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DNA barcodes for Great Salt Lake brine flies establish a baseline for monitoring changes in biodiversity

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ABSTRACT

Great Salt Lake (GSL) is the center of a valuable wetland ecosystem in the Great Basin of North America. The lake is an important site for millions of migratory birds that feed on 2 principal invertebrates, brine shrimp and brine flies (Diptera: Ephydridae). Despite their ecological and economic importance, no genetic studies have been published for either resident GSL invertebrate. The family Ephydridae (shore flies and brine flies) is one of the largest in the order Diptera, with nearly 2000 described species. Members of this family are prominent in a variety of aquatic environments and are particularly interesting because of their adaptation to several marginal habitats, including hot springs, oil ponds, highly saline lakes, and inland alkaline pools and marshes. This report provides cytochrome *c* oxidase I (COI) DNA barcodes for 5 species of GSL shore flies, distributed among 5 genera and 3 subfamilies. The phylogenetic content of these DNA sequences is explored by comparing a molecular phylogeny to those based on morphological features. Over the past decade, urbanization and inflow diversion have reduced the surface area of GSL by nearly 50%, with unknown consequences for the ecosystem. This study establishes a genetic framework to assess changes in GSL invertebrate diversity important in monitoring the effects of anthropogenic and climate pressures on this important natural resource.

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Introduction

Great Salt Lake (GSL), one of the world's largest hypersaline lakes, is a shallow remnant of Lake Bonneville, a body of water that covered ~50 000 km^2 of the Great Basin during the Pleistocene (Currey 1990). As with many terminal lakes, its depth, volume, and salinity fluctuate widely, but it is generally regarded as the fourth largest terminal lake in the world, covering ~4500 km^2 when measured at its historical average elevation (Arnow and Stephens 1990). The total watershed area of the present-day GSL is ~97 000 km^2, supporting a population of ~2 million people. A combination of increased urbanization, agricultural runoff, and decreasing freshwater inflow has resulted in a worrisome burden on this and other saline lake ecosystems (Wurtsbaugh et al. 2017, Null and Wurtsbaugh 2020). On 5 July 2022, the average daily surface water elevation was 1277 m, the lowest elevation recorded since measurements began in 1847 (USGS 2022).

> Urgent action is needed to help protect and preserve this critical resource. It's clear the lake is in trouble. We recognize more action and resources are needed, and we are actively working with the many stakeholders who value the lake. (Joel Ferry, Utah Department of Natural Resources Executive Director; USGS 2022)

Great Salt Lake has long served as a model for managing saline lake resources around the world (Hammer 1986). The principle economic activities associated with GSL include recreation, mineral extraction, and brine shrimp cyst harvesting, which together account for ~US$1.32 billion per year (Bioeconomics 2012). Salinity in the lake's south arm (~13%) supports large populations of 2 principal macroinvertebrates, pelagic brine shrimp (*Artemia fransciscana*) and benthic brine flies (Ephydridae). Increasing salinity potentially threatens both populations, with concomitant effects on the entire GSL food web (Null and Wurtsbaugh 2020). The loss of *Artemia* from most of Lake Urmia, Iran, as it receded to ~10% of its maximum size, provides an ominous preview of the cascading effects of increasing salinities beyond a level that can support these invertebrates (Stone 2015).

The GSL ecosystem represents one of the world's most important bird habitats, with >300 species of shorebirds, waterfowl, and other birds utilizing the open waters, wetlands, and uplands throughout the year (Conover and

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Bell 2020, Sorensen et al. 2020). An estimated one-third of all waterfowl in in the Pacific and Central Flyways, some 3 million individuals, spend part of the year in the GSL ecosystem, either nesting or feeding in preparation for spring and summer migrations (Paul and Manning 2002). Along with brine shrimp, brine flies (family Ephydridae; flies associated with GSL and other saline environments are commonly referred to "brine flies"; the broader term "shore flies" describes the entire family) are the dominant macroinvertebrates associated with the lake and serve as important sources of biomass for birds that rely on this ecosystem. In addition to their ecological benefit as a food source, brine flies remove an estimated 100 million kg of organic matter from GSL each year (GSL Comprehensive Management Plan 2013). One study estimated the brine fly biomass production at 7.9 g/m^2 (3.8 g/m^2 on sand to 49 g/m^2 on rock; Collins 1980b), the maximum value reported for saline lakes (Paterson and Walker 1974) and the high end for all lakes (Deevey 1941).

