Aquatic Conservation: Marine and Freshwater Ecosystems

WILEY

ARTICLE OPEN ACCESS

Estimating Species Distributions of Sphaeriid Clams in the Western United States: Implications for Conservation

Steven R. Hein[1,4] | Daniel A. Trujillo[2] | McKenna P. A. Burns[1,5] | David J. Berg[3]

[1]Department of Biology, Miami University, Oxford, Ohio, USA | [2]New Mexico Department of Game and Fish, Albuquerque, New Mexico, USA | [3]Department of Biology, Miami University, Hamilton, Ohio, USA | [4]United States Fish and Wildlife Service, Anchorage, Alaska, USA | [5]Division of Cardiology, Department of Medicine, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA

Correspondence: Steven R. Hein (steven_hein@fws.gov)

Received: 22 May 2024 | **Revised:** 17 February 2025 | **Accepted:** 25 February 2025

Funding: This work was funded by the New Mexico Department of Game and Fish.

Keywords: cosmopolitan | endemic | freshwater bivalves | isolation | Linnean shortfall | peaclams | *Pisidium sanguinichristi*

ABSTRACT

Species distributions are structured by dispersal potential and responses to vicariance events. Populations of less-vagile species can become spatially isolated in insularised habitats leading to allopatric speciation. In heavily fragmented aquatic systems, such species often contribute to high rates of endemism. Sphaeriid clams are a globally distributed, yet vastly understudied, group of bivalves. They occupy nearly every type of freshwater system, including many isolated water bodies. Studies on sphaeriid diversity in North America suggest many of the species are cosmopolitan. An exception is the Sangre de Cristo peaclam, *Pisidium sanguinichristi*, considered endemic to a single lake in New Mexico, USA. However, the taxonomic validity of the species is debated, incurring significant conservation implications as the species of conservation concern at the state and federal levels. We examined sphaeriid genetic diversity in New Mexico and Texas to characterise endemicity and test the identity of *P. sanguinichristi*. Sequences of the mitochondrial 16S gene and the nuclear 28S gene were compared amongst localities and with sequences deposited in GenBank. Our results support previous inferences that much of the sphaeriid diversity across this region comprises a few cosmopolitan species, with some exception. Furthermore, we did not find evidence to support *P. sanguinichristi* as a unique species and therefore not a valid taxon. This study implies that sphaeriid clams do not follow the common paradigm of high endemism in isolated water bodies shown by many small aquatic species, rather they present a mixed case. The widespread species of sphaeriids in this region are not of high-conservation priority in contrast to many aquatic invertebrates found throughout arid western North America.

1 | Introduction

Species distributions are determined by historical vicariance events and the dispersal capabilities of the species. The former can alter the suitability of a region for a particular species by transforming a formerly large area of contiguous habitat into a patchy matrix of suitable and unsuitable habitat (Avise 2000). The ability of a species to traverse the unsuitable matrix, and therefore migrate between patches of suitable habitat, determines how much influence a vicariance event can have on distribution. In species with low vagility, unable to move amongst patches or across barriers, populations become isolated from each other and theory predicts the effects of insularisation (Avise 2000; McKelvy and Burbrink 2017) will drive populations to diverge along separate evolutionary paths (De Queiroz 2007). Alleles will be lost in some populations and fixed in others through either nonadaptive mechanisms, primarily genetic drift, or adaptive processes such as local selection. Over time, disjunct populations can become genetically distinct, leading to allopatric speciation (Pavlova et al. 2017; Adams et al. 2018).

While dissimilar in many ways, both montane lakes and desert springs are examples of patchily distributed, insularised habitats embedded in inhospitable landscape. In each system, water bodies are isolated from one another making dispersal difficult or impossible for many aquatic species.

Extreme isolation of individual desert waterways and montane lakes commonly results in unique aquatic communities and elevated levels of local endemicity (Stanislawczyk et al. 2018). Hydrobiid snails, for example, are a diverse taxon of minute gilled snails found across multiple continents and insularised habitat types (Stagliano 2016; Miller et al. 2018). Although, despite a large distribution of the group, many individual species have extremely small range sizes such as a single spring (Brown et al. 2008). Evidence suggests high rates of micro-endemicity exhibited by hydrobiids are the result of physiology, life history and biogeographical processes. Many hydrobiid are hypothesised to have become geographically isolated following changes to ancient waterways and further driven by high susceptibility to desiccation due to gilled breathing structures plus slow growth rates that limit population expansion (Hershler and Liu 2008; Brown et al. 2008). Similar isolating circumstances have been documented in a variety of small-bodied aquatic species, including amphipods (Murphy et al. 2013; Walters, Cannizzaro, et al. 2021; Hou et al. 2022), flat worms (Inoue et al. 2020) and mites (Blattner et al. 2022). However, the commonality of endemic species, particularly small invertebrates, is often unrecognised in-part due to minimal distinguishing morphological traits—yet, upon closer scrutiny, single 'species' occupying multiple water bodies are revelled to be complexes composed of cryptic endemic species (Riddle et al. 2011; Funk et al. 2012; Hellmich et al. 2021; Inoue et al. 2020; Walters, Taynor, and Berg 2021). Failure to identify such cryptic species greatly reduces our understanding of biodiversity patterns, underestimates species richness and ultimately decreases the ability to apply effective conservation measures to these species and communities (Lopes-Lima et al. 2021). This knowledge gap is commonly referred to as the Linnean Shortfall, which can lead to endemic species being lumped together into singular widespread or cosmopolitan species (Lomolino 2004; Trontelj and Fišer 2009). In some cases, biological processes such as niche conservatism contribute to the formation of cryptic species complexes (Trontelj and Fišer 2009; Murphy et al. 2015;). In other cases, under-sampling by researchers is a driving factor (Hortal et al. 2015).

Sphaeriid clams (family Sphaeriidae), known commonly as fingernail, pill or pea clams, are a group of small bivalves (typically <10 mm) distributed across all major continents except Antarctic. Interestingly, this family includes numerous species with transcontinental ranges (Lee and Foighil 2003). They occupy a variety of limnetic habitats such as springs, seeps, swamps, lakes and temporary pools. In many of these systems, sphaeriids can be the dominant taxa in the benthos, with densities reaching 100,000 individuals per m^2 (Sandusky et al. 1979). Because of such high biomass, sphaeriids are likely to have important effects on water quality and they may be useful for biomonitoring of ecosystems (Korniushin and Glaubrecht 2002). However, despite their ubiquitous distribution, high abundance and ecological relevance, they are poorly studied and the distribution of many species is uncertain (Halabowski et al. 2024). The Freshwater Mollusc Conservation Society (FMCS) specifically excludes sphaeriids from its *USA National Conservation Strategy* due to lack of data and expertise ([FMCS] Freshwater Mollusk Conservation Society 2016). Furthermore, Sphaeriid taxonomic structure remains a point of dispute amongst taxonomists. In our article, we will rely upon the most recent taxonomic classifications as described in Bespalaya et al. (2024), Graf and Cummings (2025) and MolluscaBase (2025).

Many sphaeriid species are considered cosmopolitan, yet it is also plausible that such inferred distributions are artefacts of knowledge gaps created by insufficient sampling and study of this group. Sphaeriids are difficult to distinguish morphologically due to high levels of intraspecific phenotypic variation, plastic traits and limited synapomorphies, which may be further compounded by niche conservatism (Guralnick 2005; Schultheiß et al. 2008; Murphy et al. 2015; Rassam et al. 2021a). Additionally, sphaeriids are excellent dispersers—making reliance of sampling locality for identification purposes unreliable. As with many small aquatic invertebrates, sphaeriid dispersal is often associated with passive mechanisms such as epizoochory and endozoochory (Kappes and Haase 2012; Coughlan et al. 2017; Martín-Vélez et al. 2022). Previous studies on sphaeriids suggest that some species can survive exposure to drying terrestrial conditions or the digestive systems of larger vertebrates, allowing them to utilise a variety of hosts for dispersal (Mackie 2007). Sphaeriids have been documented attaching themselves externally to other animals by clamping onto salamander and insect appendages (Wood et al. 2008; Zelaya and Marinone 2012) and bird feathers (Mackie 2007). Some species also possess the ability to survive the digestive tracks of both birds (Mackie 2007) and fishes (Brown 2007). This combination of factors adds to the uncertainty surrounding sphaeriid species diversity, contributes to a potential Linnean Shortfall within the group and adds uncertainty to where sphearids falls in relation to the high isolation–high endemicity paradigm observed in many other isolated aquatic species. While studies that focus on desert springs and other arid water bodies are limited, genetic-based studies of montane species from Asia and Africa have identified cryptic and endemic taxa (Clewing et al. 2020; Rassam et al. 2021b; Clewing et al. 2022; Saito et al. 2022).

In western North America, sphaeriid diversity studies are limited (*but see* Guralnick 2005) and broader taxonomic studies, which include Nearctic populations, are often focused on higher level delineation of genera, subfamilies, or families (Cooley and Foighil 2000; Park and Foighil 2000; Korniushin and Glaubrecht 2002; Lee and Foighil 2003). Populations found in this region are generally considered to be one of a small number of cosmopolitan species (Guralnick 2005). A notable exception to this pattern is *Pisidium sanguinichristi*, Taylor 1987, the Sangre de Cristo pea clam. The type (and only) locality known is Middle Fork Lake located in the Sangre de Cristo Mountain Range of New Mexico, USA (Taylor 1987). Due to this extremely limited range, the species is listed as threatened by the State of New Mexico ([NMDGF] New Mexico Department of Game and Fish 2020) and is a candidate for listing as threatened or endangered under the US Endangered Species Act ([USFWS] U.S. Fish and Wildlife Service 2009). Although, there is debate regarding the taxonomic validity of the species due to known sphaeriid variation ([USFS] US

Forest Service 2017, 2020). Extensive field surveys and collection efforts have been performed by various agency biologists since the 1990s and all have failed to produce a sample that can be reliably identified as *P. sanguinichristi* (USFWS 2009; NMDGF 2002). In a study performed by the New Mexico Department of Game and Fish, 42 sites within the Sangre de Cristo Mountains were sampled between 1996 and 1999. Over 750 voucher samples were collected from Middle Fork Lake alone. After morphological examination, not a single sample was identified as *P. sanguinichristi* (NMDGF 2002). This extensive sample effort highlights an obvious contradiction to the statement of commonality of *P. sanguinichristi* in Middle Fork Lake provided in the original species description, which was originally noted by Taylor (1987) to be a locally abundant species. As already stated, conchological studies can be very difficult to perform in sphaeriid clams, and therefore, we focused on a genetic approach, which has proved a valuable tool in delimiting many other cryptic or previously unconfirmed species (Martinsson and Erséus 2021; Shults et al. 2022; Mcguire et al. 2023; Cannizzaro and Berg 2024). Genetic information can help to verify the validity of *P. sanguinichristi* as a species so appropriate listing and other conservation actions can be implemented. In addition, further investigation of sphaeriids in this region will also increase understanding of biodiversity patterns and potential conservation status of other local species.

