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SPECIAL SECTION

Native Lampreys: Research and Conservation of Ancient Fishes

Lampreys in California (*Lampetra* spp. and *Entosphenus* spp.): Mitochondrial phylogenetic analysis reveals previously unrecognized lamprey diversity

Grace Auringer[1] | **Matthew A. Campbell**[1,2] | **Pascale A. L. Goertler**[3] | **Amanda J. Finger**[1]

[1]Department of Animal Science, University of California, Davis, Davis, California, USA

[2]University of Alaska Museum of the North, Fishes and Marine Invertebrates, Fairbanks, Alaska, USA

[3] Delta Stewardship Council, Delta Science Program, Sacramento, California, USA

Correspondence
Grace Auringer
Email: gmauringer@ucdavis.edu

Abstract

Objective: Protecting biodiversity is important for preserving ecosystem functions and services, and understanding the diversity present in a system is necessary for effective conservation. Lampreys display extensive diversity in morphology and life history strategy. The extent of this diversity and the underlying genetic patterns have not yet been fully characterized. Uncertainty about species boundaries and operational taxonomic units hinders lamprey management and conservation. Limited data have been collected on California lampreys (*Lampetra* spp. and *Entosphenus* spp.), but evidence suggests widespread population decline across the west coast of North America. Study objectives were to identify which nominal species are present at study sites across northern and central California, determine whether current taxonomic classifications accurately and sufficiently describe lamprey diversity in California, and characterize the biogeographic distribution of genetically distinct lamprey lineages across the study area.

Methods: To achieve these objectives, this study utilized DNA barcoding, phylogenetic analysis, and species delimitation analysis. Lamprey individuals ($N = 87$) from 19 sites in the Sacramento–San Joaquin River basin, San Francisco Bay, and Klamath River basin were sequenced for the mitochondrial cytochrome *b* (*cyt b*) gene, and the data were combined with publicly available lamprey *cyt b* sequences for analysis.

Result: Results showed relatively deep phylogenetic divergence between *Lampetra* and *Entosphenus*. Distinct and genetically divergent lineages were observed within *Lampetra*, while distinct but genetically similar lineages were observed within *Entosphenus*. This study revealed novel *Lampetra* lineages in the Napa River and Alameda Creek, and results suggest that the biogeographic distribution of lamprey diversity may follow patterns observed in other native fishes. Species delimitation results indicated that at least seven separate candidate species of *Lampetra* can be found in California, suggesting that California holds more species-level diversity than the expected three nominal *Lampetra* species known to occur in the state.

Conclusion: These results highlight the underestimated diversity of lampreys in California and the need for further assessment of taxonomic classifications and operational taxonomic unit designations of California lampreys.

KEYWORDS

California, conservation, *Entosphenus*, *Lampetra*, lamprey, molecular species delimitation, phylogenetics

INTRODUCTION

Lampreys are jawless, boneless fish characterized by eel-like bodies with seven gill pores on each lateral side, oral discs with keratinized plates, and the absence of paired fins. The lamprey life cycle consists of a prolonged larval phase that is variable in duration (~3–9 years), followed by a period of metamorphosis, after which the life history types diverge (Renaud 2011; Lucas et al. 2021). Despite high similarity across all species during the larval phase, substantial diversity is observed in adult lampreys, especially in migration type (anadromous and freshwater resident), feeding strategy (parasitic and nonparasitic), oral disc dentition patterns, body size, and pigmentation (Renaud 2011; Potter et al. 2015). Although lampreys are part of an ancient lineage, contemporary lamprey diversity (41 species; Page et al. 2013) has a relatively recent origin; the diversification of extant lamprey species has occurred primarily in the last 20 million years of the over 450-million-year evolutionary history (Brownstein and Near 2023). Twenty-five percent of extant lamprey species are considered to be at risk of extinction, and this is likely an underestimate due to the lack of data regarding distributions, population trends, and overall species diversity and distribution (Endangered and Threatened Wildlife and Plants 2004; Maitland et al. 2015; Docker and Hume 2019; Lucas et al. 2021).

Across the globe, native lamprey species provide substantial benefits to freshwater systems and the surrounding communities. During the filter-feeding larval phase, all lampreys improve water quality, maintain streambed conditions, and cycle nutrients, thus boosting primary productivity (Shirakawa et al. 2013; Boeker and Geist 2016). Juvenile and adult lampreys are valuable prey items for many birds, fishes, and aquatic mammals; additionally, anadromous adults deposit abundant marine-derived nutrients far upstream upon completion of their spawning migration, contributing valuable resources to stream food webs (Close et al. 2002; Dunkle et al. 2020). Anadromous Pacific Lampreys *Entosphenus tridentatus* are a dietary staple and cultural resource for many Indigenous peoples along the west coast (Close et al. 2002). These fish have been highly valued, monitored, and managed by Indigenous nations since time immemorial, but population declines over the past 50–75 years jeopardize Indigenous culture and food sovereignty (Sheoships 2014; Wicks-Arshack et al. 2018). Evidence suggests that the decline in lamprey populations across North America is due to loss of larval rearing and spawning habitat, pollution, damming and channelization of rivers, and alteration of natural flow regimes (Renaud 1997; Maitland et al. 2015; Lucas et al. 2021). However, a lack of historical data and the geographically fragmented nature of contemporary data prevent a robust rangewide assessment of population declines (Lucas et al. 2021; Parker 2022). Data collection is further complicated by challenges in species identification due to the lack of distinguishing features between larval lampreys of different species (Docker 2009; Parker 2022). These barriers to lamprey conservation are compounded by a lack of basic biological knowledge and taxonomic uncertainty (Moser et al. 2007; Maitland et al. 2015; Docker et al. 2016; Lucas et al. 2021).

Conservation actions based on inaccurate taxonomy can have unintended negative consequences for the target taxa and could reduce biodiversity (McNeely 2002; Ely et al. 2017). In most lamprey genera, "paired" or "stem-satellite" species occur, in which one (or more) freshwater-resident, nonparasitic lamprey species is derived from a migratory, parasitic species, and the resultant species have overlapping ranges and are closely related (Docker 2009).

Impact statement

Lampreys are ecologically and culturally significant. Populations are thought to be declining across the west coast of North America, but a lack of research impedes monitoring and conservation of lampreys in California. This study investigated lampreys in California using DNA sequences and found more diversity than expected. By determining how many genetically different types of lampreys are present and where these various lampreys are found across the study area, this research paves the way for conservation and protection of lampreys and the numerous benefits they contribute.

Current taxonomy, primarily based on morphological evidence, recognizes paired lamprey species as distinct species (Knebelsberger et al. 2015). However, taxonomic uncertainty is prevalent throughout the entire lamprey lineage, in part due to this confusion surrounding paired species (Docker 2009).

Recent studies have demonstrated that these lamprey pairs do not always form reciprocally monophyletic groups (Schreiber and Engelhorn 1998; Espanhol et al. 2007; Docker 2009; Boguski et al. 2012) and may represent alternative life history strategies within one freely interbreeding species (Rougemont et al. 2021; Carim et al. 2023). However, other pairs are considered correctly classified as distinct species under the biological and phylogenetic species concepts (Docker 2009; Clemens and Wade 2023). Molecular phylogenetic analysis and molecular species delimitation analysis can help to resolve taxonomic relationships and inform operational taxonomic unit designation (Rannala and Yang 2020; Young et al. 2021). In molecular phylogenetic analysis, DNA sequences are analyzed to infer evolutionary relationships between related individuals, which can help to determine taxonomic classifications (Yang and Rannala 2012). The goal of molecular species delimitation is to sort sequences from related individuals into groups that reflect species boundaries, and these methods typically base delimitations on differences between DNA sequences (distance-based methods) or the topology of phylogenetic trees (tree-based methods; Zhang et al. 2013).

Knowledge gaps about west coast lampreys are prevalent, but a surge of new research on the Pacific Lamprey has been driven by the formation of the Pacific Lamprey Conservation Initiative in 2007 (Wang and Schaller 2015). However, most of the new research focuses on a single species, and lamprey populations in California watersheds are particularly understudied (Moyle et al. 2015). Current taxonomy describes eight lamprey species (*Lampetra* spp.: $N=3$; *Entosphenus* spp.: $N=5$) present in California watersheds (Table 1), and all are listed as California species of special concern (Moyle et al. 2015). Moyle (2002) described six major zoogeographic provinces with 22 subprovinces, and five of the eight nominal lamprey species found in California have geographically restricted ranges that correlate to these zoogeographic subprovinces. This suggests that the biogeographic distribution of lamprey diversity may follow the same patterns observed in other native fish taxa. Although the abundance and distribution of lamprey species have not been systematically assessed throughout California, evidence strongly suggests that both range contractions (Goertler et al. 2019) and population declines (Moyle et al. 2009) have occurred. No previous studies have explored genetic diversity across all nominal species of California lamprey, and most prior research has had a very limited geographic scope within California.

This study utilized mitochondrial DNA (mtDNA) sequence data to assess the species composition and genetic diversity of lamprey populations throughout the Sacramento–San Joaquin River basin, San Francisco Bay, and the Klamath River basin of California. The three main objectives of this study were to (1) identify which nominal species were present at each sampled

TABLE 1 California lamprey species names, life history types, and status in California. "Other" migratory type indicates that the species is considered migratory but migration behavior is not well understood. The taxonomic classification of the Western Brook Lamprey *Lampetra richardsoni* as a distinct species from the Western River Lamprey *L. ayresii* is currently in doubt based on phylogenetic evidence from this study and others (Boguski et al. 2012; Carim et al. 2023).

