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Molecular data validate historical and contemporary distributions of *Pleurobema riddellii* (Bivalvia: Unionidae) and help guide conservation and recovery efforts

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ABSTRACT: Accurate taxonomic and distributional information are arguably the most critical components of conservation status assessments but can be greatly affected by misidentifications. The Louisiana pigtoe *Pleurobema riddellii* is a freshwater mussel proposed as threatened under the US Endangered Species Act. The species belongs to the tribe Pleurobemini, which includes multiple taxa that are inherently challenging to identify without molecular data. We validated historical and recent survey records of *P. riddellii* using a combination of DNA sequence data and morphological characters to provide a more definitive assessment of range and spatiotemporal trends in distribution. Our comprehensive assessment identified specimens collected from the Pearl drainage as *P. riddellii*, extending the species' known range into eastern Gulf of Mexico drainages. Contemporary records were unavailable from the Trinity drainage; however, we designed novel minibarcode PCR primers and used historical DNA from a specimen collected in the late 1800s to confirm the historical presence of *P. riddellii* at the species' type locality in the Trinity River near Dallas, Texas, USA. Our range-wide genetic diversity assessment provides strong support for 2 main geographic groups, the Ouachita and all remaining populations, with individuals from the Pearl and Trinity drainages sharing haplotypes with conspecifics from other drainages. Available data suggest *P. riddellii* has been extirpated from a significant portion of the historical range, including the entire Trinity drainage. Additional surveys in Lake Pontchartrain, Trinity, and other drainages in the eastern periphery of the species' range may provide additional clarity on the distribution and conservation status of *P. riddellii*.

KEY WORDS: Phylogeography · Range extension · Historical DNA · DNA barcoding · Misidentification · Ambleminae · Pleurobemini · Louisiana pigtoe

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1. INTRODUCTION

Accurate distributional information is critical for the management and conservation of imperiled species, considering that range reductions are among the most important criteria when assessing conservation status (Smith et al. 2018, Grace et al. 2019). The utility of the occurrence data, however, can be greatly affected by misidentifications due to false-positive and false-negative errors (Shea et al. 2011). Freshwater mussels (Bivalvia: Unionida) are a diverse group with approximately 70% of the 350 species recognized from North America designated as extinct or of conservation concern (Williams et al. 1993, 2017, Haag & Williams 2014, Lopes-Lima et al. 2018, Graf & Cummings 2021). Despite high imperilment levels, extensive morphological variation and convergence in many lineages has confounded the ability to establish accurate distributional information for many unionid species without using molecular data to confirm identifications (Pfeiffer et al. 2016, Inoue et al. 2018, Johnson et al. 2018, Smith et al. 2019, 2021a, 2022, Patterson et al. 2021). The utility of molecular tools to resolve taxonomic uncertainties and facilitate the development of effective conservation and recovery strategies for several taxa (Smith & Johnson 2020, Garrison et al. 2021, Geist et al. 2021, Smith et al. 2021b, Gladstone et al. 2022) has prompted many researchers to consider molecular research as a priority for imperiled freshwater mussels (FMCS 2016, Lopes-Lima et al. 2018, Ferreira-Rodríguez et al. 2019).

The freshwater mussel genus *Pleurobema* Rafinesque, 1819 consists of 23 recognized species (Williams et al. 2017). The genus was once widespread in North America, from the Mississippi River and Great Lakes drainages to Gulf of Mexico drainages from Florida to Texas (Williams et al. 2008, 2014, Watters et al. 2009). Today, *Pleurobema* spp. have experienced both localized and widespread declines, leading to the extinction of 4 species, with several others on the brink of extinction (Garner et al. 2004, Williams et al. 2008). Causes of extinction and decline for some *Pleurobema* spp. have been attributed to large-scale habitat modifications like the construction of the Tennessee-Tombigbee Waterway (Williams et al. 2008). In addition to being among the most imperiled groups of unionids, *Pleurobema* spp. often exhibit high levels of intraspecific variation and interspecific convergence in morphology (Williams et al. 2008, 2017, Watters et al. 2009, Inoue et al. 2018). This variation has led to inaccurate taxonomic hypotheses in *Pleurobema* when relying on morphology alone for identification, while molecular data have proven useful in delineating species ranges and boundaries (Campbell & Lydeard 2012, Inoue et al. 2018, Morrison et al. 2021, Olivera-Hyde et al. 2023).

The Louisiana pigtoe *Pleurobema riddellii* (Lea, 1861) is currently listed as threatened in Texas (TPWD 2020) and is proposed for listing as threatened under the US Endangered Species Act (ESA) (USFWS 2009, 2023). Despite its imperilment, uncertainty remains regarding the distribution of the species due to difficulties identifying specimens morphologically, including its historical presence at the type locality in the Trinity River and eastern Gulf of Mexico drainages (Frierson 1911, Ortmann 1912, Randklev et al. 2020). The distribution of *P. riddellii*, as currently understood, includes populations in the Mississippi Embayment (i.e. Bayou Teche, Ouachita, and Red drainages) and Sabine-Trinity (i.e. Calcasieu, Sabine, Neches, San Jacinto, and Trinity drainages) provinces (Vidrine 1993, Howells et al. 1996, Haag 2010, Randklev et al. 2020). *P. riddellii* has not been collected in the Trinity drainage since the early 1900s (Simpson 1914, Strecker 1931, Athearn 1970, Howells et al. 1996), which has led researchers to presume it is extirpated, or even question its recent presence, in the Trinity River drainage (Randklev et al. 2020). The utility of historical DNA (hDNA) from museum specimens has increased in recent years; in particular, sequencing mitochondrial genes (reviewed by Raxworthy & Smith 2021) and confirming the historical presence of *P. riddellii* in the Trinity drainage will likely require a hDNA approach (Randklev et al. 2020).

The distribution of *P. riddellii* in Gulf drainages east of the Mississippi River remains unclear. Early malacologists reported *P. riddellii* from the Pearl River (Frierson 1911, Ortmann 1912, 1914); however, subsequent researchers adopted a different concept of the species and its distribution that did not include eastern Gulf drainages (Vidrine 1993, Howells et al. 1996, Jones et al. 2005, 2021). Similarly, an undescribed species of *Pleurobema* was also reported from the Amite and Pearl rivers in Louisiana but ultimately considered to be *P.* cf. *beadleianum* (Hartfield 1988). Confirming the identity of *Pleurobema* specimens in the Trinity River and eastern Gulf of Mexico is a critical research objective to assess the conservation status of *P. riddellii*.

In this study, we used DNA sequences and occurrence data from field and museum collections to better inform the ESA listing process by assessing the range and spatiotemporal trends in the distribution of *P. riddellii*. The specific objectives of this study were to (1) compile historical and contemporary distribution records for *P. riddellii* using field surveys

and museum collections; (2) confirm identifications using DNA sequences to validate records, including the use of hDNA to determine the identity of specimens collected from the Trinity River drainage (type locality for *P. riddellii*) in the late 1800s; (3) evaluate spatiotemporal changes in the historical and contemporary distribution while revising the known distribution of *P. riddellii*; (4) assess phylogeographic structure and genetic diversity within and among extant populations; and (5) discuss implications of findings on future conservation and management options.

