Journal of Fish Biology (2008) **72,** 400–417
doi:10.1111/j.1095-8649.2007.01731.x, available online at http://www.blackwell-synergy.com

Mitochondrial DNA evidence for high levels of gene flow among populations of a widely distributed anadromous lamprey *Entosphenus tridentatus* (Petromyzontidae)

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(Received 18 February 2007, Accepted 3 October 2007)

Mitochondrial DNA variation among 1246 individuals of Pacific lamprey (*Entosphenus tridentatus*) from 81 populations spanning 2600 km from the Skeena River, British Columbia, to the Ventura River, California, was surveyed using five restriction enzymes. A total of 29 composite haplotypes was detected in two gene fragments (ND2 and ND5). The three most common haplotypes, occurring in 91% of all samples, were present at similar frequencies in all regions. Samples were divided into six biogeographic regions based on sample distribution and geographical landmarks to assess geographic genetic structure. Analysis of molecular variance indicated that 99% of the genetic variation was explained by variability within drainages. The lack of geographical population structure is likely related to a life-history pattern that includes a prolonged larval freshwater stage, migration to oceanic feeding and return to fresh water to spawn. The lack of strong natal homing apparently promotes gene flow among drainages and regions.

Key words: *Entosphenus tridentatus*; genetic variation; *Lampetra tridentata*; mitochondrial DNA; Pacific lamprey; phylogeography.

INTRODUCTION

Fishes that migrate between marine and freshwater environments exhibit different levels of genetic differentiation among populations. Many anadromous salmonids (*Oncorhynchus* spp.), for example, exhibit relatively high levels of among-population genetic differentiation (Allendorf & Seeb, 2000; Waples

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et al., 2004). This structure has been used to infer biogeographical processes (McCusker *et al.*, 2000; Nilsson *et al.*, 2001; Smith *et al.*, 2001; Waples *et al.*, 2004) and guide management strategies (Wenburg *et al.*, 1998; Quinn *et al.*, 2000; Waples *et al.*, 2001). In contrast, catadromous anguillid eels exhibit relatively low levels of among-population genetic variation (Avise *et al.*, 1986; Wirth & Bernatchez, 2001). The American eel, *Anguilla rostrata* (Lesueur), exhibits no significant genetic variability over geographic distances of up to 4000 km along the North American Atlantic coastline (Avise *et al.*, 1986).

The genetic population structures of migratory fishes reflect the degree of gene flow among populations, which can often be attributed to differences among species in life-history strategies. For example, anadromous salmonids (*Oncorhynchus* spp.) exhibit high-site fidelity, migrating from adult oceanic feeding areas to natal riverine spawning habitats through the use of chemical cues (Hasler & Wisby, 1951; Hasler & Scholz, 1983; Dittman & Quinn, 1996). The relatively high level of among-population genetic differentiation in many salmonids is attributed to homing to natal streams to spawn (Taylor, 1991). Spawning-site fidelity and low gene flow among populations promote localized adaptation and genetic differentiation. Alternatively, the lack of population structure in anguillids has been attributed to a life-history strategy that facilitates high gene flow among populations. The catadromous American eel migrates from adult feeding areas in coastal rivers of eastern North America to a hypothesized single spawning population in the Sargasso Sea, and after hatching, pelagic larvae disperse to coastal rivers (Williams & Koehn, 1984). The single spawning population of the American eel facilitates high gene flow, homogenizing genetic differences and producing a panmictic population structure (Avise *et al.*, 1986).

The objective of the current study was to assess genetic population structure in the anadromous Pacific lamprey, *Entosphenus tridentatus* (Gairdner in Richardson). The Pacific lamprey is a predatory lamprey with distinct larval and adult life stages (Beamish, 1980). After hatching, larval lampreys (ammocoetes) spend *c.* 5 years in a microphagous stage, filter feeding in freshwater sediment (Moore & Mallatt, 1980; Potter, 1980). During the ammocoete stage, lampreys lack functional eyes, teeth and a suctorial disk. The ammocoete stage is followed by a rapid transformation into macrophthalmia with dramatic morphological changes including the development of eyes, teeth and suctorial disk (Potter, 1980). After transformation, macrophthalmia migrate to the Pacific Ocean to begin a predatory life stage (Beamish, 1980). Adults attach to prey, extracting body fluids and blood for subsistence (Beamish, 1980; Richards & Beamish, 1981). After feeding in the Pacific Ocean, adults migrate from the marine environment into coastal rivers and streams to spawn and subsequently die (Beamish, 1980).