The density achieved by brine flies is unmistakable to anyone who has ever visited GSL in the summer months (Fig. 1), with an early estimate of 592 million adults/km shoreline (Aldrich 1912a, 1912b). Although the GSL shorelines are dynamic and transitory, an average of 4800 km yields an estimated 2.8 trillion adults. At peak densities, the number of adults is remarkable: "… the roar of the rising flies is such to drown out the noise of the railroad trains passing by" (Schwarz 1891). Brine fly larvae are found above the anoxic water layer, along substrates of bioherms and mud (Collins 1980b).

Monitoring and quantifying changes in ecosystem community structure are important for assessing the effects of human impacts on all aquatic ecosystems. While awareness of the importance of evaluating loss of biodiversity in freshwater ecosystems is growing (e.g., Mueller et al. 2013, Macher et al. 2016), saline lakes have received far less attention, possibly due to the perception that they are less common than freshwater lakes, or that their biological constituents and food webs are relatively simple (Hammer 1986, Belovsky et al. 2011). However, the effect of anthropogenic pressures on saline lakes may be even more acute because of the specialization of organisms adapted to high salt environments. The potential impacts of changes to the invertebrate components of saline ecosystems may be more significant than those observed for many freshwater ecosystems (Wollheim and Lovvorn 1995).

This report provides cytochrome *c* oxidase subunit I (COI) DNA barcodes for 5 of the more abundant brine fly species associated with the GSL ecosystem: *Cirrula hians*, *Ephydra gracilis*, *Mosillus bidentatus*, *Paracoenia bisetosa*, and *Schema salinum*. Sequence differentiation was compared to that of other barcoding studies, and a molecular phylogeny was constructed to provide an initial examination of the evolutionary diversification of this important group of insects. In addition to enhancing our understanding of shore fly diversification, this study is the first genetic investigation of invertebrates from this iconic saline ecosystem. This first step is important for monitoring both short-term and long-term changes in brine flies, which are understudied because they lack the economic importance of brine shrimp.

Figure 1. Example of brine fly densities from the Great Salt Lake ecosystem: (top) the dark mass along the shoreline comprises adults and empty puparia; (bottom) *E. gracilis* on rocky substrate of Antelope Island, Utah.

Materials and methods

Sample collection and DNA isolation

Adult flies were collected between 2017 and 2019 from the south arm of GSL, either from Antelope Island or from sites along the mainland shore (Table 1). Individuals were separated to species based on gross morphology, and total DNA was isolated from a single individual

Table 1. Great Salt Lake sampling sites.

Species	Sampling Site	Number of Sequences
Cirrula hians	Antelope Island 41°03′57″N 112°13′52″W	8
Ephydra gracilis	Antelope Island 40°57′33″N 112°13′52″W	11
Mosillus bidentatus	Great Salt Lake State Park 40°44′02″N 112°12′40″W	6
Paracoenia bisetosa	Saltair 40°44′51″N 112°11′17″W	5
Schema salinum	Saltair 40°44′50″N 112°11′18″W	5

of each species using the Qiagen DNeasy Blood and Tissue kit (https://www.qiagen.com/us).

DNA amplification

The targeted mitochondrial COI region was amplified with standardized conditions and primers:

LCO1490F: 5′-GGTCAACCAATCATAAAGATATTGG

HC02198R: 5′-TAAACTTCTGGGATGTCCAAAAAATCA

One nanogram of total DNA was used in a 25 µL reaction with AmpliTaq Gold (ThermoFisher Scientific; https://www.thermofisher.com) reagents. The cycling profile consisted of an initial denaturation at 94 °C (4 min), 30 cycles of 94 °C (1 min), 50 °C (1 min), 72 °C (1 min), and a final incubation at 72 °C for 4 min.

