve this remaining population in the Rio Grande between Eagle Pass and Laredo would be to ensure a constant stream flow from reservoirs upstream, to prevent any damming of the river, as well as to prevent any other activity that can increase streambed sedimentation, pollution and suspended sediment and nutrient loading in the Rio Grande.

Texas pimpleback *Quadrula petrina*

This Central Texas endemic was found only at 4 locations since 1992 (Howells, 1995). In our 2005 survey we found a population of live Texas pimpleback in the Concho River (Concho Co.).

Status of Texas pimpleback in the San Saba River

In March 2011 we surveyed 2 sites on the lower San Saba River (near San Saba, San Saba County), and found 39 Texas pimpleback within quite abundant and diverse unionid assemblages.

In July 2011, we sampled 38 additional sites in the San Saba River (in San Saba and Menard counties). Of these, 42 sites at 5 locations were sampled using quadrats and strip transect sampling with random start at every 50 m of the river stretch (Photo 3). We collected a total of 135 live mussels during this survey. The average density of Texas pimpleback at survey sites was 0.29 mussel m^{-2} (maximum 3.5 m^{-2}), and it was found at 29% of the total 42 strata we surveyed using strip-transect method. The population size of Texas pimpleback in these sampled locations was 9,092 ± 1,817 mussels (mean ± 95% confidence interval here and elsewhere unless noted). Using our calculations, we estimated that the total population size of Texas pimpleback in the San Saba River may be 220,731 mussels (from 176,607 to 264,856 mussels, 95% confidence interval). Above the confluence with Bardy Creek, one of the major tributaries of the river, there was little flow, and large parts of the river dried out (Photo 5, 7). Eleven more very recently dead Texas pimpleback were found at CR 1311 crossing where a large part of the river was completely dry during our survey (Photo 5, 7).

Overall, we found quite abundant and diverse unionid assemblages in lower San Saba River (Photo 4). It was very encouraging to find a second population of Texas pimpleback in Central Texas, in addition to the known population in the Concho River. In contrast to the Concho River, this population consisted of several age-classes, including small mussels suggesting the recruitment of juvenile mussels and presence a host fish population. This may be due to the fact that the San Saba River remains relatively undeveloped and natural, since little residential development has appeared and no impoundments other than low water crossings exist (TPWD, 1974). Rich mussel assemblages found in the San Saba River are unique for Central Texas. Several other waterbodies in the area which historically had similar fauna (e.g., Llano River, Elm Creek, Concho River) are known to have lost most of their unionid diversity in last decades. We consider Central Texas as the priority region for national conservation where many environmental and anthropogenic factors contributed to the degradation of unionid fauna

including the water over-extraction, lack of forests, overgrazing, overpopulation and urbanization (Burlakova et al., 2011a, b). Most importantly, Central Texas suffers from acute droughts, and the recent droughts of 2007-2009 and 2011 were the most severe since the all-time record drought of the 1950s (Lower Colorado River Authority, 2010). Due to severe drought in 2011, many gravel bars that we surveyed in March and July were barely covered with water (Photo 3, 5), large areas of the river were completely dry (Photo 4), and the situation got even worse at the end of summer.

Photo 3. Texas pimpleback (on left) and smooth pimpleback (on right) found in one quadrat in the San Saba River gravel bar.

As the drought continued in the fall of 2011, many of these populations may no longer exist. The next 2012 year was dry as well. Nevertheless, water withdrawal for agriculture etc. continues leaving the small gravel rapids rich in unionid diversity (as in Photo 4) dry, with the only water left in the big pools (that are typically low in mussel diversity and abundance) lacking sufficient water flow for the second year in a row (Photo 5). Changes in natural water flow regime, including excessive water withdrawal, water diversion, and impoundment operations result in altered hydrology, habitat destruction, and fragmentation, and are among the most significant threats to mollusc fauna (Richter et al., 1997). The high diversity of freshwater molluscs we found in the San Saba River is most likely due to the fact that it remains relatively undeveloped and largely maintains natural flow regime, since little residential development has appeared and no impoundments other

than low water crossings exist (TPWD, 1974). However, it is critical to protect the San Saba River against significant water diversions and withdrawals, especially considering the predictions of another decade of drought conditions. The maintenance of a natural flow regime within a river or stream is one of the most important measures to protect aquatic diversity (Darwall and Vie, 2005).

Photo 4. Molluscs found in one transect 0.5 x 19 m (9.5 m^2 surface area) in the San Saba River at the San Saba Golf Course, San Saba, July 2, 2012. Total density: 5.5 mussel m^{-2}, density of Texas pimpleback (group of mussels on right): 1.4 mussel m^{-2}.

Photo 5. Low water level in San Saba River near San Saba (left, San Saba County) and dry river bed at CR 1311 crossing (right, Menard County).

Thus, it is critical to have a mechanism for regulating pumping and impoundment activities in order to ensure an unimpaired flow in the river. To preserve the river's unique biodiversity, we would strongly suggest controlling the water usage in the river. For example, the Texas Commission on Environmental Quality' Webmaster Program that works successfully in other areas of Texas, including the nearby Concho River (Concho River Watermaster) may be used to efficiently protect the natural flow regime of the San Saba River.

Texas pimpleback in the Sab Marcos River

Thirty seven live Texas pimpleback were found in the San Marcos River, west and south of Luling, in Caldwell/Gonzales counties, during our kayak survey of a 13 km stretch of the river below US-90 crossing (Photo 6). Dr. Y. Zhang. Mr. T. Noble (Texas State University, San Marcos) and Vadim Karatayev (University at Buffalo) participated in this survey and helped with survey logistics. One live Texas pimpleback was found in our survey of Guadalupe River near Cuero, at FM 766 crossing in July 2011.

Photo 6. Golden orb (upper) and Texas pimpleback (lower) collected in the San Marcos River near Luling, Caldwell/Gonzales counties, on July 7, 2011.

Smooth pimpleback *Quadrula houstonensis*

This Texas endemic is native to the Brazos and Colorado Rivers basins of Central Texas. Singley (1893) reports presence of this species in the Brazos and Little Brazos Rivers, Elm Creek, Little River, and Colorado River. Strecker (1931) found smooth pimpleback in Chambers Creek (Navarro Co.), the Elm Fork of Trinity River, the Leon River, and Richland Creek (Navarro Co.), the Llano River, and Onion and West Yegua Creeks. From 1992 to 2001 less than 30 live mussels were found in Brazos and Colorado rivers (Howells, 1994, 1996, 2000, 2002). Therefore, NatureServe Explorer concluded that "although found over the past decade at several sites in at least two, perhaps three, drainage basins, no large

or stable populations are known and the effects of declines from recent habitat loss continues to affect the species."

We found smooth pimpleback at 40% of locations surveyed in 2009 in the lower Colorado River (at densities from 0.31 ± 1.1 to 1.33 ± 2.3 m^{-2}), and at 32% of total sites in the Brazos River drainage (including the Brazos, Navasota, Little Brazos, Little rivers, and Yegua Creek) surveyed 2006-2007. Mean density over all surveyed sites was 1.4 ± 0.5 m^{-2} (range: 0.03 - 4.8 m^{-2}, 8 sites, 321 quadrats total).

In March 2011 we found 29 live smooth pimpleback much further west, in the San Saba River (San Saba Co., see above). Over 70% of the mussels found were < 50 mm in length, indicating a healthy reproducing population.

In July 2011 we found 199 smooth pimpleback during our survey of the San Saba River. The average density of smooth pimpleback at survey sites was 0.31 mussel m^{-2} (maximum 3.4 m^{-2}), and it was found at 33% of the total 42 strata we surveyed using strip-transect method. The population size of smooth pimpleback in these sampled locations was 9,594 ± 2,051 mussels (mean ± 95% confidence interval here and elsewhere unless noted). Using our calculations, we estimated that the total population size of smooth pimpleback in the San Saba River may be 232,920 mussels (from 183,117 to 282,725 mussels, 95% confidence interval).

False spike *Quincuncina mitchelli*

Historically, this species was known from central and southern Texas to Nuevo Leon, Mexico (Simpson, 1914), which included the Brazos, Colorado, and Guadalupe River systems, as well as the Rio Grande River system in New Mexico, Texas, and Mexico (Johnson, 1999; Metcalf, 1982). However, Metcalf (1982) suggested that the Rio Grande forms should be assigned to *taumilapana* and the Central Texas populations to *mitchelli* based on their conchological differences. Other than locality records, little information is available for this species. It was listed by (Stansbery, 1971) as "rare and endangered". The last recorded live specimens were collected in Texas before the 1950s (Wurtz, 1950), however there are data that the last live specimens were seen later, in 1970s: Joseph Bergmann has found ca. 20 live individuals in Llano River in Castell (Llano Co.). In 2009 we found one subfossil shell in the bank of Guadalupe River in Comfort (Kendall Co.), 6 valves (very long-dead to subfossil), as well as one relatively recently dead valve (external colors of the shell were not faded), in the San Marcos River in Palmetto State Park (Gonzales Co) (Burlakova et al., 2011a).

In March 2011 we sampled extensively the same location where live False spike were seen last time in Texas in 1970s by J. Bergmann (in Castell, Llano Co.). Joseph Bergmann participated in this survey. During 15.5 man hours of extensive surveys at this and two downstream sites, we fail to find even dead shells of the species.

In July 2011 we sampled many additional sites on San Saba, San Marcos, and Guadalupe River (including the site where the mussel was recorded alive in 1949, in the Guadalupe River above Sequin, Guadalupe Co., Wurtz, 1950). We found two valves of false spike in the San Saba River. No live false spike was found at the sites.

During the summer of 2011, C. Randklev, E. Tsakiris, M. Johnson (Texas A&M Institute of Renewable Natural Resources), and Joseph Skorupski (University of North Texas, Denton) collected the first very recently dead individual (with tissue present within the shell) of *Q. mitchelli* to be found in the last 30 years at a riffle 12 km east of San Saba, San Saba Co. (Randklev et al., 2012). This discovery may suggest that false spike is not extinct, and a small population of false spike may exist in this portion of the San Saba River. Further surveys are needed to locate live specimens in the San Saba River, and to study this species before it actually becomes extinct.

Texas Fatmucket *Lampsilis bracteata*

This species is endemic to the San Antonio, Guadalupe and Colorado River drainages of Central Texas (Howells et al., 1997). Texas fatmucket was listed as a species of special concern by Athearn (1970), however Neck (1984), referring to Bergmann, Boone, Horne, and Murray's data, noted that large populations of this species occur at numerous localities. Many of these populations seemed devastated by significant flooding and/or other events in Central Texas in late 1970-s (J. Bergmann, personal communication). Live specimens were found at only 6 locations since 1992 (Howells et al., 2003). During our 2004-2009 surveys we found only 13 live and 3 very recently dead specimens of Texas fatmucket at a total of 6 locations: 6 live mussels in the Guadalupe River (Kerr Co), 2 in Live Oak Creek (Gillespie Co), 1 in the San Saba River (Menard Co), 1 live and 1 very recently dead in Elm Creek (Runnels Co), and 3 live and 2 recently dead mussels in the Llano River (in Kimble and Mason Counties) (Burlakova et al., 2011a). In March 2011 we found 8 live Texas fatmucket during 15.5 man hours of surveys of Llano River at and below Castell, Llano Co. Almost all molluscs were found in soft sediments along the shores, in macrophytes; 1 specimen was found in tree roots at the shore, and two more in sand and gravel in the middle of the river. Interestingly, Texas fatmucket was the only live unionid species we found in Llano River in 2009-2011. Unfortunately, due to the low density of molluscs, we were not able to carry out any quantitative surveys in the Llano River.

In July 2011 we found 12 live Texas fatmucket in the upper San Saba River, west of Menard (Beyer (or Bois d'Arc) low water crossing) upstream and downstream from the crossing (Photo 6). Molluscs were found in macrophytes, but most of them – in the bedrock's ledges in the pool downstream from the crossing (Photo 7). Considering the total area of the 3 searched pools (above and below the crossing) where we found live mussels, the density of Texas fatmucket at this location was very low: app. 0.00045 mussels/m^2.

In the middle part of the San Saba River, at CR 1311 crossing (north-east of Hext, Menard Co.) the river bed was completely dry, with a few pools of water left (Photo 8, and 5 on right). This part of the river used to be populated with Texas fatmucket: we found 65 very recently dead mussels on this site. The density of Texas fatmucket at this site before it went dry was 0.005 mussels m^{-2} (total area searched was 12,400 m^2). In addition, we found there shells of Texas pimpleback (total 11, density 0.0009 m^{-2}), 1 pistolgrip, 2 Tampico pearlymussel, and 1 fragile papershell.

Using Texas fatmacket densities in the upper and middle San Saba River (where the molluscs were found) and area of these parts of the river, we calculated that total size of the

population may be from 8,000 to 10,000 mussels. However, a large part of the population may have not survived the 2011-2012 drought. More studies are needed to estimate the former and current population size to quantify the decline of the species over the last century.

Photo 7. Texas Fatmucket *Lampsilis bracteata* (left) found in San Saba River west of Menard at Bois d'Arc crossing (right and below).

Some of the differences in unionid distribution could potentially be explained by the difference in geology and soils. Edwards Plateau soils formed on mesas and plateaus of erosion-resistant limestone. Llano, Mason and part of Menard counties where Llano and part of San Saba rivers were dry in many locations, are mainly represented by Keese-Ligon-Rock granite outcrop of very low water capacity and rapid water permeability.

The San Saba County soils are Reagan-Conger, which is black and gray sandy loam, and alluvial soils. We found Texas fatmucket inhabiting exclusively pools in barren or nearly barren gneiss or granite bedrock that are prone to dryout. Texas pimpleback and smooth pimpleback were found in very different substrate, at gravel bars on riffles mainly in San Saba County, on loams and alluvial soils with higher water capacity.

Photo 8. Dead Texas fatmucket at the dry San Saba River bed at CR 1311 crossing (Menard County).

Overall, we found that Texas fatmucket still have small populations left in the San Saba and Llano rivers. Unfortunately, the specific habitat for the species are bedrocks characterized by very low water capacity and rapid water permeability that quickly go dry during low water and drought events. That is why this species is especially prone to the changes in water regime and water over-extraction.

The National Climatic Data Center reported that 55% of the continental United States was in moderate to extreme drought at the end of June 2012, the largest percentage since December 1956, when 58% was covered by drought, and comparable to some of the Dust Bowl events of the 1930s. According to the National Weather Service records, more than 77% of Texas is experiencing moderate to extreme drought. The current outbreaks, occurring simultaneously across western North America, are the largest and most severe in recorded history (Bentz, 2008). Current climate model simulations suggest that the American southwest could experience a 60-year stretch of heat and drought unseen since the 12th century and that the region is likely to become drier and experience more frequent droughts, with changes accelerating toward the end of the century (Woodhouse et al., 2010 et al). The principal mechanism for these changes is accelerating warming with associated dry periods, changing storm dynamics off the oceans, increased soilmoisture deficits in spring and summer, and reduced spring snowpack and accelerated spring snow melt. From the perspective of this drought conditions and water supply, unconstrained and unmanaged growth in southwestern cities and suburbs can no longer be accommodated, and the irrigation of certain crops in certain places no longer makes sense, even with economic subsidy (MacDonald, 2010). For regions like the southwestern United States, where water resources are especially scarce and where climatic changes may cause significant changes in water availability, quality, and demand, new approaches are needed to simultaneously meet human and environmental demands for water (Gleick, 2010).

Texas fawnsfoot *Truncilla macrodon*

Texas fawnsfoot is a very rare Central Texas endemic. Singley (1893) recorded it from the Brazos and Colorado Rivers, Strecker (1931) gave additional records in Aquilla Creek, the Bosque and North Bosque Rivers (McLennan Co.), and the Leon and Llano rivers. Less

than 300 specimens have been documented since this species was described by Lea in 1859, and only 15 living (moribund) and a number of recently dead shells have been found in recent decades (Randklev et al., 2010; R. Howells, personal communication). The Texas Parks and Wildlife Department sampled over 190 sites within the range of Texas fawnsfoot and found no evidence of it at nearly all locations (Howells et al., 1997). Ten live Texas fawnsfoot were collected in 2008 in the Brazos River at mean density 0.24 m^{-2} (Randklev et al., 2010). We found one live and few recently dead Texas fawnsfoot from all 27 locations surveyed in 2006-2007 in the Brazos River Drainage. However, in 2009 we found an abundant population of Texas fawnsfoot in the lower Colorado River (Colorado Co.), at an average density of 0.62 ± 0.13 m^{-2} (225 quadrats); the total population size was 2,794 mussels (± 1,379, 95% confidence interval). The average size of the mussels was 28.5±0.6 mm (*n* = 52), range: 21 – 38 mm. All mussels were found in a sandy shore, at low depths (0.1 – 1.0 m). Unfortunately, there is some evidence that this population may have not survived the drought in 2011 (Charrish Stevens, U.S. Fish and Wildlife, personal communication).

In our survey in March of 2011 we found one live Texas fawnsfoot in the San Saba River (San Saba Co.), at CR 208 (near San Saba). In July 2011 we found 6 more Texas fawnsfoot during our survey of the lower San Saba River (Photo 9). The average density of Texas fawnsfoot at survey sites was 0.03 mussel m^{-2} (maximum 0.5 m^{-2}), and it was found only at 12% of the total 42 strata we surveyed using strip-transect method. The population size of Texas fawnsfoot in these sampled locations was 782 ± 779 mussels (mean ± 95% confidence interval here and elsewhere unless noted). Using our calculations, we estimated that the total population size of Texas fawnsfoot in the San Saba River may be 18,995 mussels (± 18,900 mussels, 95% confidence interval).