Pacific lamprey occurs in oceanic waters of the North Pacific and coastal drainages in Japan, Asia and North America (Rohde, 1980; Yamazaki *et al.*, 2005). The North American distribution includes most coastal streams between the Aleutian Islands of Alaska and the Rio Santo Domingo, Baja California, Mexico (Larkins, 1964; Morrow, 1980; Rohde, 1980; Ruiz-Campos & Gonzalez-Guzman, 1996), although records of populations south of Point Conception are sporadic (Hubbs, 1967; Swift *et al.*, 1993; Ruiz-Campos & Gonzalez-Guzman,

1996; Chase, 2001). However, little is known about the oceanic distribution or behaviour of Pacific lampreys (Beamish, 1980). In particular, it is not known how far individual lampreys migrate during the oceanic phase and whether they exhibit fidelity to their natal streams. Sea lamprey, *Petromyzon marinus* L., in the Great Lakes show no evidence of homing behaviour (Bergstedt & Seelye, 1995), which is consistent with their rapid colonization across the Great Lakes, but the importance of homing in other lamprey species is unknown. Significant morphological (*e.g.* drainage-specific size differences; Beamish, 1980) and genetic (*e.g.* allele frequency differences; Beamish & Withler, 1986) differences among some Pacific lamprey populations suggest the possibility of natal stream fidelity in this species. The objective of this study was to determine if Pacific lampreys exhibit high levels of among-population genetic differentiation that would indicate fidelity to natal streams.

Although lampreys are widely distributed along the Pacific coast, observations of declining populations have raised concern over the long-term persistence of Pacific lamprey and several other western lamprey species (Renaud, 1997; Close *et al.*, 2002). The status of Pacific lamprey and three other lamprey species was brought to the attention of the United States Fish and Wildlife Service (USFWS) in January 2003, with a petition to list under the Endangered Species Act of 1973. The petition was declined in a 90 day review due to lack of information on population structure, distinct units and quantitative evidence of population declines; however, concern persists over their conservation status (United States Fish and Wildlife Service, 2004). The results of the current study will guide decision makers in management and conservation efforts, such as the potential designation of management units.

MATERIALS AND METHODS

SAMPLE COLLECTION

A total of 1246 ammocoete and adult Pacific lamprey were collected between 1995 and 2005 from 81 localities encompassing a diverse range of stream habitats, flow regimes, topography and climate conditions (Fig. 1). Collection localities were distributed over *c.* 2600 km of the Pacific coastline, from the Skeena River, BC, Canada, south to the Ventura River, CA, USA. Multiple collections were made in larger drainages, including the Skeena, Fraser, Columbia, Umpqua, Coquille, Rogue, Klamath, Sacramento–San Joaquin and San Francisco Bay. Substantial effort was focused on collecting specimens south of Point Conception, including 32 localities in 12 rivers as far south as the Rio Santo Domingo, Baja California, Mexico. Although these efforts were guided by records of historical collections and advice from local biologists, all collection attempts were unsuccessful. Five ammocoetes were collected by a fish biologist south of Point Conception from the Ventura River, but this sample was not large enough to quantify diversity in the region south of Point Conception. The collection from the Ventura River, California (34·30° N; 119·30° W, collected by Camm Swift, March 2005), is at the Natural History Museum of Los Angeles County (LACM 56277-1). Effort was also focused on sampling at the northern extent of their North American distribution. A collection of ammocoetes was obtained from north of the Aleutian Islands in King Salmon Creek, King Salmon, AK (58·693° N; 156·707° W, collected by USFWS, King Salmon), but all 30 specimens belonged to the lamprey genus *Lethenteron* (see Ammocoete Identification).