	Migratory			Status in California
Species	**Anadromous**	**Other**	**Parasitic**	**(Moyle et al. 2015)**
Pacific Lamprey *Entosphenus tridentatus*[a,b]	✓		✓	Moderate concern
Klamath Lamprey *E. similis*[b]		✓	✓	Moderate concern
Pit-Klamath Brook Lamprey *E. lethophagus*[b]				Moderate concern
Northern California Brook Lamprey *E. folletti*[b]				High concern
Goose Lake Lamprey *Entosphenus* sp.		✓	✓	High concern
Western River Lamprey *Lampetra ayresii*[a,b]		✓	✓	Moderate concern
Western Brook Lamprey *L. richardsoni*[a,b]				Moderate concern
Kern Brook Lamprey *L. hubbsi*[a,b,c]				High concern

[a]Included in an Endangered Species Act petition to list as endangered in 2003.

[b]Recognized by the American Fisheries Society (Page et al. 2013).

[c]Endemic to California.

location using genetic species identification, (2) determine whether current taxonomic classifications accurately and sufficiently describe California's lamprey diversity through molecular phylogenetic and species delimitation analyses, and (3) compare the biogeographic distribution of lamprey diversity in California (based on the results of objectives 1 and 2) to the patterns observed for other native fish taxa. Based on species range descriptions for the eight nominal species known to occur in California (i.e., Table 1; Moyle 2002), we expected that all eight species would be present in our study area. Previous studies have demonstrated that lamprey systematics are complex and that taxonomic classifications can be discordant with molecular phylogenetic relationships (Docker et al. 2012; Rougemont et al. 2021), so we expected to observe similar contradictions across the *Lampetra* and *Entosphenus* genera in California. Due to the unique hydrology and rugged topography of the state (Moyle 2002; Ball et al. 2013), we predicted that the biogeographic patterns of lamprey diversity would be consistent with those of other native fish taxa. New sequencing data from lamprey individuals were integrated with publicly available data to clarify higher level relationships within and between nominal lamprey taxa. This study provides a foundation for understanding the diversity of California's lamprey populations, which is a necessary step in protecting lampreys and their ecosystem functions.

METHODS

Study system

In California's Central Valley, adult lampreys are thought to spawn throughout the Sacramento and San Joaquin rivers and their tributaries (Moyle 2002). These two rivers join to form a freshwater delta and exit toward the ocean through a series of large bays (Suisun, San Pablo, and San Francisco bays), hereafter collectively termed "the San Francisco Estuary" (Figure 1). Additionally, lampreys are widely distributed in the lower Klamath River basin and anadromous Pacific Lampreys have been documented in the upper Klamath River basin (above Iron Gate Dam; Hamilton et al. 2005). California is one of the most hydrologically altered regions in the world; extensive water infrastructure was designed to maintain a consistent water supply to the state's multi-billion-dollar (1 billion = 10^9) agricultural industry and meet the needs of municipal, residential, and other industrial water uses, but the resultant altered flows and habitat reduction are known to be detrimental to native fish populations (Brown and Bauer 2010; Zimmerman et al. 2018). Historically, the range of anadromous species encompassed a drainage area equal to roughly two-thirds of the state of California (Whipple et al. 2012). Currently, impassible dams block 72% of upstream historical habitat (Yoshiyama et al. 2001) from access by anadromous forms of returning lamprey. California's watersheds form the southernmost boundaries of several lamprey species' ranges, and evidence of southern range contractions (Reid et al. 2016) underscores the need to evaluate lamprey populations in California.

Sample collection and DNA sequencing

Tissue samples (fin clips) were collected opportunistically from lamprey individuals ($N = 87$) by collaborators at fish monitoring programs (Table S.1 available in the Supplement in the online version of this article) from 19 sites across the San Francisco Estuary and the Sacramento, San Joaquin, and Klamath River basins in 2018–2020 (Figure 1). Sampling covered 15 subbasins (eight-digit hydrologic unit code [HUC8]) and occurred at least once in all nominal species' ranges to increase the likelihood that all species diversity was captured in this study. The majority (70%) of samples were from larval individuals, and 50% of samples were unidentified, even to the genus level (Table S.2). Additionally, reference samples ($N = 6$) were included in sequence data collection for this study. These archived tissue samples had previously been collected from adult lampreys (Pacific Lamprey and Western Brook Lamprey) with confident species identifications and included sampling locations from Oregon, Washington, and British Columbia (Table S.2). Genomic DNA was extracted from tissue samples ($N = 95$) using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's protocol. Samples were prepared for Sanger sequencing of the cytochrome *b* (*cyt b*) gene, a mitochondrial marker previously used for lamprey species identification and phylogenetic analysis (Boguski et al. 2012). Cytochrome *b* sequences were amplified using forward primer Cytb-196F (5′-GCCTTYTCTTCAGTTATACA CATTTG-3′) and reverse primer Pro-R (5′-TAATTTAA TGTTAAGATRCTAGCTTTGG-3′; Boguski et al. 2012). The thermocycling profile used was as follows: 95°C for 2 min; followed by 45 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 2 min; and then a final hold at 72°C for 5 min. Chromatograms were manually inspected to verify base calls, and forward and reverse sequencing reads were trimmed and aligned into consensus sequences (hereafter, "contig sequences") using Geneious version 2023.0.4 (https://www.geneious.com).

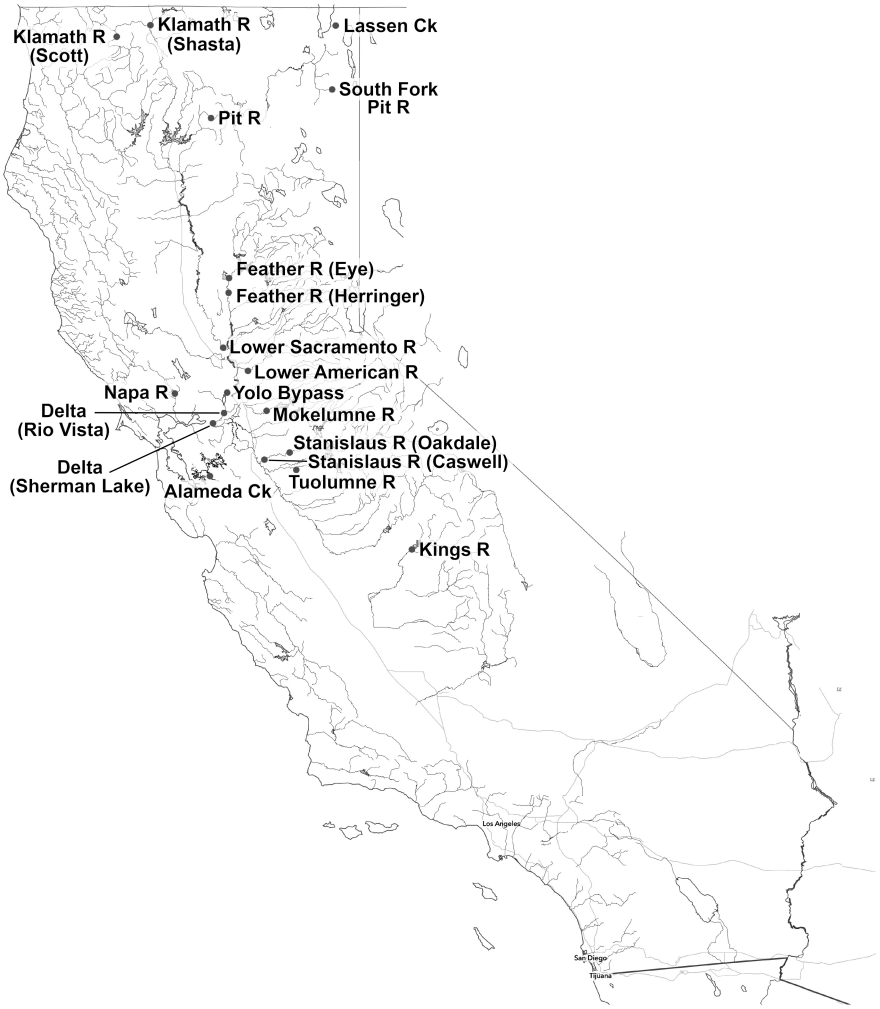

FIGURE 1 Map of California depicting sampling locations for all lamprey individuals that were sequenced for this study. Ck, Creek; R, River.

Alignment

Cytochrome *b* contig sequences from all individuals were assembled and aligned using Geneious. To enable comparisons to sequences from voucher specimens and to make comparisons across a broader geographic range, *cyt b* sequences ($N = 145$) produced by Boguski et al. (2012) were downloaded from the GenBank database (Accession Numbers GU120726 – GU120870) and incorporated into the alignment, resulting in 930-base-pair (bp) sequences for downstream analyses. After the addition of publicly available sequences, all individual sequences ($N = 238$) were eligible for downstream analyses (Figure S.1 shows alignment in FASTA format [available in the Supplement in the online version of this article]). Notably, Boguski et al. (2012) included many samples outside of California; since this study is primarily focused on lamprey diversity within California, many subsequent analyses utilized specific subsets of the total eligible samples.