Golden orb *Quadrula aurea*

Central Texas endemic golden orb is native to the Guadalupe, Colorado, San Antonio, and Nueces River systems. During our 2005 survey Golden orb was found alive at only 5 sites: two sites in the Guadalupe River upstream of Gonzales, lower San Marcos River, one small area in the Guadalupe River at Kerrville, and in Lake Corpus Christi. In 2008 we surveyed different locations in the San Marcos and lower San Antonio River, and we found golden orb to be very abundant at several sites. In 2006 in the Guadalupe River below Lake Wood dam we found 91 live golden orb at average density of 0.7±1.7 mussel m^{-2} (n = 55) within an abundant and diverse unionid assemblage (9 species, 391 molluscs found total).

We repeated sampling of the same site in 2008 and found total 33 live Golden orb at average density of 0.81 ± 2.35 m^{-2} (n = 154 quadrats, range: 0 – 20 m^{-2}). Fourteen live golden orb were found during our sampling of the San Marcos River in Palmetto State Park, and 56 molluscs at the crossing with CR 232 (Gonzales Co) (at average densities 5.1 ± 4.3 m^{-2}). The highest densities of golden orb were found in the lower San Antonio River, at Goliad, in Goliad State Park and downstream from the park, on private land of Dr. Brett Hensley Mueller (10.6 ± 10.2 golden orb m^{-2}, and 16.8 ± 2.5 m^{-2} respectively, total 285 live golden orb collected).

Photo 9. Texas fawnsfoot *Truncilla macrodon* found in the San Saba River.

On July 6, 2011 we sampled the Nueces River at 3 sites above Lake Corpus Christi (at HW 16, McMullen Co., no live mussels found, and at HW 59 and Airport Rd crossings, north-east of George West, Live Oak Co.). We found 8 live Golden orb, along with 6 other species (15 Tampico pearlymussel, 43 yellow sandshell, 7 giant floater, 5 Texas lilliput, 26 threeridge, 1 southern mapleleaf) at two last sites. The majority of mussels were found at the last site adjacent to a small public park (the least disturbed site) (Photo 10).

Photo 10. The Nueces River near George West, Live Oak Co.; Golden orb found in the river.

To survey populations of golden orb in the Guadalupe River, we sampled upper sites at Center Point (Kerr Co., 5.3 mh), near Comfort (Kendall Co.), and lower sites near Cuero (De Witt Co.). We found only one live mussel at each of the first two upper sites, but at very low densities (during 5.3 man hours of time search effort at the first site, and 2.5 mh at the second site). Mr. Thomas Miller, and Don, Regan, Jesse, and David Barclay participated in these surveys of the upper Guadalupe and Medina rivers. The density of golden orb was higher in the lower Guadalupe River, where we found total 24 mussels per

2.8 mh of time search at FM 766 crossing, and 5 more mussels during 2 mh of time search at SR 72 (both in De Witt Co.). Additional mussels found at the two last sites in the lower Guadalupe River near Cuero were threeridge (75), Tampico pearlymussel (5), yellow sandshell (5), washboard (2), and 1 Texas pimpleback. Vadim Karatayev and Trey Noble also participated in the survey of the Guadalupe River.

Twenty three golden orb were found during survey of a 13-km stretch of the San Marcos River near Luling (Caldwell/Gonzales counties, Photo 6). Additional mussels found during this survey were Texas pimpleback (37), threeridge (2), and shells of pistolgrip.

Three sites were sampled on Frio River in March and July 2011: at Choke Canyon Reservoir (FM 3445 crossing, at St. Miguel Creek), in Tilden (at SR 16) (McMullen Co.), and below the Canyon Choke Reservoir, in Three Rivers (SR 72, Tips Park, below the falls, Live Oak Co.) (Photo 11). Live mussels (3 golden orb, Photo 11, and 4 yellow sandshell) were found only at the last site. The river above the Canyon Choke Reservoir was dry at SR 16 crossing near Tilden both in March and July 2011. The water was stagnant and very polluted, brown-orange in color in the Frio River west of Fowlerton (at the end of Park Rd, La Salle Co.) (not sampled) (Photo 11).

Picture 11. Sampling the Frio River in Tips Park (Three Rivers Live Oak Co.) (upper left), golden orb found in the Frio River in Tips Park (upper right), dry Frio River at SR 16 near Tilden (McMullen Co.) (lower left), and the Frio River west of Fowlerton (La Salle Co.) (lower right).

No live mussels were found during 9.3 mh of time search at 3 sites sampled in the Medina River (at Old Pearsall Road, north of Von Ormy, at Pue Road (SR 1604) north of Macdona, Bexar Co. and at English Crossing Road, Bandera Co.).

The Colorado River was sampled at 2 sites in March 2011: at Bare Foot Camp, near Bend and Colorado Bend State Park, and at Colorado Bend State Park (Lampasas and San Saba counties), total 8 mh of search effort. We found 4 live pistolgrip, 2 fragile papershell and 2 threeridge at the first site only. Joseph Bergmann, Charles Randklev, Julie Groce, Matthew Johnson, Eric Tsakiris (Texas A&M Institute of Renewable Natural Resources), and Joe Skorupski (University of North Texas, Denton) also participated in this survey.

Texas pigtoe *Fusconaia askewi* and Louisiana pigtoe *Pleuroblema riddelli*

Our collaborators Don and Steve Barclay invited us to survey a site on the Neches River, and to make an airboat survey on the Trinity River in July 2011.

The Neches River was sampled at CR 354 (off US 79, toward Rocky Point), between Palestine and Jacksonville, in Anderson/Cherokee counties, on July 15, 2011. We sampled part of the river just below an old dyke, below the small impoundment. Twenty two live unionid species were found in the 3.9 man/hour time search at the site (Table 1). After the end of the time search we found a large bed of Texas pigtoe in the middle of the river channel. Don, Regan, Jesse, and David Barclays helped us in this sampling.

At the same site Don Barclay previously found live Texas heelsplitter (*Potamilus amphichaenus*) and creeper (*Strophitus undulatus*). Therefore, species richness at the site is 24 species. So far this site is one of the few richest in diversity and abundance of unionids in the Neches River and the whole state of Texas, and has to be protected from potential destruction (e.g., impoundments, etc.).

On July 16, 2011 we sampled the Trinity River at 9 sites downstream of SR7 (west of Crockett, east of Malvern, in Leon/Houston Co.) at historically low water level, using an airboat kindly provided by Steve Barclay.

In total, we found 353 live and recently dead mussels belonging to 14 species in the Trinity River (Table 2). The most extensive was the mussel bed at site 6 (31.25355^{o}N, 095.72358^{o}W), where hundreds of mussels, mostly western pimpleback, Texas pigtoe, and gulf mapleleaf were found. This site would be impossible to discover if not for the low water lever, as it is located in the middle of the river, where depth and high current velocity prevent successful sampling at normal water levels. There is some evidence that Texas fawnsfoot was reproducing in the river (Don Barclay personal observation), however we did not find live mussels, probably due to the dewatering of the former mussel bed located in shallow sandy areas.

Table 1. Unionid species found live during survey of Neches River at Rocky Point on July 15, 2011.

Species name	Common name	Number found in 3.9 mh time search
Amblema plicata	threeridge	11
Arcidens confragosus	rock pocketbook	1
Fusconaia askewi	Texas pigtoe	1
Lampsilis hydiana	Louisiana fatmucket	54
Lampsilis satura	sandbank pocketbook	3
Leptodea fragilis	fragile papershell	1
Megalonaias nervosa	washboard	5
Obliquaria reflexa	threehorn wartyback	239
Obovaria jacksoniana	southern hickorynut	7
Plectomerus dombeyanus	bankclimber	10
Pleurobema riddelli	Louisiana pigtoe	11
Potamilus purpuratus	bleufer	5
Pyganodon grandis	giant floater	3
Quadrula (Tritogonia) verrucosa	pistolgrip	45
Quadrula apiculata	southern mapleleaf	8
Quadrula mortoni	western pimpleback	120
Quadrula pustulosa?	pimpleback?	2
Toxolasma texasensis	Texas lilliput	1
Truncilla donaciformis	fawnsfoot	1
Truncilla truncata	deertoe	36
Villosa lienosa?	little spectaclecase?	3
Utterbackia imbecillis	paper pondshell	1
Total mussels		**568**
Total species		**22**

Table 2. Unionid species found live during survey of the Trinity River below SR 7 on July 16, 2011.

Species name	Common name	Total Live	Total Shells
Amblema plicata	threeridge	3	present
Lampsilis teres	yellow sandshell	4	present
Leptodea fragilis	fragile papershell	2	1
Megalonaias nervosa	washboard	21	present
Plectomerus dombeyanus	bankclimber	4	present
Potamilus purpuratus	bleufer	27	1
Quadrula houstonensis	smooth pimpleback	0	present
Quadrula mortoni	western pimpleback	124	present
Quadrula nobilis	gulf mapleleaf	58	present
Truncilla macrodon	Texas fawnsfoot	0	7
Potamilus amphichaenus	Texas heelsplitter	1	0
Obliquaria reflexa	threehorn wartyback	69	present
Fusconaia askewi	Texas pigtoe	30	3
Quadrula verrucosa	pistolgrip	3	1
Total live mussels		**346**	
Total live and recently dead mussels		**353**	
Total species found live		**12**	
Total species found live and dead		**14**	

Taxonomic identification of Texas pigtoe (*Fusconaia askewi*), Triangle pigtoe (*F. lananensis*), and Louisiana pigtoe (*Pleurobema riddellii*)

Taxonomic identification of endemic species based on shell morphology is challenging and complicates conservation efforts. We analyzed historic and current distributional data for three rare Texas species, Texas pigtoe (*Fusconaia askewi*), Triangle pigtoe (*F. lananensis*), and Louisiana pigtoe (*Pleurobema riddellii*) collected during our 2003-2011 state-wide surveys and tested the genetic affinities of *Fusconaia* and similar species collected from eastern Texas and western Louisiana using *cox1* and *nad1* sequences (Burlakova et al. 2012).

We found that *F. askewi* still inhabits four river basins in eastern and northeastern Texas and can be locally abundant, while *Pleurobema riddellii* was found only in one river basin.

Pleurobema riddellii was well-separated from *F. askewi* and grouped with the *P. sintoxia* clade.

The sequences for *F. lananensis* were very similar to those for *F. askewi*, with less than 1% difference, similar to the variation between *F. askewi* alleles. However, the sequences for *F. askewi* from the Sabine and Neches drainages differed from almost all more eastern species by over 3%. Our study suggested that *F. lananensis* is not a valid species, and it is likely that only one *Fusconaia* species (*F. askewi* or its probable senior synonym *F. chunii*) is currently present in Texas, thus simplifying conservation efforts. The distribution range of both these regional endemics (*F. askewi* and *P. riddellii*) has been reduced in the last 80 years (Burlakova et al., 2012, Appendix 2).

V. Conclusions: The results of this study indicated that several rare Texas endemic species Texas pimpleback, Texas fatmucket, golden orb, smooth pimpleback, Louisianna pigtoe, Texas fawnsfoot and Mexican fawnsfoot still exist in Texas, and estimated the size of the Texas pimpleback, Texas fatmucket, Texas fawnsfoot, and smooth pimpleback populations in the San Saba River. However some of them (e.g., Texas fatmucket, Texas and Mexican fawnsfoot) are presently in dangerously low numbers. Considering the critical state of the Rio Grande River, and a number of Central Texas rivers suffering from drought and dewatering, all possible conservation measures to save the remnant populations and preserve their remaining habitat should be designed and carried out as soon as possible. We located sites on the Neches and Trinity rivers that are among the richest in the state in terms of diversity and abundance of unionid bivalves, and found additional sites for Texas endemic golden orb, smooth pimpleback and Texas pimpleback in the San Saba, Nueces, San Marcos and Guadalupe rivers. We recommend these sites for future monitoring and conservation. In addition, we found that triangle pigtoe (*Fusconaia lananensis*) is not a valid species, and it is likely that only one *Fusconaia* species (Texas pigtoe) is currently present in Texas, thus simplifying conservation efforts. Distribution range of both Texas and Louisiana pigtoe has been reduced in the last 80 years. The present survey provided data required for successful management and conservation of freshwater molluscs (family Unionidae) in Texas.

VI. Presentations and Publications:

Peer-reviewed publications.

Two papers were published based on the results of this study and our previous SWG funding:

Karatayev, A. Y., T. D. Miller, and L. E. Burlakova. 2012. Long-term changes in unionid assemblages in the Rio Grande, one of the World's top 10 rivers at risk. *Aquatic Conservation: Marine and Freshwater Ecosystems.* 22(2): 206-219.

Burlakova L. E., D. Campbell, A. Y. Karatayev, and D. Barclay 2012. Distribution, genetic analysis and conservation priorities for rare Texas freshwater molluscs in the genera Fusconaia and Pleurobema (Bivalvia: Unionidae). *Aquatic Biosystems* 8(1):12. Open Access article available at: http://www.aquaticbiosystems.org/content/8/1/12

Please find the paper attached (Appendix 1).

Presentations.

We presented 4 oral talks and one poster at the local, national and international meetings:

(1) One oral presentation at the 7th Biennial Symposium of the Freshwater Mollusk Conservation Society (April 11 – 15, 2011. Louisville, Kentucky): **Burlakova, L. E., A. Y. Karatayev, V. A. Karatayev, M. E. May, D. L. Bennett, and M. J. Cook. Biogeography and conservation of freshwater mussels (Bivalvia: Unionidae): drivers of diversity and threats.**

(2) One oral presentation at the IV International Scientific Conference "Lake Ecosystems: Biological Processes, Anthropogenic Transformation, Water Quality", September 12-17, 2011, Minsk-Naroch, Belarus. **Burlakova, L. E., and A. Y. Karatayev. Biogeography and conservation of freshwater mussels (Bivalvia: Unionidae) in Texas.**

(3) Two oral presentations at the International Meeting on Biology and Conservation of Freshwater Bivalves in Braganca, Portugal (September, 4-7, 2012):

a. Burlakova, L., and Karatayev, A. Biogeography and conservation of freshwater mussels (Bivalvia: Unionidae) in Texas: drivers of diversity and threats.

b. Karatayev, A., Miller, T, and L. Burlakova. Long-term changes in unionid assemblages in the Rio Grande, one of the World's top 10 Rivers at Risk. International Meeting on Biology and Conservation of Freshwater Bivalves, Braganca, Portugal, 4-7 September 2012.

(4) One poster at the 12th Annual 2011 Faculty and Staff Research and Creativity Fall Forum, Buffalo State College, October 2011. **Karatayev, A. Y., Miller, T. D., and L. E. Burlakova. Long-term changes in unionid assemblages in the Rio Grande, one of the World's top 10 rivers at risk.**

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surveys of the Llano River. Charles Randklev, Julie Groce, Matthew Johnson, Eric Tsakiris (Texas A&M Institute of Renewable Natural Resources), and Joe Skorupski (University of North Texas, Denton) helped us during survey on the Llano and some of the sites on the San Saba River in March 2011.

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SWG/LIP Administrator

Date: 26 September 2012

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APPENDICES:

Papers published based on the results of this project and previous State Wildlife Grant funding.

APPENDIX 1.

Karatayev, A. Y., T. D. Miller, and L. E. Burlakova. 2012. Long-term changes in unionid assemblages in the Rio Grande, one of the World's top 10 rivers at risk. *Aquatic Conservation: Marine and Freshwater Ecosystems.* 22(2): 206-219.

APPENDIX 2.

Burlakova, L. E., D. Campbell, A. Y. Karatayev, and D. Barclay. 2012. Distribution, genetic analysis and conservation priorities for rare Texas freshwater molluscs in the genera Fusconaia and Pleurobema (Bivalvia: Unionidae). *Aquatic Biosystems* 8(1):12.

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Long-term changes in unionid assemblages in the Rio Grande, one of the World's top 10 rivers at risk

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ABSTRACT

1. According to the World Wildlife Fund, the Rio Grande is the most endangered river system in the North American continent and one of the World's top 10 rivers at risk, but is globally important for freshwater biodiversity. Unionid bivalves of the Rio Grande river basin used to be represented by a unique assemblage, including four endemic species (*Truncilla cognata, Potamilis metnecktayi*, *Popenaias popeii*, and *Quadrula couchiana*); however, surveys from 1998–2001 failed to recover any live endemic unionid species suggesting a sharp decrease in their populations and potential of extinction.

2. Intensive surveys (162 sites sampled) conducted by the authors from 2001–2011 on the Rio Grande and its tributaries in Texas recovered live *T. cognata, P. metnecktayi*, and the largest population of *P. popeii* ever reported. Overall the unionid assemblage of the Rio Grande basin has changed considerably during the last century.

3. Decline in species diversity, range fragmentation, local extirpations, and introduction of widespread common species were documented. Two species (*Q. couchiana* and *Quincuncina mitchelli*) are locally extinct. *Potamilus metnecktayi* and *T. cognata* have been extirpated from the Pecos River and their ranges in the Rio Grande have been reduced. *Popenaias popeii* has been extirpated from the Pecos River and Las Moras Creek along with the reduction and fragmentation of its range in the Devils River and Rio Grande.

4. Among the environmental factors responsible for the degradation of unionid assemblages in the Rio Grande river basin, the most important are impoundments, habitat degradation, salinization, pollution, and over-extraction of water.