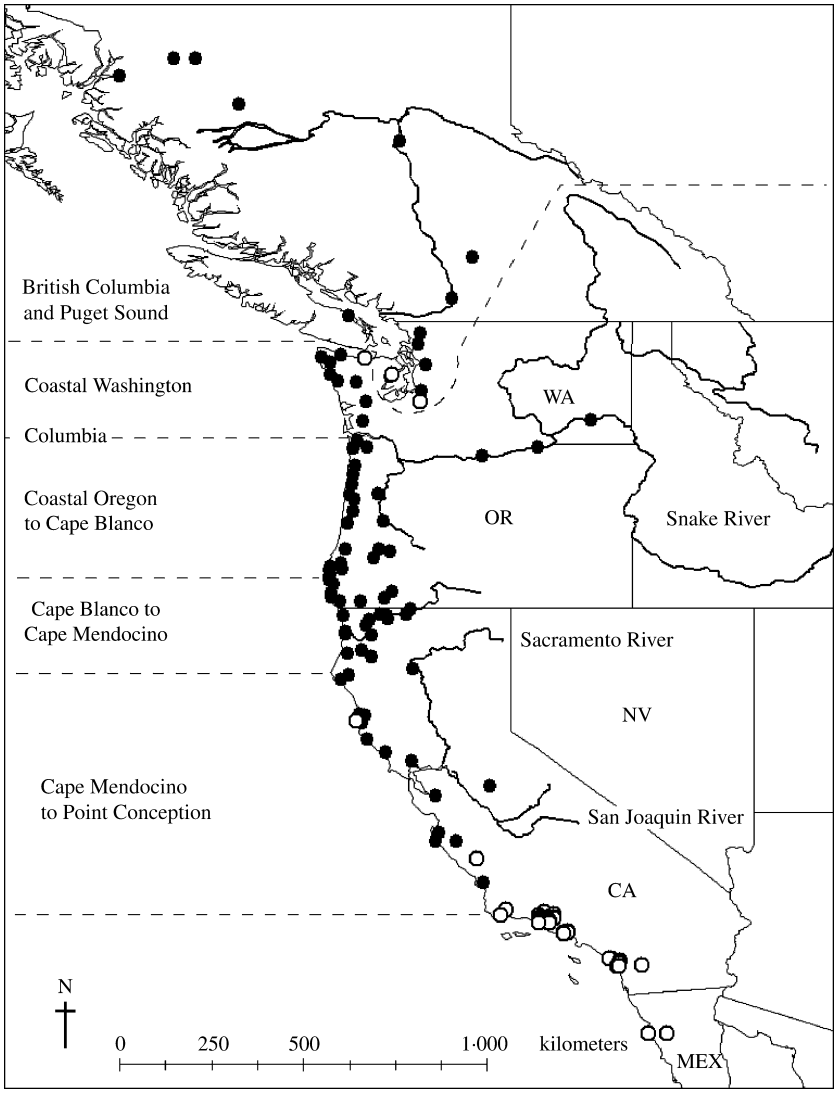

FIG. 1. Map showing locations of *Entosphenus tridentatus* sampling localities (●) and six biogeographical regions indicated by bold text and separated by dashed lines except for the Columbia Drainage, which was designated as a unique region. ○, sampling localities with no target lampreys collected. Not shown is the collection locality from the King Salmon Creek in Alaska with no target lampreys (see text).

Ammocoetes were the primary target of collections because of their year-round freshwater residence that facilitated the collection of large sample sizes. A range of size classes was collected to ensure that individuals were not siblings. Most samples were collected using a slow pulse electroshocker (University of Wisconsin Model ABP-2). Additional specimens were collected with downstream migrant traps. Specimens were preserved in 95–99% ethanol and most were deposited in the Humboldt State University Fish Collection (Table I).

TABLE I. Biogeographical regions (see text), drainage, river, sample size (*n*), latitude and longitude and Humboldt State University (HSU) voucher collection numbers of *Entosphenus tridentatus* samples used in this study. Collections without an HSU voucher number were collected by other individuals and agencies (see acknowledgement section) and are identified with asterisks