Objective 1: Identify nominal lamprey species present at study sites

To verify morphologically based field species identifications and provide species (or at least genus) identification for unknown samples, an mtDNA barcoding approach was utilized. Database searches were performed to determine which sequences in the database most closely matched the sequences from study samples. Sequences for each sample collected in California for this study ($N = 87$) were compared to all sequences in the National Center for Biotechnology Information Nucleotide collection database using the Basic Local Alignment Search Tool (BLAST; Altschul et al. 1990) to assess statistical confidence of sequence similarity. The "BLASTN" program option was selected with default parameters. Species identifications were considered reliable if database records had top match statistics for lamprey *cyt b* sequences (i.e., >99.5% sequence identity, >99.5% complete query coverage, and Expect (E) values < 0.005; E-values

are the BLASTN program's estimation of the statistical significance of a match; Meiklejohn et al. 2019). To prevent potential misidentifications due to errors in the database, we validated all genetic species identifications for unknown samples by ensuring that each unknown sequence was a sequence match to at least one reference sequence with a known species identification. Reference samples for this study were (1) voucher specimens from a previous study (Boguski et al. 2012), (2) adults collected for this study with confident species identification, or (3) archived samples with confident species identification that were shared for this study ($N = 6$; mentioned above).

Objective 2: Determine whether current taxonomic classifications accurately and sufficiently describe California's lamprey diversity

Phylogenetic analysis

Maximum likelihood phylogenetic analysis of the partial *cyt b* gene was conducted in RAxML version 8.2.10 (Stamatakis 2014). Specifically, rapid bootstrap analyses (option = -f a) were run with 1000 bootstrap replicates under a general time-reversible (GTR) model with a gamma distribution for incorporating rate variation among sites specified with four discrete rate categories (option = GTRGAMMA). Maximum likelihood phylogenies were estimated for (1) all *Lampetra* individuals collected within California ($N = 65$), including publicly available sequences (Boguski et al. 2012); (2) all *Entosphenus* individuals collected within California ($N = 62$), including publicly available sequences (Boguski et al. 2012); (3) all samples (*Entosphenus* and *Lampetra*) collected within California as well as out-of-state reference samples for the Western Brook Lamprey, Pacific Brook Lamprey *L. pacifica*, and Pacific Lamprey ($N = 136$), including publicly available sequences from Boguski et al. (2012); and (4) unique haplotypes from all samples (*Entosphenus* and *Lampetra*) collected along the west coast of North America ($N = 75$), including publicly available sequences from Boguski et al. (2012). See Table S.3 for additional information about the publicly available sequences used in this study. The resultant trees were visualized using the R package ggtree (Yu et al. 2017). Nodes with bootstrap proportions less than 50% were collapsed, and all nodes with greater than 70% support were displayed.

Genetic distance

All samples collected for this study within California ($N = 87$) were included in the analysis of genetic distance. Pairwise genetic distances were calculated for all pairwise comparisons of all individual sequences using the dist.dna function in the R package ape (Paradis and Schliep 2019) under the Kimura two-parameter (K2P) model of base substitution (also frequently called the Kimura 80 [K80] model). To calculate average pairwise K2P distances within and between two lineages, genetic distance values for all individual pairwise comparisons made between lineages were averaged (by taking the arithmetic mean). For example, to obtain the average pairwise K2P distance between Western River Lamprey and Pacific Lamprey, all pairwise genetic distance values produced from pairwise comparisons between Western River Lamprey and Pacific Lamprey individuals were averaged. Typically considered standard in barcoding analyses, the widespread use of K2P distances has faced criticism, primarily for a lack of justification of model choice (Srivathsan and Meier 2012). However, when comparing results from the best model determined by rigorous model selection to results from the K2P model, genetic distance estimates are highly similar (Collins et al. 2012). Thus, the consequences of using the K2P model, even when the model is least supported, are very minimal.

Molecular species delimitation

To delimit individuals into candidate species groupings based on *cyt b* sequences, this study utilized a distance-based method (Assemble Species by Automatic Partitioning [ASAP]; Puillandre et al. 2020) and a tree-based method (Bayesian Poisson Tree Processes [bPTP] model; Zhang et al. 2013). For these analyses, "candidate species" are defined as "mitochondrial lineages delimited as separate species by statistical methods." Each method was applied to sequence data from all individuals in the *Lampetra* genus collected in California ($N = 66$), including publicly available sequences (Boguski et al. 2012; see Table S.3). This analysis was also run for the *Entosphenus* genus, but the methods performed poorly for those data, likely due to high sequence similarity across the genus. Therefore, haplotype network analysis was conducted (described below) to investigate relationships between *cyt b* haplotypes for *Entosphenus*. The ASAP program implements a hierarchical clustering algorithm that does not rely on phylogenetic reconstruction or on prior biological insight; instead, it only considers genetic distances to identify a threshold between small intraspecific differences and large interspecific differences (Puillandre et al. 2020). The other approach, bPTP, estimates branching rates from an input phylogenetic tree, determining which portions

of the tree (with certain branching rates) best fit a speciation model versus a coalescent model, and species are partitioned by maximizing the likelihood of a shift between the two categories of branching rates (Zhang et al. 2013). Both maximum likelihood and Bayesian approaches for statistical inference of species delimitations were performed separately within bPTP. To eliminate meaningless intraspecific branching events in the tree, sequences were reduced before bPTP analysis such that each unique haplotype was only represented once ($N = 23$). The underlying assumption in the bPTP model is that the number of substitutions between species is significantly larger statistically than the number within species. Both programs are designed for single-locus bar code data, such as the *cyt b* sequences generated in this study, but each approach has limitations. Empirical evidence indicates that tree-based methods (i.e., bPTP) tend to oversplit species, while distance-based methods (i.e., ASAP) often undersplit species, which highlights the importance of employing multiple approaches (Luo et al. 2018).

Haplotype network

Genetic relationships within the genus *Entosphenus* were explored using a haplotype network constructed from sequences of all *Entosphenus* individuals collected for this study within California ($N = 62$) via the R package pegas (Population and Evolutionary Genetics Analysis System; Paradis 2010). Sequences from Boguski et al. (2012) were not included in this analysis because that study focused on *Lampetra* and had very limited sampling of *Entosphenus*. Unique haplotypes were identified, and the number of individuals belonging to each haplotype was calculated. The network is displayed such that each uniquely colored circle represents a unique haplotype, the size of the circle is proportional to the number of individuals with that haplotype, and the length of the line between circles depends on how closely related the haplotypes are.

RESULTS

The alignment of *Lampetra* sequences had 132 variable sites and 120 parsimony-informative sites and contained 65 sequences from two nominal species. Overall nucleotide diversity at *cyt b* was 0.032 for *Lampetra* sequences. Among *Lampetra* sequences from individuals collected within California ($N = 65$), 23 total unique haplotypes were identified, 13 of which were from previously published data (Boguski et al. 2012) and 10 of which were from novel sequencing for this study. The alignment of *Entosphenus* sequences was 930 bp long, had 18 variable sites and 11 parsimony-informative sites, and contained 64 sequences from three nominal species. Nucleotide diversity at *cyt b* was 0.002 for *Entosphenus* sequences. Across all *Entosphenus* sequences from individuals collected within California ($N = 62$), 14 unique haplotypes were identified: one from previously published data (Boguski et al. 2012) and 13 from novel sequencing for this study.

Objective 1: Identify nominal lamprey species present at study sites

In *Lampetra*, two nominal species (Western River Lamprey and Kern Brook Lamprey) were found within their described ranges (Figure 2). Individuals identified in the field as Western River Lampreys were verified by genetic identification, which revealed that only two individual Western River Lamprey had been correctly identified in the field; five out of seven samples that were morphologically identified as Western River Lamprey were genetically identified as Pacific Lamprey (Table S.4). All individuals from the Kings River ($N = 5$) were a sequence match to Kern Brook Lamprey sequences in the database. The sequences from the Napa River and Alameda Creek samples were not a match to any publicly available sequences and were most similar to *Lampetra* samples of unknown species, confirming their placement in the genus *Lampetra*.

In *Entosphenus*, three nominal species (Pacific Lamprey, Pit-Klamath Brook Lamprey, and Klamath Lamprey) were found within their described ranges (Figure 3). Genetic verification of field species identification showed that 31 out of 34 individuals identified as Pacific Lampreys in the field were also genetically identified as Pacific Lampreys (Table S.4). The *cyt b* sequences from the adult Pit-Klamath Brook Lamprey and other individuals from the Pit River, the South Fork Pit River, and Lassen Creek best matched Pit-Klamath Brook Lamprey sequences in the database but were also highly similar (≥99.68%) to Pacific Lamprey sequences. Similarly, *cyt b* sequences from individuals identified as Klamath Lamprey in the field matched Klamath Lamprey sequences in the database but also showed high similarity (≥99.5%) to both Pacific Lamprey and Pit-Klamath Brook Lamprey sequences.

In total, the mitochondrial *cyt b* sequence identity BLAST search confirmed 82% of the total field species identifications ($N = 37$), contradicted 17% of the field species identifications ($N = 8$), confirmed genus-level identity for all genus-level field identifications ($N = 12$), and provided genus- or species-level identity for all unidentified samples ($N = 30$; Table S.4).