In this study, we addressed two distinct questions: (1) Do sphaeriids in the American Southwest follow the pattern of narrow distribution and high endemism common in other desert and montane aquatic species and (2) is *P. sanguinichristi* a valid species based on genetic analysis? We hypothesised, based on the high dispersal potential, that sphaeriids in the isolated water bodies of the western United States would present an example counter to most isolated aquatic species by displaying broadly distributed genetic diversity and not supporting the current recognition of *P. sanguinichristi* as a distinct species. The results of this study will add to our understanding of how aquatic species

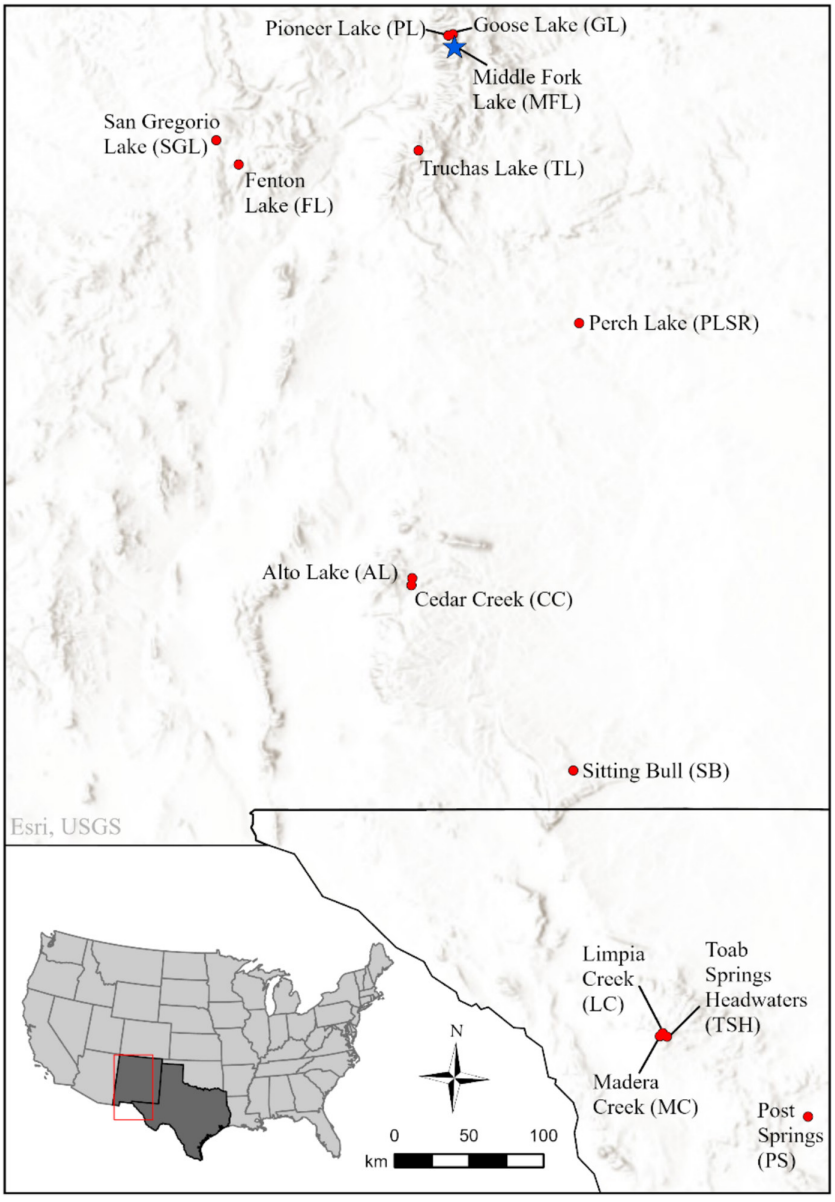

FIGURE 1 | Sphaeriid sampling localities. Middle Fork Lake (indicated by blue Star) is the type locality of *Pisidium sanguinichristi*.

TABLE 1 | NCBI GenBank BLASTn results for the 16s gene. The 'Haplotype number' column corresponds to the shared haplotypes in the haplotype network (Figure 2). The 'Localities' column displays collection localities (Figure 1) that share the haplotype, and the 'Number of individuals' column displays how many individuals share the haplotype. The 'Species identified on NCBI GenBank' column displays the top three results, in order, along with accession numbers in parentheses. The three genera identified include Euglesa (*Pisidium*), *Musculium*, and *Sphaerium*. The 'Percent sequence identities' column indicate the sequence identities, in order, for the species displayed in the previous column. When discrepancies in nomenclature existed between the species name recorded in GenBank and the most recent taxonomic classification we provide both formatted as 'current name (name as recorded on GenBank [accession number])'.

Haplotype number	Localities	Number of individuals	Species identified on NCBI GenBank	Percent sequence identities
1	CC, SB, AL	12	*E. fallax* (*P. fallax* [AY093561.1]) *E. fallax* (*P. fallax* [AY957816.1]) *E. fallax* (*P. fallax* [AY957824.1])	99.57 99.31 98.87
2	LC, TSH	8	*E. fallax* (*P. fallax* [AY957813.1]) *P. sp RPG-5* [AY957870.1] *P. sp RPG-2005c* [AY957869.1]	100 97.28 97.28
3	TSH	1	*E. fallax* (*P. fallax* [AY957813.1]) *P. sp RPG-5*[AY957870.1] *P. sp RPG-2005c* [AY957869.1]	100 97.28 97.28
4	LC	1	*E. fallax* (*P. fallax* [AY957813.1]) *P. sp RPG-5* [AY957870.1] *P. sp RPG-2005c* [AY957869.1]	100 97.28 97.28
5	AL	1	*E. fallax (P. fallax* [AY093561.1]) *E. fallax (P. fallax* [AY957816.1]) *E. fallax (P. fallax* [AY957824.1])	99.35 99.31 98.87
6	AL	2	*E. fallax (P. fallax* [AY093561.1]) *E. fallax (P. fallax* [AY957816.1]) *E. fallax (P. fallax* [AY957824.1])	99.57 99.31 98.87
7	FL	1	*E. compressa* [KY202871.1] *E. compressa* [KY202870.1] *E. compressa* [AY957810.1]	99.78 99.77 99.77
8	FL, PS	8	*E. compressa* [KY202871.1] *E. compressa* [KY202870.1] *E. compressa* [AY957810.1]	99.78 99.77 99.77
9	PLSR	1	*P. sp RPG-2005a* [AY957814.1] *P. sp RPG-2005b* [AY957815.1] *P. sp RPG-2005c* [AY957869.1]	97.28 96.83 96.83
10	PLSR	19	*P. sp RPG-2005a* [AY957814.1] *P. sp RPG-2005b* [AY957815.1] *P. sp RPG-2005c* [AY957869.1]	97.51 97.06 97.05
11	PLSR	2	*P. sp RPG-2005a* [AY957814.1] *P. sp RPG-2005b* [AY957815.1] *P. sp RPG-2005c* [AY957869.1]	97.51 97.06 97.05
12	CC	4	*P. sp RPG-2005a* [AY957814.1] *P. sp RPG-2005b* [AY957815.1] *P. sp RPG-2005c* [AY957869.1]	99.55 98.87 98.64
13	MFL	1	*E. variabilis* (*P. variabile* [AY957802.1]) *E. variabilis* (*P. variabile* [AF152030.1]) *E. variabilis* (*P. variabile* [AY957803.1])	100 99.78 99.78
14	MFL, SGL	12	*E. variabilis* (*P. variabile* [AY957802.1]) *E. variabilis* (*P. variabile* [AF152030.1]) *E. variabilis* (*P. variabile* [AY957803.1])	100 99.78 99.77

(Continues)

TABLE 1 | (Continued)

Haplotype number	Localities	Number of individuals	Species identified on NCBI GenBank	Percent sequence identities
15	MFL	1	*E. variabilis* (*P. variabile* [AY957802.1])	99.77
			E. variabilis (*P. variabile* [AF152030.1])	99.57
			E. variabilis (*P. variabile* [AY957803.1])	99.55
16	MFL, PL	5	*E. casertana* [KY126452.1]	100
			E. casertana [KY126458.1]	99.79
			E. casertana [AY957788.1]	99.79
17	TL	3	*E. casertana* [AY957796.1]	99.32
			E. casertana [AY957795.1]	99.32
			E. casertana [AY957785.1]	99.32
18	GL, PL	3	*E. casertana* [KY126452.1]	99.15
			E. casertana [KY126455.1]	99.15
			E. casertana [KY126457.1]	99.15
19	SGL	2	*E. casertana* [AY957792.1]	99.77
			E. casertana [AY957791.1]	99.55
			E. casertana [AY957789.1]	99.09
20	PL	1	*E. casertana* [AY957796.1]	99.77
			E. casertana [AY957795.1]	99.77
			P. sp CC-2104 [KF483282.1]	99.56
21	PL	2	*E. casertana* [AY957796.1]	99.55
			E. casertana [AY957795.1]	99.55
			E. atkinsoniana (*P. atkinsonianum* [KF483307.1])	99.35
22	PL	1	*E. casertana* [AY957796.1]	99.32
			E. casertana [AY957795.1]	99.32
			P. sp CC-2104 [KF483282.1]	99.12
23	MC	3	*S. lacustre* (*M. lacustre* [AY093551.1])	97.41
			S. occidentale [AF152046.1]	97.41
			S. lacustre (*M. lacustre* [AF152035.1])	97.41

Note: Identified samples without species epithets were collected in either Colorado, USA ('RPGxxxxx' see Guralnick 2005), or Tibet ('P. sp CC-xxxxx', see Clewing et al. 2013).

are restricted by, or able to overcome, dispersal barriers across inhospitable landscapes. Furthermore, it will aid in resolving the conservation status of *P. sanguinichristi*.