2. MATERIALS AND METHODS

2.1. Taxon sampling

We compiled or generated molecular data for specimens tentatively identified as *Pleurobema riddellii* collected from 8 river drainages: Calcasieu, Neches, Ouachita, Pearl, Red, Sabine, San Jacinto, and Trinity (Fig. 1, Table 1). This included DNA sequence data obtained from a dried tissue sample residing within a historical specimen (pre-1900) putatively identified as *P. riddellii* from the Trinity River near Dallas, Texas (University of Michigan Museum of Zoology [UMMZ] 113389, Fig. 2A). We also generated DNA sequences from specimens collected from the Pearl River, Louisiana, that could not be readily identified and assigned to a known species during tactile surveys for freshwater mussels conducted 2017–2022 (Fig. 2B, Table 1). After further examination, internal and external conchological characters suggested the specimens belonged to the genus *Pleurobema* but appeared to be morphologically distinct from *P. beadleianum* (Lea, 1861), which, at the time of collection, was the only member of *Pleurobema* recognized in the Pearl River basin (Vidrine 1993, Jones et al. 2005, 2021). The unidentified specimens have a deeper umbo pocket when compared

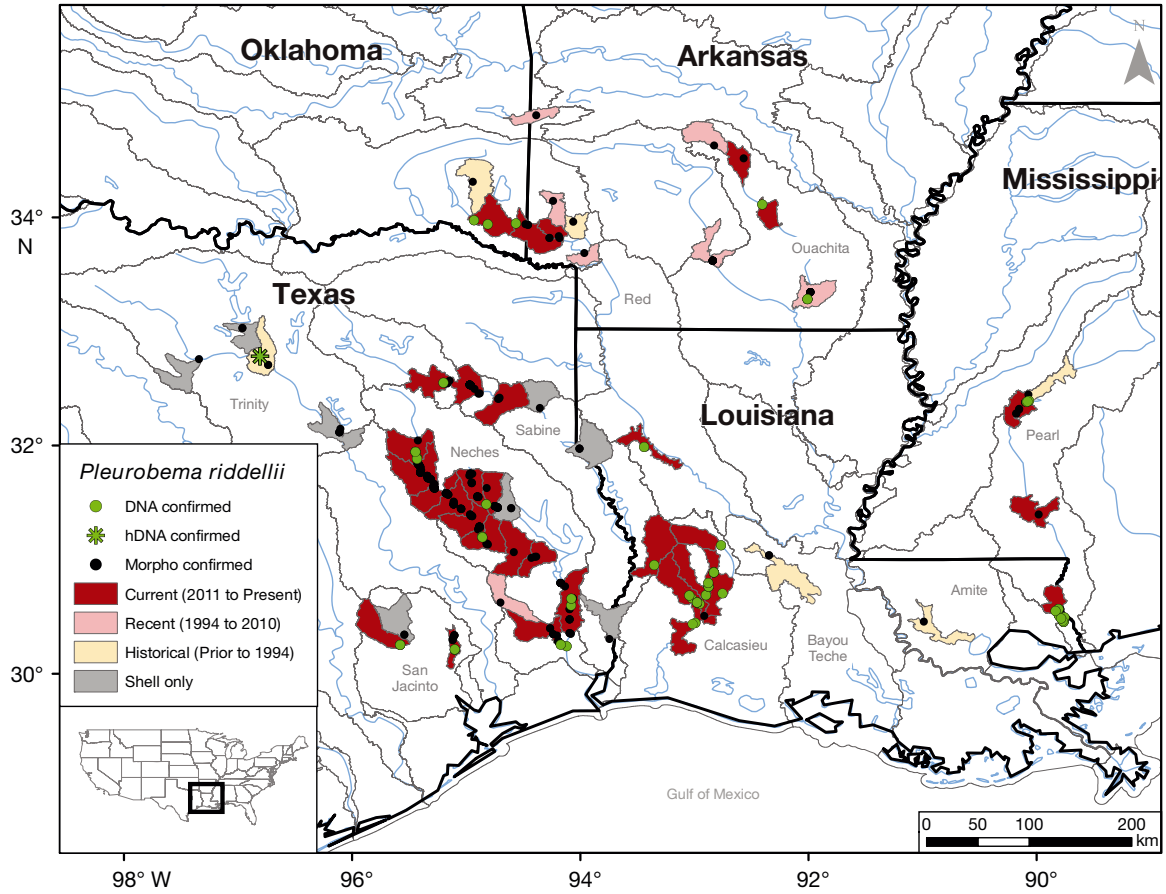

Fig. 1. Map providing spatiotemporal distribution information of *Pleurobema riddellii* at the hydrologic unit code (HUC) 10-level. Color shading of HUCs indicate the most recent date of collection for *P. riddellii*. All known *P. riddellii* records are plotted with colors to indicate locations where DNA data were used to confirm identifications. Green star denotes the type locality for *P. riddellii* (Trinity River near Dallas, Texas), which is the same collection location for the specimen identified using historical DNA (hDNA)

Table 1. Collection details for specimens included in genetic analyses with associated GenBank accession numbers for COI sequences. The taxon field corresponds to labels in Fig. 1, and additional metadata for each specimen are available from Johnson et al. (2023). LA: Louisiana; PA: Pennsylvania; TX: Texas; MS: Mississippi; AR: Arkansas; OK: Oklahoma; ASUMZ: Arkansas State University Museum of Zoology; JBFMC: Joseph Britton Freshwater Mussel Collection; MMNS: Mississippi Museum of Natural Sciences; UF: Florida Museum; UAUC: University of Alabama Unionid Collection; UMMZ: University of Michigan Museum of Zoology