DNA sequencing and phylogenetic analysis

Amplified targets were cloned using the TOPO cloning kit (Invitrogen, Themo Fisher Scientific, Waltham, MA, USA) and plasmids purified with the Wizard (Promega, Madison, WI, USA) miniprep system. The COI barcoding sequences were determined by pyrosequencing (Genewiz.com) and deposited under accession numbers OP971535–OP971569. As an internal quality control, sequences were determined from both strands of each barcode. Amplification of mitochondrial DNA may be associated with 2 artifacts related to homology and erroneous sequences (Buhay 2009), which include amplification of nonfunctional nuclear mitochondrial pseudogenes (NUMTs). Each of the COI barcodes was screened for deletions and intact reading frames. The alignment of 39 sequences (Supplemental Fig. S1) show intact reading frames and no gaps. Sequence comparisons and phylogenetic analysis were performed with MEGA (Kumar et al. 2018). Trees were reconstructed using maximum likelihood and the GTR + G + I model for rate heterogeneity. For comparison, trees were also produced by parsimony and neighbor-joining, and statistical support for the phylogenies was assessed with bootstrapping.

Voucher specimens

Voucher specimens are maintained in the Department of Zoology, Weber State University, Ogden, Utah. Note that Sturtevant and Wheeler (1954) established the use of the name *E. cinerea* (Jones 1906) for *E. gracilis* but, as outlined by Mathis and Zatwarnicki (1990), the latter name has precedent. The species originally named *Ephydra hians* (Say 1830) is now recognized as part of the genus *Cirrula* (Mathis and Zatwarnicki 1995).

Results

Brine fly diversity and DNA sequence variation

Barcodes were obtained from a minimum of 5 different individuals for each of the 5 GSL species sampled (Table 1). Before considering the sequence comparisons of the COI barcodes, a review of the classification of the 5 shore flies sampled from GSL is useful (Table 2). As discussed in detail later, the family Ephydridae comprises 2 main groups, the Hydrelliine (2 subfamilies) and Gymnomyzine (3 subfamilies; Zatwarnicki 1992, Mathis and Zatwarnicki 1995). *S. salinum* is the only representative of the Hydrelliine group associated with the GSL ecosystem. The 4 other species sampled here are part of the Gymnomyzine group, with 3 species belonging to the subfamily Ephydrinae and *M. bidentatus* to the subfamily Gymnomyzinae.

Additional shore fly barcodes are available in the genetic databases (Table 3), and some of those provide context for the comparisons described here. Of the 5 GSL species examined here, COI barcodes are available in the database for only *C. hians* (HM374264; Saskatchewan) and *P. bisetosa* (JF874926; Manitoba). Sequences are available for the related taxa, *P. fumosa* (JF873397; Ontario), as well as 2 species in the genus *Ephydra*: *E. riparia* (JF867574; Alberta KU496737; Kenai, Alaska)

Table 2. Phylogenetic context of Great Salt Lake species examined (after Zatwarnicki 1992).

Family Group	Hydrelliine	Gymnomyzine	Gymnomyzine
Subfamily	Hydrelliinae	Gymnomyzinae	Ephydrinae
Species	*Schema salinum*	*Mosillus bidentatus*	*Cirrula hians* * *Ephydra gracilis* * *Paracoenia bisetosa*

*See voucher specimens discussion in materials and methods for historical references to the names *C. hians* and *E. gracilis*.

Table 3. Classification of select North American *Ephydra* (Wirth 1971).

Subgenus (group)	Described from Great Salt Lake	Barcoding database	This study
Ephydra			
(Riparia)			
E. macellaria		X	
E. packardi	X	X[#]	
E. riparia		X	
(Glauca)			
E. auripes	X		
E. pectinulata	X		
Halephydra			
E. gracilis	X		X
Hydropyrus			
E. hians *	X	X	X

#Incomplete sequence not included in this analysis.
*Now *Cirrula hians*.
Note: Only species associated with Great Salt Lake or represented in the barcoding database are listed.

and *E. macellaria* (MF059325; Malta). This information enables some intra-genus comparisons (Tables 4 and 5). COI sequence variation within the genus *Ephydra* (3 species, mean = 5.7%) and *Paraceonia* (2 species, mean = 6.9%) are similar and on par with intra-generic comparisons seen in other insect barcoding studies (Smith et al. 2005, 2006, Cywinska et al. 2006, Hajibabaei et al. 2006).