Region	Drainage	Population	*n*	Date	North latitude	West longitude	HSU numbers
British Columbia and Puget Sound	Skeena/Nass	Cranberry	4	November 1999	55·42	128·22	*
		Quilgauw	1	April 1999	55·00	129·55	*
		Buck	12	June 2000	54·30	126·63	*
		Kispiox	4	June 1999	55·42	127·70	*
	Fraser	Fraser	1	May 1999	53·40	122·70	*
		Thompson	31	May 1999	50·57	120·92	*
		Gordon	9	October 2000	49·55	121·43	*
	Vancouver Island	Chase	3	Not available	49·13	123·97	*
	Nooksack	South Fork Nooksack	10	13 July 2004	48·72	122·20	3932
	Skagit	East Fork Nookachamps	8	13 July 2004	48·45	122·25	3930
	Snohomish	Pilchuck	10	13 July 2004	47·94	122·08	3928
	Green River	Green	9	13 July 2004	47·30	122·17	3925
	Duckabush	Duckabush	10	14 July 2004	47·66	122·95	3934
	Pysht	Pysht	20	14 July 2004	48·19	124·14	3936
Coastal Washington	Lake Ozette	Big	14	14 July 2004	48·13	124·61	3952
	Quillayute	Soleduc	20	14 July 2004	47·98	124·40	3954
	Cedar	Cedar	5	14 July 2004	47·71	124·41	3956
	Queets	Salmon	20	15 July 2004	47·55	124·22	3957
	Lake Quinault	Quinault	5	15 July 2004	47·53	123·77	3960
	Chehalis	Satsop	19	16 July 2004	47·04	123·53	3961
	Willapa	Forks	20	16 July 2004	46·56	123·60	3963

TABLE I. Continued

Region	Drainage	Population	*n*	Date	North latitude	West longitude	HSU numbers
Columbia River	Snake	Lower Granite Dam	10	2000	46·66	117·43	*
		Goose Dam	11	June 2000	46·58	118·03	*
	Umatilla	Umatilla	53	January 2001	45·91	119·34	*
	Columbia	Bonneville Dam	13	August 2000	45·72	120·70	*
	Willamette	Luckiamute	20	24 May 2004	44·78	123·24	3855
		McKenzie	59	December 2000	44·12	123·10	*
	Youngs	North Fork Klaskanine	18	25 May 2004	46·09	123·74	3858
Oregon Coast to Cape Blanco	Nehalem	Fishawk	10	25 May 2004	45·93	123·50	3848
	Necalicum	Necalicum	12	25 May 2004	45·90	123·86	3861
	Tillamook	Wilson	20	25 May 2004	45·48	123·81	3865
	Nestuca	Nestuca	20	25 May 2004	45·25	123·85	3868
	Salmon	Salmon	10	26 May 2004	45·01	123·89	3871
	Siletz	Siletz	20	26 May 2004	44·76	123·91	3872
	Yaquina	Yaquina	19	26 May 2004	44·65	123·82	3874
	Alsea	Alsea	10	26 May 2004	44·35	123·84	3877
	Siuslaw	North Fork Siuslaw	5	26 May 2004	44·05	123·99	3881
	Umpqua	Calapooya	30	20 October 2003	43·41	123·21	3399
		Rock	30	20 October 2003	43·37	122·96	3402
		South Fork Umpqua	30	21 October 2003	43·21	123·35	3397
	Coos	East Fork Milacoma	9	27 May 2004	43·43	124·03	3885
	Coquille	Coquille	11	June 2000	43·08	124·14	*
		South Fork Coquille	10	20 May 2004	42·95	124·11	3853
	4-Mile	4-Mile	5	27 May 2004	43·00	124·40	3888
	Floras	Floras	10	27 May 2004	42·91	124·43	3891
	Sixes	Sixes	10	27 May 2004	42·82	124·41	3894
Cape Blanco to Cape Mendocino	Elk	Elk	4	October 1995	42·76	124·43	*
	Brush	Brush	20	27 May 2004	42·69	124·43	3895
	Euchre	Euchre	12	27 May 2004	42·58	124·33	3897