Taxon	Drainage	State	Source	Catalog no.	GenBank
Fusconaia cerina	Pearl	LA	Inoue et al. (2018)	UF439077	MF961897
Pleurobema beadleianum	Pearl	LA	This study	UF439433	OR177673
Pleurobema clava	Ohio	PA	Campbell et al. (2005)	UAUC1477	AY655013
Pleurobema riddellii					
CpusSaJ081	San Jacinto	TX	This study	JBFMC11020.1	OR177663
FcerPrl029	Pearl	MS	This study	MMNS15702	OR177664
FcerPrl031	Pearl	MS	This study	MMNS15702	OR177665
FcerPrl032	Pearl	MS	This study	MMNS15702	OR177666
FflaCal090	Calcasieu	LA	This study	JBFMC12067.3	OR177667
FspeSal027	Ouachita	AR	This study	UF439572	OR177668
FspeSal029	Ouachita	AR	This study	UF439572	OR177669
FspeSal030	Ouachita	AR	This study	UF439572	OR177670
FspeSal032	Ouachita	AR	This study	UF439572	OR177671
FspeSal033	Ouachita	AR	This study	UF439572	OR177672
Prid_01_AR_SA_MF961974	Ouachita	AR	Inoue et al. (2018)	No voucher	MF961974
Prid_02_AR_SA_MF961975	Ouachita	AR	Inoue et al. (2018)	No voucher	MF961975
Prid_03_AR_SA_MF961976	Ouachita	AR	Inoue et al. (2018)	No voucher	MF961976
Prid_04_AR_SA_MF961977	Ouachita	AR	Inoue et al. (2018)	No voucher	MF961977
Prid_05_AR_SA_MF961978	Ouachita	AR	Inoue et al. (2018)	No voucher	MF961978
Prid_JF326434	Neches	TX	Campbell & Lydeard (2012)	Unavailable	JF326434
Prid_Little4630_MF961991	Red	OK	Inoue et al. (2018)	ASUMZ4630	MF961991
Prid_Little4917_MF961992	Red	OK	Inoue et al. (2018)	ASUMZ4917	MF961992
Prid_Little4934_MF961993	Red	OK	Inoue et al. (2018)	ASUMZ4934	MF961993
Prid_MF961990	Red	OK	Inoue et al. (2018)	ASUMZ4629	MF961990
Prid_NEC01	Neches	TX	Pieri et al. (2018)	JBFMC8149.1	MH133603
Prid_NEC03	Neches	TX	This study	JBFMC8154.2	OR177712
Prid_NEC04	Neches	TX	Pieri et al. (2018)	JBFMC8025.1	MH133604
Prid_NEC05	Neches	TX	Pieri et al. (2018)	JBFMC8025.2	MH133605
Prid_NEC06	Neches	TX	Pieri et al. (2018)	JBFMC8025.3	MH133606
Prid_NEC07	Neches	TX	Pieri et al. (2018)	JBFMC8025.4	MH133607
Prid_NEC09	Neches	TX	This study	JBFMC8042.2	OR177723
Prid_NEC10	Neches	TX	Pieri et al. (2018)	JBFMC8042.3	MH133608
Prid_NEC11	Neches	TX	This study	JBFMC8042.4	OR177724
Prid_NEC12	Neches	TX	This study	JBFMC8042.5	OR177725
Prid_NEC13	Neches	TX	This study	JBFMC8316.1	OR177726
Prid_NEC14	Neches	TX	This study	JBFMC8316.2	OR177727
Prid_NEC15	Neches	TX	This study	JBFMC8316.3	OR177728
Prid_NEC16	Neches	TX	This study	JBFMC8316.4	OR177729
Prid_NEC17	Neches	TX	This study	JBFMC8316.5	OR177730
Prid_Ouachita2765_MF961979	Ouachita	AR	Inoue et al. (2018)	ASUMZ2765	MF961979
Prid_Ouachita2771_MF961980	Ouachita	AR	Inoue et al. (2018)	ASUMZ2771	MF961980
Prid_Ouachita2773_MF961981	Ouachita	AR	Inoue et al. (2018)	ASUMZ2773	MF961981
Prid_Ouachita2788_MF961982	Ouachita	AR	Inoue et al. (2018)	ASUMZ2788	MF961982
Prid_RED01	Red	OK	This study	ASUMZ1378	OR177747
Prid_RED02	Red	OK	This study	ASUMZ1378	OR177748
Prid_RED04	Red	OK	This study	ASUMZ1378	OR177749
Prid_RED07	Red	OK	This study	ASUMZ1378	OR177750
Prid_RED08	Red	OK	This study	ASUMZ1378	OR177751
Prid_RED09	Red	OK	This study	ASUMZ1378	OR177753
Prid_RED10	Red	OK	This study	ASUMZ1378	OR177754
Prid_RED11	Red	OK	This study	ASUMZ1378	OR177755
Prid_RED12	Red	OK	This study	ASUMZ1378	OR177756
Prid_RED13	Red	OK	This study	ASUMZ1378	OR177757
Prid_RED15	Red	OK	This study	ASUMZ1378	OR177758
Prid_RED16	Red	OK	This study	ASUMZ1378	OR177759

(*Table 1 continued on next page*)

Taxon	Drainage	State	Source	Catalog no.	GenBank
Prid_RED17	Red	OK	This study	ASUMZ1378	OR177760
Prid_RED19	Red	OK	This study	ASUMZ1378	OR177761
Prid_RED20	Red	OK	This study	ASUMZ1378	OR177762
Prid_RED21	Red	OK	This study	ASUMZ1380	OR177763
Prid_Saline3253_MF961983	Ouachita	AR	Inoue et al. (2018)	ASUMZ3253	MF961983
Prid_Saline3257_MF961984	Ouachita	AR	Inoue et al. (2018)	ASUMZ3257	MF961984
Prid_Saline3264_MF961985	Ouachita	AR	Inoue et al. (2018)	ASUMZ3264	MF961985
Prid_Saline3274_MF961986	Ouachita	AR	Inoue et al. (2018)	ASUMZ3274	MF961986
Prid_Saline3780_MF961987	Ouachita	AR	Inoue et al. (2018)	ASUMZ3780	MF961987
Prid_Saline4640_MF961988	Ouachita	AR	Inoue et al. (2018)	ASUMZ4640	MF961988
Prid_Saline4641_MF961989	Ouachita	AR	Inoue et al. (2018)	ASUMZ4641	MF961989
Prid_VillageTX09_MF962002	Neches	TX	Inoue et al. (2018)	No voucher	MF962002
Prid_VillageTX10_MF962003	Neches	TX	Inoue et al. (2018)	No voucher	MF962003
Prid_VillageTX11_MF962004	Neches	TX	Inoue et al. (2018)	No voucher	MF962004
PridCal041	Calcasieu	LA	This study	JBFMC9593.1	OR177674
PridCal042	Calcasieu	LA	This study	No voucher	OR177675
PridCal047	Calcasieu	LA	This study	No voucher	OR177676
PridCal050	Calcasieu	LA	This study	No voucher	OR177677
PridCal052	Calcasieu	LA	This study	No voucher	OR177678
PridCal071	Calcasieu	LA	This study	JBFMC12030.1	OR177679
PridCal072	Calcasieu	LA	This study	JBFMC12030.2	OR177680
PridCal073	Calcasieu	LA	This study	JBFMC12030.3	OR177681
PridCal074	Calcasieu	LA	This study	JBFMC12030.4	OR177682
PridCal075	Calcasieu	LA	This study	JBFMC12030.5	OR177683
PridCal076	Calcasieu	LA	This study	JBFMC12067.1	OR177684
PridCal077	Calcasieu	LA	This study	JBFMC12067.2	OR177685
PridCal078	Calcasieu	LA	This study	JBFMC12067.4	OR177686
PridCal079	Calcasieu	LA	This study	JBFMC12067.5	OR177687
PridCal080	Calcasieu	LA	This study	JBFMC12067.6	OR177688
PridCal081	Calcasieu	LA	This study	JBFMC12067.7	OR177689
PridCal083	Calcasieu	LA	This study	JBFMC12135.1	OR177690
PridCal084	Calcasieu	LA	This study	JBFMC12135.2	OR177691
PridCal086	Calcasieu	LA	This study	JBFMC12137.1	OR177692
PridCal087	Calcasieu	LA	This study	JBFMC12137.2	OR177693
PridCal088	Calcasieu	LA	This study	JBFMC12137.3	OR177694
PridCal089	Calcasieu	LA	This study	JBFMC12137.4	OR177695
PridCal090	Calcasieu	LA	This study	JBFMC12139.1	OR177696
PridCal091	Calcasieu	LA	This study	JBFMC12139.2	OR177697
PridCal092	Calcasieu	LA	This study	JBFMC12118.1	OR177698
PridCal093	Calcasieu	LA	This study	JBFMC12119.1	OR177699
PridCal094	Calcasieu	LA	This study	JBFMC12120.1	OR177700
PridCal095	Calcasieu	LA	This study	JBFMC12120.2	OR177701
PridCal096	Calcasieu	LA	This study	JBFMC12121.1	OR177702
PridCal097	Calcasieu	LA	This study	JBFMC12121.2	OR177703
PridCal098	Calcasieu	LA	This study	JBFMC12121.3	OR177704
PridCal099	Calcasieu	LA	This study	JBFMC12121.4	OR177705
PridCal100	Calcasieu	LA	This study	JBFMC12121.5	OR177706
PridCal101	Calcasieu	LA	This study	JBFMC12121.6	OR177707
PridCal103	Calcasieu	LA	This study	JBFMC12117.1	OR177708
PridCal106	Calcasieu	LA	This study	JBFMC12108.3	OR177709
PridNec003	Neches	TX	Inoue et al. (2018)	UF438929	MF961994
PridNec004	Neches	TX	Inoue et al. (2018)	UF438929	MF961995
PridNec005	Neches	TX	Inoue et al. (2018)	UF438929	MF961996
PridNec006	Neches	TX	Inoue et al. (2018)	UF438929	MF961997
PridNec007	Neches	TX	Inoue et al. (2018)	UF438934	MF961998
PridNec011	Neches	TX	Inoue et al. (2018)	No voucher	MF961999
PridNec012	Neches	TX	Inoue et al. (2018)	No voucher	MF962000
PridNec015	Neches	TX	This study	JBFMC8322.1	OR177710
PridNec029	Neches	TX	This study	JBFMC9513.1	OR177711
PridNec030	Neches	TX	This study	JBFMC9513.2	OR177713
PridNec031	Neches	TX	This study	JBFMC9513.3	OR177714