2 | Methods

We collected whole body samples from sites of various habitat types spanning New Mexico and west Texas, which included the type locality of *P. sanguinichristi*, Middle Fork Lake in Taos County, New Mexico (Figure 1). Sphaeriids were collected by sifting through aquatic sediments by hand or with aquarium nets to expose the clams. Whole specimens were preserved in 95%–100% ethanol and stored at −80°C. We attempted multiple methods of DNA extraction to determine the best protocol for obtaining sufficient high-quality DNA, while preserving the very delicate valves for future morphological work. Tissue preservation is known to be particularly problematic in sphaeriids (Frankiewicz 2018); however, we found a standard CTAB extraction followed by ethanol precipitation to be the most effective for maximising yield of high-quality DNA (Maroof et al. 1984). We selected two genes commonly utilised during sphaeriid phylogenetic studies: the mitochondrial 16S ribosomal subunit gene (16S; Cooley & Ó Foighil 2000; Lee and Foighil 2003) and the nuclear 28S ribosomal subunit gene (28S; Bößneck et al. 2016). Both genes were sequenced following previously described methods (Clewing et al. 2013). Samples were sequenced at Miami University's Center for Bioinformatics and Functional Genomics on an Applied Biosystems 3730 DNA Analyser (Thermo Fisher Scientific, Waltham, MA) following a Big Dye terminator and cycle sequencing protocol (available at http://miamioh.edu/cas/academics/centers/cbfg/tools-protocols/index.html). Both forward and reverse sequencing products were visualised, curated and combined into a single consensus strand using CLC Genomics Workbench v 21.0.3 (https://digitalinsights.qiagen.com/). We used the National Center for Biotechnology Information's (NCBI) BLASTn tool to compare our sample sequences to those published in the GenBank sequence repository. This allowed us to determine sequence identity and establish how similar the samples from amongst the collection localities were to each other, as well as to species identified on

TABLE 2 | NCBI GenBank BLASTn results for the 28S gene. The 'Haplotype number' column corresponds to the shared haplotypes in the haplotype network (Figure 3). The 'Localities' column displays collection localities (Figure 1) that share the haplotype, and the 'Number of individuals' column displays how many individuals share the haplotype. The 'Species identified on NCBI GenBank' column displays the top three results, in order, along with accession numbers in parentheses. The three genera identified include *Pisidium*, *Musculium*, and *Sphaerium*. The 'Percent sequence identities' indicates the sequence identities, in order, for the top three identified species displayed in the previous column. When discrepancies in nomenclature existed between the species name recorded in Genbank and the most recent taxonomic classification we provide both formatted as 'current name (name as recorded on GenBank [accession number])'.

Haplotype number	Localities	Number of individuals	Species identified on NCBI GenBank	Percent sequence identities
1	CC	2	*P. walkeri* (*P. walkeri* [KX713421.1]) *E. floresiana* (*P. floresianum* [MN913632.1]) *E. casertana* (*P. casertanum* [KF483338.1])	99.84 99.68 99.68
2	SGL, TL, AL, FL, GL, MFL, PL, PLSR, PS	34	*E. floresiana* (*P. floresianum* [MN913632.1]) *E. casertana* (*P. castertanum* [KF483338.1]) *E. subtruncata* (*P. subtruncatum* [MN913651.1])	100 100 99.84
3	LC	1	*E. floresiana* (*P. floresianum* [MN913632.1]) *E. casertana* (*P. castertanum* [KF483338.1]) *E. subtruncata* (*P. subtruncatum* [MN913651.1])	99.68 99.68 99.52
4	AL, CC, SB	7	*E. floresiana* (*P. floresianum* [MN913632.1]) *E. casertana* (*P. castertanum* [KF483338.1]) *E. subtruncata* (*P. subtruncatum* [MN913651.1])	99.84 99.84 99.68
5	CC	1	*E. floresiana* (*P. floresianum* [MN913632.1]) *E. casertana* (*P. castertanum* [KF483338.1]) *E. subtruncata* (*P. subtruncatum* [MN913651.1])	99.68 99.68 99.52
6	TSH, LC	3	*E. floresiana* (*P. floresianum* [MN913632.1]) *E. casertana* (*P. castertanum* [KF483338.1]) *E. subtruncata* (*P. subtruncatum* [MN913651.1])	99.68 99.68 99.52
7	SGL, MFL	14	*P. sp* [KF483324.1] *E. floresiana* (*P. floresianum* [MN913632.1]) *E. casertana* (*P. casertanum* [KF483338.1])	99.84 99.68 99.68

(Continues)

TABLE 2 | (Continued)

Haplotype number	Localities	Number of individuals	Species identified on NCBI GenBank	Percent sequence identities
8	SGL	1	*E. floresiana* (*P. floresianum* [MN913632.1])	99.84
			E. casertana (*P. castertanum* [KF483338.1])	99.84
			E. subtruncata (*P. subtruncatum* [MN913651.1]	99.68
9	MC	1	*S. sp* [KY513183.1]	99.52
			S. indicum (*M. indicum* [KF483345.1])	99.52
			S. lacustre [AM779712.1]	99.52
10	MC	1	*S. sp* [KY513183.1]	99.36
			S. indicum (*M. indicum* [KF483345.1])	99.36
			S. lacustre [AM779712.1]	99.36

GenBank. Using MEGAX (Kumar et al. 2018), sequences were aligned by MUSCLE (Edgar 2004) for each gene individually. In PopART (Bandelt et al. 1999; Leigh and Bryant 2015), we built minimum spanning haplotype networks to visualise sequence similarities and shared haplotypes amongst samples for each gene.

For samples in which both genes were successfully sequenced, we used SequenceMatrix (Vaidya et al. 2011) to generate a concatenated sequence matrix. A maximum-likelihood phylogeny was generated in IQtree v 1.6.12 (Nguyen et al. 2015) from these concatenated sequences. Prior to conducting the phylogenetic analysis, we determined the best-fit evolutionary model and partitioning scheme also using IQtree. Node support was assessed using 10,000 ultrafast bootstraps (Hoang et al. 2018) and an SH-aLRT test (Guindon et al. 2010).

3 | Results

We successfully collected and sequenced samples from 14 locations across west Texas and New Mexico (Figure 1), including samples from Middle Fork Lake. A total of 94 and 64 samples were sequenced for 16S and 28S, respectively (Tables 1 and 2). Final sequence lengths, after processing, resulted in 471 bp for 16S and 629 bp for 28S.

The BLASTn results displayed high sequence identities at both the 16S and 28S genes when comparing the individuals sampled in this study with other described and undescribed species of sphaeriids (Tables 1 and 2). The previously described species identified by 16S include *Euglesa fallax*, *E. compressa*, *E. variabilis*, *E. casertana*, *Sphaerium lacustre* and *S. occidentale*. The two non-*Euglesa* species were only found in Madera Creek in west Texas. However, these samples shared equal 16S sequence identity (97.41%) with both *S. lacustre* and *S. occidentale*, casting doubt on the reliability of this identification (Table 1). The undescribed species identified by 16S include individuals mostly identified from a previous study of sphaeriid diversity in the state of Colorado, USA (see Guralnick 2005) The described species identified by 28S include *E. walkeri*, *E. floresiana*, *E. casertana*, *E. subtruncata*, *S. indicum* and *S. lacustre*. The non-*Euglesa* samples identified by 28S are the same samples from Madera Creek identified by 16S as either *S. lacustre* or *S. occidentale*. Using 28S, the samples from Madera Creek were identified as either *S. indicum* or *S. lacustre*.

Identical haplotypes were shared broadly across the region. For example, the same haplotypes are shared between a small spring near Marathon, Texas and Fenton Lake in northern New Mexico, sites located over 700 km apart across arid landscapes with no physical water connections (Figures 1, 2 and 3). Furthermore, we did not find support for the pea clams collected at Middle Fork Lake being an endemic species (Figures 4, 5 and 6). The '*P. sanguinichristi*' 16S haplotypes collected from this site were identical to those from San Gregorio Lake and Pioneer Lake (Figures 2 and 5), localities that are approximately 150 and 10 km from Middle Fork Lake, respectively. While these distances may not seem large, other similarly sized desert aquatic invertebrates show population and species divergences at much smaller distances (Walters et al. 2022). We also found that 28S haplotypes were shared between Middle Fork Lake and eight other locations broadly distributed across the region (Figures 3 and 6). The GenBank results based on mtDNA indicate that the samples collected from Middle Fork Lake are either *E. variabilis* or *E. casertana*, with 99%–100% sequence identities (Table 1). The nDNA indicates that the samples from Middle Fork Lake are either *E. floresiana*, *E. casertana* or *E. subtruncata*, again with 99%–100% sequence identities (Table 2).

A similar result is displayed by the maximum-likelihood tree. There are multiple well-supported clades but only one monophyletic locality: Perch Lake, NM (Figure 4). These samples were also identified by the mtDNA GenBank analysis to be most closely related to the undescribed species noted by Guralnick (2005), albeit less strongly, with only 96.83%–97.51% sequence identity (Table 1), while the nDNA samples were identified as either *E. floresiana*, *E. casertana* or *E. subtruncata* (Table 2) with 99%–100% sequence identity. Most of the other clades found in the tree are composed of samples from multiple localities. The samples from Middle Fork Lake are found in two well-supported clades that also include individuals collected at San Gregorio Lake or Pioneer Lake.