The COI sequences from *C. hians* show considerable differentiation compared to those from the genus *Ephydra*, consistent with the reclassification of the former species to the genus *Cirrula* (Mathis and Zatwarnicki 1995). For *C. hians*, the variation of the GSL sequences compared to HM374264, from Saskatchewan, is modest (1.3%) but greater than intraspecies variation among the 8 sequences obtained from GSL (mean = 0.53%). Furthermore, the sequence variation seen with *P. bisetosa* isolated from GSL and that from JF874926 isolated from Manitoba is only 0.68%, whereas variation among the 5 GSL sequences is 0.22%. In general, COI divergences of <1% characterize members of the same species, whereas divergences <5% are associated with members of the same genus (Ratnasingham and Hebert 2007, Park et al. 2011).

These sequence comparisons can be extended to include all sequences (35 total) obtained from the 5 species sampled from GSL (Table 6). The degree of differentiation among COI sequences within species was low, with values consistent with other insect studies (e.g., Smith et al. 2005 [ants], 2006 [tachinid flies], Cywinska et al. 2006 [mosquitoes], Hajibabaei et al. 2006 [moths and butterflies]). Sequence divergences within the Gymnomyzine group are similar (mean = 12.1% among 4 species), whereas the divergence between the Gymnomyzine and Hydrelliine group (the latter represented here by a single species, *S. salinum*) is somewhat greater (mean = 13.9%; Table 7). Comparing our results (Tables 6 and 7) to those of other Diptera is interesting. Across 1058 COI sequences from 68 species from the family Drosophilidae, maximum intraspecific sequence divergences ranged from 0% to 11% (mean = 1.9%) and interspecific differences from 0% to 12% (mean = 5.1%; Yassin et al. 2010). Barcodes from *Belvosia* (family Tachinidae) showed intraspecific divergences between 0% and 1.5% (mean = 0.278%) and interspecific differences between 1.6% and 4.9% (mean = 3.25%; Smith et al. 2006). In a study of 37 mosquito species (family Culicidae), intraspecific COI divergences ranged from 0% to 3.9% (mean = 0.5%) and congeneric interspecies differences from 0.2% to 17.2% (mean = 10.4%; Cywinska et al. 2006).

Phylogenetic content of barcode sequences

To examine the phylogenetic content of the COI barcodes, phylogenetic trees were generated using maximum likelihood. We chose the likelihood method of inference because of the numerous pitfalls associated with distance methods usually used for barcoding analyses (summarized in DeSalle and Goldstein 2019). Although not fully resolved, a number of studies strongly support the dipteran superfamily Ephydroidea, which includes the major families Ephydridae, Camillidae, Diastidae, Drosophilidae, and Curtonidae (summarized in McAlpine 1989, Grimaldi 1990). For the analyses shown here, a COI sequence from Diastidae (KM928824) was used as an outgroup. The use of other outgroups from within Ephydroidea (Drosophilidae, Camillidae) and beyond (Bombyliidae, Sphaeroceridae) did not change the branching of the major lineages.

Table 4. Cytochrome *c* oxidase I sequence divergence within the genus *Ephydra**. Sequence data from this study (GSL) and others (GenBank accession number).

	E. gracilis-03 (GSL)	*E. macellaria* (MF059325)	*E. riparia* (JF867574)	*C. hians* (HM374264)	*C. hians*-02 (GSL)
E. gracilis-03 (GSL)	—	5.7%	5.7%	9.3%	9.4%
E macellaria (MF059325)		—	1.1%	10.9%	10.4%
E. riparia (JF867574)			—	12.2%	11.9%
C. hians (HM374264)				—	1.3%
C. hians-02 (GSL)					—

*Because *C. hians* has historically been part of the genus *Ephydra*, it is included here.

Table 5. Cytochrome *c* oxidase I sequence divergence within the genus *Paracoenia*.