(*Table 1 continued on next page*)

Taxon	Drainage	State	Source	Catalog no.	GenBank
PridNec032	Neches	TX	This study	JBFMC9513.4	OR177715
PridNec033	Neches	TX	This study	JBFMC9513.5	OR177716
PridNec034	Neches	TX	This study	JBFMC9562.3	OR177717
PridNec035	Neches	TX	This study	JBFMC9562.4	OR177718
PridNec036	Neches	TX	This study	JBFMC9562.1	OR177719
PridNec037	Neches	TX	This study	JBFMC9562.2	OR177720
PridNec039	Neches	TX	This study	JBFMC9580.1	OR177721
PridNec040	Neches	TX	This study	JBFMC9580.2	OR177722
PridPrl013	Pearl	LA	This Study	UF439345	OR177731
PridPrl014	Pearl	LA	This study	UF439345	OR177732
PridPrl020	Pearl	LA	This study	UF439412	OR177733
PridPrl021	Pearl	LA	This study	UF439405	OR177734
PridPrl023	Pearl	LA	This study	UF439408	OR177735
PridPrl024	Pearl	LA	This study	UF439411	OR177736
PridPrl025	Pearl	LA	This study	UF561636	OR177737
PridPrl026	Pearl	LA	This study	UF561637	OR177738
PridPrl027	Pearl	MS	This study	MMNS17490	OR177739
PridPrl029	Pearl	MS	This study	MMNS17490	OR177740
PridPrl030	Pearl	MS	This study	MMNS17490	OR177741
PridPrl031	Pearl	MS	This study	MMNS17491	OR177742
PridPrl032	Pearl	MS	This study	MMNS17491	OR177743
PridPrl033	Pearl	MS	This study	MMNS17491	OR177744
PridPrl035	Pearl	MS	This study	MMNS17492	OR177745
PridPrl036	Pearl	MS	This study	MMNS17492	OR177746
PridRed082	Red	LA	This study	JBFMC12098.1	OR177752
PridSab002	Sabine	LA	Pfeiffer et al. (2016)	UF441165	KT285646
PridSab038	Sabine	TX	This study	JBFMC9578.1	OR177764
PridSaJ026	San Jacinto	TX	This study	JBFMC9503.1	OR177765
PridSaJ027	San Jacinto	TX	This study	JBFMC9503.2	OR177766
PridSaJ028	San Jacinto	TX	This study	JBFMC9503.3	OR177767
PridSaJ060	San Jacinto	TX	This study	JBFMC11064.1	OR177768
PridSaJ061	San Jacinto	TX	This study	JBFMC11064.2	OR177769
PridSaJ062	San Jacinto	TX	This study	JBFMC11064.3	OR177770
PridSaJ063	San Jacinto	TX	This study	JBFMC11055.1	OR177771
PridSaJ064	San Jacinto	TX	This study	JBFMC11055.2	OR177772
PridSaJ065	San Jacinto	TX	This study	JBFMC11055.3	OR177773
PridSaJ066	San Jacinto	TX	This study	JBFMC11055.4	OR177774
PridSaJ067	San Jacinto	TX	This study	JBFMC11055.5	OR177775
PridSaJ068	San Jacinto	TX	This study	JBFMC11055.6	OR177776
PridSaJ069	San Jacinto	TX	This study	JBFMC11055.7	OR177777
PridTri025	Trinity	TX	This study	UMMZ113389	OR177778
Psin_RED10	Red	OK	This study	ASUMZ1382	OR177788
PsinOua214	Ouachita	AR	This study	UF439547	OR177779
PsinOua215	Ouachita	AR	This study	UF439547	OR177780
PsinOua216	Ouachita	AR	This study	UF439547	OR177781
PsinOua218	Ouachita	AR	This study	UF439547	OR177782
PsinOua219	Ouachita	AR	This study	UF439547	OR177783
PsinOua221	Ouachita	AR	This study	UF439547	OR177784
PsinOua222	Ouachita	AR	This study	UF439547	OR177785
PsinOua223	Ouachita	AR	This study	UF439547	OR177786
PsinOua224	Ouachita	AR	This study	UF439547	OR177787

Table 1 (*continued*)

to *P. beadleianum* (Fig. 2C) and lack the wide, shallow sulcus, which is diagnostic for *Fusconaia cerina* (Conrad, 1838) (Fig. 2D). After comparing the unknown individuals to specimens in museum collections, including the holotype (USMN 84635, Fig. 2E) and material identified by Hartfield (1988) as *Pleurobema* sp. (Fig. 2F), we tentatively identified the specimens from the Pearl River as *P. riddellii*. Given the potential impact of a possible range extension, all identifications were pending verification using a combination of DNA sequencing and morphological assessment.