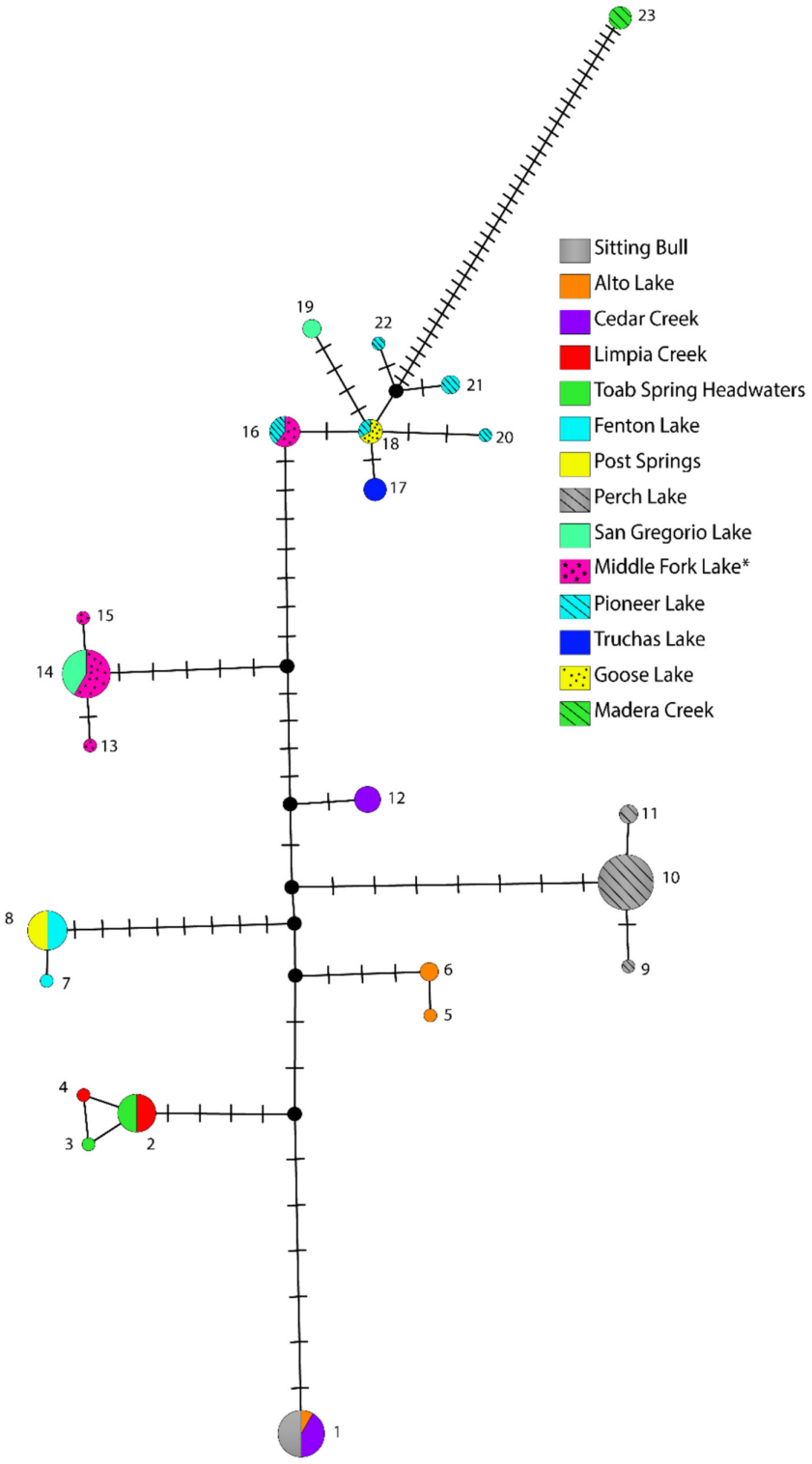

FIGURE 2 | Minimum-spanning haplotype network for the 16S gene. Coloured circles represent unique haplotypes shared amongst locations. The size of circle is proportional to total number of individuals sharing the haplotype. Black dots represent ancestral or unsampled haplotypes. Hash marks represent the number of nucleotide substitutions between haplotypes. Numbers next to haplotypes indicate haplotype numbers that correspond to Table 1.

4 | Discussion

Species distributions are commonly explained by the interplay of two fundamental concepts in biogeography: vicariance and dispersal (Zink et al. 2000). Major geographical changes associated with climatic shifts (Heimburger et al. 2022), changing hydrological patterns (Adams et al. 2018) and the creation of geological barriers (Frantz et al. 2018) can disrupt dispersal and fragment populations, leading to allopatric speciation. Highly isolated waterbodies, such as desert springs and mountain

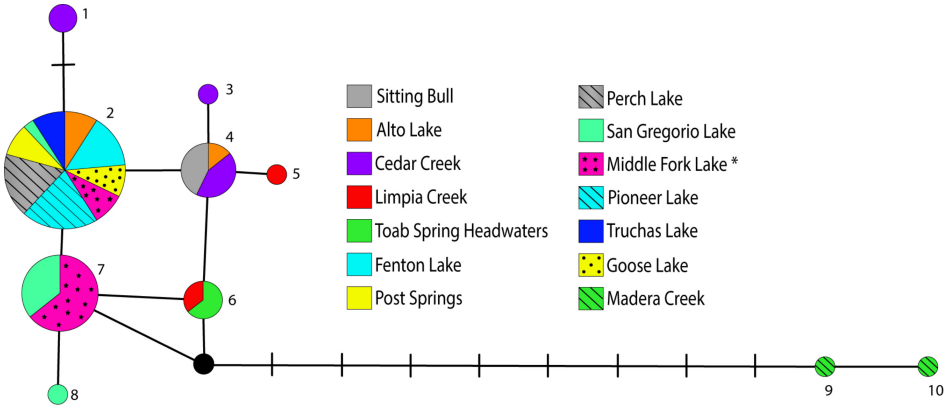

FIGURE 3 | Minimum-spanning haplotype network for the 28S gene. Coloured circles represent unique haplotypes shared amongst locations. The size of circle is proportional to the total number of individuals sharing the haplotype. Black dots represent ancestral or unsampled haplotypes. Hash marks represent the number of nucleotide substitutions between haplotypes. Numbers next to haplotypes indicate haplotype numbers that correspond to Table 2.

lakes, are well known for harbouring especially high numbers of endemic aquatic species with extremely limited ranges (Davis et al. 2006; Seidel et al. 2009; McKelvey et al. 2020; Work 2023). Endemic species or populations that have been isolated due to historic vicariance event are characterised by unique haplotypes and increased genetic dissimilarity compared with other populations (Hein et al. 2024). In the western United States, this pattern is both common and well-documented across a breadth of aquatic species that lack terrestrial life stages (Hershler et al. 2014; Morningstar et al. 2014; Culumber et al. 2016; Sağlam et al. 2016; Cannizzaro et al. 2017; Stevens et al. 2020; Walters, Trujillo and Berg 2022). For example, Ancient hydrographic connections, that have long since been altered, are hypothesised to be a driving factor in initial distributions and subsequent isolation of multiple aquatic invertebrate species. For example, in the American West, shifts in rivers and lake systems during the Miocene, Pliocene and Pleistocene contributed to increases in diversity amongst the hydrobiid snails (Hershler et al. 1994; Hershler and Liu 2008). Looking even further back in biogeographical history, during the late-Cretaceous, the western United States was submerged under an inland sea. The retreat of this sea is considered a primary driving factor to the dispersal and subsequent isolation of many recently described amphipod species (Adams et al. 2018). Our results suggest relatively high dispersal amongst sphaeriid populations based on shared DNA sequences. Therefore, it is harder to make the connection between contemporary genetic diversity in sphaeriids. Although, we do find at least one population that harbours unique genetic variation, which may have resulted from historical hydrogeographic changes.

In contrast to species or populations that have been isolated for extended periods of time, populations with shared haplotypes and low genetic dissimilarity likely experienced either recent or continuing gene flow (Du et al. 2020). The greater a species' dispersal ability, the greater the genetic similarity amongst geographically distant populations (Krauss et al. 2004; Lv et al. 2019). Our results show that the majority of sphaeriid 16S haplotypes are shared across multiple locations and that they are either identical to, or very similar to, sequences from described species previously deposited in GenBank. This includes the cosmopolitan species *E. casertana* (*P. casertanum*), considered the most widely distributed mollusc in the world (Mackie 2007; Bespalaya et al. 2019). The other species identified by 16S as inhabiting Middle Fork Lake was *E. variabilis* (*P. variable*), another species found throughout much of North America. The additional species identified by 16S were *E. compressa* and *E. fallax*, even though *E. fallax* is typically found at the higher latitudes of southern Canada and the Great Lakes (Mackie 2007) but is also found at more southerly portions of the United States such as the state of Alabama. Individuals collected at Perch Lake contained *Euglesa* (*Pisidium*) 16S haplotypes found nowhere else and not matching any species on GenBank. However, further investigation is required to determine if these samples potentially represent an undescribed species. The samples collected from Madera Creek were the only samples not identified as *Pisidium* on GenBank. The identity of these samples remains unclear as they were all equally identified (97.41%) as either *S. lacustre* or *S. occidentale*.

The degree of diversity amongst the 28S sequences was low and inconsistent with information from 16S. We found a single haplotype accounting for 52% of the samples. Interestingly, this haplotype is associated with three separate species showing 99.84%–100% sequence identity on GenBank: *E. casertana*, *E. floresiana* and *E. subtruncata*. Only *E. casertana*is considered native to North America; the latter two species are native to Europe, Africa or Asia (Korniushin 2000; Mackie 2007; Clewing et al. 2020; Rassam et al. 2020). Another 22% of the 28S samples were of a haplotype identified as either *E. casertana* or *E. floresiana*. These two widely distributed 28S haplotypes were also the only haplotypes found in Middle Fork Lake. Consistent with results for 16S, the only non-*Pisidium* samples identified by 28S were from Madera Creek and were identified as a species of *Sphaerium*. Nuclear gene sequences are often conserved and provide relatively low resolution amongst species in invertebrate taxa (Zhang and Hewitt 2003; Adams et al. 2018; Kocot-Zalewska et al. 2021), and it is possible that the same is true for sphaeriids. The increased genetic distances between the Madera Creek populations, which are not *Pisidium*, and all

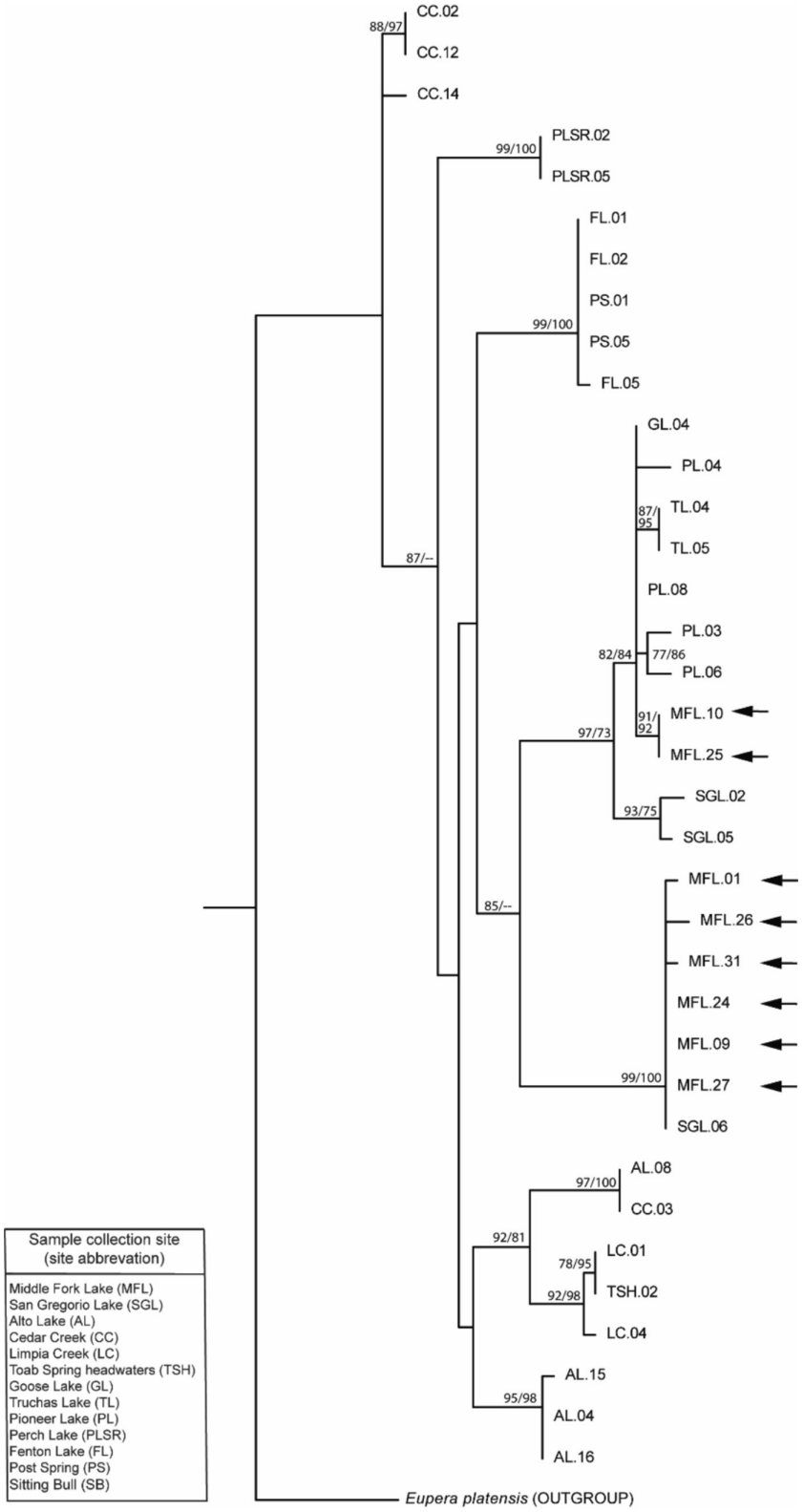

FIGURE 4 | Maximum-likelihood phylogeny for concatenated 28S and 16S sequences. Support is displayed for both SH-aLRT (left) and ultra-fast bootstrap (right) values at each node. Support values < 70 are not displayed.

other populations suggest that 28S does however provide resolution at higher taxonomic levels.