TABLE I. Continued

Region	Drainage	Population	*n*	Date	North latitude	West longitude	HSU numbers
	Rogue	Bear	10	20 October 2003	42·38	122·90	3401
		Applegate	30	21 October 2003	42·24	123·07	3398
		Illinois	30	20 October 2003	42·15	123·66	3400
	Hunter	Hunter	10	28 May 2004	42·36	124·38	3899
	Pistol	Pistol	20	28 May 2004	42·27	124·38	3900
	Chetco	Chetco	20	28 May 2004	42·14	124·18	3901
	Smith	Smith	20	11 July 2004	41·81	124·08	3922
	Klamath	Klamath	4	2004	41·92	122·44	3843, 3617, *
		Scott	6	2004	41·81	123·01	3847, 3851, 3619
		Shasta	9	Not available	41·73	123·01	*
		Salmon	10	9 May 2004	41·34	123·41	3902
		Clear	1	June 1998	47·71	123·45	*
		Dillon	3	June 1998	41·58	123·54	*
		Trinity	23	2004 & 2005	40·95	123·63	3920, *
	Redwood	Prairie	5	2004	41·39	124·02	3560, 3616, 3852
	Mad	Mad	20	8 July 2005	40·85	123·99	3919
	Eel	South Fork Eel	19	12 June 2004	40·34	123·94	3903, *
Cape Mendocino	Mattole	Mattole	20	12 June 2004	40·24	124·13	3904
to Point	Noyo	South Fork Noyo	20	21 June 2004	39·39	123·68	3908
Conception	Big	North Fork Big	20	13 June 2004	39·35	123·56	3907
	Navarro	Navarro	15	13 June 2004	39·17	123·64	3905
	Gualala	Gualala	20	21 June 2004	38·77	123·50	3909
	Russian	Austin	20	21 June 2004	38·47	123·05	3910
	San Francisco	Sonoma	10	22 June 2004	38·24	122·42	3911
	Bay and	Penitencia	10	22 June 2004	37·39	121·83	3913
	Sacramento–	Clear	30	23 November 2004	40·51	122·38	3938
	San Joaquin	Tuolumne	30	24 November 2004	37·65	120·49	3940
	Salinas	Arroyo Seco	20	25 June 2004	36·28	121·32	3915
	Carmel	Carmel	20	25 June 2004	36·48	121·75	3916
	Big Sur	Big Sur	20	25 June 2004	36·28	121·83	3917
	San Luis Obispo	San Luis Obispo	20	23 June 2004	35·28	120·66	3914

BIOGEOGRAPHICAL REGIONS

Samples were grouped into drainages and biogeographical regions for data analyses due to small sample sizes at several localities (Table I). Biogeographic regions were based on sample distribution and geographical landmarks. Several of the biogeographical regions are similar to the ecological regions used by Waples *et al.* (2001) in the characterization of diversity of anadromous salmon in the Pacific North-west. The northernmost region, British Columbia and Puget Sound, included all rivers draining into the Strait of Juan de Fuca and Puget Sound, as well as British Columbia coastal drainages. Coastal Washington and Coastal Oregon to Cape Blanco regions were separated by the Columbia River. The Columbia River itself was designated as a distinct region due to its large size and penetration east of the Cascade Mountains. Southern coastal regions were separated by coastal capes or points of biogeographic importance (*i.e.* Cape Blanco, Cape Mendocino and Point Conception) (Burton, 1998; Waples *et al.*, 2004).

AMMOCOETE IDENTIFICATION

Several lamprey species are distributed within the range of the current study and are distinguished from *E. tridentatus* through a combination of morphological and genetic characters. *Entosphenus tridentatus* ammocoetes were distinguished from sympatric *Lampetra* species primarily by morphology, as described in Richards *et al.* (1982). Individuals of *Lampetra*, misidentified by morphology, were subsequently identified by digestion patterns in the five *E. tridentatus* restriction digests (Docker *et al.*, 2007). Where other *Entosphenus* species were present, such as in the Klamath Basin, *E. tridentatus* ammocoetes could not be reliably distinguished from non-target species by morphology. *Entosphenus tridentatus* could be distinguished from other *Entosphenus* species in the Klamath Basin, however, by a restriction digest with *Hpa*I of a 432 base pair (bp) fragment of the cytochrome *b* (cyt *b*) gene (Docker *et al.*, 1999; Lorion *et al.*, 2000; unpubl. data). This digest was performed on individuals from the Klamath Basin and on a single individual from each composite haplotype, identified by the five restriction enzyme assays (Docker *et al.*, 2007). In Alaska, where *E. tridentatus* ammocoetes co-occur with *Lethenteron* species, the two genera were distinguished with *Hae*III digests of cyt *b* (Docker *et al.*, 1999). Cyt *b* polymerase chain reaction (PCR) primer sequences were 5′-CCA CCM ACC RWC YAT TMT TCG AAA AAC-3′ (unpubl. data) and 5′-CCC TCA GAA TGA TAT TTG TCC TCA-3′ (Palumbi, 1996). Thermocycle conditions were one cycle of 94° C for 2 min, 35 cycles of 94° C for 1 min, 57° C for 1 min and 72° C for 1·5 min and a final extension cycle of 72° C for 5 min. The resulting gene fragments were visualized on a 1·8% agarose gel after separation by electrophoresis. The digest fragment sizes were *Entosphenus* 92, 166 and 174 bps and *Lethenteron* 432 bp PCR and restriction fragment length polymorphism (RFLP) assay protocols are described below.