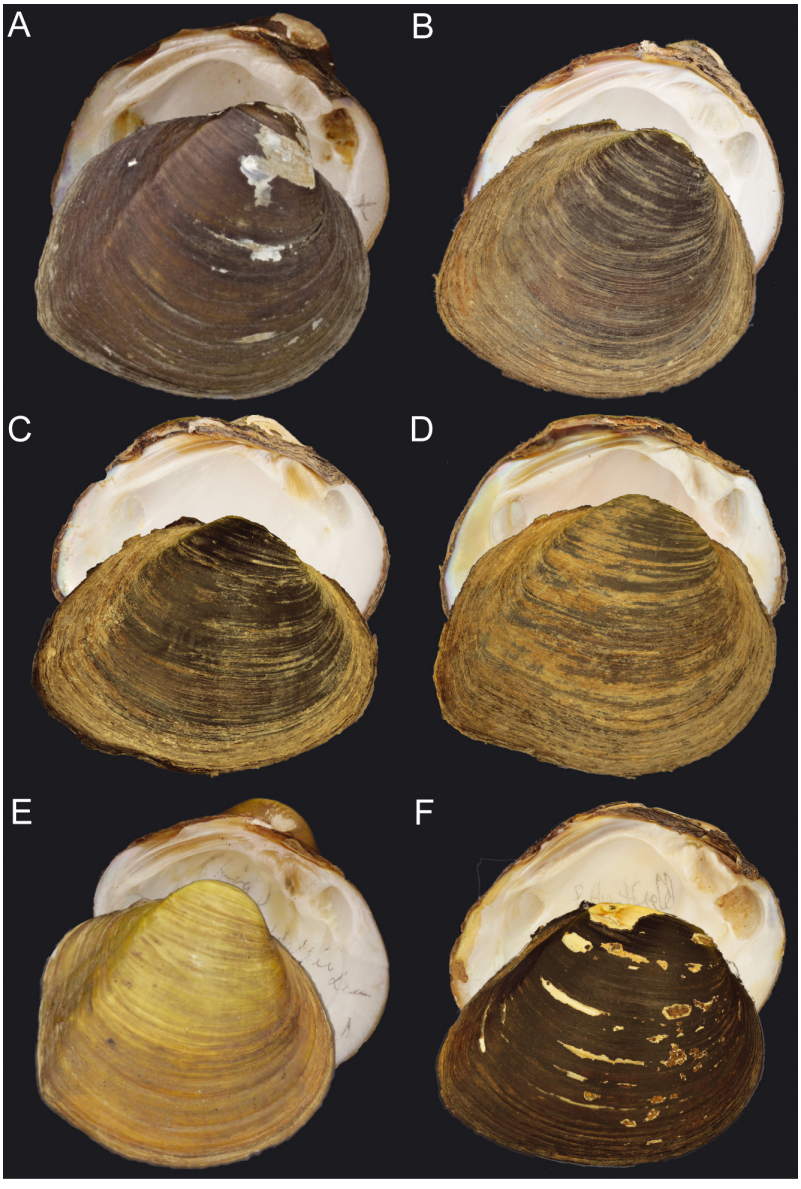

Fig. 2. Representative photos of *Pleurobema riddellii*, *P. beadleianum*, and *Fusconaia cerina* specimens showing a range of intraspecific variation and interspecific similarity. (A) *P. riddellii* historical DNA (hDNA) sample, Trinity River near Dallas, Texas (47 mm, UMMZ 113389.1). (B) *P. riddellii* West Pearl River near Picayune, Mississippi (58 mm, UF 439408). (C) *P. beadleianum*, West Pearl River near Picayune, Mississippi (66 mm, UF 439409). (D) *F. cerina*, Bogue Chitto River (Pearl River drainage) near Picayune, Mississippi (50 mm, UF 439077). (E) *P. riddellii* Holotype, Trinity River near Dallas, Texas (33 mm, USNM 84635). (F) *P. riddellii,* Amite River near Port Vincent, Mississippi (61 mm, MMNS 2485)

2.2. DNA extraction, PCR, and sequencing

We extracted DNA from buccal swabs, fresh mantle tissue stored in cell lysis buffer, mantle tissue preserved in 95% EtOH, and desiccated tissue from a specimen collected in the late 1800s using the Qiagen PureGene extraction kit following the manufacturer's protocols. We amplified and sequenced a 658 bp segment of cytochrome *c* oxidase subunit I (COI) using the primers reported in Campbell et al. (2005). Due to DNA degradation in the isolation from the museum specimen originally collected from the Trinity River (UMMZ 113389), reliable COI sequences could not be obtained using previously published primers (Campbell et al. 2005). We designed 4 minibarcode primers based on published *Fusconaia* and *Pleurobema* COI sequences to identify the museum specimen: FB-R1, AGG RAT AAG CCA ATT ACC AA; FB-F2, CGC ATG CTT TTA TAA TRA TT; FB-R2, CGG AAT TCG CTC AGC AAC; and FB-F3, CTT GCT GGT GCA TCT TCT AT. Individual PCRs were performed using the following primer pairs: (1) the forward primer reported in Campbell et al. (2005) and FB-R1, (2) FB-F2 and FB-R2, and (3) FB-F3 and the reverse primer reported in Campbell et al. (2005). For all PCR reactions (both minibarcodes and full amplicons), thermal cycling conditions and PCR chemistry followed Johnson et al. (2018). The thermal cycling profile consisted of an initial denaturation at 95°C for 3 min followed by 5 cycles of 95°C for 30 s, 45°C for 40 s, 72°C for 45 s, then 35 cycles of 95°C for 30 s, 51°C for 40 s, 72°C for 45 s, with a final elongation at 72°C for 10 min, and hold at 4°C. Our PCR reactions consisted of 12.5 µl mixture containing distilled deionized water (4.25 µl), MyTaq™ Red Mix (6.25 µl) (Bioline), primers (0.5 µl each at 10 µM) and DNA template (1 µl at 50 ng). Bidirectional sequencing was performed at the Molecular Cloning Laboratories (South San Francisco, CA, USA) on an ABI 3730XL (Life Technologies). Geneious v. 10.2.1 (Kearse et al. 2012) was used to edit chromatograms and assemble consensus sequences. All COI sequences were aligned in Mesquite v. 3.7.0 (Madison & Madison 2021) using the L-INS-i method in MAFFT v. 7.299 (Katoh & Standley 2013) and translated into amino acids to ensure absence of stop codons and

gaps. All sequences were submitted to GenBank, and detailed information regarding individuals are available electronically (Johnson et al. 2023).

2.3. Molecular analyses

Phylogenetic inference was performed in BEAST v. 2.6.7 (Bouckaert et al. 2019). We included sequences for *F. cerina*, *P. beadleianum*, and *P. clava* to serve as an outgroup following relationships depicted in previous phylogenetic studies (Campbell & Lydeard 2012, Inoue et al. 2018). Before the analysis, the best partitioning scheme for each codon position and nucleotide substitution models were determined using ModelFinder (Kalyaanamoorthy et al. 2017). A strict molecular clock was fitted to each partition and the Yule process was used as the tree prior. The BEAST analysis was run for 2×10^7 MCMC generations sampling every 5000 generations with an initial 10% burn-in. Effective sample size (ESS) greater than 200 was ensured using Tracer 1.7 (Rambaut et al. 2018), and a maximum clade credibility tree was created using TreeAnnotator v. 2.6 (Bouckaert et al. 2019).

To visualize the geographic distribution of genetic variation within *P. riddellii*, we constructed a TCS haplotype network (Clement et al. 2002) using PopArt (Leigh & Bryant 2015). Missing data were handled using complete deletion. To evaluate intraspecific and inter-drainage genetic variation within *P. riddellii*, we calculated pairwise genetic distance values using uncorrected p-distances and pairwise deletion of missing data in MEGA11 (Tamura et al. 2021). Individuals of *P. riddellii* were grouped according to drainage of capture (Calcasieu, Neches, Ouachita, Pearl, Red, Sabine, San Jacinto, and Trinity) for both haplotype networks and pairwise genetic distance calculations.