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KEY WORDS: river; biodiversity; distribution; rare species; invertebrates; impoundments agriculture; salinity; Unionidae; threats

INTRODUCTION

A continuing dramatic increase in pollution, habitat destruction and introduction of invasive species is resulting in simplification and homogenization of ecosystems and a loss of biodiversity worldwide (Mckinney and Lockwood, 1999). Biodiversity loss is especially large in fresh waters, where many species are far more imperilled than their marine or terrestrial counterparts (Jackson *et al.*, 2001; Strayer and Dudgeon, 2010). This loss of diversity results from widespread habitat degradation, pollution, flow regulation, and water extraction, and these activities are predicted to increase in the

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future (Naiman and Turner, 2000; Jackson *et al*., 2001; Strayer and Dudgeon, 2010). The opportunity to conserve much of the remaining biodiversity in fresh waters may vanish if trends in human demands for fresh water remain unaltered and species losses continue at present rates (Dudgeon *et al*., 2006).

The Rio Grande is a globally important river for freshwater biodiversity, supporting numerous endemic fishes, birds, and molluscs (Groombridge and Jenkins, 1998; Revenga *et al*., 1998, 2000; Johnson, 1999); however, because of the level of impacts affecting the Rio Grande at present (Dahm *et al*., 2005), many of these species are now extinct and others are facing a sharp decrease in their population density or fragmentation in their range. Focusing analysis on river basins with high ecological importance and those with large human populations, the World Wildlife Fund recognized the Rio Grande River as the most endangered river in the North American continent, and one of the world's top 10 rivers at risk (Wong *et al*., 2007).

The Rio Grande/Rio Bravo River (length: 2830 km, river basin area: 870 000 km^2) is one of the longest in North America, flowing from its headwaters in Colorado through New Mexico and then forming the shared border between Texas and Mexico before it empties into the Gulf of Mexico near Brownsville, Texas (Dahm *et al*., 2005). It traverses seven physiographic provinces with a variety of habitats, but most of the basin is arid or semiarid with either desert shrubland or desert grassland (Dahm *et al*., 2005). The Rio Conchos, the Pecos River and the Devils River historically contributed the main flow of the Rio Grande in the stretch between their confluences and Amistad Reservoir, although these flows have been reduced substantially and are stored at Amistad International Reservoir. Amistad Dam (completed in 1969) and Falcon Dam (completed in 1953) impound the Rio Grande along the border for irrigation and flood control. Evaporation from major reservoirs has been estimated to exceed the quantity of water used for municipal purposes in the basin, which constitute up to 5% of the agricultural consumption. From Laredo to the mouth of the Rio Grande, the river constitutes the primary source of drinking water for communities in both Mexico and the USA (Dahm *et al*., 2005). Over 10 million people live in the Rio Grande basin, and urban areas are growing fast, particularly in border towns between the USA and Mexico. By 2060 the area from Eagle Pass to Brownsville is projected to almost triple in population (Texas Water Development Board, 2007). Irrigated agriculture is the primary use of the Rio Grande surface flow throughout the basin and accounts for more than 80% of all water taken from the river (Dahm *et al*., 2005). The river bed between El Paso and Presidio frequently is dry, owing to water over-extraction for irrigation and domestic consumption, and since 2001 the river often fails to reach the Gulf of Mexico (Edwards and Contreras-Balderas, 1991; Contreras-Balderas *et al*., 2002; Dahm *et al*., 2005; Wong *et al*., 2007; Douglas, 2009). Many other factors have contributed to the recent status of the Rio Grande, including persistent drought, increase in border populations, and subsequent declines in water quantity and quality (Dahm *et al*., 2005; Wong *et al*., 2007; Douglas, 2009).

Freshwater bivalves in the order Unionoida are considered to be one of the most endangered groups of animals in North America (Bogan, 1993; Lydeard *et al*., 2004) with over 76% of the North American Unionidae and Margaritiferidae presumed extinct, threatened, endangered, or deemed of special concern (Williams *et al*., 1993). Unionid bivalves of the Rio Grande river basin represent a unique assemblage and are distinct from the rest of Texas (Neck, 1982; Neck and Metcalf, 1988; Burlakova *et al*., 2011a, b). The first data on unionid bivalves of the Rio Grande and its tributaries were published at the turn of the 19th century (Singley, 1893; Simpson, 1900, 1914). In the second half of the 20th century, numerous studies conducted on the Rio Grande system were summarized by Johnson (1999), who provided a detailed description of historical records and current distribution of all 15 species of unionids reported from this system. Extensive surveys done by Texas Parks and Wildlife Department in 1998–2001 failed to recover any live endemic unionid species from the Rio Grande, and Howells (2001) suggested that a sharp decrease in their populations may have put them on the edge of extinction. However, subsequent intensive surveys done by the authors in 2001–2011 recovered live *Truncilla cognata, Potamilis metnecktayi*, and the largest population of *Popenaias popeii* ever reported, proving that at least three endemic unionid species are still present in the river. The goals of this paper are: (1) to analyse the changes in the unionid assemblage of the Rio Grande river basin over 100 years; (2) to study the current distribution of the endemic species and estimate, whenever possible, their population densities; (3) to discuss major factors affecting unionid diversity and distribution in the Rio Grande.

METHODS

Data collection

To assess the current distribution of unionids, mussels were surveyed at 162 sample sites (subsites) pooled into 28 larger sites within the Rio Grande system during 2001–2011 (Figure 1). Fifteen of these sites were sampled once, while 13 sites were sampled from 2–25 times. Survey sites were often selected within state parks, near public boat ramps, or based on accessibility from roads that either crossed or approached a water body owing to the prevalence of private land in Texas, where only 2% of the lands remain in public ownership (Texas Parks and Wildlife Department, 1974). In addition, numerous sites were reached by canoe or kayak. When site surveys were conducted from private land, a Landowner Permission for wildlife research was acquired from each property owner before entering the property. The work was carried out with an appropriate Scientific Research Permit issued by the Texas Parks and Wildlife Department.

Sampling was completed by hand collection of both live and dead mussels, by wading in low water and by snorkelling or diving. Reconnaissance sampling (timed searches) and random searches were used at most sites to reveal the presence of mussels and species diversity (Strayer *et al.*, 1997; Vaughn *et al.*, 1997) and to compare with historical data. If significant mussel assemblages were present, quantitative methods (randomly placed 0.25 m^2 quadrats, mark-and-recapture surveys, or area searches) were used for assessments of density (Dunn, 2000; Strayer and Smith, 2003). Collected live mussels and shells were taxonomically identified, counted, and measured with calipers to the nearest millimetre. After measurements live mussels were carefully bedded into the sediment from which they were taken. Shell condition of dead mussels was recorded for each specimen.

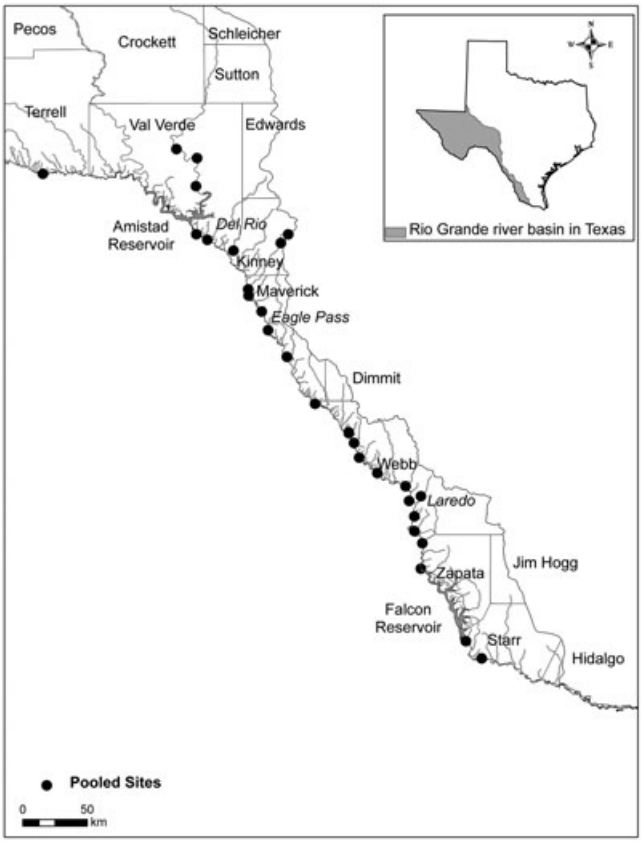

Figure 1. Map of the Rio Grande river basin in Texas with 28 pooled sampling sites surveyed during 2001–2011. Texas counties, major cities (in italics) and reservoirs are indicated.

A mark-and-recapture census was conducted at the La Bota Ranch site in Northern Laredo (Webb County) in March 2011 using methods described by Lang (2001) and Villella *et al.* (2004). Following recommendations by Villella *et al.* (2004), three consecutive days were sampled to estimate capture probabilities using closed population models. All mussels present (new captures, and recaptures) were measured (shell length, width, height ($\pm$ 0.1 mm)), and wet-weighed. First-time captured individuals were marked with unique numbers assigned by embedding oval (4×10 mm) Floy laminated flex tags in Super Glue Gel along the valve hinge posterior to the umbo, to one valve.

Specimens were identified using published taxonomic keys and descriptions (Howells *et al.*, 1996; Johnson, 1998). Voucher specimens were deposited into the Great Lakes Center Invertebrate Collection at Buffalo State College, Buffalo, NY. Each specimen was labelled with a unique number and catalogued in a database with the following information: specimen number, species name, name of person who collected and identified the specimen, date of collection, and detailed site information.

Data analysis

To estimate population density at the mark–recapture site the Schnabel method, an extension of the Petersen method to analyse a series of samples, was used (Krebs, 1999). To evaluate the total size of the *P. popeii* population the average density in the mark–recapture site and the estimation of available habitat area at the La Bota site near Laredo were used.

Differences in community structure were assessed with nonparametric multivariate statistical techniques on data matrices of all live species and their relative

densities (as catch-per-unit of effort data, i.e. the number of live mussels for each species found per time search effort at each sampling site (mussels per person per hour). A square root transformation was used to normalize relative densities for the analysis. Similarity of the community composition was summarized by calculating Bray–Curtis distances – a measure of similarity with values ranging from 0 (identical samples) to 1, which is not influenced by rare species as other indices (Bray and Curtis, 1957; Clarke, 1993). To visualize the differences among assemblages, a non-metric multi-dimensional scaling (NMDS) was used, which calculates a set of metric coordinates for samples, most closely approximating their non-metric distances. Differences among communities were assessed by analysis of similarities (ANOSIM), a resampling technique that uses permutation/randomization methods on Bray–Curtis similarity matrices to identify differences among groups of samples (Clarke, 1993). These analyses were performed using PRIMER 6 software (Plymouth Routines in Multivariate Ecological Research, Version 6.1.6, Primer E-Ltd. 2006). All tests effects were considered significant if $P < 0.05$.

To analyse the historical data a database containing information of unionid species name, water body name, location, recorded date, and the collector's name was created using all available data including published records, museum collections, and web-based searches. Unionid assemblages in the Rio Grande system were analysed using the following time periods: (1) initial reports (before 1931), including collections made by the United States and Mexico Boundary Surveys mostly conducted in 1892 (Taylor, 1967), and data from Singley (1893), Ellis *et al.* (1930), and Strecker (1931); (2) 1968–1990 based mostly on data from Metcalf and Neck (Metcalf, 1974, 1982; Murray, 1975; Neck, 1984; 1987; Neck and Howells, 1984; Neck and Metcalf, 1988); (3) 1992–1999 based on Howells' data (Howells 1994, 1996a, b, 1997a, 1998, 1999, 2000); and (4) 2001–2011 based on the authors' data. Several assumptions were made in the analysis. If the status of a recorded unionid was not reported in the paper used for historical analysis, it was assumed that the specimen was found alive; if the date of collection was not reported in the paper, it was assumed that the mussel was recorded one year earlier preceding the publication year (excluding papers where museum collections were analysed).

RESULTS

Unionid diversity

This study showed that the Rio Grande still supports most of the unionid species previously reported from this river, including the regional endemics *Potamilus metnecktayi, Popenaias popeii*, and *Truncilla cognata* (Table 1). During the current study the most common unionid species were *Cyrtonaias tampicoensis* and *Quadrula*

Table 1. Historical and current records of live unionids (L) and their dead shells (D) from the Rio Grande drainage (excluding the Rio Grande River itself, RGD) and the Rio Grande River (including Falcon and Amistad reservoirs, RG) in Texas. n. r. - not recorded. Total number of species found dead is in parentheses

	Before 1931		1968–1990		1992–1999		2001–2011	
Species	RGD	RG	RGD	RG	RGD	RG	RGD	RG
Cyrtonaias tampicoensis	L	n. r.	L	L	L	L	L	L
Lampsilis teres	L	L	L	L	D	D	n. r.	L
Megalonaias nervosa	L	n. r.	n. r.	D	n. r.	D	n. r.	L
Potamilus metnecktayi[a]	n. r.	n. r.	L	L	n. r.	D	n. r.	L
Popenaias popeii[a]	L	n. r.	L	L	D	D	L	L
Potamilus purpuratus[b]	n. r.	n. r.	n. r.	n. r.	n. r.	L	n. r.	n. r.
Pyganodon grandis[b]	L	n. r.	L	n. r.	n. r.	n. r.	L	n. r.
Quadrula apiculata[b]	n. r.	n. r.	L	L	L	L	L	L
Quadrula couchiana[a]	L	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.
Quincuncina mitchelli[a]*	D	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.
Toxolasma parvus	L	n. r.	n. r.	n. r.	L	L	n. r.	n. r.
Toxolasma texasensis	L	n. r.	L	n. r.	n. r.	n. r.	L	n. r.
Truncilla cognata[a]	n. r.	n. r.	L	L	n. r.	n. r.	n. r.	L
Uniomerus sp.	n. r.	n. r.	L	n. r.	n. r.	n. r.	n. r.	n. r.
Utterbackia imbecillis	L	n. r.	L	L	L	L	L	L
Total	9 (1)	1	10	7 (1)	4 (2)	5 (4)	6	8

[a]Regional endemics
[b]Introduced species
*Only fossil and greatly weathered specimens are known from Texas part of Rio Grande drainage.

apiculata, found alive at 28.6% of sites sampled (Table 2). The percentage of sites where live molluscs were found compared with the total number of sites where live and dead specimens were found was the greatest for *Q. apiculata* (73%), *Megalonaias nervosa* and *P. popeii* (58% each), and the lowest for *T. cognata* (17%) and *P. metnecktayi* (13%). The rarest species was *P. metnecktayi*, which was found alive at only one location. Other rarely recorded species were *Utterbackia imbecillis* and *Toxolasma texasensis*, which were found mostly in tributaries (Table 2). The highest diversity of unionids was found in a 24 km stretch of the Rio Grande above Laredo (Figure 2(C), 3(B), 4(D)). No live mussels were found below Amistad Reservoir and few below Laredo. Two distinct unionid assemblages depending on the substrate type were found in the Rio Grande above Laredo (Figure 5, $R = 0.942$, $P = 0.001$, one-way ANOSIM). On soft and unconsolidated sediments (silt, sand, small gravel, and combinations of these) unionid assemblages were dominated by *Q. apiculata*, and *C. tampicoensis*; additional species were *M. nervosa* and *T. cognata*. On bedrock and boulders the dominant species was *P. popeii*. This species was most commonly found in crevices under flat boulders resting on the bedrock. Often up to 10 individuals were found under one rock. Additional unionids found in this habitat included *Lampsilis teres, Q. apiculata*, and *T. cognata*.