The phylogeny based on concatenated sequences displays results similar to the 16S haplotype network, which is unsurprising given the minimal resolution provided by 28S sequences. Samples from Perch Lake (PLSR) form a monophyletic clade not closely related to any species documented on GenBank, suggesting that this locality potentially contains a different species than other sites or that this species has not been previously sequenced,

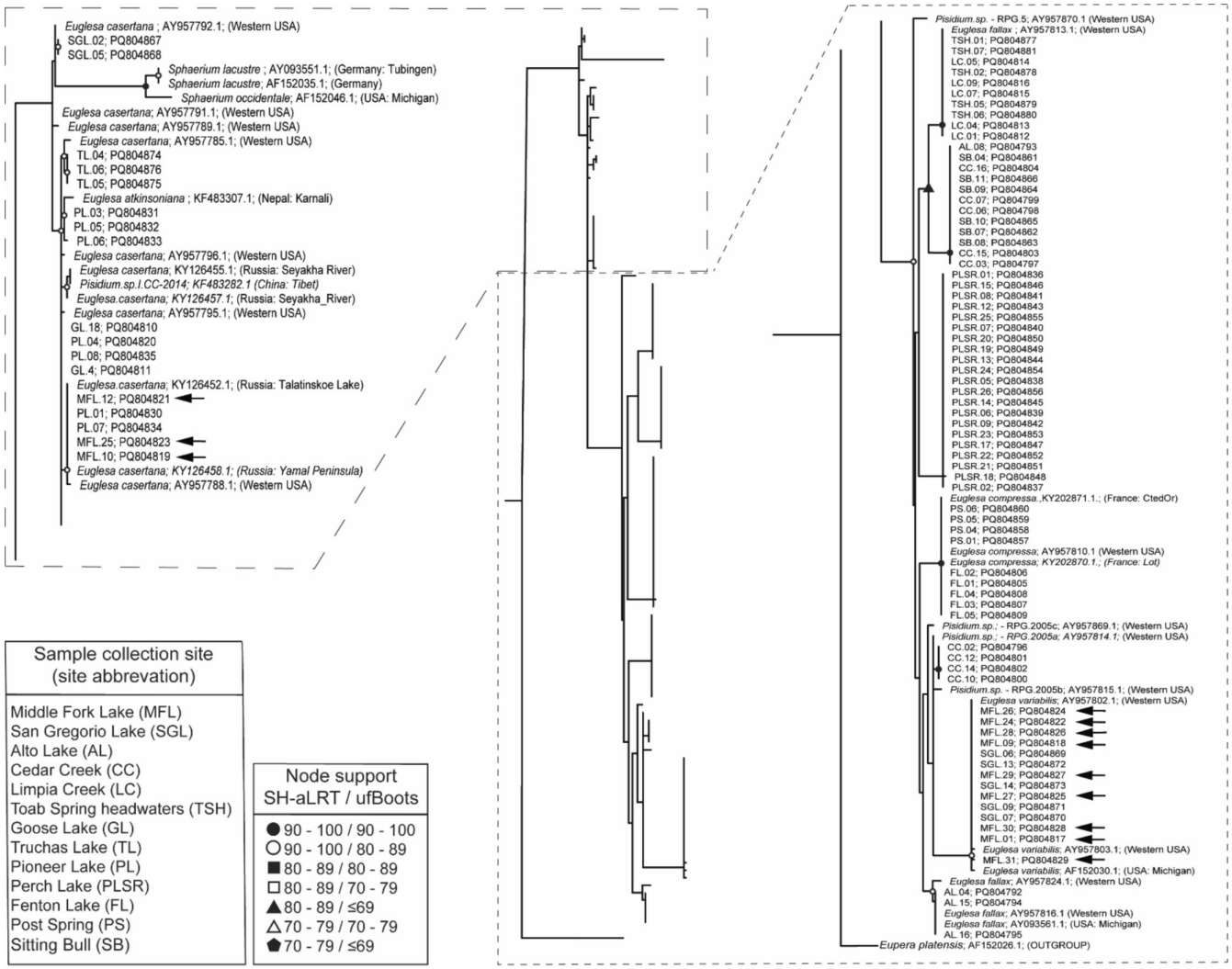

FIGURE 5 | Maximum likelihood phylogeny for 16S sequences including GenBank samples referenced in Table 1. GenBank samples are display with species name, GenBank accession number, and collection locality as provided in GenBank or associated study. Node support values, expressed as percentages, are displayed for both SH-aLRT and ultra-fast bootstrap (ufBoots). Support values less than 70 are not displayed. Arrows highlight samples collected at Middle Fork Lake (i.e., putative *Pisidium sanguinichristi* samples).

but this hypothesis requires further investigation. All other locations, including Middle Fork Lake (*P. sanguinichristi* holotype locality), contain at least two clades. Overall, the high degree of shared haplotypes amongst sphaeriid populations indicates that gene flow remains relatively high despite physical isolation of the waterbodies. In other regions of the world, authors have described multiple endemic species of sphaeriids that likely have low dispersal capabilities (Korniushin 2000; Bößneck et al. 2016; Clewing et al. 2020, 2022; Saito et al. 2022). However, in the western United States, the distribution of many sphaeriids is consistent with extensive ranges and high dispersal capabilities.

In much of our study region, availability of amphibians or fishes to act as passive dispersers is unlikely due to a combination of arid environments and physical isolation of habitats. Birds or even flying insects are more likely to serve as vectors. While we were unable to locate any studies from the immediate region, in other areas of the western United States, the distribution of a widespread snail is associated with waterfowl migration flyways (Martin et al. 2020). The location of our study is within the Central Flyway, which could explain the movement of clams amongst disparate locations. In addition to natural dispersal mechanisms, humans may also serve as a means of dispersal for sphaeriids in this region. Human-mediated movement of sphaeriids has been well documented, including the introduction of multiple invasive species across continents (Grigorovich et al. 2000; Mouthon et al. 2018). None of the species identified in this study are known invasive species, but the majority of the sampling locations serve as recreational fishing and/or swimming areas. We cannot rule out that humans have unintentionally moved sphaeriids between locations.

4.1 | Conservation Implications

The Linnean Shortfall exists because much biodiversity remains undescribed. This underestimation of species diversity typically limits the ability to accurately assess species needs and to pursue

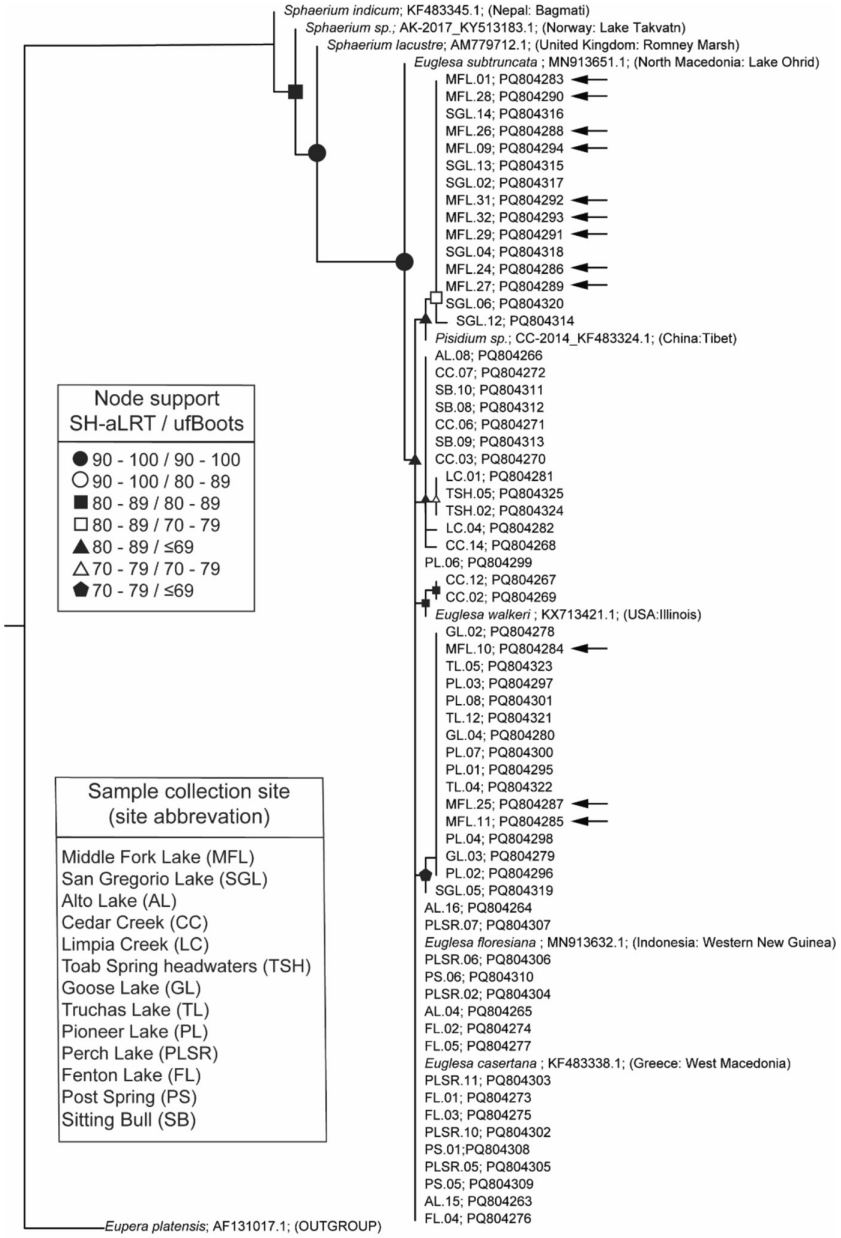

FIGURE 6 | Maximum likelihood phylogeny for 28S sequences including GenBank samples referenced in Table 2. GenBank samples are display with species name, GenBank accession number, and collection locality as provided in GenBank or associated study. Node support values, expressed as percentages, are displayed for both SH-aLRT and ultra-fast bootstrap (ufBoots). Support values less than 70 are not displayed. Arrows highlight samples collected at Middle Fork Lake (i.e., putative *Pisidium sanguinichristi* samples).