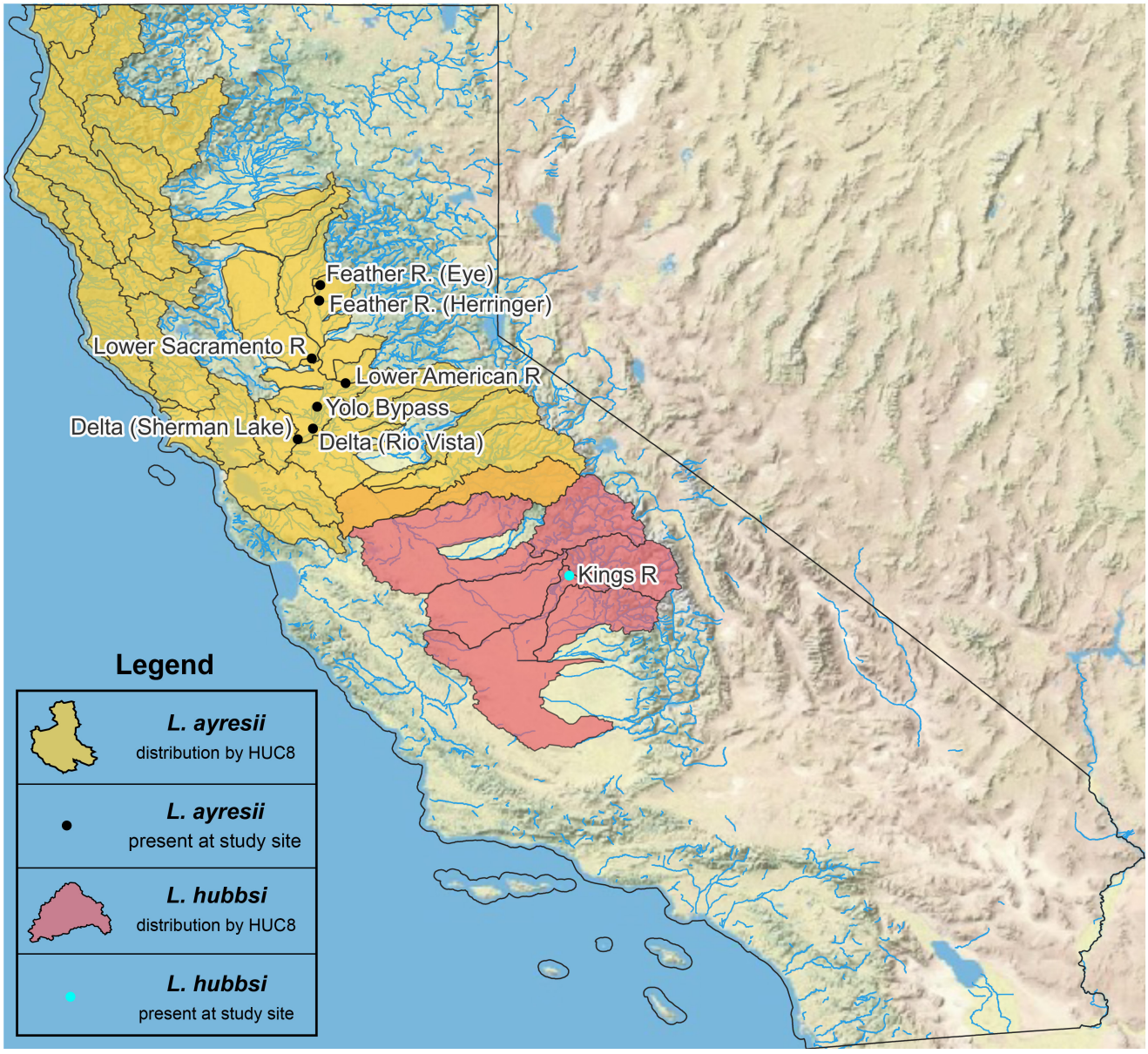

FIGURE 2 Map of California displaying all ranges for nominal species within *Lampetra* and study sites where each nominal species was found. Eight-digit hydrologic unit code (HUC8) range data are from the Western Native Fishes Committee (databasin.org). R, River.

Objective 2: Determine whether current taxonomic classifications accurately and sufficiently describe California's lamprey diversity

Phylogenetic analysis

The maximum likelihood phylogeny supported both the *Lampetra* and *Entosphenus* genera as monophyletic groups with maximal (100%) bootstrap support (Figure S.2). The genera were then split, and trees were reconstructed for each genus separately (Figures 4 and 5). All Western River Lamprey in this study were part of the same clade (89% bootstrap support) that included previously sequenced individuals from the San Francisco Bay and the Feather River (Boguski et al. 2012). The previously sequenced *Lampetra* sp. individuals from the Klamath River, Redwood Creek, and the Navarro River formed a monophyletic clade (100% bootstrap support) that was sister to Western River Lamprey collected from San Francisco Bay, the Sacramento River, and the Feather River. Kern Brook Lamprey from the Kings River and previously sequenced Kern Brook Lamprey from the Merced River formed a monophyletic group (100% bootstrap support) that was sister to one *Lampetra* sp. individual from Paynes Creek in the upper Sacramento River basin. *Lampetra* spp. from Alameda Creek, the Napa River, and the Russian River, which were previously sequenced by

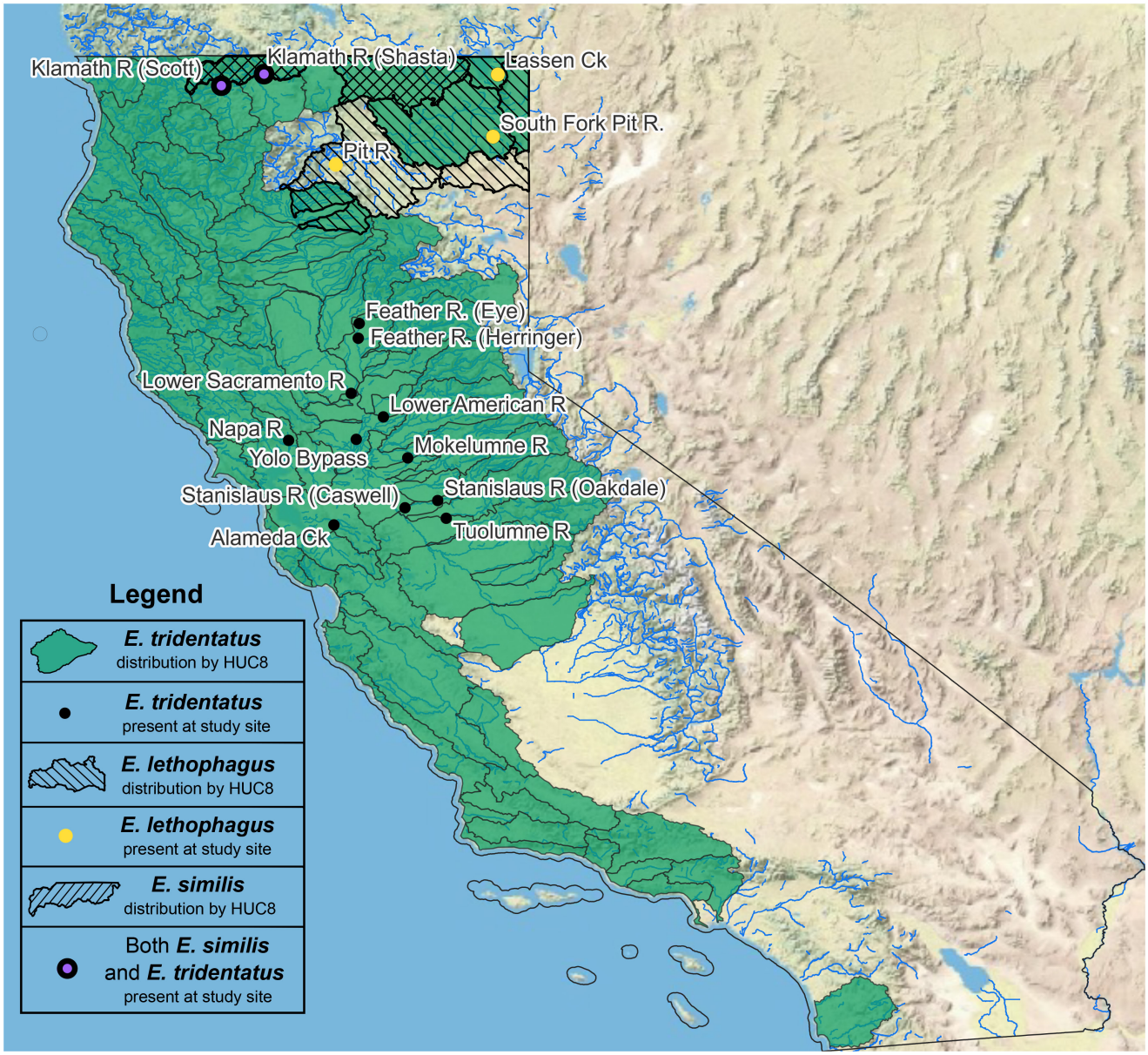

FIGURE 3 Map of California displaying all ranges for nominal species within *Entosphenus* and study sites where each species was found. Eight-digit hydrologic unit code (HUC8) range data are from the Western Native Fishes Committee (databasin.org). Ck, Creek; R, River.

Boguski et al. (2012), were part of a distinct clade (100% bootstrap support). Previously sequenced *Lampetra* sp. from Kelsey Creek in the Clear Lake basin (Boguski et al. 2012) formed a distinct monophyletic clade that was sister to all other *Lampetra* lineages in California (100% bootstrap support). Klamath Lamprey and Pit-Klamath Brook Lamprey both formed monophyletic groups (each 100% bootstrap support); within the Pit-Klamath Brook Lamprey, the individuals from the main-stem Pit River formed a distinct clade (100% bootstrap support). Several polytomies were observed throughout the *Entosphenus* phylogenetic tree, and most nodes were collapsed due to having less than 50% bootstrap support.