Endemic species account

Potamilus metnecktayi

Nineteen live *P. metnecktayi* were found in the Rio Grande at the John's Marina site, south of Dryden, Terrell County in 2003–2008 (Figure 2(C)). Mussels were generally found along the shores, in soft sediments (in a mixture of silt and clay) at 0.5–1.2 m depth (at low flows ~30 m^3 s^{-1}). Their size varied from 63 to 124 mm, averaging 87.1 mm (±17.6 standard deviation). Dead shells of *P. metnecktayi* were found at seven more sites. *P. metnecktayi* had the lowest percentage of sites where live mussels were found, from the total number of sites where shells of the species were recorded (13%). At 15 sites below Lake Amistad, only 50 long-dead or sub-fossil valves

Table 2. Occurrence of unionid species in the Rio Grande river drainage, and separately in the river main stem and its tributaries based on 2001–2011 surveys. In total, 28 pooled sites were studied in the Texas part of the drainage, including 21 sites in the Rio Grande River (excluding reservoirs) and seven sites on tributaries. Species occurrence was calculated as a number of sites where the species was found, and percentage occurrence was calculated as the percentage of sites where the species was found. Single valves were counted as half of a shell

	Rio Grande drainage			Rio Grande River			Tributaries only		
Species	Total found	Occurrence (number of pooled sites)	Percentage occurrence	Total	Occurrence (number of pooled sites)	Percentage occurrence	Total	Occurrence (number of pooled sites)	Percentage occurrence
Live mussels									
Cyrtonaias tampicoensis	89	8	28.6	29	7	33.3	60	1	14.3
Lampsilis teres	17	2	7.1	17	2	9.5	0	0	0
Megalonaias nervosa	34	7	25.0	34	7	33.3	0	0	0
Popenaias popeii	656	7	25.0	649	5	23.8	7	2	28.6
Potamilus metnecktayi	19	1	3.6	19	1	4.8	0	0	0
Quadrula apiculata	204	8	28.6	129	7	33.3	75	1	14.3
Toxolasma texasensis	11	1	3.6	0	0	0	11	1	14.3
Truncilla cognata	19	2	7.1	19	2	9.5	0	0	0
Utterbackia imbecillis	7	1	3.6	0	0	0	7	1	14.3
Total live mussels	**1056**	**14**	**50.0**	**896**	**11**	**52.4**	**160**	**3**	**42.9**
Shells									
Cyrtonaias tampicoensis	789	20	71.4	788	19	90.5	1	1	14.3
Lampsilis teres	84.5	9	32.1	84.5	9	42.9	0	0	0
Megalonaias nervosa	180.5	12	42.9	180.5	12	57.1	0	0	0
Popenaias popeii	473.5	12	42.9	465	11	52.4	8.5	1	14.3
Potamilus metnecktayi	159.5	8	28.6	159.5	8	38.1	0	0	0
Quadrula apiculata	533.5	11	39.3	533	10	47.6	0.5	1	14.3
Toxolasma texasensis	1	1	3.6	0	0	0	1	1	14.3
Truncilla cognata	291	12	42.9	291	12	57.1	0	0	0
Utterbackia imbecillis	57	10	35.7	17	7	33.3	40	3	42.9
Total shells	**2569.5**	**21**	**75.0**	**2518.5**	**19**	**90.5**	**51**	**3**	**42.9**

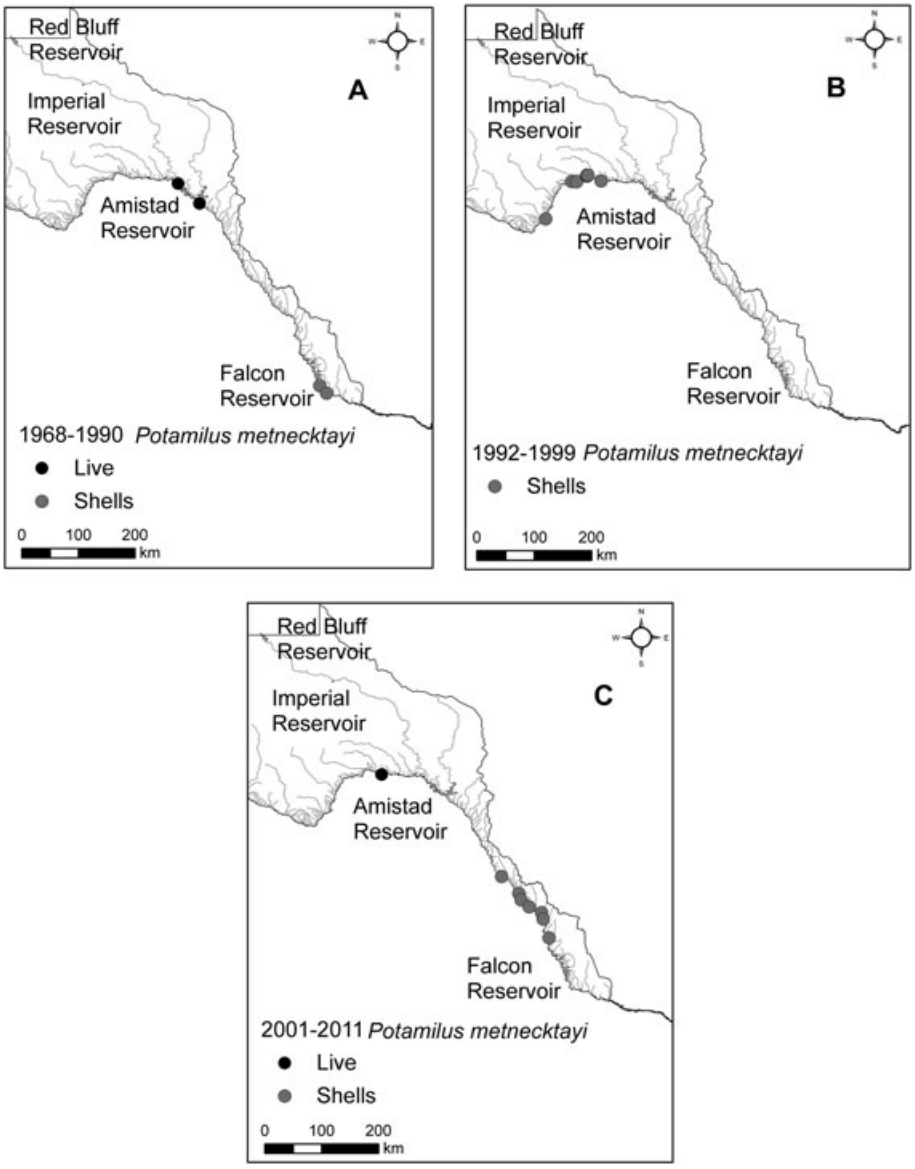

Figure 2. Map of the Rio Grande river basin in Texas with sites where live and/or dead shells of *Potamilus metnecktayi* were found in 1968–1990 (Metcalf, 1974, 1982; Murray, 1975; Neck and Howells, 1984; Neck, 1987; Neck and Metcalf, 1988) (A); in 1992–1999 (Howells 1994, 1996a,b, 1997a, 1998, 1999, 2000) (B); and from 2001 to 2011 (authors' data) (C).

were found, possibly indicating a once widespread population.

Truncilla cognata

In total, 19 live *T. cognata* were found from 2001 to 2011 in the Rio Grande River in Laredo, Webb County. Most molluscs were found down to 15–20 cm deep in a mixture of gravel and sand, and between large boulders. Because of its small size, it was difficult to distinguish *T. cognata* from gravel, adding to the difficulty of detecting this cryptic species. Many excavations were made below the Water Treatment Plant in Laredo, but no live mussels were found there. In 2011 12 *T. cognata* were found at five subsites examined in and above Laredo. Most of them were found in unconsolidated sediments (sand with some silt), captured in shallow protected areas adjacent to gravel riffles. Their size varied from 20.5 mm to 33 mm (average 28.4 ± 4.1 mm). Dead shells of *T. cognata* were found at 12 sites (Figure 3(B)). Very recently dead specimens (i.e. shells with flesh, to 51 mm) were found at four subsites below Laredo into Zapata County. Based on these data, it is likely that additional specimens may be found in Pinto Valle Creek (Webb County) and Dolores Creek (Zapata County). All of the 19 live *T. cognata* from the current study have been found at the confluences of Santa Isabel, Sombrerito, and Zacate Creeks above

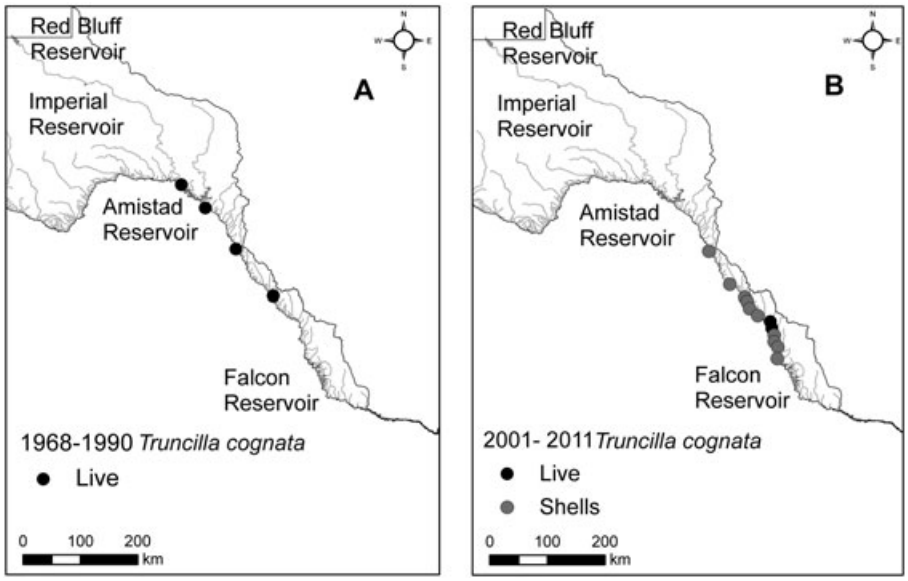

Figure 3. Map of the Rio Grande river basin in Texas with sites where live and/or dead shells of *Truncilla cognata* were found in 1968–1990 (data from Metcalf, 1974, 1982; Murray, 1975, Neck and Howells, 1984; Neck, 1987; Neck and Metcalf, 1988) (A), and from 2001 to 2011 (authors data) (B).

Laredo. Their presumed habitat preference of small gravel/sand/silt mixed substrates is also well known as each of these areas has or had a sand and gravel excavation site nearby.

Popenaias popeii

During 10 years of the current survey, one live *P. popeii* was found in the Rio Grande River in Terrell County (John's Marina), seven live in the Devils River, and 648 live in the Rio Grande near Laredo. Live mussels were found at seven sites, and dead shells were found at a further five sites (Figure 4(D)). Most live mussels were found at the La Bota mark-and-recapture subsite (in Laredo) which had an abundance of low-flow refuges occurring under large boulders, where sand and clay seams provide substrates for mussels. At this mark–recapture site (area sampled *c.* 1000 m^2) 406 live *P. popeii* were found. The recovery rate was 11.7% (18 of 154 mussels marked) on the second day, and was 6.5% (17 of 260 mussels marked) on the third day (9.1% in average). Therefore, the total population may be near 1500 at the site, with a density of ~1.5 m^{-2}. This population consisted of multiple age-classes, with shell lengths ranging from 33.2 to 87 mm (63 ± 1, mean $\pm$ 95% confidence interval). Over a third of the mussels measured were less than 60 mm, and 12 individuals were less than 45 mm in length. Considering that the total area of similar substrate upstream of this site was ~ 3200 m^2, and assuming similar densities, up to 4700 individuals of this species may be in this area. At three other subsites located *c.* 1.6 km downstream from this mark-and-recapture locality, 182 live *P. popeii* were found in 3 person-hours of timed searches. These subsites were located along a 280 m river stretch, and may contain up to 4000 more mussels. Therefore, the total population of *P. popeii* in the La Bota area may contain up to 8700 mussels.

DISCUSSION

Long-term changes in unionid diversity

The unionid assemblage of the Rio Grande drainage has changed significantly over the last century (Table 1, 3). Although the Rio Grande itself still supports the majority of unionid species ever reported alive in this river, its unionid assemblage has faced decline in species diversity, range fragmentation, local extirpations, and introduction of widespread common species. Two species (*Quadrula couchiana* and *Quincuncina mitchelli*) are already extinct from the Texas part of the Rio Grande basin. The most drastic changes were recorded during the last 40 years (Table 3).

Several streams and rivers of the Rio Grande drainage have lost all or a significant number of unionid species, including Las Moras Creek in Fort Clark (Brackettville, Kinney County), the Devils River and the Pecos River (Table 4). Along with the local extirpation of rare and endemic species from the Rio Grande drainage, the unionid

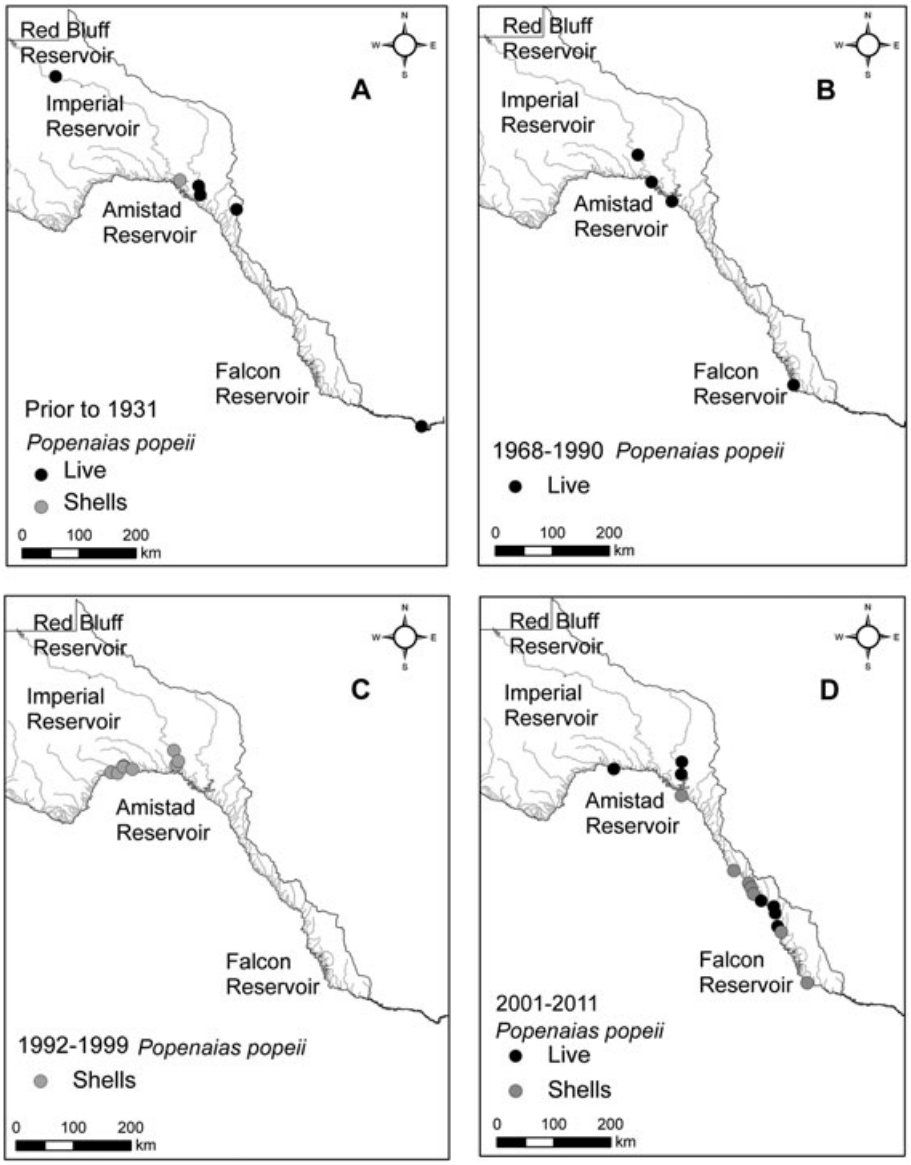

Figure 4. Map of the Rio Grande river basin in Texas with sites where live and/or dead shells of *Popenaias popeii* were found before 1931 (based on data from Singley, 1893; Ellis *et al*., 1930; Strecker, 1931; Taylor, 1967) (A); in 1968–1990 (Metcalf, 1974, 1982; Murray, 1975, Neck and Howells, 1984; Neck, 1987; Neck and Metcalf, 1988) (B); in 1992–1999 (Howells 1994, 1996a, b 1997a, 1998, 1999, 2000) (C); and from 2001 to 2011 (authors' data) (D).

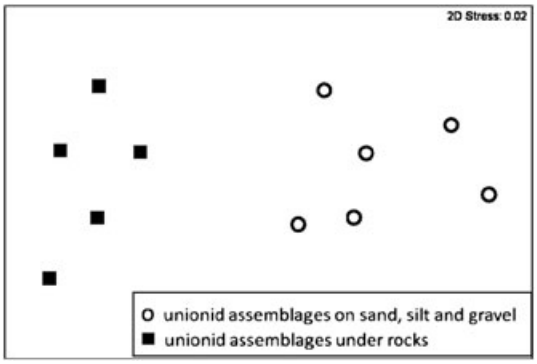

Figure 5. NMDS ordination plot of the unionid assemblages in the Rio Grande near Laredo found on sand, silt and gravel and under rocks. Relative density data (mussels per person per hour) for live molluscs collected at all sampled sites (excluding sites where fewer than two species were collected) were square-root transformed and converted to a similarity matrix using the Bray–Curtis similarity index. There was a significant difference in assemblage structure among the two substrates (Global R = 0.942, $P = 0.001$, one-way ANOSIM).

assemblage was reshaped by the introduction of common species (*Q. apiculata, P. purpuratus*, and *P. grandis*) non-native to this drainage (Metcalf and Smart, 1972; Metcalf, 1982; Johnson, 1999). In the 20th century *Q. apiculata* became very common in the Rio Grande and its tributaries. Previous research noted a lack of fossil *Q. apiculata* (Metcalf, 1982), and no fossil specimens were found during this study. Current data indicate slow, upriver range extension of *Q. apiculata* with greater abundance in Casa Blanca and Falcon reservoirs. *Potamilus purpuratus* has been recorded in the Amistad Reservoir in 1994, 1995 and 1998 (Howells, 1997b, 1999). Historical records of *P. purpuratus* from the Rio Grande river basin (Singley, 1893) have been shown to be

Table 3. Long-term changes in unionid diversity in the Texas part of the Rio Grande drainage

Time period	Changes
Before 1900	Extinction of *Quadrula couchiana* from the Rio Grande drainage
	Introduction of *Pyganodon grandis*
1900–1970	Extinction of *Q. mitchelli* from the Rio Grande drainage
	Introduction of *Q. apiculata*
1970–2010	Local extirpations of *Popenaias popeii, Potamilus metnecktayi, Truncilla cognata*
	Range fragmentation of *P. popeii, P. metnecktayi, T. cognata*
	Introduction of *Potamilus purpuratus*
	Range expansion of *Q. apiculata*

misidentified specimens of *C. tampicoensis* (Neck and Metcalf, 1988; Johnson, 1999). Another introduced species, *P. grandis*, was reported from the Granjeno Lake in 1892 (Singley, 1893) and canals in Hidalgo County (Ellis *et al.*, 1930), from the El Toro Cement Agency Lake in El Paso in 1969 (Johnson, 1999), and in the Topaz Power Plant cooling pond, Laredo in 2006 (T. Miller unpublished data).