INTRASPECIFIC VARIABILITY IN PACIFIC LAMPREY

Genomic DNA was extracted from a *c.* 2 mm^3 piece of muscle tissue with the Wizard® Genomic DNA Kit (Promega, Madison, WI, U.S.A.) following manufacturer's instructions or with a solution of 0·01 g chelex (Sigma, St Louis, MO, U.S.A.), 0·2 ml H_20, 1·2 μl Proteinase K solution (Qiagen, Valencia, CA, U.S.A.), digested overnight and boiled for 8 min at 100° C (Miller & Kapuscinski, 1996).

Genetic variability among 1246 *E. tridentatus* specimens was assessed with five RFLP assays described in Docker *et al.* (2007). Two mtDNA fragments were amplified using PCR (1) ND2, a 1599 bp fragment comprising the 3′end of the ND1 gene (129 bp), the entire 1044 bp of the ND2 gene, six tRNA genes (42–71 bp) and small non-coding regions (2–31 bp) and (2) ND5, a 1086 bp fragment comprising the 3′end of the ND4 gene (134 bp), 744 bp at the 5′end of the ND5 gene and three tRNA genes (69–71 bp). The ND2 segment was digested with *Hin*fI, *Hae*III and *Dde*I, and the

ND5 segment with *Eco*RI and *Dde*I. Restriction fragment patterns were visualized on a 1·8% agarose gel and included 29 composite haplotypes. Nucleotide substitutions associated with gains or losses of restriction enzyme recognition sites were identified from a total of 34 sequences, providing nucleotide variation at 18 positions (GenBank accession numbers DQ508267–DQ508300).

DATA ANALYSIS

Population structure was investigated with an analysis of molecular variance (AMOVA) (Excoffier *et al.*, 1992) using ARLEQUIN 2.0 (Schneider *et al.*, 2000) and a hierarchical structure including biogeographic regions and drainages (Table I). AMOVA estimated variance components and Φ-statistics (F-statistic analogous) among regions (Φ_{CT}), among drainages within regions (Φ_{SC}) and within drainages (Φ_{ST}). Significance of the variance components and Φ-statistics were tested through a non-parametric permutation of the data at $\alpha = 0{\cdot}05$. Many alternative hierarchical structures were evaluated, all of which resulted in similar partitioning of variance components; thus, only the above hierarchical structure will be discussed. Haplotypic richness and regional haplotypic richness were calculated using rarefaction implemented in HP-RARE (Kalinowski, 2005).

RESULTS

Twelve hundred and forty six *E. tridentatus* were assayed with the five restriction enzymes, producing 29 composite haplotypes (Tables II and III), of which three were common to every region and most samples. Haplotype 1 occurred in 62%, haplotype 11 in 18% and haplotype 18 in 11% of the total sample. Eleven of the haplotypes were 'rare', occurring at low frequencies (7% of total sample) in multiple drainages. Fifteen haplotypes were 'private', found in only a single drainage (1% of total sample).

Haplotypic richness and region-specific haplotypic richness *v.* biogeographic region revealed a trend of reduced richness in northern regions (Fig. 2). No drainage-specific haplotypes were found in the two most northern regions, and only one was found in the Columbia region (near the mouth of the Columbia), whereas 3, 5 and 6 private haplotypes were found in the three more southern regions. AMOVA indicated that 99% of haplotype variation in *E. tridentatus* was explained by variation within drainages and a small amount of genetic variation (<1·5%) was explained by variation among regions or among drainages within regions (Table IV).

DISCUSSION

EVIDENCE FOR HIGH LEVELS OF GENE FLOW IN PACIFIC LAMPREY

Entosphenus tridentatus exhibited low levels of mtDNA differentiation among widely separated collections, providing little evidence of among-population genetic differentiation. This was supported by similar abundances and distributions of three common haplotypes in surveyed regions and by an AMOVA indicating that 99% of the total sample genetic variance was due to genetic variation within samples. The low levels of population structure in *E. tridentatus* are consistent with sufficient gene flow among populations to genetically

TABLE II. Haplotype frequencies in samples of *Entosphenus tridentatus* in the six biogeographical regions from British Columbia to Point Conception, CA. Asterisks indicate private haplotypes found in a single sample