appropriate conservation actions (Bini et al. 2006). Because conservation resources in the forms of capital assets, labour, consumables and other expenses are limited, allocation of those resources needs to be prioritised (Joseph et al. 2009; Gerber et al. 2018; Pienkowski et al. 2021; Wiedenfeld et al. 2021). It is equally important from an investment perspective to assess the potential impacts of overestimation of biodiversity in developing cost-effective conservation decisions (Folt et al. 2019; Chan et al. 2022). There are a variety of logistical, cultural and societal factors that must be considered when allocating resources towards setting conservation priorities, including minimising extinction risk (Miller et al. 2006). In taxa that are widespread and with high dispersal capabilities, extinction risk is likely relatively low compared with narrow endemic species and those that are poor dispersers. While this study provides insight into sphaeriid biodiversity, it is also important to point out two potential shortfalls. First, the geographic extent of sampling is limited and there may yet still be undiscovered diversity in the western United States. For instance, sphaeriids from Perch Lake, New Mexico, require more investigation to determine whether they are an undescribed endemic species. Secondly, while sampling efforts at Middle Fork Lake were extensive during our study period and multiple large scale efforts have been made previously to collect the species ([NMDGF] New Mexico Department of Game and Fish 2002; [USFS] U.S. Forest Service 2020), it is still possible that *P. sanguinichristi* is present and was simply missed

in the collecting process. However, in the original species description, Taylor (1987) suggests that *P. sanguinichristi* is found 'in quantity' so the potential of missing individuals is highly unlikely.

When cosmopolitan species are more deeply investigated, a typical result is to find cryptic diversity which serves to decrease the Linnean Shortfall (McLeod 2010; Scherz et al. 2019; Nori et al. 2022). While this is a common pattern, there are exceptions (Morningstar et al. 2018; Chan et al. 2022). Sphaeriids present a mixed case—where both endemic and cosmopolitan species are commonplace (Clewing et al. 2022; Saito et al. 2022). We found that many sphaeriids in the American Southwest are widely distributed; however, we do find some exception, which is consistent with the results of Guralnick (2005).

Conservation is subjected to resource availability. Therefore, widely distributed species that appear also to be abundant should have a lower priority compared with less abundant and/or minimally distributed species when allocating these limited conservation resources. This is an especially important conclusion in the case of '*P. sanguinichristi*.' Our results are consistent with the presence of only widely distributed and previously described sphaeriid species inhabiting Middle Fork Lake. We found no evidence of an endemic sphaeriid taxon at this locality, nor did we find evidence of a unique species of sphaeriid anywhere that should be considered *P. sanguinichristi*. Consequently, our genetic study, taken in combination with previous morphological based studies (*see* [NMDGF] New Mexico Department of Game and Fish 2002), does not support the existence of *P. sanguinichristi* as a valid taxon. Furthermore, this suggests that conservation of the Middle Fork Lake population of sphaeriids, which appears to be comprised of species genetically identified as lineages of either *E. casertana*or or *E. variabilis*, should not be a high priority action. While this study does not draw many of the typical conservation conclusions, our findings are still equally important for informing strategic implementation of limited conservation resources.

Author Contributions

Conceptualisation: SRH, MPAB, DAT, DJB. Developing methods: SRH, MPAB, DAT, DJB. Conducting research: SRH, MPAB, DAT. Data analysis: SRH, MPAB. Data interpretation: SRH, MPAB, DAT, DJB. Preparation of figures and table: SRH. Writing: SRH, DJB.

Acknowledgements

We would like to thank past and present members of the Berg Lab for assistance with sample collection and lab work including Mary Jones, Ethan Devillez, Andrew Cannizzaro, Ashley Walters, Jake Almeda, Alyssa Bley and Ashley Stanke. Our colleagues Tereza Jezkova, Thomas Crist, Bart Grudzinski, Bruce Cochrane and student members of the Berg lab provided valuable input on an early version of this manuscript. This work was funded by the New Mexico Department of Game and Fish. This research was completed as a component of SRH's dissertation research at Miami University, Oxford, OH. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the US Fish and Wildlife Service. Any use of trade, product or firm names is for descriptive purposes only and does not imply endorsement by the US Government.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Sequence data are publicly available in Genbank (https://www.ncbi.nlm.nih.gov/genbank/) – Accession Numbers PQ804263–4325 (28S) and PQ804792–4881 (16S).

References

Adams, N. E., K. Inoue, R. A. Seidel, B. K. Lang, and D. J. Berg. 2018. "Isolation Drives Increased Diversification Rates in Freshwater Amphipods." *Molecular Phylogenetics and Evolution* 127: 746–757.

Avise, J. C. 2000. *Phylogeography: The History and Formation of Species*. Harvard University Press.

Bandelt, H. J., P. Forster, and A. Röhl. 1999. "Median-Joining Networks for Inferring Intraspecific Phylogenies." *Molecular Biology and Evolution* 16: 37–48.

Bespalaya, Y., J. Joyner-Matos, I. Bolotov, et al. 2019. "Reproductive Ecology of *Pisidium Casertanum* (Poli, 1791) (Bivalvia: Sphaeriidae) in Arctic Lakes." *Journal of Molluscan Studies* 85: 11–23.

Bespalaya, Y. V., M. V. Vinarski, O. V. Aksenova, et al. 2024. "Phylogeny, Taxonomy, and Biogeography of the Sphaeriinae (Bivalvia: Sphaeriidae)." *Zoological Journal of the Linnean Society* 201: 305–338.

Bini, L. M., J. A. F. Diniz-Filho, T. F. Rangel, R. P. Bastos, and M. P. Pinto. 2006. "Challenging Wallacean and Linnean Shortfalls: Knowledge Gradients and Conservation Planning in a Biodiversity Hotspot." *Diversity and Distributions* 12: 475–482.

Blattner, L., K. Lucek, N. Beck, D. Berner, and S. von Fumetti. 2022. "Intra-Alpine Islands: Population Genomic Inference Reveals High Degree of Isolation Between Freshwater Spring Habitats." *Diversity and Distributions* 28: 291–305.

Bößneck, U., C. Clewing, and C. Albrecht. 2016. "Exploring High-Mountain Limnic Faunas: Discovery of a Novel Endemic Bivalve Species (Sphaeriidae: *Pisidium*) in the Nepal Himalayas." *Invertebrate Systematics* 30: 588–597.

Brown, K. M., B. Lang, and K. E. Perez. 2008. "The Conservation Ecology of North American Pleurocerid and Hydrobiid Gastropods." *Journal of the North American Benthological Society* 27: 484–495.

Brown, R. J. 2007. "Freshwater Mollusks Survive Fish Gut Passage." *Arctic* 60: 124–128.

Cannizzaro, A. G., and D. J. Berg. 2024. "Molecular Phylogenetic Analyses Reveal a Radiation of Freshwater Gammarus (Amphipoda: Gammaridae) in the Northern Chihuahuan Desert." *Zoological Journal of the Linnean Society* 202: zlae108.

Cannizzaro, A. G., A. D. Walters, and D. J. Berg. 2017. "A New Species of Freshwater *Gammarus* Fabricius, 1775 (Amphipoda: Gammaridae) From a Desert Spring in Texas, With a Key to the Species of the Genus *Gammarus* From North America." *Journal of Crustacean Biology* 37: 709–722.

Chan, K. O., C. R. Hutter, P. L. Wood Jr., Y. C. Su, and R. M. Brown. 2022. "Gene Flow Increases Phylogenetic Structure and Inflates Cryptic Species Estimations: A Case Study on Widespread Philippine Puddle Frogs (*Occidozyga Laevis*)." *Systematic Biology* 71: 40–57.

Clewing, C., U. Bössneck, P. V. von Oheimb, and C. Albrecht. 2013. "Molecular Phylogeny and Biogeography of a High Mountain Bivalve Fauna: The Sphaeriidae of the Tibetan Plateau." *Malacologia* 56: 231–252.

Clewing, C., T. Geertz, H. Rassam, T. H. Woldekiros, and C. Albrecht. 2022. "Freshwater Diversity at a Biogeographic Edge Zone: The

High-Mountain Pea-Clams of Ethiopia." *Systematics and Biodiversity* 20: 1–15.

Clewing, C., B. Stelbrink, U. Bößneck, et al. 2020. "Freshwater Biogeography in Wallacea: The Case of Sphaeriid Bivalves in the Malili Lake System (Sulawesi, Indonesia)." *Journal of Great Lakes Research* 46: 1176–1186.

Cooley, L. R., and D. Ó. Foighil. 2000. "Phylogenetic Analysis of the Sphaeriidae (Mollusca: Bivalvia) Based on Partial Mitochondrial 16S rDNA Gene Sequences." *Invertebrate Biology* 119: 299–308.

Coughlan, N. E., T. C. Kelly, J. Davenport, and M. A. Jansen. 2017. "Up, Up and Away: Bird-Mediated Ectozoochorous Dispersal Between Aquatic Environments." *Freshwater Biology* 62: 631–648.

Culumber, Z. W., G. W. Hopper, N. Barts, et al. 2016. "Habitat Use by Two Extremophile, Highly Endemic, and Critically Endangered Fish Species (*Gambusia Eurystoma* and *Poecilia Sulphuraria*; Poeciliidae)." *Aquatic Conservation: Marine and Freshwater Ecosystems* 26: 1155–1167.

Davis, S. K., A. A. Echelle, and R. A. Van Den Bussche. 2006. "Lack of Cytonuclear Genetic Introgression Despite Long-Term Hybridization and Backcrossing Between Two Poeciliid Fishes (*Gambusia Heterochir* and *G. Affinis*)." *Copeia* 2006: 351–359.

De Queiroz, K. 2007. "Species Concepts and Species Delimitation." *Systematic Biology* 56: 879–886.

Du, H., M. Liu, S. Zhang, F. Liu, Z. Zhang, and X. Kong. 2020. "Lineage Divergence of *Dendrolimus Punctatus* in Southern China Based on Mitochondrial Genome." *Frontiers in Genetics* 11: 65.