Endemic species accounts

Potamilus metnecktayi

This regional endemic was reported to be extremely rare in the Rio Grande in Texas (Neck and Metcalf, 1988), uncommon even at the fossil localities sampled in New Mexico and Mexico (Metcalf, 1982), and it has been recently added to the state's list of threatened species (Texas Register 35, 2010). Live specimens in the USA were collected in Texas only by Metcalf on the Rio Grande 9.7 km west of Del Rio in 1972, and by Taylor in the Pecos River 1.28 km above its mouth at the former crossing of US Hwy 90 in 1968 (Metcalf, 1982) (Table 4, Figure 2). No live or dead *P. metnecktayi* were found in the Del Rio area during sampling in 2008. Only dead shells of *P. metnecktayi* were found in Texas since the mid-1970s (Howells, 1994, 1999, 2000; Howells *et al.*, 1997; Figure 2(B)). Our discovery of 19 live and numerous shells of *P. metnecktayi* in the Rio Grande by Johnson Marina, Terrell County, proves that this species still exists in the middle Rio Grande, although its distribution range was significantly reduced during the 20th century. Additional studies are urgently needed to estimate the current distribution and population size of *P. metnecktayi* in the Rio Grande considering the subsequent catastrophic floods in 2008 and 2010, and to develop appropriate measures for the species' conservation.

Truncilla cognata

Truncilla cognata is another regional endemic that was described from the Devils River, Texas, and Rio Salado, Nuevo Leon, Mexico (Lea, 1857; Johnson, 1999). This species has a NatureServe

Table 4. Historical and current records of live *Potamilus metnecktayi, Truncilla cognata*, and *Popenaias popeii* in the Texas part of the Rio Grande drainage

Water body	Historical collections	Current status
Potamilus metnecktayi		
Rio Grande, 9.7 km West of Del Rio	1972 (Metcalf, 1982)	No live mussels were found
Rio Grande, Johnson Marina, Terrell County	No historical records from this location	19 live specimens were collected by the authors 2003–2008
Pecos River, 1.28 km above its mouth at the former US Hwy 90 crossing	1968 (Metcalf, 1982)	Flooded by Amistad Reservoir. No live mussels were found
Truncilla cognata		
Rio Grande, 9.7 km West of Del Rio	1972 (Metcalf, 1982)	No live mussels found
Rio Grande, Laredo	No historical records from this location	19 mussels total were found by the authors at two sites 2001–2011
Pecos River, 1.28 km above its mouth at the former US Hwy 90 crossing	1968 (Metcalf, 1982)	Flooded by Amistad Reservoir (population probably extirpated)
Popenaias popeii		
Las Moras Creek, Kinney County	1892 (Taylor, 1967)	No live mussels were found. Population extirpated (Murray, 1975)
Devils River, Val Verde County	1892 (Singley, 1893)	7 live mussels were found by authors 2008–2011
Pecos River, Val Verde County	1903, 1968, 1972, 1973 (Metcalf, 1982)	Flooded by Amistad Reservoir. No live mussels were found
Rio Grande, 9.7 km West of Del Rio	1972 (Metcalf, 1982)	No live mussels were found
Rio Grande, 2.3 km downstream of Falcon Dam, Starr County	1975 (Neck and Metcalf, 1988)	No live mussels were found
Rio Grande, Laredo	No historical records from this location	645 live mussels were found by the authors in 2002–2011
Rio Grande, Johnson Marina, Terrell County	No historical records from this location	1 live specimen was collected by the authors in 2008

global status of 'critically imperilled' (NatureServe, 2009), is considered endangered by the American Fisheries Society, and has recently been added to the state's list of threatened species (Texas Register 35, 2010). *Truncilla cognata* is currently under consideration for federal listing by the US Fish and Wildlife Service (USFWS) (Federal Register 74, 2009). In the USA, *T. cognata* was reported only from a few sites in Texas (Table 4, Figure 3) with no living or dead specimens collected since 1972 (Howells *et al.*, 1997; Howells, 2001). Again it is likely that the Pecos River population of *T. cognata* is already extirpated and the 19 live specimens that were found in the Rio Grande near Laredo in 2001–2011 represent the only known population of this species left in the US.

Popenaias popeii

Popenaias popeii is known from the Rio Grande drainage in Texas (Singley, 1893; Taylor, 1967; Neck, 1987), Black River in New Mexico (Lang, 2001; Carman, 2007), and several Mexican tributaries of the Rio Grande (Simpson, 1914; Johnson, 1999; Strenth *et al.*, 2004). Strecker (1931) reported that *P. popeii* 'seems to be rather scarce', Stansbery (1971) listed this species as 'rare and endangered', and Neck (1984) included it in his list of restricted and declining species of Texas. NatureServe ranks *P. popeii* as critically imperilled across its range (NatureServe, 2009). This species has recently been added to the state's list of threatened species (Texas Register 35, 2010), and is currently considered a candidate for listing (priority 8) under the federal Endangered Species Act.

In Texas, live *P. popeii* were reported from Las Moras Creek (Taylor, 1967), the Devils (Singley, 1893) and Pecos Rivers (Metcalf, 1982), and from two distinct areas in the Rio Grande (Metcalf, 1982; Neck and Metcalf, 1988) (Table 4, Figure 4). Only two dead shells of *P. popeii* were reported in Texas outside the Rio Grande drainage in the South Concho and Llano Rivers (Strenth *et al.*, 2004). There is no evidence that these records represent extant populations of *P. popeii*.

No live *P. popeii* had been found in the Rio Grande since the mid-1970s (Howells, 2001). Our discovery of seven live *P. popeii* in the Devils River in 2008–2011, and 45 live *P. popeii* in 2002–2008 in the Rio Grande River confirmed that the species was still present in Texas. However, more significant was the discovery of a large population (604 live specimens recorded) of *P. popeii* in 2011 in Laredo. The conservative estimate of more than 8000 individuals made this Laredo population by far the largest ever reported from Texas, New Mexico or Mexico. This population consisted of multiple age-classes suggesting the recruitment of juvenile mussels and thus a healthy reproducing population. This also implies that a healthy host fish population occurs in this reach of the river, which is very important for unionid reproduction, and future population survival.

These particular refuges in upper Laredo may be vulnerable to excess water fluctuations including periods of low water and flood. During a low-flow period ($22.6\,m^3\,s^{-1}$) in December, 2002, snowy egrets (*Egreta thula*) were observed feeding on *P. popeii*. Another site on Zacate Creek (Las Palmas Park, a TPWD mussel sanctuary) where more than 50 live mussels of six species (including numerous *P. popeii*) were found over the years, has been smothered by cobble deposited by the July 2010 flood. No live mussels were recorded at this site since this last flood. Specimens of *P. popeii* do not appear to survive well in the Rio Grande downstream of Zacate Creek (Las Palmas Park, Laredo). Only one live mussel and two shells have been found in numerous shore surveys along the 80 km downstream reach of the river to Falcon Lake.

Another important finding was suitable habitat for *P. popeii* in the Rio Grande. This is similar to the preferred habitat for this species in the Black River: low-flow refuges characterized by aggregations of mussels under large boulders of limestone conglomerates, where clay seams provide stable substrates for mussels in low-velocity microhabitats (Lang, 2010). This habitat is different from the soft substrate type preferred by other species such as *C. tampicoensis*, *T. cognata*, *M. nervosa*, and *Q. apiculata* (Figure 5).

Environmental factors affecting unionids

The Rio Grande is at present one of the most impaired rivers in the world, with both water quantity and water quality issues being the major concerns (Dahm *et al.*, 2005). We suggest that among various types of human activities on the Rio Grande drainage, most destructive for unionid assemblages are impoundments, habitat degradation, salinization, pollution, and over-extraction of water (Table 5).

Impoundments

Some species may now be extinct in the Pecos system because of impoundment of its

lowermost part by Amistad Reservoir (Metcalf and Stern, 1976). Creation of Falcon Reservoir most likely decimated the lotic habitat of the bivalves in the lower Rio Grande (Neck and Metcalf, 1988). In south-eastern New Mexico, the construction of impoundments (Lake MacMillan, Brantley and Avalon reservoirs) was one of the many factors responsible for extirpation of *P. popeii* from the Pecos River mainstem (Taylor, 1967). The construction of reservoirs also facilitated the introduction and range expansion of common species (*Q. apiculata, P. purpuratus*, and *P. grandis*) non-native to the Rio Grande river drainage (Metcalf and Smart, 1972; Metcalf, 1982; Johnson, 1999). Low-head dams on the Black River apparently preclude opportunities for recolonization by *P. popeii* in upstream riverine reaches and with downstream recolonization potentially limited by altered physicochemical (salinity gradient) and flow regimes (Lang, 2001). Any future projects to construct a new dam, or to modify existing low-head dams and associated water diversion structures, both on the Black River or in the Rio Grande River in Laredo, could potentially have impacts on *P. popeii*.

Salinity

Salinity concentrations in the Rio Grande are the result of both human activities and natural conditions: the naturally salty waters of the Pecos River are a major source of the salts that flow into Amistad Reservoir and continue downstream. Salinity may be the major factor limiting *P. popeii* distribution in the Pecos River and in the Rio Grande below its confluence with the Pecos River. In laboratory studies *P. popeii* shows behavioural signs of physiological stress, followed by death, at a salinity of 7.0 psu (Lang, 2001). Salinity in the area of the Black River occupied by *P. popeii* is approximately 0.9 psu. It increases significantly downstream to 2.8 psu and, in the Pecos River, ranges from 6.0–7.0 psu downstream of the confluence with the Black River (Lang, 2001). This increased salinity may have prevented populations becoming established in the main stem of the Pecos River even before its impoundment.

Table 5. Environmental pressures affecting unionid assemblages in the Texas part of the Rio Grande drainage

Environmental pressure	Effect
Impoundments	• Extirpation of *P. metnecktayi, T. cognata* and *P. popeii* from the lower Pecos River flooded by Amistad Reservoir • Decreased range of *P. metnecktayi* and *T. cognata* in the Rio Grande • Introduction of *P. grandis*, and *P. purpuratus*
Habitat degradation and pollution	• Extirpation of all unionids, including *Q. mitchelli* and *P. popeii* from Las Moras Creek • Decreased or fragmented range of all unionids, including *P. popeii, P. metnecktayi*, and *T. cognata* in the Rio Grande
Salinization	• Extirpation of all unionids, including *P. popeii* from the Pecos River
Over-extraction of water	• Decreased or fragmented range of all unionids, including *P. popeii, P. metnecktayi*, and *T. cognata* in the Rio Grande

Over-extraction, habitat destruction, and pollution

Water diversion from the middle Rio Grande is so high that the river bed between El Paso and Presidio/Ojinaga often lies dry (Dahm *et al.*, 2005; Wong *et al.*, 2007; Douglas, 2009). Evapotranspiration, groundwater recharge, and human appropriation of Rio Grande water has resulted in less than 1% of basin precipitation reaching the mouth, and failures to reach the Gulf of Mexico were recorded in much of 2002 and 2003 (Dahm *et al.*, 2005). Growth in water demand from agricultural economic activity near the Mexican border and regional maquiladoras (manufacturing or export assembly plants in northern Mexico that produce parts and products for the USA) resulted in more than a 5-fold loss of lower Rio Grande stream flow between 1905–1934 and 1951–1980 (reviewed in Douglas, 2009). The population in the basin was about 13 million inhabitants in 1990, and increased along the Texas border by 27% between 1980 and 1990, and by 26% on the Mexican side. As a result of low water levels, the concentration of pollutants is very high; salinization has already displaced 32 native freshwater fish species, while marine fish species are invading as far as 400 km upstream (Contreras and Lozano, 1994). In addition to salinization, water quality problems include elevated nutrients, bacteria, metals, pesticides, herbicides, and organic solvents (Dahm *et al.*, 2005). Another major change in the Rio Grande in recent years has been the disconnection of the river from the floodplain (Molles *et al.*, 1998);

the fragmentation of river channels by dams, diversions and depletions has eliminated the natural flood pulse, reducing productivity and altering the structure of riparian ecosystems.

Protection

All three endemic species (*P. metnecktayi, T. cognata*, and *P. popeii*) have been added by the Texas Parks and Wildlife Department (TPWD) to the list of state-threatened species in 2010 (Texas Register 35, 2010). However, the state protection only prohibits the 'take' of a state-threatened species. Since 2009 these species are under consideration for federal listing by the USFWS (Federal Register 74, 2009), but listing of these species has not yet been warranted (Federal Register 76, 2011). *Popenaias popeii* was petitioned to the Candidate list as a Federally Endangered Species with Critical Habitat in 2004, and is currently considered a Candidate Species under the Federal Endangered Species Act (Federal Register 71, 2006). Although USFWS encourages conservation of these species, candidate species receive no statutory protection under the Endangered Species Act.

In 1978, a 315 km stretch of the United States side of the Rio Grande along the Mexican border was designated as a National Wild and Scenic River (National Parks and Recreation Act of 1978, Public Law 95–625, November 10, 1978). The river segment begins in, and is administered from, the Big Bend National Park in Brewster County and continues to the Terrell and Val Verde County border, thereby encompassing the area where the extremely rare *P. metnecktayi* and a few specimens of *P. popeii* were found. The Wild and Scenic Rivers Act prohibits federal support for construction of dams, water conduits, reservoirs, or other instream activities that would harm the river's free-flowing condition, water quality, or outstanding resource values. However, the designation neither prohibits development nor gives the federal government control over private property, and does not affect existing water rights. Although the part of the Rio Grande in and above Laredo where we found the only large known population of *P. popeii* has the status of a mussel sanctuary (where mussel harvest is prohibited) (Texas Register 31, 2006), additional protection is urgently necessary as any activity associated with water flow alteration could potentially damage the remaining habitat of *P. popeii*.

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AQUATIC BIOSYSTEMS

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Distribution, genetic analysis and conservation priorities for rare Texas freshwater molluscs in the genera *Fusconaia* and *Pleurobema* (Bivalvia: Unionidae)

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Abstract

Background: Freshwater bivalves in the order Unionoida are considered to be one of the most endangered groups of animals in North America. In Texas, where over 60% of unionids are rare or very rare, 15 species have been recently added to the state's list of threatened species, and 11 are under consideration for federal listing. Due to insufficient survey efforts in the past decades, however, primary data on current distribution and habitat requirement for most of these rare species are lacking, thus challenging their protection and management. Taxonomic identification of endemic species based on shell morphology is challenging and complicates conservation efforts. In this paper we present historic and current distributional data for three rare Texas species, *Fusconaia askewi, F. lananensis,* and *Pleurobema riddellii*, collected during our 2003–2011 state-wide surveys and suggest appropriate conservation measures. In addition, we tested the genetic affinities of *Fusconaia* and similar species collected from eastern Texas and western Louisiana using *cox1* and *nad1* sequences.

Results: We found that *F. askewi* still inhabits four river basins in eastern and northeastern Texas and can be locally abundant, while *P. riddellii* was found only in one river basin. *Pleurobema riddellii* was well-separated from *F. askewi* and grouped with the *P. sintoxia* clade. The sequences for *F. lananensis* were very similar to those for *F. askewi*, with a maximum difference of just over 1% for *nad1* and only 0.7% for *cox1*, similar to the variation between *F. askewi* alleles. Except for one low difference (1.55%) with the partial *cox1* sequence for *F. burkei*, all other *Fusconaia* populations, including those from the Calcasieu drainage, differed by over 2.3% for both genes.

Conclusions: Our study suggested that *F. lananensis* is not a valid species, and it is likely that only one *Fusconaia* species (*F. askewi* or its probable senior synonym *F. chunii*) is currently present in East Texas, thus simplifying conservation efforts. Distribution range of both these regional endemics (*F. askewi* and *P. riddellii*) has been reduced in the last 80 years.

Keywords: Freshwater molluscs, *Fusconaia askewi*, *Fusconaia lananensis*, *Pleurobema riddellii*, Molecular identification, Taxonomy, Distribution, Habitat requirements, Conservation priorities

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Background

Molluscs are among the most threatened groups of animals on the planet [1], and freshwater bivalves in the order Unionoida are considered to be one of the most endangered groups of animals in North America [2-4]. Our long-term state-wide study of Texas mussels revealed that 65% of all Texas unionid species are rare, including all state and regional endemics, and most endemic species are very rare [5]. Being one of the top states in species diversity and endemism, Texas ranks fourth in terms of the number of species extinctions [6]. Damming, pollution, water extraction, and urban development have all negatively affected the freshwaters of Texas [7]. Fifteen rare freshwater mussel species were recently added to the state's list of threatened species [8], and 11 of those are currently under consideration for federal listing by the U. S. Fish and Wildlife Service [9,10].

Biodiversity is a fundamental component of evolutionary potential, and species are the primary targets of the U.S. Endangered Species Act. Conservation laws and methods cannot be implemented until the endangered organism is properly clarified and its geographical range is known [11,12]. In particular, some of these rare species, *Fusconaia flava* (Rafinesque), *F. askewi* (Marsh), and *F. lananensis* Frierson, are currently reported from several drainages west of the Mississippi [13-15], but identifying specimens using shell morphology is challenging. Morphological variation in *Fusconaia* in the lower Mississippi drainage is especially complex [16]. Burdick and White [17] reported an unusual genetic type in *Fusconaia* from the northern and western Ozark region, which could represent a northern extension of *F. askewi*. *Pleurobema riddellii* (Lea) can also be very similar in shell features to *F. askewi* [16]. Johnson [18] synonymized *F. askewi* with *F. flava* (under the name *F. undata*).

In light of the difficulties, we used genetic data as an additional line of evidence. We sampled *Fusconaia* and similar species from river systems in eastern Texas and western Louisiana to test the genetic affinities of the species, using *cox1* and *nad1* sequences. In this paper we describe the geographical distribution and habitat requirements of rare *Fusconaia* spp. and *P. riddellii* and results of molecular genetic analyses to define their biogeography, proper taxonomic status, and suggest appropriate conservation measures.