	P. bisetosa-02 (GSL)	*P. bisetosa* (JF874926)	*P. fumosa* (JF73397)
P. bisetosa-02 (GSL)	—	0.68%	6.9%
P. bisetosa (JF874926)		—	6.9%

The molecular phylogeny is consistent with traditional classification (Fig. 2). A clear distinction exists between the main groups of Ephydridae, Hydrelliine (represented here by *S. salinum*), and Gymnomyzine (represented here by *Mosillus*, *Cirrula, Ephydra,* and *Paracoenia*). The phylogeny also clearly differentiates barcodes from the subfamily Gymnomyzinae (*Mosillus*) and those obtained from Ephydrinae (*Cirrula, Ephydra,* and *Paracoenia*). Moreover, among the 3 genera sampled here from the subfamily Ephydrinae, an affiliation between *Cirrula* and *Ephydra* is strongly supported to the exclusion of *Paracoenia*. Regardless of the performance of COI sequences in resolving phylogenetic relationships, each species is characterized by a unique and stable barcode, properties that make barcodes useful for assessing species diversification.

Ephydrids are well represented in broad barcoding surveys of insect diversity, especially those of Nearctic regions. To further explore the potential utility of COI in examining shore fly taxonomy, 12 barcode sequences from the subfamilies Ephydrinae, Gymnomyzinae, and Hydrelliinae were added to the phylogenetic analysis (Fig. 3). The sequences form 3 monophyletic groups that correspond to the subfamily designations, although the statistical support for the deep branches is not compelling. Sequence differentiation among *Allotrichoma filiforme* (JF867835.1), *A. bezzi* (KU496679.1), and an unspecified *Allotrichoma* sp. (MG081085.1) ranges from 0.34% to 2.5% (mean = 1.78%); and among *Scatella picea* (JN582245), *S. stagnalis* (KT959999.1), and an unspecified *Scatella* sp. (KM912977), COI divergence ranges from 3.2% to 6.5% (mean = 5.0%). These values are consistent with those given earlier for GSL shore flies. Thus, it seems the genetic signal inherent in COI barcodes can be used to monitor GSL brine fly dynamics over time.

Discussion

The mitochondrial cytochrome *c* oxidase subunit I gene (COI) is the standard sequence used for DNA barcoding of animals (Ratnasingham and Hebert 2013, Wilson et al. 2017, Adamowicz et al. 2019). Because of their abundance in aquatic ecosystems, ephydrids are commonly reported in insect barcoding surveys from a variety of studies, especially the extensive barcoding efforts for Canadian insects. However, to our knowledge, this is the first study to use DNA sequences in a systematic way to examine the diversity of the ephydrids from a single ecosystem. The family Ephydridae is one of the largest in the order Diptera, with nearly 2000 species described (Mathis et al. 2016, 2021). Members of this family are often abundant in a variety of aquatic environments and are particularly interesting because of their adaptation to marginal habitats (Nemenz 1960, Wirth and Mathis 1979, Barnby 1987, Foote 1995). In some highly stressful habitats, such as GSL, shore flies are the dominant macroinvertebrates.

The most comprehensive phylogenetic study of Ephydridae is that of Zatwarnicki (1992), who examined 390 species with a focus mainly on male genitalia structure. That phylogeny includes 2 main groups of subfamilies, Hydrelliine (subfamilies Discomyzinae and

Table 6. Intraspecies sequence divergence (Kimura 2-parameter) of cytochrome *c* oxidase I barcodes from Great Salt Lake.

Species	*C. hians*	*E. gracilis*	*M. bidentatus*	*P. bisetosa*	*S. salinum*
No. sequences	8	11	6	5	5
Range	0–1.0%	0–0.84%	0.01–0.85%	0–0.34%	0–0.18%
Mean	0.53%	0.44%	0.47%	0.22%	0.076%

Table 7. Sequence divergence (Kimura 2-parameter) of cytochrome *c* oxidase I barcodes among species examined in this study (mean values for multiple sequences).

Species	*C. hians*	*E. gracilis*	*M. bidentatus*	*P. bisetosa*	*S. salinum*	Species average*
C. hians	—	9.6%	13.8%	13.1%	15.2%	12.9%
E. gracilis		—	11.3%	10.2%	12.3%	10.9%
M. bidentatus			—	11.4%	13.9%	12.6%
P. bisetosa				—	14.1%	12.2%
S. salinum					—	13.9%

*Average divergence of this species from all other sequences.