Edgar, R. C. 2004. "MUSCLE: Multiple Sequence Alignment With High Accuracy and High Throughput." *Nucleic Acids Research* 32, no. 5: 1792–1797. https://doi.org/10.1093/nar/gkh340.

Folt, B., J. Bauder, S. Spear, et al. 2019. "Taxonomic and Conservation Implications of Population Genetic Admixture, Mito-Nuclear Discordance, and Male-Biased Dispersal of a Large Endangered Snake, *Drymarchon couperi*." *PLoS one* 14: e0214439.

Frankiewicz, A. 2018. *Guidelines for Field Collecting and Preserving Sphaeriidae Clams, for DNA and Taxonomic Research*. Detailed Sampling Procedures and Quality Control. Institute for Great Lakes Research, Central Michigan University.

Frantz, L. A., A. Rudzinski, A. M. S. Nugraha, et al. 2018. "Synchronous Diversification of Sulawesi's Iconic Artiodactyls Driven by Recent Geological Events." *Proceedings of the Royal Society B: Biological Sciences* 285: 20172566.

[FMCS] Freshwater Mollusk Conservation Society. 2016. "A National Strategy for the Conservation of Native Freshwater Mollusks." *Freshwater Mollusk Biology and Conservation* 19: 1–21.

Funk, W. C., M. Caminer, and S. R. Ron. 2012. "High Levels of Cryptic Species Diversity Uncovered in Amazonian Frogs." *Proceedings of the Royal Society B: Biological Sciences* 279: 1806–1814.

Gerber, L. R., M. C. Runge, R. F. Maloney, et al. 2018. "Endangered Species Recovery: A Resource Allocation Problem." *Science* 362: 284–286.

Graf, D. L., and K. S. Cummings. 2025. "The Freshwater Mussels (Unionoida) of the World (And Other Less Consequential Bivalves)." MUSSEL Project web Site, http://www.mussel-project.net/. Accessed January 17, 2025.

Grigorovich, I. A., A. V. Korniushin, and H. J. MacIsaac. 2000. "Moitessier's Pea Clam *Pisidium Moitessierianum* (Bivalvia, Sphaeriidae): A Cryptogenic Mollusc in the Great Lakes." *Hydrobiologia* 435: 153–165.

Guindon, S., J. F. Dufayard, V. Lefort, M. Anisimova, W. Hordijk, and O. Gascuel. 2010. "New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0." *Systematic Biology* 59: 307–321.

Guralnick, R. P. 2005. "Combined Molecular and Morphological Approaches to Documenting Regional Biodiversity and Ecological Patterns in Problematic Taxa: A Case Study in the Bivalve Group *Cyclocalyx* (Sphaeriidae, Bivalvia) From Western North America." *Zoologica Scripta* 34: 469–482.

Halabowski, D., R. Sousa, M. Lopes-Lima, et al. 2024. "Off the Conservation Radar: The Hidden Story of Europe's Tiny pea Clams (Bivalvia: Sphaeriidae)." *Biodiversity and Conservation* 33: 1–15.

Heimburger, B., L. Schardt, A. Brandt, S. Scheu, and T. R. Hartke. 2022. "Rapid Diversification of the Australian Amitermes Group During Late Cenozoic Climate Change." *Ecography* 2022, no. 9: e05944. https://doi.org/10.1111/ecog.05944.

Hein, S. R., K. Farleigh, and D. J. Berg. 2024. "Riverscape Genomics of the Endangered Freshwater Mussel *Lampsilis Rafinesqueana*." *Freshwater Biology* 69: 1438–1453.

Hellmich, D. L., A. B. Saidenberg, and T. F. Wright. 2021. "Genetic, But Not Behavioral, Evidence Supports the Distinctiveness of the Mealy Amazon Parrot in the Brazilian Atlantic Forest." *Diversity* 13: 273.

Hershler, R., T. J. Frest, E. J. Johannes, P. A. Bowler, and F. G. Thompson. 1994. "Two New Genera of Hydrobiid Snails (Prosobranchia: Rissooidea) From the Northwestern United States." *Veliger* 37: 221–243.

Hershler, R., and H. P. Liu. 2008. "Ancient Vicariance and Recent Dispersal of Springsnails (Hydrobiidae: Pyrgulopsis) in the Death Valley System, California-Nevada."

Hershler, R., H. P. Liu, and J. Howard. 2014. "Springsnails: A new Conservation Focus in Western North America." *Bioscience* 64: 693–700.

Hoang, D. T., O. Chernomor, A. Von Haeseler, B. Q. Minh, and L. S. Vinh. 2018. "UFBoot2: Improving the Ultrafast Bootstrap Approximation." *Molecular Biology and Evolution* 35: 518–522.

Hortal, J., F. de Bello, J. A. F. Diniz-Filho, T. M. Lewinsohn, J. M. Lobo, and R. J. Ladle. 2015. "Seven Shortfalls That Beset Large-Scale Knowledge of Biodiversity." *Annual Review of Ecology, Evolution, and Systematics* 46: 523–549.

Hou, Z., P. Jin, H. Liu, et al. 2022. "Past Climate Cooling Promoted Global Dispersal of Amphipods From Tian Shan Montane Lakes to Circumboreal Lakes." *Global Change Biology* 28: 3830–3845.

Inoue, K., A. L. Pohl, M. Sei, B. K. Lang, and D. J. Berg. 2020. "Use of Species Delimitation Approaches to Assess Biodiversity in Freshwater Planaria (Platyhelminthes, Tricladida) From Desert Springs." *Aquatic Conservation: Marine and Freshwater Ecosystems* 30: 209–218.

Joseph, L. N., R. F. Maloney, and H. P. Possingham. 2009. "Optimal Allocation of Resources Among Threatened Species: A Project Prioritization Protocol." *Conservation Biology* 23: 328–338.

Kappes, H., and P. Haase. 2012. "Slow, But Steady: Dispersal of Freshwater Molluscs." *Aquatic Sciences* 74: 1–14.

Kocot-Zalewska, J., P. J. Domagała, and B. Lis. 2021. "Living in Isolation for Almost 40 Years: Molecular Divergence of the 28S rDNA and COI Sequences Between French and Polish Populations of the Cave Beetle *Speonomus Normandi hydrophilus* (Jeannel, 1907)." *Subterranean Biology* 37: 75–88.

Korniushin, A. V. 2000. "Review of the Family Sphaeriidae (Mollusca: Bivalvia) of Australia, With the Description of Four new Species." *Records-Australian Museum* 52: 41–102.

Korniushin, A. V., and M. Glaubrecht. 2002. "Phylogenetic Analysis Based on the Morphology of Viviparous Freshwater Clams of the Family Sphaeriidae (Mollusca, Bivalvia, Veneroida)." *Zoologica Scripta* 31: 415–459.

Krauss, J., T. Schmitt, A. Seitz, I. Steffan-Dewenter, and T. Tscharntke. 2004. "Effects of Habitat Fragmentation on the Genetic Structure of the Monophagous Butterfly *Polyommatus Coridon* Along Its Northern Range Margin." *Molecular Ecology* 13: 311–320.

Kumar, S., G. Stecher, M. Li, C. Knyaz, and K. Tamura. 2018. "MEGA X: Molecular Evolutionary Genetics Analysis Across Computing Platforms." *Molecular Biology and Evolution* 35: 1547–1549.

Lee, T., and D. O. Foighil. 2003. "Phylogenetic Structure of the Sphaeriinae, a Global Clade of Freshwater Bivalve Molluscs, Inferred From Nuclear (ITS-1) and Mitochondrial (16S) Ribosomal Gene Sequences." *Zoological Journal of the Linnean Society* 137: 245–260.

Leigh, J. W., and D. Bryant. 2015. "PopART: Full-Feature Software for Haplotype Network Construction." *Methods Ecol Evol* 6: 1110–1116.

Lomolino, M. V. 2004. "Conservation Biogeography." In *Frontiers of Biogeography: New Directions in the Geography of Nature*, edited by L. Lomolino and L. R. Heaney, 293–296. Sinauer Associates.

Lopes-Lima, M., N. Riccardi, M. Urbanska, et al. 2021. "Major Shortfalls Impairing Knowledge and Conservation of Freshwater Molluscs." *Hydrobiologia* 848: 2831–2867.

Lv, K., J. R. Wang, T. Q. Li, et al. 2019. "Effects of Habitat Fragmentation on the Genetic Diversity and Differentiation of *Dendrolimus Punctatus* (Lepidoptera: Lasiocampidae) in Thousand Island Lake, China, Based on Mitochondrial COI Gene Sequences." *Bulletin of Entomological Research* 109: 62–71.

Mackie, G. L. 2007. "Biology of Freshwater Corbiculid and Sphaeriid Clams of North America." *Ohio Biological Survey Bulletin New Series* 15: ix–436pp.

Maroof, M. S., K. M. Soliman, R. A. Jorgensen, and R. W. Allard. 1984. "Ribosomal DNA Spacer-Length Polymorphisms in Barley: Mendelian Inheritance, Chromosomal Location, and Population Dynamics." *Proceedings of the National Academy of Sciences* 81: 8014–8018.

Martin, K. R., P. T. Johnson, J. Bowerman, and J. Li. 2020. "Biogeography of the Freshwater Gastropod, *Planorbella Trivolvis*, in the Western United States." *PLoS ONE* 15: e0235989.

Martinsson, S., and C. Erséus. 2021. "Cryptic Clitellata: Molecular Species Delimitation of Clitellate Worms (Annelida): An Overview." *Diversity* 13: 36.

Martín-Vélez, V., M. I. Sánchez, Á. Lovas-Kiss, F. Hortas, and A. J. Green. 2022. "Dispersal of Aquatic Invertebrates by Lesser Black-Backed Gulls and White Storks Within and Between Inland Habitats." *Aquatic Sciences* 84: 1–10.

Mcguire, J. A., X. Huang, S. B. Reilly, et al. 2023. "Species Delimitation, Phylogenomics, and Biogeography of Sulawesi Flying Lizards: A Diversification History Complicated by Ancient Hybridization, Cryptic Species, and Arrested Speciation." *Systematic Biology* 72: 885–911.

McKelvey, K. S., C. Kallstrom, J. Ledbetter, D. W. Sada, K. L. Pilgrim, and M. K. Schwartz. 2020. "An Inventory of Springsnails (*Pyrgulopsis* spp.) in and Adjacent to the Spring Mountains, Nevada." *Western North American Naturalist* 80: 183–193.

McKelvy, A. D., and F. T. Burbrink. 2017. "Ecological Divergence in the Yellow-Bellied Kingsnake (*Lampropeltis Calligaster*) at Two North American Biodiversity Hotspots." *Molecular Phylogenetics and Evolution* 106: 61–72.