Methods

Field surveys

In this manuscript we use results of our state-wide survey of unionids in Texas, USA (latitudes 33°50′ - 26°56′, longitudes 102°08′ - 93°31′) from 2003 to 2011 [5,19]. Mussels were surveyed at 463 sub-sites that were pooled into141 major sites, distributed among 66 waterbodies belonging to 11 major drainages in Texas. The study was carried out with an appropriate Scientific Research Permit issued by the Texas Parks and Wildlife Department (TPWD), and landowner permission for wildlife research was acquired from each property owner before entering their property, if the land was privately owned. Abiotic parameters (physical and chemical) were recorded at the sites using a HACH Hydrolab Quanta, measured parameters included: temperature (°C), pH, total dissolved solids (g/L), conductivity (μS/cm), and turbidity (ed. NTU). In addition, we recorded depth and the dominant substrate type using the following classification by particle size: bedrock; large boulders (>45 cm); boulders (>25 - 45 cm); cobble (>6 - 25 cm); gravel (>6 - 60 mm); sand (0.06 - 6 mm); mud/silt (<0.06 mm). Substrates in sampled East Texas sites were represented by sand (32%), sand and gravel (21%), silt (15%), clay (6%), and combinations of these. Unionid sampling was conducted via hand collection of both live and dead mussels, by wading in shallow water and by snorkeling. Due to poor water visibility, tactile searches (running fingers over the sediment, usually up to 15 cm deep, depending on substrate type) were used at all sites. Timed searches were used to detect the presence of mussels and species diversity [20,21] at each site, and if mussel assemblages were present, quantitative methods (from 5 to 28 randomly placed 0.25 m^2 quadrats at a site, in average 9 quadrats covering area of 3.75 m^2), or area -constrained searches (area searched were from 4 to 66 m^2) were used for assessments of density [22,23]. Relative species abundance was calculated as a percentage of live specimens belong to this species collected at a site from the total number of all live mussels found at the same site, and used as an indicator of the species' dominance in mussel assemblages. Collected mussels were identified based on shell morphology, counted, measured with calipers to the nearest mm, and then carefully rebedded into the sediment from which they were taken. Ten specimens of *Fusconaia* sp. from the Neches drainage and 5 from the Sabine drainage were sequenced for *cox1*. Five *Fusconaia* specimens from the Neches drainage (including one not amplified for *cox1*) and 3 from the Sabine drainage were sequenced for *nad1*. Two specimens of *P. riddellii* from the Neches drainage were sequenced for *cox1,* with one of them also sequenced for *nad1*. Voucher specimens were deposited in the Great Lakes Center (Buffalo State College) Invertebrate Collection, in the North Carolina State Museum of Natural Sciences (Raleigh, NC), and in the Invertebrate Zoology Collection of the National Museum of Natural History (Smithsonian Institution, Washington, D.C.). All *Fusconaia* species identified during our study (*F. askewi* and *F. lananensis*) and historical data reported from East Texas (*F. askewii* [24,25], *F. askewi* [15,26-30], *F. flava* [15], *F. lananensis* [31-33], *Quadrula askewi* [34,35], *Q. askewii* [25], *Q. chunii* [25,35], *Q. flava nasuta* [34], *Q. lananensis*

[25,34,36], *Q. undata chunii* [34], *Unio askewii* [24], *U. cerinus* [24,37], *U. chunii* [24,37,38], were considered to be *F. askewi*. For justification see sections "Genetic analysis" in Results and Discussion.

Genetic analysis

Specimens were preserved in ethanol in the field. DNA extraction used Qiagen DNA extraction kits. Portions of the *cox1* and *nad1* genes were amplified. Primers for *cox1* were 5'–GTTCCACAAATCATAAGGATATTGG–3' and 5'–TACACCTCAGGGTGACCAAAAAACCA–3', adapted from Folmer *et al.* [39] and primers for *nadh1* were 5'–TGGCAGAAAAGTGCATCAGATTTAAGC–3' and 5'–GCTATTAGTAGGTCGTATCG–3' [40,41]. The primer LoGlyR (5'–CCTGCTTGGAAGGCAAGTGT ACT–3′) [42] served as an alternate reverse primer for *nadh1*. The forward primer UNIOCOII.2 from Walker *et al.* [43] and/or the reverse primer HCOout (CCAGG TAAAATTAAAATATAAACTTC [44]) provided good amplification for *cox1* for some species. PCR cycles were: 92°C 2 min; 92°C 40 sec 40°C 40 sec 72°C 90 sec 5x; 92°C 40 sec 50°C 40 sec 72°C 90 sec 25x; 72°C 10 min; hold 4°C. PCR products were purified using Qiagen QIAquick PCR purification kits and, if necessary, Qiagen gel extraction kits. Cycle sequencing used ABI Big Dye Terminator kits with thermal cycle parameters of 1°C per second ramp speed, starting with 1 min at 96°C followed by 26 cycles of 96°C for 10 sec, 49°C for 5 sec, and 60°C for 4 min, then 10 min at 60°C and hold at 4°C. The cycle sequencing products were purified with Qiagen DyeEx kits and then run on an automated sequencer.

The results for each strand were compared and aligned using BioEdit [45]. We analyzed the sequences, along with previously published sequences for other representatives of Pleurobemini with TNT [46]. An Additional file 1 contains sequences used for genetic analysis [see Additional file 1]. Maximum parsimony analyses used 500 random replicates, using all the "new technology" methods (sectorial searching, ratchet, drift, and tree fusing), which greatly speed up the process of finding optimal trees over older approaches [46]. Jackknife analyses used 500 replicates, each using a random "new technology" parsimony search of 10 replicates.

Results

Genetic analysis

The sequences for *F. lananensis* were very similar to those for *F. askewi*, with less than 1% difference, similar to the variation between *F. askewi* alleles (Tables 1, 2). However, the sequences for *F. askewi* from the Sabine and Neches drainages differed from all other *Fusconaia* species by over 2.3% for both genes, except for the partial *cox1* sequence for *F. burkei*. In particular, the *cox1* sequences differed by no more than 0.7% between *F. askewi* and *F. lananensis*, typical of within-species variation, but differed by a minimum of over 2.5% from all other *Fusconaia* sequences, except the short sequence for *F. burkei*, fairly normal for species-level differences. The *cox1* sequences from putative *F. askewi* from the Calcasieu River system in Louisiana [47] differed from sequences for *F. flava* and *F. cerina* by less than 2% and in most cases by less than 1% (Table 1). One published sequence for *F. flava* (AF231733, [48] was identical to one of the Calcasieu sequences. Figures 1, 2 and 3 show the phylogenetic analyses. Jackknife percentages close to 100 show strong support for a particular group. As cladograms, their branching sequence provides the important information. Thus, in Figure 1, *Pleurobema (Sintoxia) riddellii* 186TS is modestly supported (51%) as being most closely related to the strongly supported (100%) group including *P. (Sintoxia) sintoxia*, *P. (Sintoxia) cordatum*, and *P. (Sintoxia) rubrum*. Those four in turn are most closely related to the group of the three *Pleuronaia* species. However, this association of *Pleuronaia* and *P. (Sintoxia)* received less than 50% jackknife support and was not supported by all of the analyses. The two *Fusconaia lananensis* have good support (84%) as being each other's closest relative, and there is very strong support (100%) for a group including the Sabine and Neches *F. askewi* as well as *F. lananensis*. In turn, this *F. askewi-lananensis* group has fairly good support (78%) as being most closely related to the group including *F. masoni, F. cerina, F. flava*, the putative *F. askewi* from the Calcasieu, *F. burkei*, and *F. escambia*. The Calcasieu *Fusconaia* specimens are strongly supported (92%) as being most closely related to *F. flava*. In Figure 2, *P. riddelli* again appears to be most closely related to *P. rubrum, P. sintoxia*, and *P. cordatum* 2572, but yet again this result is not well-supported. Multiple branches coming from a single vertical line indicates that the relationship among those branches is unresolved. Figure 2 shows strong support (95%) for a group including the Sabine and Neches *F. askewi* and the *F. lananensis* specimens, but does not tell anything about relationships among those eight sequences. Relationships among the different groups within *Fusconaia* are not well-resolved in Figure 2. Similarly, Figure 3 has strong support (99%) for a group of all of the *F. lananensis* and Sabine and Neches *F. askewi*, but apart from strong support (99%) for a group of *F. askewi* Sab1 and Sab2, does not support any particular relationships within that group. Again, *P. riddellii* receives weak support as being most closely related to *P. sintoxia, P. rubrum*, and *P. cordatum*.

Distribution, densities, size structure, and habitat

Fusconaia askewi

A total of 931 live individuals was collected during our surveys (including 774 mussels originally identified as *F. askewi* and 157 identified as *F. lananensis*) at 25 sites

Table 1 Percent differences in *cox1* sequence for *Fusconaia* species

	***F. askewi* 3392**	***F. askewi* 3395**	***F. askewi* Sab1 2**	***F. askewi* Sab3**	***F. askewi* Sab4**	***F. askewi* Sab5**	***F. askewi* TS131 133**	***F. askewi* TS166**	***F. askewi* TS233 130 204**	
F. askewi 3395	0.16									
F. askewi Sab1 2	3.94	4.12								
F. askewi Sab3	4.23	4.41	0.36							
F. askewi Sab4	4.48	4.68	0.57	0.19						
F. askewi Sab5	4.03	4.23	0.59	0.20	0.39					
F. askewi TS131, 133	4.08	4.24	0.35	0.54	0.57	0.59				
F. askewi TS166	2.72	2.64	0.53	0.55	0.60	0.32	0.43			
F. askewi TS233 130 204	3.73	3.91	0.35	0.18	0.19	0.20	0.30	0.22		
F. burkei	2.47	2.69	2.51	3.07	3.05	3.48	2.93	1.55	2.70	
F. cerina	1.16	1.54	4.49	4.80	5.09	4.65	4.59	3.57	4.26	
F. cerina LA	0.66	0.92	3.76	4.04	4.29	3.83	3.76	2.87	3.44	
F. cor	4.77	4.65	4.88	5.20	5.53	5.53	5.03	4.05	4.85	
F. cor 2606	4.60	4.55	4.71	5.02	5.34	5.33	4.92	3.97	4.75	
F. cuneolus	4.26	4.24	3.60	3.88	3.91	3.85	3.94	2.65	3.62	
F. escambia	10.37	10.63	10.03	10.63	10.61	10.84	10.40	7.39	10.40	
F. flava H1681	0.16	0.47	3.76	4.04	4.28	3.82	3.73	2.55	3.40	
F. flava MO	0.33	0.61	3.94	4.23	4.48	4.03	3.92	2.86	3.59	
F. flava 1	0.66	0.62	4.14	4.62	4.91	4.46	4.13	2.92	3.97	
F. hebetata? Ff8	3.73	4.14	3.32	3.42	3.68	3.07	3.39	3.73	3.00	
F. hebetata? Ff9	3.09	3.56	3.56	3.90	4.20	3.87	3.59	3.99	3.20	
F. lananensis TS129 132 179 203	3.73	3.91	0.70	0.54	0.57	0.59	0.61	0.43	0.30	
F. masoni	2.51	2.78	3.58	3.48	3.69	3.62	3.44	2.87	3.12	
F. ozarkensis	4.24	4.22	4.32	4.62	4.90	4.87	4.41	3.79	4.08	
F. ozarkensis 3501	4.76	4.70	4.87	5.18	5.50	5.50	4.89	4.02	4.57	
F. subrotunda 1554	4.25	4.39	4.52	4.82	5.11	4.67	4.42	3.56	4.42	
F. subrotunda PA l	4.07	4.56	4.33	4.62	4.91	4.67	4.59	3.79	4.59	
F. subrotunda PA s	4.77	4.87	4.88	4.80	5.09	4.87	4.41	3.55	4.40	
	F. burkei	*F. cerina*	*F. cerina* LA	*F. cor*	*F. cor* 2606	*F. cuneolus*	*F. escambia*	*F. flava* H1681	*F. flava* MO	*F. flava* 1
F. cerina	3.15									
F. cerina LA	2.69	1.24								
F. cor	4.36	4.83	4.65							
F. cor 2606	4.36	4.59	4.39	0.17						
F. cuneolus	4.11	4.27	4.08	2.55	2.25					
F. escambia	8.61	11.68	10.63	11.53	11.53	11.23				
F. flava H1681	2.24	0.95	0.48	4.47	4.22	3.91	10.13			
F. flava MO	2.24	1.23	0.61	4.65	4.39	4.08	10.13	0.16		
F. flava 1	2.69	1.56	0.93	4.82	4.44	4.14	10.11	0.48	0.62	
F. hebetata Ff8	2.99	3.41	3.76	5.09	5.16	4.55	9.52	3.33	3.76	4.22
F. hebetata Ff9	2.38	2.82	3.18	4.43	4.54	4.15	8.84	2.73	3.18	3.62
F. lananensis TS129 132 179 203	2.93	4.26	3.44	4.85	4.74	3.61	10.67	3.40	3.59	3.97

Table 1 Percent differences in *cox1* sequence for *Fusconaia* species *(Continued)*

F. masoni	2.24	3.12	2.47	4.65	4.55	4.40	9.89	2.24	2.47	2.82
F. ozarkensis	3.39	4.25	3.90	4.84	4.57	4.25	10.91	3.89	3.90	4.28
F. ozarkensis 3501	3.84	4.73	4.38	5.37	5.07	4.73	11.16	4.38	4.38	4.77
F. subrotunda 1554	3.64	4.59	4.24	3.95	4.06	4.08	10.13	3.90	4.23	4.46
F. subrotunda PA l	3.40	4.59	4.41	3.59	4.07	4.25	10.13	3.90	4.40	4.46
F. subrotunda PA s	4.10	5.07	4.72	3.95	4.06	4.24	10.91	4.39	4.71	4.95

	F. hebetata Ff8	*F. hebetata* Ff9	*F. lananensis* TS129 132 179 203	*F. masoni*	*F. ozarkensis*	*F. ozarkensis* 3501	*F. subrotunda* 1554	*F. subrotunda* PA l
F. hebetata Ff9	1.30							
F. lananensis TS129 132 179 203	3.00	3.20						
F. masoni	2.99	2.41	3.43					
F. ozarkensis	4.15	3.57	4.40	3.58				
F. ozarkensis 3501	4.54	3.95	4.89	4.06	0.46			
F. subrotunda 1554	4.36	4.16	4.42	3.91	4.24	4.72		
F. subrotunda PA l	4.76	4.17	4.59	3.76	4.41	4.89	1.24	
F. subrotunda PA s	4.75	4.35	4.40	4.23	4.72	5.21	1.23	1.24

in 17 East Texas counties (Anderson, Angelina, Cherokee, Hardin, Harrison, Houston, Jasper, Leon, Nacogdoches, Panola, Rusk, San Augustine, Shelby, Smith, Titus, Tyler, and Upshur) (Table 3, Figure 4B). We found *F. askewi* in four drainages (Neches, Trinity, Sabine, and Red river basins) in eastern and northeastern Texas. *Fusconaia askewi* was locally very abundant in Village Creek (Neches River basin), Neches, Sabine, Trinity and Angelina (Neches River basin) rivers, and in the Big Cypress Bayou (Red River basin). On average, *F. askewi* was the third most abundant species, and the number of live *F. askewi* collected at a particular site, on average, comprised 22% of the total number of all live mussels found at that site. Average density in mussel aggregations was 6.7 m^{-2} (Table 3). Sites with the greatest abundance were on Village Creek and the Neches and Sabine rivers. The most typical substrate for the species was sand, then a mixture of sand and silt, and gravel with sand. Average shell length of live *F. askewi* was 59.2 ± 0.6 mm (mean ± standard error here and elsewhere unless noted). Based on the presence of juveniles (Figure 5), the populations in East Texas were reproducing (shell length varied from 17 to 90 mm). Nevertheless we failed to find *F. askewi* in several waterbodies belong to the species' former distribution range: in the San Jacinto River, its tributaries, and in Lake Houston, as well as in its historical location in Kickapoo Creek (North of Brownsboro, Henderson Co. [34] (Figure 4). Likewise, we did not find the species in any of the 6 reservoirs on the Trinity River and its tributaries. Our surveys also confirmed that *F. askewi* has been extirpated from Lanana and Bonita creeks (type localities for *F. lananensis*).

Only one dead shell and one valve of mussels identified as *F. flava* were found during our surveys, at two sites in the Sulphur River (Red River drainage), in Red River County and in Delta/Hopkins counties. Live individuals resembling *F. flava* have recently been collected in the East Fork of the Trinity River approximately 70 km from Dallas [54]. Mussels from the Sulphur River and the Trinity River have not been genetically tested yet.

Pleurobema riddellii

During our surveys, we found 132 live *P. riddellii* at 10 sites in 5 Texas counties (Anderson, Angelina, Cherokee, Hardin, and Nacogdoches), in the Neches, and Angelina rivers, and in Village Creek (Figure 6B, Table 3). Average density of *P. riddellii* was 1.9 m^{-2}, and the species was not dominant in local unionid assemblages (the average relative abundance of *P. riddellii* was 5%, Table 3). Most often *P. riddellii* was found in sand, silty sand, and sometimes in a mixture of sand and clay. Mean and median *P. riddellii* length were 52.4 ±1.1 mm, range - 39–82 mm (Figure 5). The largest density was found in the Neches River south of Neches (Anderson Co.) in sand and gravel; this population had many juveniles (< 25 mm long) in 2009 (Barclay unpublished data).