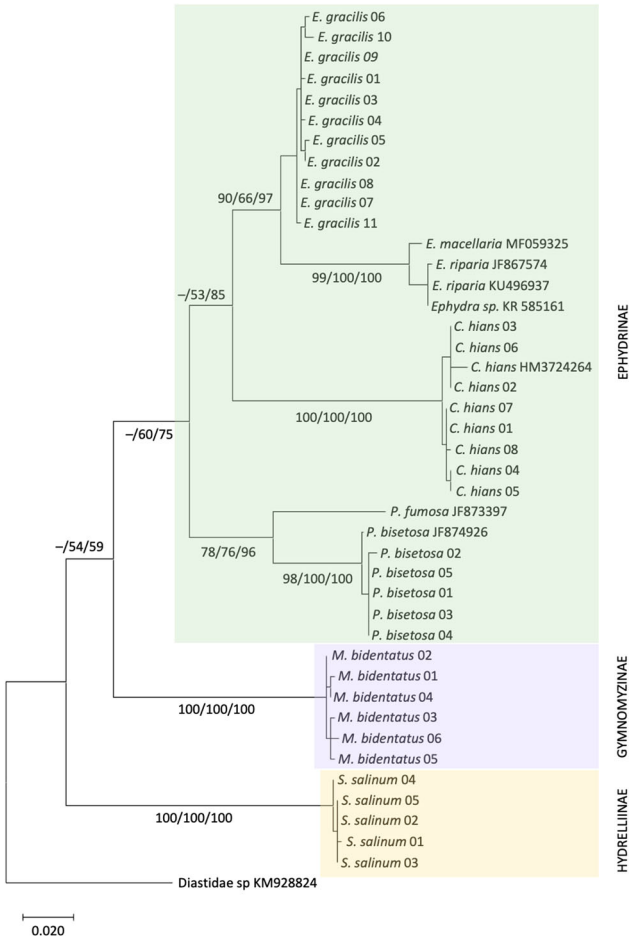

Figure 2. Cytochrome *c* oxidase I phylogeny for Great Salt Lake brine flies. Species identities and GenBank accession numbers are shown for related taxa. This tree was generated with maximum likelihood (log likelihood −2246.39) with 43 nucleotide sequences over 597 positions. Initial trees for the heuristic search were obtained with maximum parsimony, and a discrete gamma distribution was used to model evolutionary rate differences among sites. Bootstrap support (500 replicates) for major nodes is shown for 3 methods of phylogenetic inference: likelihood/parsimony/distance. The scale bar is number of nucleotide substitutions per site, and a sequence from the family Diastidae was used as an outgroup. Ephydridae subfamily designations are shaded and depicted in capital letters.