2.4. Conservation map

We compiled distributional information from museum specimens, field notes, and surveys to update the known distribution of *P. riddellii*. Sources of information included, but were not limited to, Arkansas State University Museum of Zoology (ASUMZ), Carnegie Museum of Natural History (CM), Florida Museum (UF), Joseph Britton Freshwater Mussel Collection (JBFMC), Louisiana Department of Wildlife and Fisheries (LDWF), Mississippi Museum of Natural Sciences (MMNS), Ohio State Museum (OSUM), Smithsonian National Museum of Natural History (USNM), Texas A&M Natural Resources Institute, Texas Parks and Wildlife Department (TPWD), University of Alabama Unionid Collection (UAUC), UMMZ, US Geological Survey, and US Fish and Wildlife Service. Due to the inherent uncertainties that accompany morphological identifications among members of Pleurobemini, we used DNA to confirm identifications when possible; however, our identifications for a subset of records were dependent on morphology alone. The date of collection, locality information, collector(s), initial field-based identification, and source of each record are available from Johnson et al. (2023).

We produced a conservation status assessment map using ArcMap 10.8.2 (ESRI) following the protocol produced by Georgia Department of Natural Resources (2014) to evaluate spatiotemporal changes in the distribution of *P. riddellii.* Conservation status maps play an important role in conservation planning for mussels by identifying range size, survey needs, and high priority areas for protection (Johnson et al. 2016, McLeod et al. 2017, Smith et al. 2021a). We plotted all known records using the Mussels of Texas database (MOTX; Randklev et al. 2021) and recent survey data from Arkansas, Louisiana, Mississippi, Oklahoma, and Texas. Occurrence data of *P. riddellii* were then georeferenced and mapped at the hydrologic unit code (HUC)-10 level based on last known observation. We selected 3 separate time frames to show collection history: current (2011–present), recent (1994–2010), and historical (prior to 1994). These time frames were chosen as they represent major collection efforts in the region (see Randklev et al. 2021). Occurrence data were then categorized based on the type of identification, which included DNA, hDNA, or morphology only. The input file used to generate the maps is available from Johnson et al. (2023) to facilitate reproducibility and future analyses as more records become available.

3. RESULTS

3.1. Taxon sampling and molecular analyses

Our alignment for phylogenetic analyses consisted of 163 *P. riddellii* collected across 8 major river drainages (Figs. 1 & 3, Table 1): Calcasieu (n = 37), Neches (n = 38), Ouachita (n = 30), Pearl (n = 19), Red (n = 22), Sabine (n = 2), San Jacinto (n = 14), and Trinity (n = 1). The total alignment length was 658 nucleotides (3.43% missing data), and

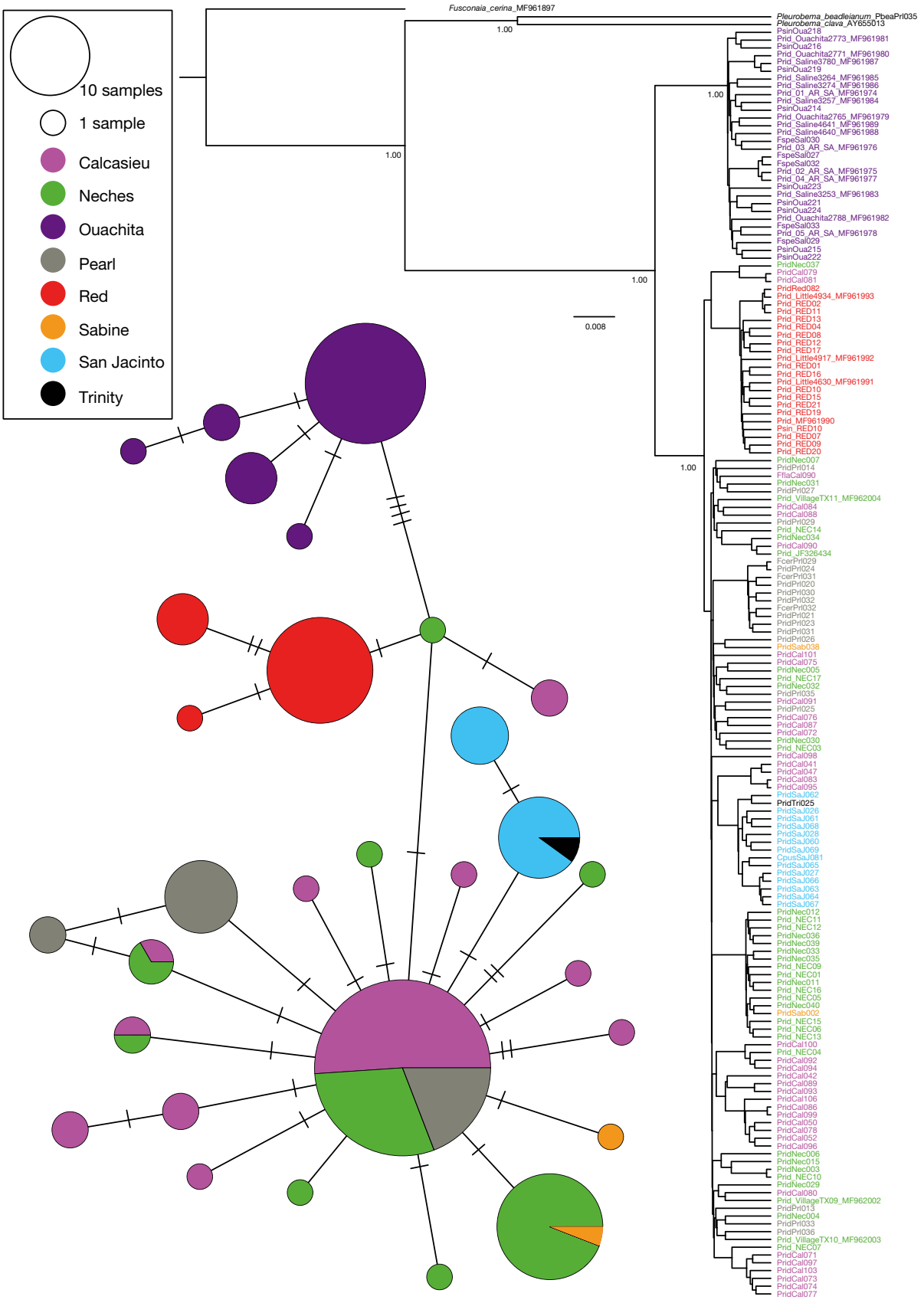

Fig. 3. TCS haplotype network and Bayesian phylogeny with posterior probability (PP) nodal support values for well-supported clades (PP = 1.00) based on mitochondrial COI data for *Pleurobema riddellii*. Colors correspond to drainage of capture

no stop codons or indels were present. Our final dataset included 125 COI sequences generated as part of our study (GenBank accessions: OR177663–OR177788) and 38 COI sequences obtained from GenBank representing *P. riddellii* (Table 1). Included were specimens confirmed to be *P. riddellii* using DNA sequences that were initially identified as the following: *F. cerina* (n = 3), *Fusconaia flava* (n = 1), *Fusconaia* sp. (n = 5), *Pleurobema sintoxia* (n = 10), and *Pustulosa pustulosa* (n = 1). Our final DNA alignment and detailed collection information, including initial and DNA identifications, are available electronically (Johnson et al. 2023).