Genetic distance

Within the genus *Lampetra*, the average K2P distance was 3.25% (Figure 6), an order of magnitude higher than that within the genus *Entosphenus*. Genetic distances between lineages in *Lampetra* ranged from 2.95% to 5.54%, much higher than interspecific values in *Entosphenus*. The smallest average K2P distance between distinct lineages within *Lampetra* was between the Napa River and Alameda Creek lineages (2.95%), and the largest difference was between the Napa River lineage and the Western River Lamprey (5.54%; Figure 6). Average genetic distance within each species in the genus *Lampetra* ranged from

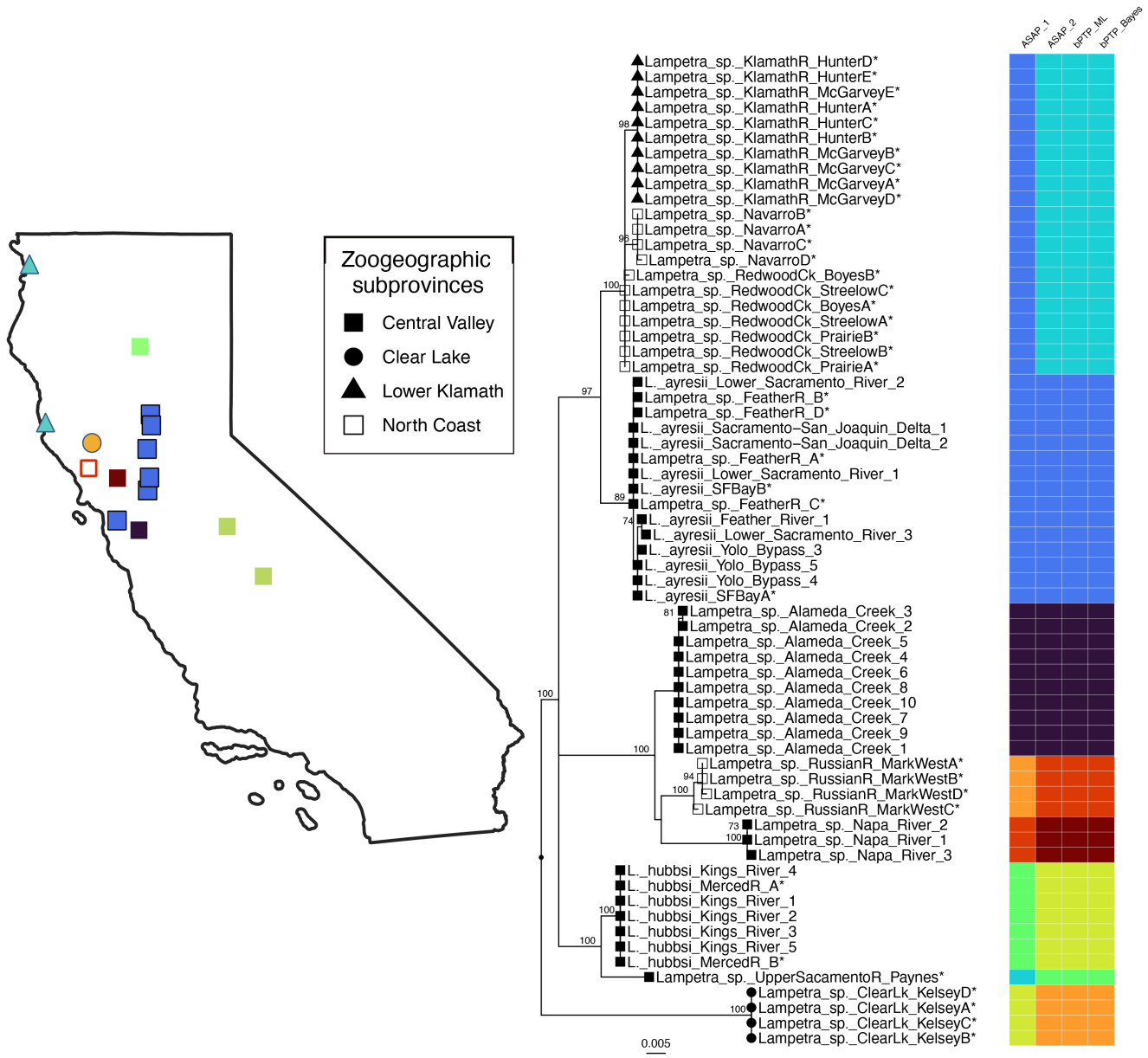

FIGURE 4 Maximum likelihood phylogeny showing estimated evolutionary relationships at the cytochrome *b* (*cyt b*) gene of *Lampetra* spp. in California. Bootstrap proportions are displayed for nodes with over 70% support, and nodes with less than 50% support are collapsed to polytomies. Colored boxes to the right of the tree show species delimitation results from Assemble Species by Automatic Partitioning (ASAP; first and second most supported partitions: $n = 7$ and $n = 8$ species, respectively) and Bayesian Poisson Tree Processes (bPTP; maximum likelihood [ML] and Bayesian [Bayes] results: $n = 8$ species for both). In each column, unique colors represent distinct candidate species. The inset map displays sample locations; point shape corresponds to zoogeographic subprovince, and point color corresponds to the candidate species delimited by ASAP and bPTP. Publicly available sequences are marked with asterisks.

0.00% (Kern Brook Lamprey) to 0.17% (Western River Lamprey).

Within *Entosphenus*, the average K2P distance was 0.21% (Figure 6). The smallest average K2P distance between lineages was between the Klamath Lamprey and Pacific Lamprey (0.30%), while the largest difference was between the Klamath Lamprey and Pit-Klamath Brook Lamprey (0.49%). Average genetic distance within each species in the genus *Entosphenus* ranged from 0.00% (Klamath Lamprey) to 0.18% (Pit-Klamath Brook Lamprey; Figure 6).

Lampetra species delimitation

All *Lampetra* sequences from individuals collected within California for this study and previously sequenced individuals collected in California from the Boguski et al. (2012)

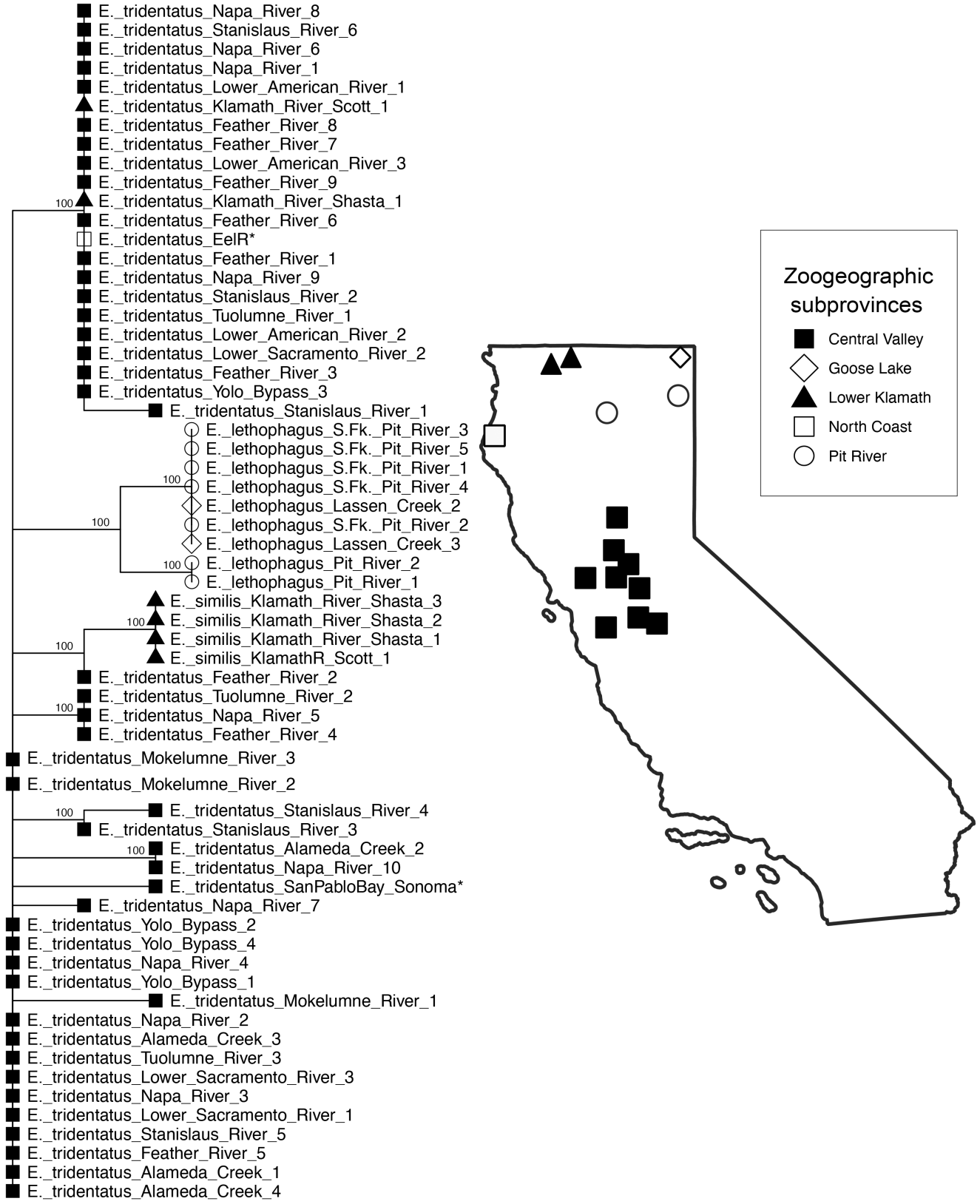

FIGURE 5 Maximum likelihood phylogeny showing estimated evolutionary relationships at the cytochrome b (*cyt b*) gene of *Entosphenus* spp. in California. The shape of each tree tip point reflects the zoogeographic subprovince from which the sample was collected. Bootstrap proportions are displayed for nodes with over 70% support, and nodes with less than 50% support are collapsed to polytomies. The inset map displays sample locations; point shape and fill indicate the zoogeographic subprovince that each sampling location falls under. Publicly available sequences are marked with asterisks.