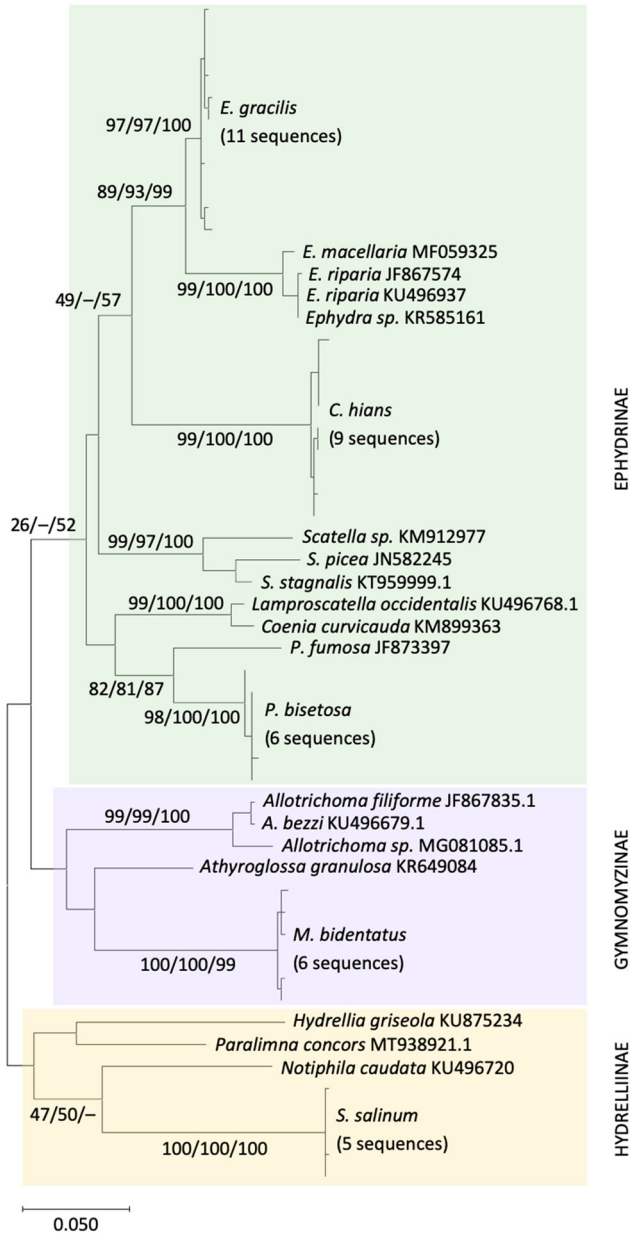

Figure 3. Expanded cytochrome *c* oxidase I phylogeny for 3 subfamilies of shore flies. Species identities and GenBank accession numbers are shown for related taxa. This tree is unrooted and was generated with maximum likelihood (log likelihood −3365.93) with 54 nucleotide sequences over 597 positions. Initial trees for the heuristic search were obtained with maximum parsimony, and a discrete gamma distribution was used to model evolutionary rate differences among sites. Bootstrap support (500 replicates) for major nodes is shown for 3 methods of phylogenetic inference: likelihood/parsimony/distance. Values are only shown if at least 2 of 3 methods recovered a particular clade. The scale bar is number of nucleotide substitutions per site. Ephydridae subfamily designations are shaded and depicted as in Fig. 2.

Hydrelliinae) and Gymnomyzine (subfamilies Gymnomyzinae, Ithyeinae, and Ephydrinae; framework in Table 2). Although comprising only 5 species, the molecular phylogeny presented here (Fig. 2) is consistent with that outlined by Mathis and Zatwarnicki (1995) and Zatwarnicki (1992). There is reasonable resolution of the 2 main lineages of shore flies, Hydrelliine and Gymnomiziine (because *S. salinum* is the only representative of the Hydrelliinae group reported from the GSL ecosystem and was thus the only species sampled here.) Within the Gymnomyzine group, the COI phylogeny clearly distinguishes the subfamily Gymnomyzinae, represented here by *M. bidentatus*, from members of the subfamily Ephydrinae. Within the subfamily Ephydrinae, 4 main monophyletic groups

are apparent: (1) *C. hians*, (2) *E. gracilis*, (3) *E. riparia* + *E. macellaria*, and (4) the genus *Paracoenia*. The distinction among (1), (2), and (3) parallels the classical taxonomic view of *Ephydra* (Table 3), and the COI phylogeny provides support for the reclassification of *C. hians* to the genus *Cirrula*, as opposed to the genus *Ephydra*. Based on morphology, evidence strongly indicates that the genera *Cirrula* and *Ephydra* form a monophyletic group closely related to the genus *Paracoenia* (Wirth 1971, Mathis 1979). Mathis (1979) placed the former 2 genera in an informal Group I, with *Paracoenia* and related genera belonging to a Group II.

DNA barcodes can be used to evaluate the stability of taxonomic groups proposed by morphological studies (DeSalle and Goldsteain 2019). Expanding the analysis to include other shore flies shows that the barcoding data are largely consistent with phylogenetic hypotheses for this well-studied family (Fig. 3). However, the lack of statistical support for clades at the tribe and subfamily levels indicates that additional sequence data will be needed to examine shore fly phylogeny with better resolution. Regardless of their utility for inferring phylogenies, closely related species can clearly be distinguished by stable barcodes, which is the tangible utility of DNA barcoding (Will and Rubinoff 2004). Within the subfamily Ephydrinae, *E. packardi*, *E. auripes*, and *E. pectinulata* have been reported from the GSL ecosystem (Jorgenson 1956, Wirth 1971), but these species were not encountered during our sampling period. The barcodes established here will be useful in distinguishing and verifying the identities of these species when they are collected. In addition, barcodes obtained from adults can serve as important tools to identify larval forms, which, for most taxa, are not well characterized.