ModelFinder determined that 2 partitions and nucleotide substitution models best fit our data for the BEAST analysis: COI codon 1 and 2 – HKY + F + I, and COI codon 3 – HKY + F + G4. The phylogenetic reconstruction depicted 2 main geographic groups of *P. riddellii* with strong support (PP = 1.0): (1) Ouachita drainage; and (2) Calcasieu, Neches, Pearl, Red, Sabine, San Jacinto, and Trinity drainages (Fig. 3). Specimens from the Pearl and Trinity drainages showed little divergence from *P. riddellii* specimens collected in the Calcasieu, Neches, Red, Sabine, and San Jacinto drainages (Fig. 3). Haplotype networks showed similar patterns of divergence as phylogenetic analysis, with specimens from the Pearl and Trinity drainages depicting little differentiation from, and even shared haplotypes with, *P. riddellii* from other drainages (Fig. 3). Intra-drainage genetic p-distances ranged from 0.09–0.33% with the highest level of variation in the Sabine River population, followed by Neches, Pearl, Calcasieu, Red, and San Jacinto drainages (Table 2). Genetic distance values were not calculated for the Trinity River drainage due to the low sample size (n = 1). Inter-drainage genetic p-distances ranged from 0.07% (San Jacinto and Trinity drainages) to 1.87% (Ouachita and San Jacinto drainages), with an average p-distance of 1.77% between the Ouachita River samples and all other populations averaged. Inter-drainage comparisons across samples from the other sampled drainages were much lower (average 0.44%) (Table 2).

3.2. Distribution

We compiled distributional data from freshwater mussel survey and museum records from the known range of *P. riddellii* to assess its contemporary and historical geographic distribution. In total, 467 records of both live individuals and shell were obtained for *P. riddellii* collected from pre-1900–2022 (Table 1; also see Johnson et al. 2023). The exact collection date for the Trinity River specimen (UMMZ 113389) could not be determined; however, the specimen originally resided in the collection of James Lewis, who was actively publishing research on unionids from 1854–1879 (Simpson 1900). The specimen was later transferred to the Bryant Walker Collection and eventually to UMMZ in the late 1930s. Based on these dates, we conservatively estimate the Trinity River specimen was collected before 1900.

Overall, the distributional information we compiled included 9 river basins spanning from the Trinity and San Jacinto rivers in Texas, east to the Pearl River in Mississippi, and north to the Red and Ouachita rivers in Oklahoma and Arkansas, respectively (Fig. 1). Using DNA sequences, we confirmed the presence of extant populations and recent records for the Calcasieu, Neches, Ouachita, Pearl, Red, Sabine, and San Jacinto drainages. Records of *P. riddellii* were distributed across 54 HUC-10 boundaries (Arkansas 11, Louisiana 13, Mississippi 4, Oklahoma 4, Texas 27; note some HUC-10 boundaries were split by state borders). The status of each HUC-10 was categorized as follows: 7 HUCs with shell only (Texas 5; shared border of Texas and Louisiana 2); 33 HUCs had cur-

Table 2. Mean uncorrected pairwise genetic distances between drainages (below diagonal) and within drainages (along diagonal) for COI sequences representing *Pleurobema riddellii* from 8 river drainages. *: intra-drainage distance could not be computed due to limited sample size

	Ouachita	Sabine	Neches	Pearl	Calcasieu	San Jacinto	Red	Trinity
Ouachita	0.0013							
Sabine	0.0182	0.0033						
Neches	0.0174	0.0025	0.0025					
Pearl	0.0180	0.0031	0.0030	0.0022				
Calcasieu	0.0168	0.0028	0.0027	0.0027	0.0021			
San Jacinto	0.0187	0.0038	0.0037	0.0037	0.0034	0.0009		
Red	0.0173	0.0068	0.0064	0.0067	0.0058	0.0074	0.0012	
Trinity	0.0180	0.0032	0.0031	0.0031	0.0027	0.0007	0.0066	*

rent (2011 to present) collections of live individuals (Arkansas 3, Louisiana 8, Mississippi 2, Oklahoma 1, Texas 17); 7 HUCs had recent (1995–2010) collections (Arkansas 6, Oklahoma 1, Texas 1); and 6 HUCs had historical (prior to 1995) collections (Arkansas 1, Mississippi 1, Louisiana 2, Oklahoma 1, Texas 1). Only historical records (pre-2000) were available for the Trinity and Amite drainages. Using hDNA, we validated the historical presence of *P. riddellii* in the Trinity River at the type locality; however, material suitable for DNA-based identifications were unavailable for specimens from the Amite River.

4. DISCUSSION

4.1. Molecular identification and revised distribution of *Pleurobema riddellii*

Prior to our study, the presumptive distribution of *P. riddellii* did not include the Pearl or Lake Pontchartrain drainages (Vidrine 1993, Howells et al. 1996, Jones et al. 2005, 2021). Using DNA sequences, we were able to confirm the presence of *P. riddellii* in the Pearl River basin; however, individuals from Lake Pontchartrain drainages were unavailable for molecular analysis. Based on morphological characters, we tentatively identify the specimen from the Amite River (MMNS 2485) reported by Hartfield (1988) as *P. riddellii* (Fig. 2). Our findings expand the known distribution of *P. riddellii* eastward to include the Amite and Pearl drainages and likely other tributaries of Lake Pontchartrain, although additional surveys and molecular confirmation are needed due to difficulties with morphological identifications in this group.

P. riddellii was described from the Trinity River near Dallas, Texas, in 1861 (USNM 84635, Fig. 2). Recent survey efforts in the Trinity River drainage have failed to locate *P. riddellii* despite extensive geographic coverage of the historical distribution and using DNA barcoding to ensure species identification (Pieri et al. 2018, Randklev et al. 2020). Previous assessments of archeological material have suggested that *P. riddellii* could have been commonly distributed throughout the Trinity River drainage in the late Holocene (Randklev et al. 2010, 2020). However, historical specimens of *P. riddellii* are difficult to confirm with morphological characters alone due to high levels of morphological variation and similarities with other sympatric species (Randklev et al. 2020). This led Randklev et al. (2020) to suggest findings from archeological material warranted molecular confirmation, presumably using ancient DNA or hDNA, to confirm the presence of *P. riddellii*. Our approach of using hDNA from the Trinity drainage validates the findings of Randklev et al. (2020) and confirms *P. riddellii* was extant in the Trinity drainage less than 200 yr ago. Confirmation of the species at the type locality is also important for confirming the validity of the species and may have conservation implications given the species is likely extirpated from the Trinity drainage (see Section 4.3).