McLeod, D. S. 2010. "Of Least Concern? Systematics of a Cryptic Species Complex: *Limnonectes Kuhlii* (Amphibia: Anura: Dicroglossidae)." *Molecular Phylogenetics and Evolution* 56: 991–1000.

Miller, J. P., M. A. Ramos, T. Hauffe, and D. Delicado. 2018. "Global Species Richness of Hydrobiid Snails Determined by Climate and Evolutionary History." *Freshwater Biology* 63: 1225–1239.

Miller, R. M., J. P. Rodríguez, T. Aniskowicz-Fowler, et al. 2006. "Extinction Risk and Conservation Priorities." *Science* 313: 441–441.

MolluscaBase. eds. 2025. "MolluscaBase." Accessed at https://www.molluscabase.org on 2025-01-17. doi: https://doi.org/10.14284/448.

Morningstar, C. R., K. Inoue, B. K. Lang, and D. J. Berg. 2018. "A Comprehensive Status, Phylogenetic, and Anatomical Review of *Stagnicola Caperata* (Say, 1829) in the South-West United States." *Aquatic Conservation: Marine and Freshwater Ecosystems* 28: 527–534.

Morningstar, C. R., K. Inoue, M. Sei, B. K. Lang, and D. J. Berg. 2014. "Quantifying Morphological and Genetic Variation of Sympatric Populations to Guide Conservation of Endangered, Micro-Endemic Springsnails." *Aquatic Conservation: Marine and Freshwater Ecosystems* 24: 536–545.

Mouthon, J., M. Forcellini, and T. Van Haaren. 2018. "*Euglesa Compressa* (Bivalvia, Sphaeriidae), Native of North America, a "Hidden" Species Introduced in Western Europe Before 1940." *Basteria* 82: 50–54.

Murphy, A. L., A. Pavlova, R. Thompson, J. Davis, and P. Sunnucks. 2015. "Swimming Through Sand: Connectivity of Aquatic Fauna in Deserts." *Ecology and Evolution* 5: 5252–5264.

Murphy, N. P., M. Adams, M. T. Guzik, and A. D. Austin. 2013. "Extraordinary Micro-Endemism in Australian Desert Spring Amphipods." *Molecular Phylogenetics and Evolution* 66: 645–653.

Nguyen, L. T., H. A. Schmidt, A. Von Haeseler, and B. Q. Minh. 2015. "IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies." *Molecular Biology and Evolution* 32: 268–274.

[NMDGF] New Mexico Department of Game and Fish. 2002. "Status of Aquatic Mollusks of New Mexico, Completion Report." Submitted to United State Fish and Wildlife Service, Division of Federal aid, Albuquerque, New Mexico.

[NMDGF] New Mexico Department of Game and Fish. 2020. "Threatened and Endangered Species of New Mexico; 2020 Biennial Review."

Nori, J., R. Semhan, C. S. Abdala, and O. Rojas-Soto. 2022. "Filling Linnean Shortfalls Increases Endemicity Patterns: Conservation and Biogeographical Implications for the Extreme Case of *Liolaemus* (Liolaemidae, Squamata) Species." *Zoological Journal of the Linnean Society* 194: 592–600.

Park, J. K., and D. Ó. Foighil. 2000. "Sphaeriid and Corbiculid Clams Represent Separate Heterodont Bivalve Radiations Into Freshwater Environments." *Molecular Phylogenetics and Evolution* 14: 75–88.

Pavlova, A., L. B. Beheregaray, R. Coleman, et al. 2017. "Severe Consequences of Habitat Fragmentation on Genetic Diversity of an Endangered Australian Freshwater Fish: A Call for Assisted Gene Flow." *Evolutionary Applications* 10: 531–550.

Pienkowski, T., C. Cook, M. Verma, and L. R. Carrasco. 2021. "Conservation Cost-Effectiveness: A Review of the Evidence Base." *Conservation Science and Practice* 3: e357.

Rassam, H., C. Albrecht, R. Sousa, M. Lopes-Lima, H. Benaissa, and M. Ghamizi. 2021a. "Intraspecific Variation in the Common pea Clam, *Pisidium Casertanum* (Poli, 1791) (Bivalvia: Sphaeriidae): A Geometric Morphometric Analysis." *Malacologia* 63: 183–194.

Rassam, H., M. Ghamizi, H. Benaissa, C. Clewing, and C. Albrecht. 2021b. "The Fingernail Clams (Bivalvia: Veneroida: Sphaeriidae) of Morocco: Diversity, Distribution and Conservation Status." *Biodiversity Data Journal* 9: e73346. https://doi.org/10.3897/BDJ.9.e73346.

Rassam, H., S. Moutaouakil, H. Benaissa, C. Albrecht, and M. Ghamizi. 2020. "First Record of *Pisidium Subtruncatum* Malm, 1855 (Bivalvia, Sphaeriidae) in an African Cave." *Subterranean Biology* 34: 99–108.

Riddle, B. R., R. J. Ladle, S. A. Lourie, and R. J. Whittaker. 2011. "Basic Biogeography: Estimating Biodiversity and Mapping Nature." In *Conservation Biogeography*, edited by R. J. Ladle and R. J. Whittaker, 45–92. Blackwell Publishing Company.

Sağlam, İ. K., J. Baumsteiger, M. J. Smith, et al. 2016. "Phylogenetics Support an Ancient Common Origin of Two Scientific Icons: Devils Hole and Devils Hole Pupfish." *Molecular Ecology* 25: 3962–3973.

Saito, T., K. Fujimoto, S. Uchida, et al. 2022. "Uncovering Overlooked Diversity Using Molecular Phylogenetic Approach: A Case of Japanese

Sphaeriid Clams (Sphaeriidae: Bivalvia)." *Molecular Phylogenetics and Evolution* 173: 107508.

Sandusky, M. J., R. E. Sparks, and A. A. Paparo. 1979. "Investigations of Declines in Fingernail Clam (*Musculium Transversum*) Populations in the Illinois River and Pool 19 of the Mississippi River." *Bulletin of the American Malacological Union* 1979: 11–15.

Scherz, M. D., F. Glaw, C. R. Hutter, et al. 2019. "Species Complexes and the Importance of Data Deficient Classification in red List Assessments: The Case of *Hylobatrachus* Frogs." *PLoS ONE* 14: e0219437.

Schultheiß, R., C. Albrecht, U. Bößneck, and T. Wilke. 2008. "The Neglected Side of Speciation in Ancient Lakes: Phylogeography of an Inconspicuous Mollusc Taxon in Lakes Ohrid and Prespa." *Hydrobiologia* 615: 141–156.

Seidel, R. A., B. K. Lang, and D. J. Berg. 2009. "Phylogeographic Analysis Reveals Multiple Cryptic Species of Amphipods (Crustacea: Amphipoda) in Chihuahuan Desert Springs." *Biological Conservation* 142: 2303–2313.

Shults, P., M. Hopken, P. A. Eyer, et al. 2022. "Species Delimitation and Mitonuclear Discordance Within a Species Complex of Biting Midges." *Scientific Reports* 12: 1730.

Stagliano, D. M. 2016. "Current Population Status of a Locally Endemic Springsnail (Hydrobiidae: *Pyrgulopsis Bedfordensis*) in Montana." *Western North American Naturalist* 76: 509–513.

Stanislawczyk, K., A. D. Walters, T. J. Haan, M. Sei, B. K. Lang, and D. J. Berg. 2018. "Variation Among Macroinvertebrate Communities Suggests the Importance of Conserving Desert Springs." *Aquatic Conservation: Marine and Freshwater Ecosystems* 28: 944–953.

Stevens, L. E., J. Jenness, and J. D. Ledbetter. 2020. "Springs and Springs-Dependent Taxa of the Colorado River Basin, Southwestern North America: Geography, Ecology and Human Impacts." *Water* 12: 1501.

Taylor, D. W. 1987. *Fresh-Water Molluscs From New Mexico and Vicinity*. Vol. 116. New Mexico Bureau of Mines & Mineral Resources.

Trontelj, P., and C. Fišer. 2009. "Cryptic Species Diversity Should Not Be Trivialised." *Systematics and Biodiversity* 7: 1–3.

[USFS] U.S. Forest Service. 2017. *Potential Species of Conservation Concern Comments and Response to Comments*. Carson National Forest.

[USFS] U.S. Forest Service. 2020. *Potential Species of Conservation Concern*. Carson National Forest.

[USFWS] U.S. Fish and Wildlife Service. 2009. "Federal Register, Department of Interior; Fish and Wildlife Service; Endangered and Threatened Wildlife and Plants; Partial 90-Day Finding on a Petition to List 475 Species in the Southwestern United States as Threatened or Endangered With Critical Habitat; Proposed Rule." Federal Register 74, 419–427.

Vaidya, G., D. J. Lohman, and R. Meier. 2011. "SequenceMatrix: Concatenation Software for the Fast Assembly of Multi-Gene Datasets With Character Set and Codon Information." *Cladistics* 27: 171–180.

Walters, A. D., A. G. Cannizzaro, D. A. Trujillo, and D. J. Berg. 2021. "Addressing the Linnean Shortfall in a Cryptic Species Complex." *Zoological Journal of the Linnean Society* 192: 277–305.

Walters, A. D., K. N. Taynor, and D. J. Berg. 2021. "Genetic Diversity in the Threatened Freshwater Mussel *Lampsilis Powellii*." *Freshwater Mollusk Biology and Conservation* 24: 26–33.

Walters, A. D., D. A. Trujillo, and D. J. Berg. 2022. "Micro-Endemic Species of Snails and Amphipods Show Population Genetic Structure Across Very Small Geographic Ranges." *Heredity* 128: 325–337.

Wiedenfeld, D. A., A. C. Alberts, A. Angulo, et al. 2021. "Conservation Resource Allocation, Small Population Resiliency, and the Fallacy of Conservation Triage." *Conservation Biology* 35: 1388–1395.

Wood, L. R., R. A. Griffith, K. Groh, E. Engel, and L. Schley. 2008. "Interactions Between Freshwater Mussels and Newts: A Novel Form of Parasitism?" *Amphibia-Reptilia* 29: 457–462.

Work, K. 2023. "The Distribution, Magnitude, and Endemic Species of US Springs." *Frontiers in Environmental Science* 10: 1022424.

Zelaya, D. G., and M. C. Marinone. 2012. "A Case of Phoresis of Sphaeriids by Corixids: First Report for the Americas." *Malacologia* 55: 363–367.

Zhang, D. X., and G. M. Hewitt. 2003. "Nuclear DNA Analyses in Genetic Studies of Populations: Practice, Problems and Prospects." *Molecular Ecology* 12: 563–584.

Zink, R. M., R. C. Blackwell-Rago, and F. Ronquist. 2000. "The Shifting Roles of Dispersal and Vicariance in Biogeography." *Proceedings of the Royal Society of London. Series B: Biological Sciences* 267: 497–503.