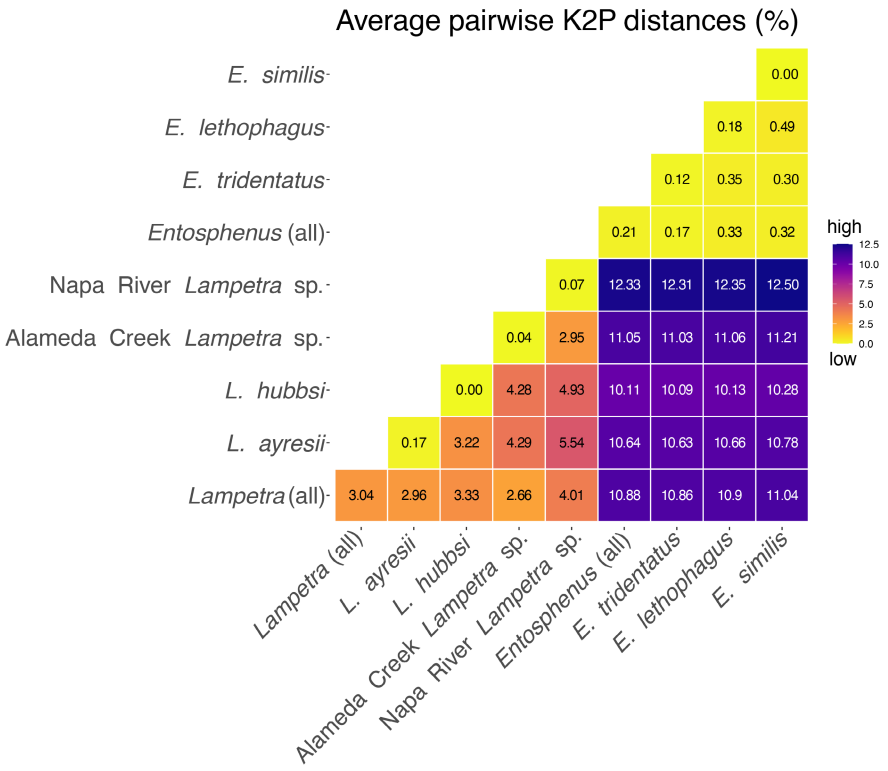

FIGURE 6 Average pairwise Kimura two-parameter (K2P) distances (%) within and between distinct lamprey lineages present in California.

study were included in this analysis ($N=65$). The highest supported result from ASAP delimited seven candidate species in *Lampetra* from California (Figure 4): (1) the Western River Lamprey from the lower Sacramento River, Feather River, and Yolo Bypass and *Lampetra* sp. from the Klamath River, Navarro River, and Redwood Creek; (2) *Lampetra* sp. from Alameda Creek; (3) *Lampetra* sp. from Mark West Creek in the Russian River basin; (4) *Lampetra* sp. from the Napa River; (5) the Kern Brook Lamprey from the middle and upper San Joaquin River basin (Merced and Kings rivers); (6) *Lampetra* sp. from Paynes Creek in the upper Sacramento River basin; and (7) *Lampetra* sp. from Kelsey Creek in the Clear Lake basin. The second most supported result from ASAP and both maximum likelihood and Bayesian results from bPTP delimited eight species (Figure 4): (1) the Western River Lamprey from the lower Sacramento River, Feather River, and Yolo Bypass; (2) *Lampetra* sp. from the Klamath River, Navarro River, and Redwood Creek; (3) *Lampetra* sp. from Alameda Creek; (4) *Lampetra* sp. from Mark West Creek in the Russian River basin; (5) *Lampetra* sp. from the Napa River; (6) the Kern Brook Lamprey from the middle and upper San Joaquin River basin (Merced and Kings rivers); (7) *Lampetra* sp. from Paynes Creek in the upper Sacramento River basin; and (8) *Lampetra* sp. from Kelsey Creek in the Clear Lake basin. The difference between the top supported and second most supported results from ASAP was the delimitation of (1) the Western River Lamprey from the lower Sacramento River, Feather River, and Yolo Bypass and (2) *Lampetra* sp. from the Klamath River, Navarro River, and Redwood Creek as either two separate species or a single species.

Entosphenus haplotype network

All *Entosphenus* sequences from individuals collected in California for this study and publicly available *Entosphenus* sequences collected in California from the Boguski et al. (2012) study were included in this analysis ($N=62$). Fourteen unique haplotypes were identified, with two common haplotypes found in 60.9% ($N=39$) of individuals from 15 different sampling locations (Figure 7). These two common haplotypes were separated by only one mutational step. Eleven haplotypes were considered rare (i.e., found in <5% of individuals). Seven of those haplotypes were unique to one individual. All individuals from the North Coast zoogeographic province shared a single haplotype. Similarly, all individuals from the Goose Lake zoogeographic subprovince shared one haplotype (Figure 7). The Pit-Klamath Brook Lamprey contained two haplotypes: one was unique to individuals from the main-stem Pit River and the other was shared by individuals from the South Fork Pit River and Lassen Creek (Figure 7).

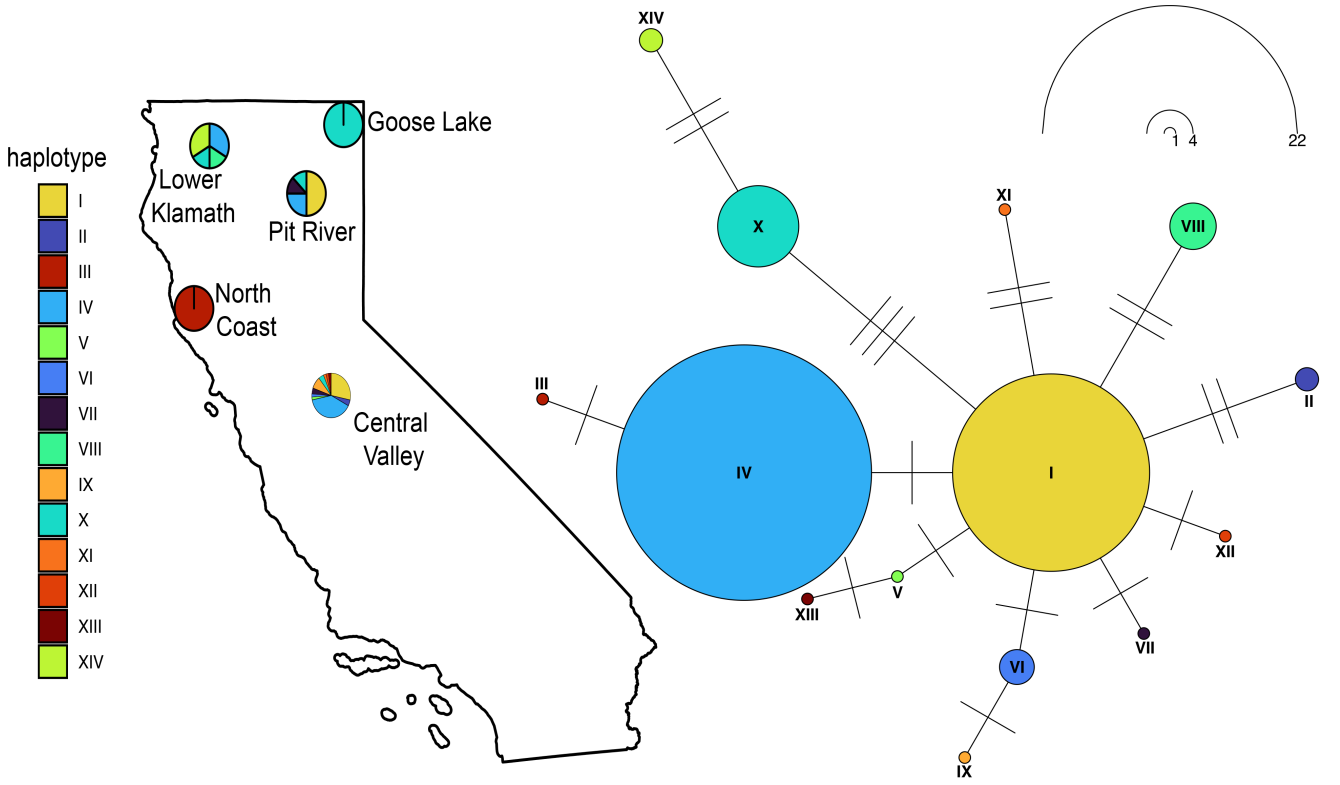

FIGURE 7 Haplotype network displaying relationships between lamprey individuals in the *Entosphenus* genus. Each uniquely colored circle represents a unique haplotype, and the size of each circle in the network is proportional to the number of individuals with each haplotype, as indicated by the scale of different-sized circles in the top right legend. Tick marks on the lines connecting circles represent mutational steps between haplotypes. The inset map shows how haplotypes are distributed across zoogeographic subprovinces in California; the circles on the map represent zoogeographic subprovinces, and the different colored segments of each circle correspond to unique haplotypes found within that zoogeographic subprovince.