Biomonitoring of indicator taxa have traditionally relied on morphological characters, which may be lacking for some taxa. Sorting and identification errors can have a dramatic effect on macroinvertebrate assessment programs (Haase et al. 2006) and can be exacerbated by lack of expertise in species identification and by the presence of cryptic species (Feckler et al. 2014). The barcodes presented here clearly distinguish the most abundant brine flies associated with the GSL ecosystem. Molecular analyses, such as DNA metabarcoding, provide a complementary approach to morphological surveys, leading to enhanced resolution of macroinvertebrate ecosystem assessments (Macher et al. 2016). In a comparison of morphological and genetic species identification from 5 study sites in California, Jackson et al. (2014) found a substantial increase in macroinvertebrate biodiversity in streams when morphological identification was augmented with barcoding. Particularly important for brine flies, which are not widely studied, is that barcodes provide a way to link adult and larval forms of the same species. In addition to monitoring changes in brine fly species compositions over time, DNA barcodes may also be used to identify ecological preferences and specific responses by each species to anthropogenic stresses. Such information is currently not available for the GSL ecosystem.

The principal brine flies associated with GSL occupy a key trophic position in the lake's biology, consuming benthic algae and detritus serving as a major food source for millions of migratory birds. Although the reproductive cycles of *E. gracilis*, *C. hians*, and *P. bisetosa* are well documented (Collins 1975, 1980a, 1980b, Zack 1983, Herbst 1990, 1999), little is known about the life cycles of the other 2 species studied here, or the extent to which they depend on the lake water. The distribution of brine flies within saline lakes in the Great Basin is influenced by salinity and water chemistry. Under conditions of high salinity (>25 g/L), *C. hians* is more abundant in alkaline waterbodies (e.g., Mono Lake, California), whereas *E. gracilis* is more prominent in chloride waters, such as GSL (Herbst 1999). At lower salinities, 2 additional species, *E. packardi* and *E. auripes*, predominate, with respective distributions influenced by solute composition. The distribution of *C. hians* in 2 alkaline Great Basin Lakes, Albert Lake (Oregon) and Mono Lake (California), changed to a measurable extent in response to natural fluctuations in salinities experienced by both in 1983–1984 (Herbst 1988). These observations are consistent with a model in which *C. hians* distribution is limited at low salinity by biotic interactions and at higher salinity by physiological limitations.

Anthropogenic changes to the GSL ecosystem potentially affect both brine shrimp and brine flies and the millions of birds that depend on them. Although *Artemia* can tolerate salinities >25%, reproduction is limited at levels >14% (Stephens and Birdsey 2002). For brine flies, estimates of salinity for optimum reproduction in the laboratory range from 25–100 g/L for *C. hians* to 100–200 g/L for *E. gracilis* (Herbst 1999). Because of their evolutionary adaptation of osmoregulation, brine flies are likely able to cope with moderate fluctuations in salinity, and at moderate salinities, evidence indicates that temperature, nutrients, and pH have a larger impact on brine fly density and reproduction than does salinity (Belovsky et al. 2011). Although we are far from a complete understanding of GSL macroinvertebrate dynamics, brine flies and brine shrimp are no longer found in the north arm of the lake (Gunnison Bay), where salinities are at or near saturation

(White et al. 2015). This reduction in macroinvertebrate availability can be detrimental to avian populations. For example, while over half of the entire GSL phalarope population was associated with Gunnison Bay relatively recently (1993), they have all but disappeared from this region, which historically comprises nearly 40% of the total lake surface area (Conover and Bell 2020). DNA barcoding may facilitate identification of avian dietary preferences and allow us to determine if certain species can shift their diets when their preferred food is not available.

In summary, this study establishes DNA barcodes for 5 species of brine flies from the GSL ecosystem. The barcodes clearly demarcate 3 species within the genus *Ephydra* and the related genus *Cirrula*. In what is likely the first examination of the molecular phylogeny of the family Ephydridae, character-based analysis recovers clades largely congruent with taxonomy based on morphological characters. Together with a growing number of sequences in the genetic databases, these barcodes can be used in future studies to corroborate the identities of adult and larval shore flies from a variety of habitats. Knowledge of brine fly diversity and abundance may also be useful in monitoring both short-term and long-term ecological dynamics of a lake under increasing stress from urbanization, mineral extraction, inflow diversion, and agricultural runoff.

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