Habitat requirements

We found that *F. askewi* and *P. riddellii* have similar distribution (Table 3) and very similar habitat requirements. All these species were found exclusively in lotic waters, in relatively shallow areas (at 0.2 - 1.5 m depth),

Table 2 Percent differences in *nad1* sequence for *Fusconaia* species

	F. askewi 3391	*F. askewi* 3392	*F. askewi* Sab1	*F. askewi* Sab2	*F. askewi* Sab5	*F. askewi* TS219	*F. askewi* TS233	*F. burkei*	*F. cerina*	
F. askewi 3392	0.24									
F. askewi Sab1	3.85	3.84								
F. askewi Sab2	3.80	3.79	0.26							
F. askewi Sab5	3.00	2.99	1.04	1.02						
F. askewi TS219	3.10	3.07	1.18	1.18	0.33					
F. askewi TS233	3.48	3.47	1.59	1.58	0.79	0.51				
F. burkei	2.39	2.39	3.34	3.10	2.58	2.51	3.19			
F. cerina	1.37	1.24	3.96	4.04	3.24	3.07	3.60	2.45		
F. cor	4.68	4.66	6.06	5.77	5.04	6.12	5.93	4.34	4.28	
F. cuneolus	4.51	4.49	6.23	6.12	5.57	6.11	6.29	4.01	4.62	
F. escambia	2.71	2.58	3.97	3.92	3.38	3.43	3.88	0.63	3.00	
F. flava	0.49	0.61	3.43	3.39	2.59	2.91	3.07	2.55	1.49	
F. lananensis TS129 TS179	2.71	2.69	0.91	0.90	0.13	0.17	0.66	2.71	3.12	
F. lananensis TS203	2.85	2.83	1.04	1.02	0.25	0.17	0.79	2.89	3.25	
F. masoni	2.55	2.54	4.17	4.17	3.34	3.24	3.92	2.32	2.81	
F. ozarkensis	4.38	4.34	5.50	5.15	4.61	5.19	4.86	4.53	4.69	
F. subrotunda	5.52	5.50	7.56	7.42	6.50	6.68	7.07	5.35	5.66	
F. subrotunda PA l	4.75	4.72	6.43	6.35	5.52	5.55	5.96	4.70	5.06	
F. subrotunda PA s	4.85	4.84	6.30	6.21	5.39	5.56	5.69	5.70	5.21	

	F. cor	*F. cuneolus*	*F. escambia*	*F. flava*	*F. lananensis* TS129 TS179	*F. lananensis* TS203	*F. masoni*	*F. ozarkensis*	*F. subrotunda*	*F. subrotunda* PA l
F. cuneolus	4.33									
F. escambia	4.50	4.17								
F. flava	4.50	4.66	2.82							
F. lananensis TS129 TS179	5.33	5.15	3.44	2.32						
F. lananensis TS203	5.54	5.36	3.59	2.46	0.12					
F. masoni	5.07	5.07	3.08	2.41	3.21	3.34				
F. ozarkensis	6.18	5.67	5.00	4.23	4.33	4.49	5.32			
F. subrotunda	6.17	5.16	5.51	5.67	6.17	6.39	6.21	7.04		
F. subrotunda PA l	6.19	5.18	5.24	4.85	5.21	5.36	5.71	6.68	1.26	
F. subrotunda PA s	6.21	5.68	5.62	4.97	5.08	5.21	5.85	6.57	1.30	1.11

and the most preferable substrates for both *F. askewi* and *P. riddellii* were sand, and combinations of sand with gravel and silt. Total dissolved solids among waterbodies studied varied from 0.10 to 0.15 g/L, turbidity – from 18.9 to 66.9 ed. NTU, pH – from 6.38 to 8.21. The lowest pH was recorded in Village Creek (average of 4 measurements in 2005 and 2007: 6.64 ± 0.24 (standard deviation), minimal 6.38 ± 0.12) and in Sandy Creek (6.69 ± 0.006). Minimal pH value for the studied rivers and creeks recorded from 1973 to 2009 was 4.8 (4.8 for Village Creek, 5.4 for the Angelina River, 5.6 for the Neches River, and 5.7 for Attoyac Bayou; data from the Texas Commission on Environmental Quality database (TCEQ Data Management and Analysis, Water Quality Planning Division), measured 4–12 times a year). This low pH caused heavy erosion of *F. askewi* shells, as it was previously recorded for *Corbicula fluminea* inhabiting acidic waters (streams with pH 5.6) [55]. In a few extreme cases, shells were eroded to the extent that the mussels' soft tissues were visible.

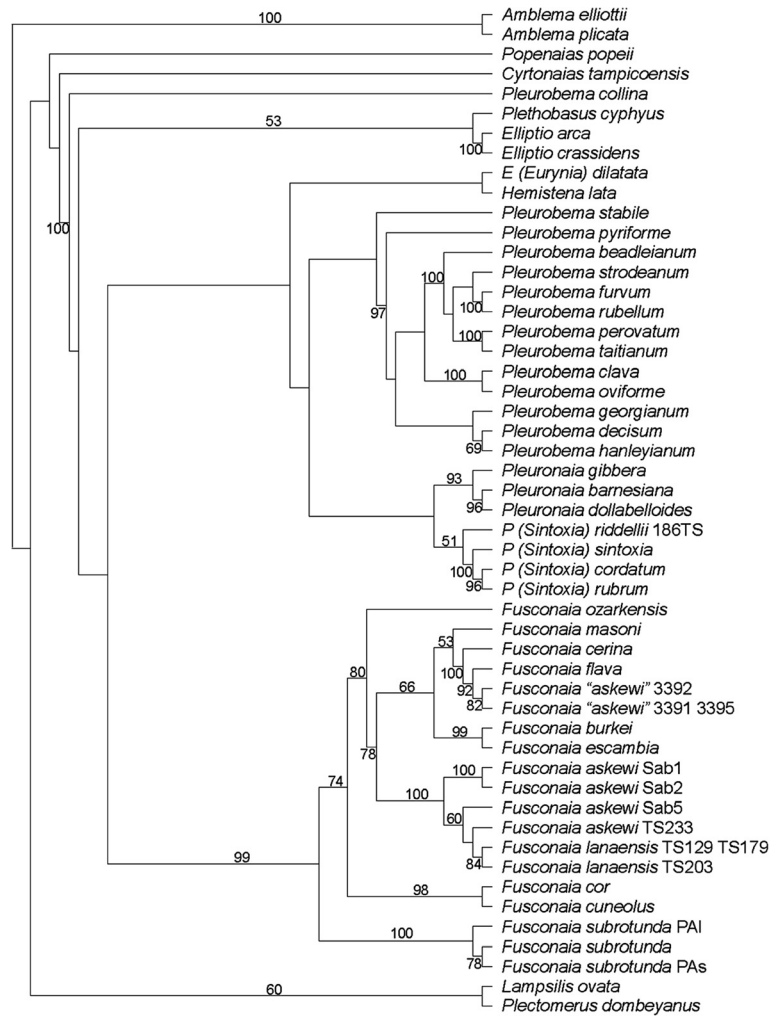

Figure 1 Strict consensus cladogram, combined *cox1* and *nad1* data, with jackknife percentages shown if over 50%. See text for discussion.

Discussion

Our surveys documented the current distribution and change in historical range, densities, and preferred habitat of rare Texas species. Genetic analysis revealed that: (1) *F. lananensis* is not a valid species; (2) it is likely that only one *Fusconaia* species (*F. askewi*) is currently found in East Texas; (3) the presence of *F. flava* in East Texas is unlikely, however the species may still persist in the Red River basin and upper Trinity River; (4) *P. riddellii* was well-separated from *F. askewi* and instead grouped with the *P. sintoxia* clade.

Genetic analysis

We found that the specimens from the Sabine and Neches drainages were genetically distinct from all other currently recognized *Fusconaia* species, as well as from the unusual sequences obtained by Burdick and White [17], and represented a distinct species. The relatively low percent difference from *F. burkei* reflects the shorter sequence for *F. burkei*, which consistently has a low difference from other sequences. Apart from it, all other *Fusconaia cox1* sequences differed from *F. askewi* and *F. lananensis* by more than 3.5 times as much as the largest difference within the *F. askewi-F. lananensis* group. In contrast, putative *F. askewi* sequences from the Calcasieu River in Louisiana matched closely sequences for *F. flava,* strongly suggesting that this population belongs in *F. flava* rather than *F. askewi*. The Calcasieu River runs between the Mississippi (specifically, the Red River) and the Sabine drainages, so faunal exchange could occur in either direction. Study of additional populations would be necessary to determine whether *F. askewi* is also present in the Calcasieu system or anywhere else east of the Sabine drainage.

All analyses strongly supported a group of *Fusconaia lananensis* and *F. askewi* (excluding the Calcasieu

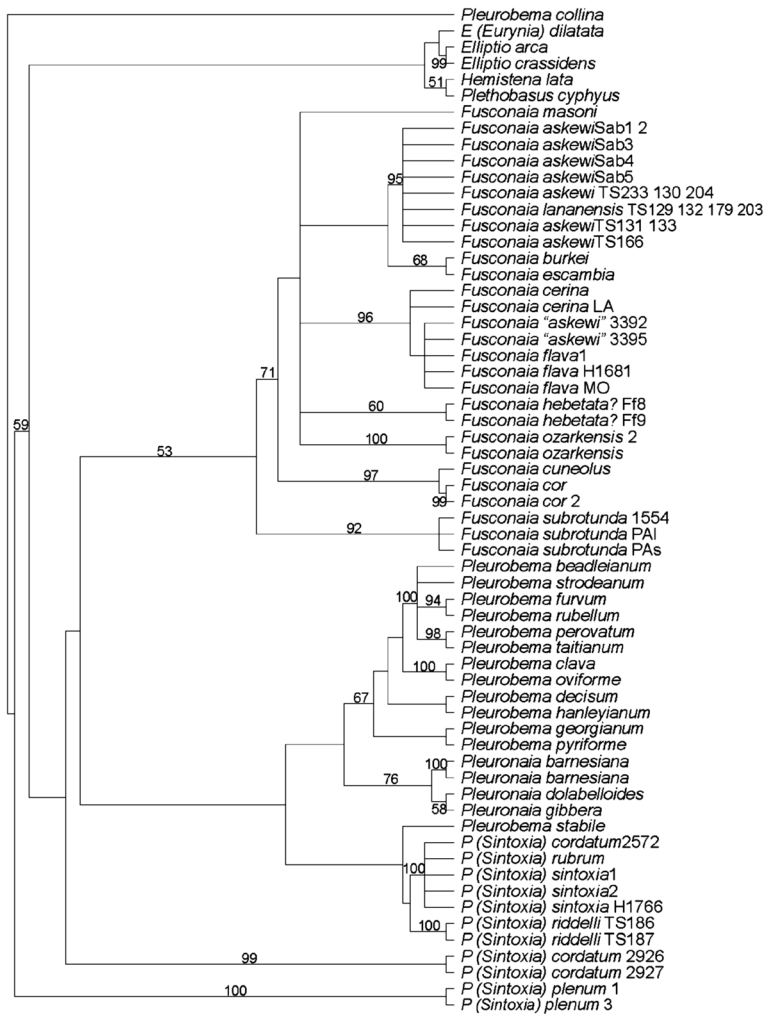

Figure 2 Strict consensus cladogram, *cox1* data, with jackknife percentages shown if over 50%.

specimens). None of the analyses separated *F. askewi* from *F. lananensis*. Along with the low percentage difference (especially within the Neches drainage) and presence of morphologically intermediate specimens, this suggests that the *F. lananensis* is a subjective junior synonym of *F. askewi.* The distinguishing features noted by Frierson [36] would represent individual variation. Conversely, the specimens from the Calcasieu drainage are consistently strongly supported as closely related to *F. flava* and *F. cerina*. Current molecular data do not clearly distinguish between *F. cerina* and *F. flava* [17,47], so the Calcasieu population should probably be regarded as representing *F. flava*. The variations between Figures 1, 2, 3 show that relationships within *Fusconaia* are not well-resolved. Although the support is not strong, all analyses agree that *F. subrotunda* is basal, followed by a clade of *F. cor* and *F. cuneolus*. The remaining *Fusconaia* species, including *F. askewi* and *F. lananensis*, form a group with generally poorly resolved internal relationships. Thus, *F. askewi* and *F. lananensis* clearly belong in *Fusconaia*, are distinct from other currently recognized species (except each other), and are most closely related to the *F. cerina-F. flava* group, the *F. escambia-F. burkei* group, *F. masoni, F. ozarkensis*, and the unidentified *flava*-like *Fusconaia* from the Ozark region (*hebetata?*). Support for the genus *Fusconaia* is modest in the *cox1* only analysis (perhaps due to the partial sequences) but very high in the others. However, relationships of *Fusconaia* to other genera of Pleurobemini are poorly resolved, and the weakly supported relationships between genera are not consistent between analyses.

Pleurobema riddellii shows consistent but weakly supported affinity for members of the subgenus *Sintoxia-P. sintoxia, P. rubrum*, and *P. cordatum*. However, the *cox1* analysis shows that other specimens identified as *P. cordatum* are more distantly related to this group. This may reflect the difficulties of identifying species in the *P. cordatum* group. Ongoing genetic work on this group [56] shows

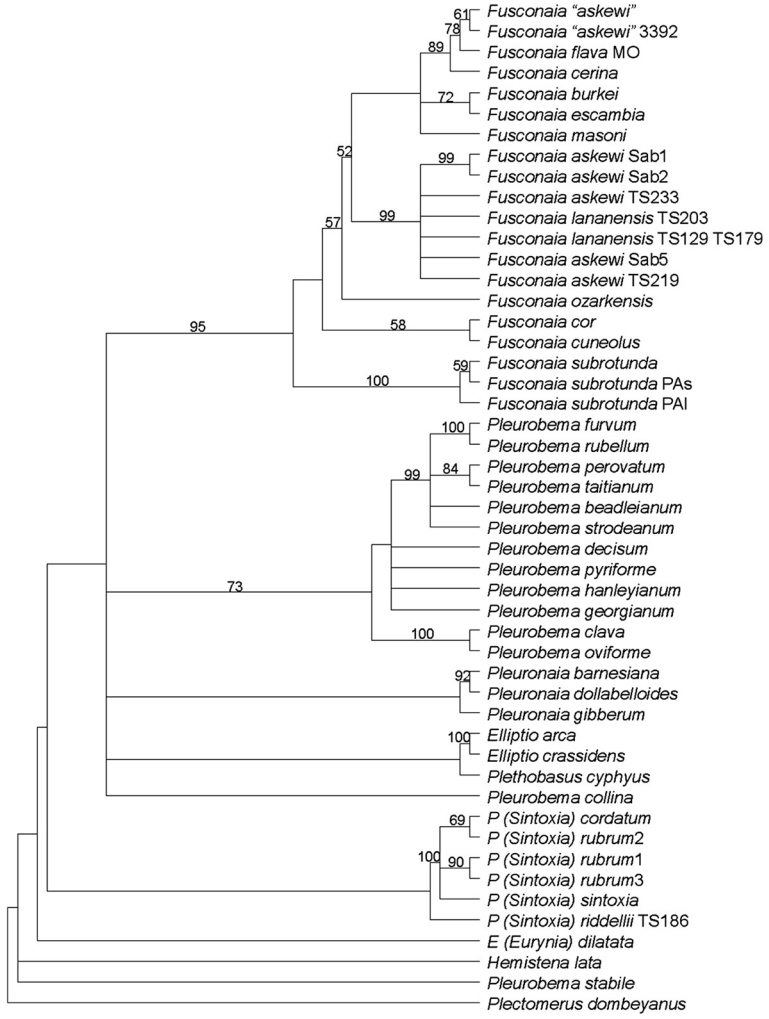

Figure 3 Strict consensus cladogram, *nad1* data, with jackknife percentages shown if over 50%.

further complications, but the morphological similarities of *P. riddellii* to the *P. cordatum* group [57] supports a relationship. Additionally, the only species of *Pleurobema* that occur in the lower Mississippi drainage are from the *P. cordatum* group [13], so the relationship also makes biogeographic sense.