DISCUSSION

Results from this study add resolution to the existing knowledge of lamprey diversity. Of the eight nominal species that are thought to occur in California (Table 1), five were observed in their described ranges (Figures 2 and 3): the Western River Lamprey, Kern Brook Lamprey, Pacific Lamprey, Pit-Klamath Brook Lamprey, and Klamath Lamprey. Results supported previous findings (Goodman et al. 2009), which demonstrated that mtDNA barcoding using the *cyt b* gene can reliably distinguish between the genera *Lampetra* and *Entosphenus* and can identify Kern Brook Lampreys to the species level. Phylogenetic and species delimitation results indicated that current taxonomic classifications likely both under- and overrepresent the genetically distinct lamprey lineages present in California. For example, results suggested that several distinct *Lampetra* lineages (i.e., those in the Napa River, Alameda Creek, Clear Lake, and the Russian River) may warrant novel species designations. Conversely, the low levels of genetic divergence between the Western River Lamprey and Western Brook Lamprey as well as the genetic similarity among the Pacific Lamprey, Pit-Klamath Brook Lamprey, and Klamath Lamprey indicated that these nominal species might be incorrectly classified as separate species and may instead be more accurately described as ecotypes or subspecies. The biogeographic distribution of nominal species and distinct lamprey lineages is similar to patterns of other native fish taxa, which highlights the importance of biodiversity conservation in regions with high rates of endemism. This study provides a valuable initial assessment of the genetic diversity of lampreys in the study area; further research is required to reach a more complete understanding of these lineages and to determine taxonomic classifications.

Objective 1: Identify nominal lamprey species present at study sites

Results corroborated previous findings that mtDNA barcoding using the *cyt b* gene can reliably distinguish between the *Entosphenus* and *Lampetra* genera and can identify the Kern Brook Lamprey (Goodman et al. 2009). However, mtDNA barcoding using the *cyt b* gene does

not provide adequate resolution to discern between all nominal species within each lamprey genus. For example, the high *cyt b* sequence similarity among the Pacific Lamprey, Pit-Klamath Brook Lamprey, and Klamath Lamprey indicated that these three nominal species are very closely related. This points to the need for further research to determine whether these are three distinct species or represent life history variants within one species. To distinguish between these species (or ecotypes) using molecular methods, additional gene sequences or higher resolution genetic markers would need to be utilized.

Although this study was not designed to quantify abundance or distribution, patterns were observed that can inform these topics. Our results confirmed the presence of nominal species within their described ranges (Figures 2 and 3). The most abundant and wide-ranging species in our data set was the Pacific Lamprey, which made up about 50% of all individuals in this study and was found at 14 of 19 sites (Table S.2). The Western River Lamprey was the next most widespread species and was found at 5 of the 19 collection sites but was not nearly as abundant as the Pacific Lamprey, making up less than 10% of all samples (Table S.2). Results indicated that other lineages have relatively restricted geographic ranges and likely have low relative abundance compared to the two anadromous species (Pacific Lamprey and Western River Lamprey).

Objective 2: Determine whether current taxonomic classifications accurately and sufficiently describe California's lamprey diversity

We confirmed the presence of two main mitochondrial lineages of lamprey in California, divided between two genera. The distribution of diversity observed in mitochondrial sequence data from *Lampetra* and *Entosphenus* was distinctive. Within *Lampetra*, geographically restricted species-level lineages were phylogenetically structured (Figure 4) and exhibited deep genetic divergences (average K2P distance = 3.252%; Figure 6). *Entosphenus* was characterized by a lack of phylogenetic structure (Figure 5) and by shallow mtDNA divergences across a broad geographic scale, with some evidence of isolation of nominal species (average K2P distance = 0.205%; Figure 6).

Lampetra

Previous phylogenetic studies have determined that the genus *Lampetra* is polyphyletic (Brownstein and Near 2023; Carim et al. 2023). The *Lampetra* species originating from Europe, Asia, and eastern North America form a lineage that is sister to *Eudontomyzon* and representative of the original description of this genus (Pereira et al. 2021; Brownstein and Near 2023; Carim et al. 2023). Another clade of *Lampetra* is present in western North America that diverged from a combined lineage of *Lethenteron*, *Eudontomyzon*, and other *Lampetra* prior to the Neogene and that merits recognition as a separate genus. In California, the western North American "*Lampetra*" genus (hereafter, *Lampetra*) was characterized by high levels of *cyt b* sequence differentiation across unique lineages and nominal species, except between the Western River Lamprey and Western Brook Lamprey. However, within the larger grouping of Western River Lamprey and Western Brook Lamprey, there were numerous deep divergences, and within California there were two clear divisions. With Western River Lamprey collected in California, little divergence was observed within major clades such that the Sacramento River, San Joaquin River, and San Francisco Estuary samples were highly similar in mtDNA sequences to each other, as were the northern California samples originating from Redwood Creek, the Navarro River, and the Klamath River (Figure 4). Like the Pacific Lamprey, there may be a lack of strong philopatry in this species (Moyle et al. 2009), but further investigation is required to understand the migratory behavior of Western River Lamprey. Rangewide studies have shown polyphyly of Western River Lamprey and Western Brook Lamprey individuals across multiple phylogenetic lineages (Boguski et al. 2012; Carim et al. 2023), indicating a lack of support for their current taxonomic classification as separate species. One possible resolution is to consider the Western Brook Lamprey as a synonymy of the Western River Lamprey. Another is the redescription of the Western Brook Lamprey to be representative of a northern river lamprey species. The Western River Lamprey appears to occupy disjunct distributions with six differentiated clades (Boguski et al. 2012), and we sampled two of these: Sacramento–San Joaquin rivers/San Francisco Bay and northern California. Additional Western River Lampreys are found in the Yaquina River, the Columbia River, and northward (Moyle 2002). Without fully representing Western River Lamprey across the full species range, this study does provide some evidence that supports splitting Western River Lamprey into two distinct species (Figure 4).

None of the samples collected in our study was genetically identified as the Western Brook Lamprey. Given that the Western Brook Lamprey likely represents a genetically similar life history variant of the Western River Lamprey, it is reasonable that it was not detected in this study. Although both species are thought to occur widely across the west coast, the type localities for these species

are at opposite ends of their mostly overlapping ranges. The original species description for the Western Brook Lamprey was based on specimens from Smith Creek in the Fraser River basin of British Columbia (Vladykov and Follett 1965), while the type locality for the Western River Lamprey is in the San Francisco Bay (Günther 1870). Sequences from the original type specimens were unavailable, but representative samples collected near the type localities were included in this study. Phylogenetic analysis indicates that Western Brook Lamprey specimens from the Fraser River, British Columbia, and the Columbia River are in the same clade as *Lampetra* sp. from the north coast of California (the Klamath River, Redwood Creek, and the Navarro River; Figure S.3). The Navarro River was reported to have spawning Western Brook Lamprey, and life history variation may exist within Western River Lamprey on the northern California coast, representing a freshwater-resident lamprey life history. The question, then, is "In the San Francisco Bay and Sacramento–San Joaquin River system, are there life history variants (i.e., freshwater-resident lampreys) within the Western River Lamprey that may be identified as Western Brook Lamprey based on morphology and current taxonomy but genetically identified as Western River Lampreys due to high genetic similarity between life history variants?" Watersheds where Western Brook Lamprey are thought to occur in this region include Kelsey Creek in the Clear Lake drainage (Sacramento River drainage; Moyle 2002), which clearly holds a distinct *Lampetra* lineage (Figure 4). Southern San Francisco Bay brook lamprey (species unknown) (Hubbs 1925) are represented by a population in Alameda Creek, which is also a distinct *Lampetra* lineage (Figure 4). The Russian River has also been indicated as a location where Western Brook Lamprey occur (Moyle 2002), and a distinctive *Lampetra* lineage occupies this system (Figure 4). The eastern San Joaquin Valley has already been demonstrated to contain a separate *Lampetra* species, the Kern Brook Lamprey. Instead of a close genetic relationship between life history variants, as observed between the Western River Lamprey and Western Brook Lamprey in more northerly regions of the west coast, perhaps the southern lineage of Western River Lamprey in the San Francisco Bay and associated drainages has a more distant genetic relationship with multiple freshwater-resident lamprey lineages that have previously been assumed to be Western Brook Lamprey. Such a conclusion would require further investigation of adult individuals' morphology, genotypes, and life history phenotypes; however, study results are consistent with the hypothesis that multiple freshwater-resident *Lampetra* lineages occur in the San Francisco Bay area and Sacramento–San Joaquin River drainages. We identified five candidate freshwater-resident lamprey species from mtDNA sequence data (Figure 4), and our findings supported the recognition of the Kern Brook Lamprey, for a total of six freshwater-resident lamprey species in the *Lampetra* genus.

Novel distinct *Lampetra* lineages were identified in this study from the north and south San Francisco Bay regions. The *cyt b* sequences from *Lampetra* sp. from the Napa River and Alameda Creek were 3% different than any publicly available sequences, which is substantial considering the short length of the sequence (930 bp) and the fact that these sequences represent a highly conserved mitochondrial protein-coding gene. For reference, the Western River Lamprey and Pacific Lamprey showed an approximately 10% sequence difference at this locus, and these species belong to different genera that share a most recent common ancestor from about 60 million years ago (Brownstein and Near 2023).