Our molecular identifications of *P. riddellii* reinforce concerns regarding misidentifications with species of *Fusconaia*, *Pleurobema*, and to a lesser extent, *Pustulosa* (Inoue et al. 2018, Johnson et al. 2018, Pieri et al. 2018, Randklev et al. 2020, Smith et al. 2022). Reevaluating morphological characters of DNA confirmed *P. riddellii* specimens that were initially identified as other taxa in the field revealed diagnostic features that may help separate these morphologically similar taxa. For example, *P. riddellii* is sympatric with *F. askewi, F. cerina*, *F. chunii*, or *F. flava* throughout most of its distribution. Most of the *P. riddellii* specimens we received that were initially identified as *Fusconaia* sp. lacked the wide, shallow sulcus and were comparatively more inflated than co-distributed *Fusconaia* sp., which are the most reliable external morphological characters that separate *P. riddellii* from sympatric *Fusconaia* sp. Additionally, the soft parts (e.g. foot, gills, mantle) of *Fusconaia* sp. are typically orange to light brown in color with all 4 gills marsupial (tetragenous), whereas soft parts in *Pleurobema* sp. are cream-colored with only the outer gills marsupial (ectobranchus). In the Pearl and Lake Pontchartrain drainages, *P. riddellii* is sympatric with congener *P. beadleianum*; however, the former has a deeper umbo pocket, thicker hinge plate, more robust lateral and pseudocardinal teeth, and tends to be less elongate and more inflated when compared to *P. beadleianum.* In the Red and Ouachita drainages, the anteriorly positioned umbo, thicker hinge plate, and lack of a shallow sulcus are useful for separating sympatric *P. rubrum* and *P. sintoxia* from *P. riddellii*. Although DNA-based identifications are not practical in all situations, it can be invaluable when accurate identification is critical, such as confirming range extensions, historical presence, or during broodstock selection for captive propagation.

4.2. Genetic variation within *P. riddellii*

Our study provides the most comprehensive genetic and geographic assessment for *P. riddellii* to date and strongly supports the existence of 2 mtDNA haplo-

groups coinciding with the Ouachita drainage and individuals throughout the remainder of the species' range (Fig. 3). Inter-population comparisons with the Ouachita drainage also yielded the highest genetic p-distances. These findings align with a previous study that hypothesized the Ouachita drainage population likely represents an undescribed species (Inoue et al. 2018). Although the Ouachita drainage population appears to be molecularly diagnosable, sampling gaps remain across the putative distribution of *P. riddellii* in tributaries of the lower Mississippi River (Fig. 1). Future efforts incorporating material throughout the range of *P. riddellii*, with an emphasis on material from the lower Mississippi River, may be necessary to assess whether the taxon in the Ouachita River drainage should be considered a separate species from *P. riddellii*.

Throughout the remainder of the range, molecular data showed moderate levels of genetic variation with respect to geography in *P. riddellii* (Fig. 3). We observed unique haplotypes in each drainage (except the Trinity due to limited sampling) and fine structuring that aligned with geographic sub-provinces as defined by Haag (2010) and de Moulpied et al. (2022): Calcasieu–Sabine–Neches, Pascagoula–Pearl–Pontchartrain, Red, and Trinity–San Jacinto (Fig. 3). We also observed a high level of haplotype sharing between eastern and western Gulf of Mexico drainages (Calcasieu, Neches, Pearl and Sabine), suggesting relatively recent contact between these populations. Samples from the Trinity–San Jacinto had unique haplotypes, aligning with other studies that have highlighted the genetic distinctiveness of the Trinity–San Jacinto sub-province (Pieri et al. 2018, Smith et al. 2019, 2021b, 2022, de Moulpied et al. 2022). However, we observed haplotype sharing between the Trinity and San Jacinto drainage, which may have implications toward recovery planning (see below).

4.3. Implications on conservation and management

Distributional and taxonomic uncertainty surrounding *P. riddellii* has complicated conservation assessments and management strategies and has potential implications as the species is proposed for listing under the ESA (USFWS 2009, 2023). Despite expanding the known range of *P. riddellii* to include several eastern Gulf of Mexico drainages, the eastern range limit of *P. riddellii* remains uncertain. Many freshwater mussel species found in the Pearl and Pontchartrain drainages also occur in the Pascagoula drainage (Haag 2010, Smith & Johnson 2020, Jones et al. 2021), and additional surveys in the Pascagoula drainage could help to determine if the Pearl drainage represents the eastern periphery of *P. riddellii*.

Our findings indicate the current distribution of *P. riddellii* in the eastern Gulf of Mexico drainages has likely been greatly reduced. It is unclear whether populations in Pontchartrain drainages (i.e. Amite, Tangipahoa) are extirpated, below detectable levels, or lack sufficient survey effort (Fig. 1). Given the species has been overlooked in eastern Gulf of Mexico drainages, we hypothesize extant populations likely exist in Pontchartrain drainages. Hartfield (1988) reported collections in the Amite River between Magnolia and Port Vincent, Louisiana, and future tactile surveys could target *P. riddellii* in suitable habitat in this stream segment. The high number of observations of *P. riddellii* in the Neches and Sabine rivers of Texas indicates these streams either continue to support subpopulations or their absence in the recent past was due to insufficient sampling. In the last 10 yr both rivers have been the focus of intensive surveys (Randklev et al. 2020). Thus, it is entirely possible the lack of distributional data for *P. riddellii* in other river systems is due to limited sampling. Because of this, targeted sampling throughout its known range could yield new records and reconfirm the existence of populations considered extirpated. Based on our maps and occurrence information, we recommend future surveys focus on the Lake Pontchartrain basin (e.g. Amite, Tangipahoa, and Tickfaw rivers), Louisiana, Lower Bayou Boeuf, Louisiana, Saline River, Arkansas and Louisiana, Red River from Texarkana, Texas to Shreveport, Louisiana, Bayou Pierre, Louisiana, and Black River, Louisiana. These areas either historically harbored *P. riddellii* or are located near reaches or basins where occurrences have been reported.

Extensive survey efforts have been performed in the Trinity drainage, yet *P. riddellii* has not been detected since the early 1900s, which suggests the species has been extirpated, similar to several other freshwater mussel species (Randklev et al. 2010, 2020, Wolverton & Randklev 2016). Our molecular assessment provides evidence that the Trinity and San Jacinto drainage populations are closely related, which may have significant implications for recovery planning. Extant populations have only been recently discovered in the San Jacinto drainage, including the East Fork San Jacinto and West Fork San Jacinto drainages. If these populations are found to be self-sustaining, captive propagation or translocation using broodstock from the San Jacinto drainage may be a promising recovery option for re-establishing popu-

lations in the Trinity drainage. However, the San Jacinto drainage had the lowest mean intra-population genetic distance values of all populations sampled (Table 2), suggesting this population may have suffered a genetic bottleneck in the past and may benefit from genetic rescue efforts (see Frankham 2015). Regardless, additional surveys and assessments of suitable habitat for *P. riddellii* in the Trinity drainage could be beneficial before inter-drainage translocations or captive propagation are performed. Our thorough sampling of range-wide genetic diversity in *P. riddellii* provides a foundation for guiding future recovery efforts involving captive propagation. Additionally, our findings and datasets facilitate the development of species-specific eDNA assays, which could serve as a rapid assessment tool to increase detection probabilities and focus additional tactile survey efforts in basins like the Trinity River where the contemporary status of *P. riddellii* remains unknown.

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