At least four names older than *F. askewi* are available for *Fusconaia* species west of the Mississippi, besides *F. flava,* which was described from the Ohio drainage but occurs also in the upper Mississippi and west of it. *Fusconaia ozarkensis* (Call) is genetically and morphologically distinctive, but the remaining species have all been synonymized with or confused with *F. flava*: *Fusconaia fulgidus* (Lea), from the Red River at Alexandria, Louisiana; *F. hebetata* (Conrad), from Missouri (unfortunately, no information on which drainage); *F. chunii* (Lea), from the Trinity River at Dallas, Texas; and *F. friersoni* (Wright), from Bayou Pierre in the Red River system, De Soto Parish, Louisiana. Although the first three are generally regarded as synonyms of *F. flava* [16], as older names they would have priority over *F. askewi*; *F. friersoni* was published just before *F. askewi*, but appears to be a synonym of *P. riddellii* instead [49]. Burdick and White [17] sampled one population from the lower Red River drainage near Alexandria and found it genetically similar to *F. flava*. The present results for the Calcasieu system also suggest that *F. flava* occurs in the lower Red River system. Graf and Cummings [57] suggested that *F. hebetata* might be a valid species. Study of the populations in the Ozark region, building on the work of Utterback [58] and Graf [16], should determine whether the conchological variation in populations in this region can be correlated with the genetic divergence found by Burdick and White [17]. If so, *F. hebetata* and other names based on material from the Ozark region can be assigned to the appropriate population. However, as Burdick and White's [17] sequences are quite distinct from those

Table 3 Historical and current distribution, and densities of *Fusconaia askewi* and *Pleurobema riddellii* in Texas

Habitat characteristics	*F. askewi*	*P. riddellii*
Distribution (Literature data)	Angelina River, Attoyac Bayou, Bonita Creek, Lanana Creek, Cypress Bayou, Cypress River, Big Lake, Big Creek, Chambers Creek, Lake Fork Creek, Navasota River, Kickapoo Creek, Neches River, Sabine River, Sandy Creek, San Jacinto River, Trinity River, Village Creek and tributaries [14,15,24,26-29,31,34-36,49-53]	Angelina River, Big Lake, Kickapoo Creek, Sabine River, San Jacinto River, Trinity River, Village Creek and tributaries, Chambers Creek [15,24,30,31,34,35,37]
Current distribution (Our data)	Angelina River (27), Attoyac Bayou (25), Sandy Creek (52), Big Cypress Bayou (2), Neches River (274), Sabine River (129), Trinity River (36), Village Creek (386)	Angelina River (9), Neches River (86), Village Creek (37)
Density, m^{-2}	6.7 ± 12.8 (data from 7 sites, 89 quadrats total)	1.9 ± 1.2 (5 sites, 49 quadrats)
Relative abundance, %	22 (1 – 58)	5 (1 – 13)

Amount of live molluscs found in each waterbody during this study is in parentheses. Densities in mussel assemblages (mean ± standard deviation) were calculated using 0.25 m^2 quadrats. Relative species abundance (mean and range in parentheses) was calculated as a percentage of live specimens belong to this species collected at a particular site from the total number of all live mussels found at this site, and used as an indicator of the species' dominance in mussel assemblages.

obtained in the present study for *F. askewi*, it seems safe to assume that *F. hebetata* is not applicable to the present material from Texas and Louisiana.

This leaves *F. chunii* as a possible senior synonym of *Fusconaia askewi* and *F. lananensis*. Howells *et al.* [14] synonymized *F. chunii* with *F. flava*, but Graf [16] identified their illustrated *F. "flava"* from Texas as different from true *F. flava*. We were unable to obtain live specimens from the Red River systems in Texas for genetic analyses. Specimens suggestive of *F. flava* from the Neches drainage,

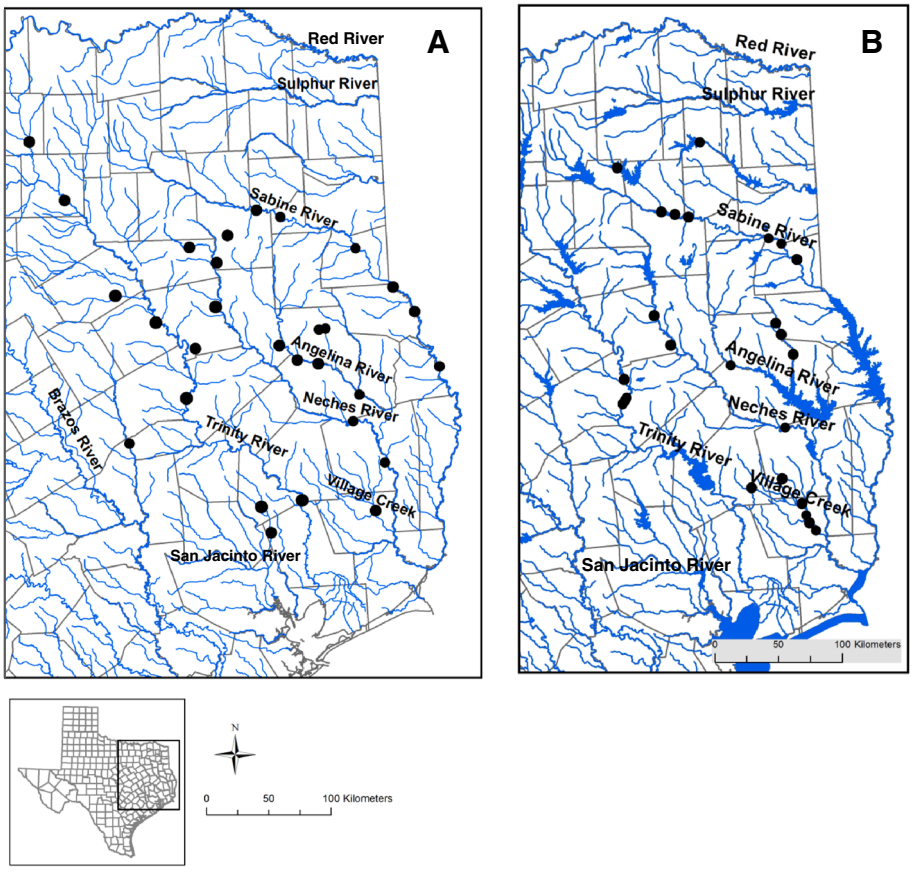

Figure 4 Historical (before 1940, A) and current (1990-present, B) distribution of *Fusconaia askewi* in East Texas. Historical data are from Pilsbry [38], Singley [37], Frierson [24], Frierson [36], Frierson [25], Strecker [34], and Bachtel [35]. Current data include authors' data and literature records [15,26-33].

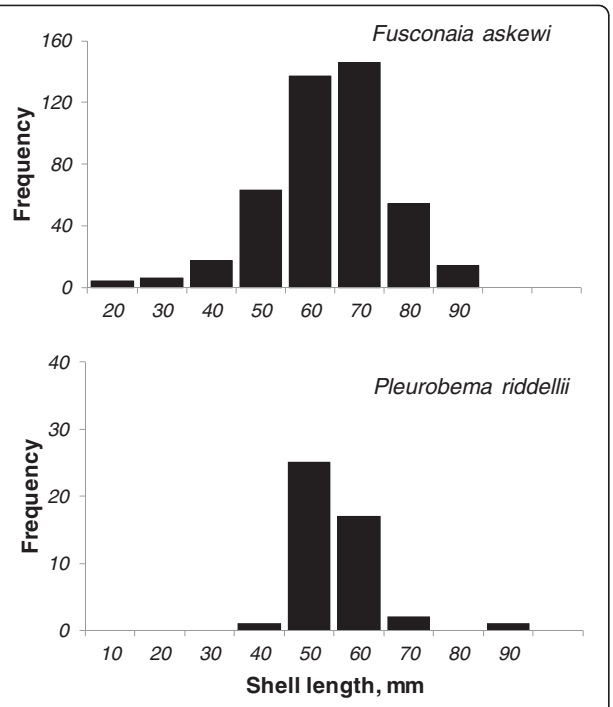

Figure 5 Size-frequency distribution of live *Fusconaia askewi*, and *Pleurobema riddellii* shell length.

sampled in the present study, placed genetically with *F. askewi*. The Trinity system is immediately west of the Neches and the headwaters of the Sabine, and could easily have exchanged species through stream capture or other interaction. Stream capture occurs when a stream previously connected to one drainage system becomes connected to another, eventually becoming a part of the second drainage system [59]. However, the Trinity River headwaters also adjoin the Red River system in northern Texas. The lower Red River system in Louisiana has *F. flava* [17]. To the north of the Red River system is the Arkansas system, and the possible *F. hebetata* haplotype occurs in an Arkansas tributary. The picture is thus very complex, but it seems most likely that *F. chunii* is a senior synonym of *F. askewi*.

In contrast to the varying opinions on *Fusconaia* species, authors have generally agreed on recognizing *Pleurobema riddellii*. However, there has been some uncertainty about its affinities [13]. The present results provided moderate support for Frierson's [60] suggestion that it is relatively closely related to the *Pleurobema cordatum* group. Most other work on this group has focused exclusively on the Mississippi drainage species and does not mention *P. riddellii*.

Distribution, densities, size structure, and habitat

Fusconaia askewi

F. askewi is a regional endemic, historically known from the Sabine, Neches, Trinity and San Jacinto rivers in Texas [38] (Table 3, Figure 4A), and from Louisiana [13]. Simpson [50] lists *F. askewi* range from western Louisiana to eastern Texas with type locality as Village Creek, Hardin Co., and the Sabine River, Texas. Strecker [34] recorded this species in the Angelina, Sabine and Navasota rivers, and from Kickapoo Creek. Neck [49] reported *F. askewi* as locally common, but noted that the status over its entire range was unclear. During our surveys we found live *F. askewi* in four drainages in eastern and northeastern Texas (Table 3, Figure 4B). This species was locally abundant, often dominated mussel assemblages, and several populations were reproducing. The most typical substrate for the species was sand, sand and silt, and gravel with sand.

Fusconaia lananensis was described by Frierson in 1901 [36], after the first account of Texas unionids was published [37]. Frierson collected 200 specimens of *F. lananensis* from Lanana and Bonita creeks near Nacogdoches, Texas [36]. Strecker [34] found live *F. lananensis* in Lanana Creek, and in the San Jacinto River. In 1990s, few live mussels were found in Attoyac Bayou and Sandy Creek (Angelina River drainage) [51], and 36 live mussels were found in Village Creek [15]. We found live mussels that fit the description of "*F. lananensis*" in several waterbodies in East Texas. Due to the similar shell morphologies of *F. askewi* and *F. lananensis*, field identification between the two nominal species was very challenging, which is not surprising considering their genetic similarity. Frierson [36] reports that "*Q[uadrula] lananensis* is closely allied to *Q. askewi* Marsh, both by its conchological and anatomical characteristics. It may be differentiated from that shell by being longer, more compressed, more oblique, and its shell is never so inflated and thickened in front as *askewi* and not so acutely angled on the posterior ridge. Internally, *lananensis* is rose-colored nearly invariably and the color is uniformly spread over its surface. *Askewi* is mostly white, and, when colored (pink) the color is almost always confined exterior to the pallial line. Finally, *Q. askewi* never possess those peculiar pearly excrescences, which seem to belong to *lananensis*". We observed several patterns in nacre coloration of *Fusconaia* from East Texas drainages. There were three forms recorded in the Neches drainage: with entirely white nacre, solid rose/pink, and the form with the pink extrapallial ring described by Frierson [36]. Practically the entire *Fusconaia* population in the Sabine River had white nacre, while almost none of the Trinity *Fusconaia* showed the pink extrapallial ring (most of them were white, and a few - solid pink). Therefore, we saw the same features (e.g., pearly excrescences and rose-colored nacre) in both species, with many intermediate forms that were impossible to separate, suggesting that *F. lananensis* may not be a valid species. This suggestion was supported by our genetic analysis. Habitat and substrate preferences of both *Fusconaia* spp. were found to be similar as well.

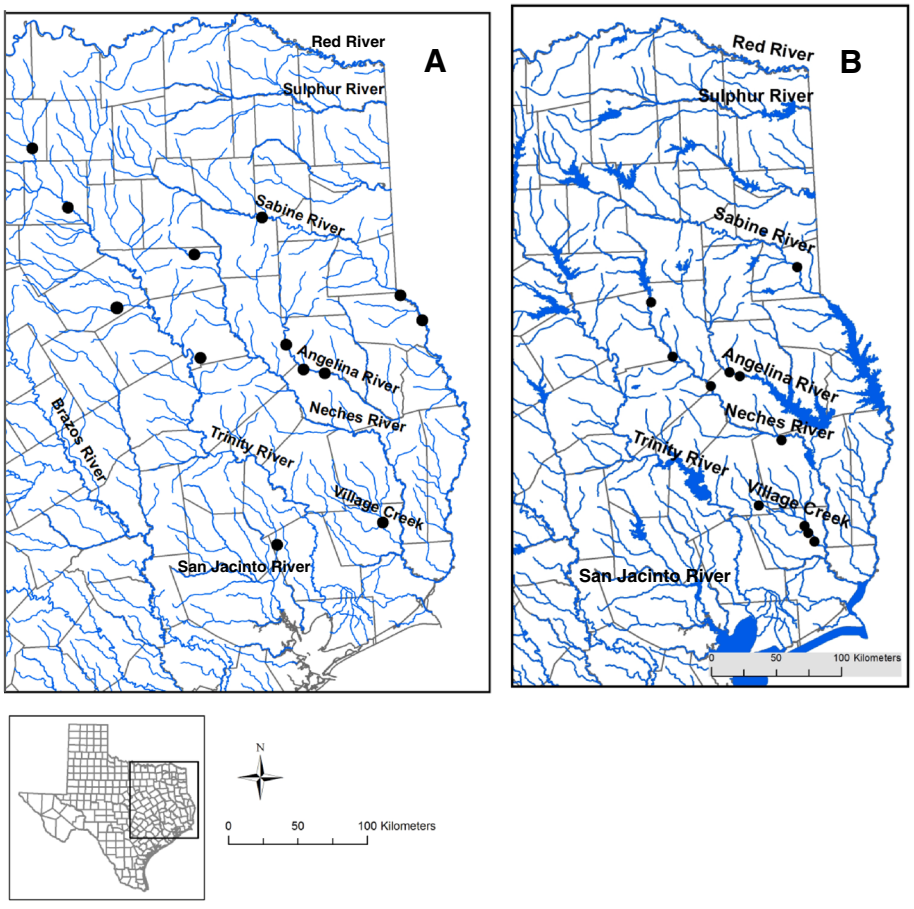

Figure 6 Historical (before 1940, A) and current (1990-present, B) distribution of *Pleurobema riddellii* in Texas. Historical data are from Frierson [24], Strecker [34], Bachtel [35]. Current data include authors' data and literature records [15,30,31].

Pleurobema riddellii

This species is a regional endemic, found in Texas and Louisiana [14,51]. Singley [37] recorded *P. riddellii* in Village Creek only; Strecker recorded the species from the Angelina, Sabine, San Jacinto and Trinity rivers in East Texas [34] (Figure 6A). NatureServe reports a substantial recent decline in this species [61]. During our surveys, we found a total of 132 live *P. riddellii* in one East Texas river basin (the Neches River), but not at the sites we surveyed on the Trinity River (Figure 6B). *Pleurobema riddellii* has probably been extirpated from the San Jacinto River. This species was not locally abundant, and not dominant in mussel assemblages. Although most populations were comprised of older animals, several populations were reproducing. *Pleurobema riddellii* was found exclusively in lotic waters, in relatively shallow areas, most often in sand, or in a mixture of sand, gravel and silt.

Conservation priorities

Fusconaia askewi

The American Fisheries Society considers *F. askewi* and *F. lananensis* to be of special concern [4], and both species are currently listed as state threatened [8] and as near-threatened by the IUCN [62]. Our recent surveys classified these species as rare (species that were found at low densities in 1 to 9 Texas waterbodies) based on their occurrence and density [5]. The U.S. Fish and Wildlife Service found that substantial scientific information was presented indicating that listing of *F. lananensis* may be warranted due to the present or threatened destruction, modification, or curtailment of its habitat or range [10], and a status review for the species was initiated in 2009. However, our study suggested that *F. lananensis* is not a valid species and it is likely that only one *Fusconaia* species (*F. askewi*, senior synonym *F. chunii*) is currently present in East Texas, thus simplifying conservation efforts. Although we found that *F. askewi* still inhabits four river basins in eastern and northeastern Texas and can be locally abundant, its distribution range has been reduced in the last 80 years: the species have been extirpated from a number of waterbodies in Texas, including Lanana and Bonita creeks, the San Jacinto and Navasota rivers, and Kickapoo Creek (Figure 4). The distribution of *F. askewi* in the Trinity River has been also reduced in the

last 40 years (Figure 4). The species has been extirpated from much of its former range in the upper Trinity River north of SR-7 (Leon/Houston Counties), and appears to be completely absent from the river south of Lake Livingston (D. Barclay, personal observations).

Pleurobema riddellii

This species was found in only one East Texas drainage (the Neches River), and at very low densities. During the last 80 years the distribution range of *P. riddellii* has been dramatically reduced, and this species has been extirpated from several East Texas waterbodies where it occurred historically (Figure 6). Notably, some of these waterbodies (e.g., San Jacinto River) that lost both *F. askewi* and *P. riddellii*, are the most highly populated in Texas [19]. At the beginning of 20th century, the San Jacinto River was a home for 29 unionid species, but due to extensive mining, deforestation, damming and urbanization, it lost almost 70% of its former unionid diversity [19]. The U.S. Fish and Wildlife Service found that listing of *P. riddellii* as threatened or endangered may be warranted due to the present or threatened destruction, modification, or curtailment of its habitat or range resulting from general human modification of the water and adjacent land, siltation, impoundments, and water pollution [9,10], however it is currently listed as threatened only at the state level [8].

Currently East Texas has predominantly forested watersheds with little urbanization, both factors being important for maintaining the health of aquatic environments [63]. Not surprisingly, this part of Texas is the hotspot for the state's unionid diversity where almost every river supports from 17 to 28 species [19]. However, Texas is one of the fastest growing states in the nation. The urban population in Texas nearly doubled in the last 30 years [64], with a 21% increase in urbanization since 1990 [65]. Along with growing urbanization, it is predicted that $>$ 20 million ha of U.S. forest will be developed over the next 50 years [66,67], and $>$ 11% of private forests, mostly in the South, could experience substantial increases in housing density by 2030 [68,69]. Considering growing development and water demand, the best measure for conservation of both *F. askewi* and *P. riddellii* would be by controlling deforestation, urbanization and water diversion in East Texas watersheds, and particularly the Neches River.

Additional file

Additional file 1: Sequences used for genetic analysis [42,47,48,56,70-78].

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LEB and AYK designed the study and surveyed sites state-wide. DB surveyed additional sites in East Texas. DC carried out the molecular genetic studies and their interpretation. LEB, AYK and DC led, and DB edited the writing. All authors read and approved the final manuscript.

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