The most highly supported partitions from bPTP and ASAP delimited populations from the Napa River and Alameda Creek as distinct species. This, combined with the high genetic distance from other California species and the lack of any similar publicly available *cyt b* sequences, suggests that these populations represent previously undescribed lamprey diversity. One caveat of these analyses relates to the low-resolution representation of phylogenetic relationships provided by sequences from a single gene (Degnan and Rosenberg 2009; Waters et al. 2010). The genetic patterns observed at the *cyt b* gene represent a very small fraction of the genome-wide patterns that species trees aim to portray. Another drawback of species delimitation based on mitochondrial sequence data is the maternal inheritance of mitochondrial genes, which can bias results due to differences in male and female dispersal (Petzold and Hassanin 2020; Lukic et al. 2021). Putative species delimited in these analyses can be used as an initial hypothesis that should be further investigated. An integrative taxonomic framework must incorporate these results with genome-wide data as well as morphological, biological, and ecological evidence to thoroughly assess species boundaries in *Lampetra* (Fujita et al. 2012).

Boguski et al. (2012) identified a unique mitochondrial lineage from the Clear Lake basin in central California. Sequences in the present study included new diversity but did not recover any individuals that were closely related to the unique *Lampetra* sp. population found in Clear Lake, thus further supporting the distinctiveness of the Clear Lake lineage. Phylogenetic results also substantiated this lineage as the earliest branching *Lampetra* in California (Figure 4). The Clear Lake basin, a region of high endemism, is thought to have been one of the first to become isolated in California (late Pleistocene–Holocene), promoting speciation and adaptation to Clear Lake's complex ecosystem (Hopkirk 1988). In more recent history, flooding, fires,

land use changes, mining, nutrient loading, nonnative species introductions, and high levels of contaminants have all threatened the multitude of endemic species in the Clear Lake basin (Suchanek et al. 2002). Human dimensions, especially tourism and agriculture, play an important role in Clear Lake management (Suchanek et al. 2002), but the distinct lamprey lineage, accumulation of endemic species, and numerous natural and environmental stressors in this ecosystem emphasize the need to prioritize protection of the rich native biodiversity that is present.

An unexpected finding from Boguski et al. (2012) was the close phylogenetic relationship between Kern Brook Lampreys from the Merced River and one *Lampetra* sp. individual from Paynes Creek in the upper Sacramento River basin. Phylogenetic analysis in our study also found that the *Lampetra* sp. individual from Paynes Creek was sister to Kern Brook Lampreys from the Kings and Merced rivers. Boguski et al. (2012) suggested that the range of the Kern Brook Lamprey extended into the upper Sacramento River; however, our additional sequencing of fish from the Sacramento River did not recover any sequences similar to those of the Paynes Creek sample. Additionally, species delimitation analysis identified the Paynes Creek sample as a species lineage, which suggests that this may be a distinct lineage warranting its own species designation. Considering that this pattern has only been observed based on mitochondrial sequences from a single individual, further sampling of the Paynes Creek region and other tributaries of the Sacramento River will be required to resolve the relationship and taxonomic classification of this Paynes Creek lineage.

Entosphenus

Contrary to the deep genetic divergence observed in *Lampetra*, high levels of *cyt b* sequence similarity were observed within and across nominal species in *Entosphenus* within California. The absence of strong genetic differentiation among the Pacific Lamprey, Klamath Lamprey, and Pit-Klamath Brook Lamprey despite stark morphological and ecological differences may be the result of ongoing gene flow between these groups, may represent a case of high phenotypic plasticity, or may indicate a relatively recent divergence. The Pacific Lamprey and Pit-Klamath Brook Lamprey, a presumed species pair, showed high similarity at the *cyt b* gene, but higher resolution genomic analyses and population genetic sampling would help to clarify the relationship between these groups. Within the Pacific Lamprey, very low to no genetic structure was observed across geographic sites, which supports the hypothesized lack of strong philopatry in this species (Hatch and Whiteaker 2009; Spice et al. 2012; Hess et al. 2020). Although this study is focused on California, prior research also found high levels of gene flow among Pacific Lamprey populations across the west coast of North America. However, at this larger geographic scale, genetic structure and local adaptation were observed (Spice et al. 2012; Hess et al. 2020). In the present study, no Pacific Lamprey individuals were found in the Pit River basin or the Goose Lake basin, suggesting that there is an impassable barrier to upstream migration between the lower Sacramento River and the Pit River. This finding is consistent with the lack of connectivity between the Pit River and lower Sacramento River for other fish populations, likely due to the construction of the Shasta Dam in 1945 (Schick and Lindley 2007).

Objective 3: Compare biogeographic distribution of lamprey diversity to patterns observed in other native taxa

At a broad scale, species diversity occurs on a latitudinal cline, being highest at the equator and declining toward the polar regions (Hillebrand 2004; van Humboldt 1828). Viewing western North American lamprey diversity in this framework, a higher amount of species diversity should occur in the southern range (i.e., California) of the group, declining to the north. Our work and other studies substantiate this trend (Boguski et al. 2012). Overall high rates of endemism occur in California; over 80% of native fishes in the state are endemic to California or watersheds shared with neighboring states (Leidy and Moyle 2021). This richness of endemic species may be further explained by other aspects of California's geography and climate. The prevalence of intermittent streams, which frequently cause fluctuating stream fragmentation, may increase the likelihood of fish populations becoming isolated, leading to high rates of endemism (Ball et al. 2013). Furthermore, the state's rugged topography results in many isolated watersheds (Moyle 2002).

The distribution of lamprey diversity across the landscape was concordant with broader zoogeographic patterns of California native fish diversity, suggesting that the diversification of extant lampreys was shaped by biogeographic characteristics similar to other fish lineages. Moyle (2002) described six major zoogeographic provinces in California: Klamath, Sacramento–San Joaquin, North Coast, Great Basin, South Coast, and Colorado River, with various subprovinces in each (22 total subprovinces). Within the Sacramento–San Joaquin province, nominal lamprey species with restricted ranges were found in the upper Kern River subprovince (Kern Brook Lamprey) and the Pit River

and Goose Lake subprovinces (Pit-Klamath Brook Lamprey). Additionally, the range-restricted Klamath Lamprey was found in the Upper and Lower Klamath subprovince. Beyond nominal species, distinct lamprey populations were found in the Clear Lake subprovince (*Lampetra* sp. from Kelsey Creek; Boguski et al. 2012), the Central Valley subprovince (*Lampetra* sp. from Alameda Creek and the Napa River), and the North Coast province (*Lampetra* sp. from Mark West Creek; Boguski et al. 2012). Additionally, species delimitation results suggested that *Lampetra* sp. from the North Coast province may be distinct from Western River Lampreys in the Sacramento–San Joaquin province. These pockets of distinct lampreys and other native fishes highlight the importance of protecting ecosystems in regions of California with high biodiversity and endemism.

CONCLUSIONS AND MANAGEMENT IMPLICATIONS

In conclusion, results of these analyses showed that the genus *Lampetra* harbors high diversity in California and contains very divergent lineages in comparison to the genus *Entosphenus*. Previously unrecognized lamprey diversity within *Lampetra* was identified in the Napa River and Alameda Creek; these populations are distinct from the novel unique lineages found in Mark West, Kelsey, and Paynes creeks by Boguski et al. (2012). Additional lines of evidence are required to determine the appropriate taxonomic classification for these populations, but the level of genetic differentiation at *cyt b* suggests that these lineages should at least be treated as independent management units. The HUCs, part of a standard system developed by the U.S. Geological Survey for defining hydrologic areas (Seaber et al. 1987), can be a helpful tool in the initial stages of management unit delineation. In this study, lamprey populations often showed genetic differentiation between basins (six-digit HUCs [HUC6]), but little to no genetic differentiation was observed between subbasins (HUC8), suggesting that basins (HUC6) are the more appropriate hydrological unit for management of lamprey populations in the study area. Notably, results indicated that multiple species frequently co-occur within basins (HUC6), so accurate species identification will be especially important in efforts to characterize the abundance and distribution of lampreys. Since lampreys are most frequently sampled in the field during the larval phase, when they may lack distinguishing morphological features, genetic species identification would improve the accuracy of data collected and would help to advance our understanding of species distributions (Docker et al. 2016; Clemens et al. 2022). The genetic marker used for DNA barcoding in this study (*cyt b*) could not distinguish between all nominal lamprey species in California, but molecular identification of different lamprey species (or ecotypes) is likely feasible with higher resolution genomic sequencing techniques. Although it also may be possible to distinguish between species by using a combination of several genetic markers for DNA barcoding, genomic sequencing provides higher resolution data and may be more cost effective due to advances in technology. Goals for addressing long-term stability of native species are complicated by the compounding impacts of human modifications, resource use, and climate warming. However, maintaining lamprey species complexity and fostering resilience cannot begin without an understanding of their underlying genetic diversity.

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CONFLICT OF INTEREST STATEMENT

The authors have declared no conflict of interest regarding the publication of this paper.

ETHICS STATEMENT

This research study was conducted in compliance with ethical principles and guidelines. Tissue samples for this project were obtained from individuals listed in the acknowledgements. Sample collections for this study followed institutional guidelines and permitting. Previously published DNA sequence data included in this study was accessed on a publicly available database.

DATA AVAILABILITY STATEMENT

All data (genetic sequences) generated as part of this study are publicly available through GenBank (Accession Numbers OQ818491–OQ818585).

ORCID

Grace Auringer https://orcid.org/0000-0002-7639-3766
Matthew A. Campbell https://orcid.org/0000-0002-5826-0329
Pascale A. L. Goertler https://orcid.org/0000-0001-6259-5108
Amanda J. Finger https://orcid.org/0000-0003-3850-3685

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SUPPORTING INFORMATION

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