	Haplotypes																												
Region	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Total
British Columbia and Puget Sound	80						1				23							14				14								132
Coastal Washington	72										17				1			10					2		1					103
Columbia	115										33				2	3		23				1	5		1		1*			184
Coastal Oregon to Cape Blanco	179		1			1					53	1*			1			28				1	2		2	1*		1*		271
Cape Blanco to Cape Mendocino	177	5	1		3*					4	46			1*		1	1*	25	1*	1		1	7		1				1*	276
Cape Mendocino to Pt. Conception	153	6		1*		4	2	1*	1*	1	54		1*		2	1		35		2	1*	1	7	1*	1					275
Total	776	11	2	1*	3*	5	3	1*	1*	5	226	1*	1*	1*	6	5	1*	135	1*	3	1*	18	23	1*	6	1*	1*	1*	1*	1241

TABLE III. Sequence map of *Entosphenus tridentatus* haplotypes identified with five restriction digests, including gene region assayed, restriction enzyme and nucleotide position within the ND2 or ND5 gene. NCA, non-coding region between tRNA-Trp and tRNA-Ala. NCB, non-coding region between the ND1 and ND2 regions

Region	ND2							ND2				ND2			ND5	ND5			
Enzyme	*Hin*fI							*Hae*III				*Dde*I			*Eco*RI	*Dde*I			
Location	96	348	612	780	936	973	NCA	231	262	540	NCB	177	862		306	277	280	426	678
Haplotype																			
1	C	T	T	C	A	C	T	G	G	G	G	G	C		C	T	G	T	G
2	C	T	T	C	A	C	T	G	G	G	G	G	C		T	T	G	T	G
3	C	T	T	C	A	C	T	G	G	G	G	G	C		C	T	A	T	G
4	C	T	T	C	A	C	T	G	G	G	G	G	C		C	T	G	C	G
5	C	T	T	C	A	C	T	G	G	G	G	G	C		C	T	G	T	A
6	C	T	T	C	A	C	T	G	G	G	G	G	T		T	T	G	T	A
7	C	T	T	C	A	C	T	G	G	G	G	G	T		T	C	G	T	G
8	C	T	T	C	A	C	T	G	G	G	G	G	T		T	T	A	T	A
9	C	T	T	C	A	C	T	G	G	G	G	G	T		T	T	G	C	G
10	C	T	T	C	A	C	T	G	G	G	G	G	T		C	T	G	T	G
11	C	T	T	C	A	C	T	G	G	G	G	G	T		T	T	G	T	G
12	C	T	T	C	A	C	T	G	G	G	G	A	C		C	T	G	T	G
13	C	T	T	C	A	C	T	G	G	G	T	G	C		C	T	G	T	G
14	C	T	T	C	A	C	T	A	G	G	G	G	C		C	T	G	T	G
15	C	T	T	C	A	C	T	A	G	G	G	G	T		T	T	G	T	G
16	C	T	T	C	A	C	T	G	G	A	G	G	C		C	T	G	T	G
17	C	T	T	C	A	C	T	G	A	G	G	G	C		C	T	G	T	G
18	C	C	T	C	A	C	T	G	G	G	G	G	T		T	T	G	T	G
19	C	C	T	C	A	C	T	G	G	G	G	G	C		T	T	G	T	G
20	C	C	T	C	A	C	T	G	G	G	G	G	T		C	T	G	T	G
21	C	C	T	C	A	C	T	G	G	G	G	G	T		T	T	G	T	A
22	C	C	T	C	A	C	T	A	G	G	G	G	T		T	T	G	T	G
23	C	T	C	C	A	C	T	G	G	G	G	G	T		T	T	G	T	G
24	C	T	C	C	A	C	T	G	G	G	G	G	T		C	T	G	T	G
25	C	C	T	C	G	C	T	G	G	G	G	G	T		T	T	G	T	G
26	C	T	T	C	A	A	T	G	G	G	G	G	C		C	T	G	T	G
27	T	T	T	C	A	C	T	G	G	G	G	G	C		C	T	G	T	G
28	C	T	T	T	A	C	T	G	G	G	G	G	C		C	T	G	T	G
29	C	T	T	C	A	C	C	G	G	G	G	G	C		C	T	G	T	G

homogenize geographically separated populations. The precise behavioural mechanisms facilitating high levels of gene flow are unknown. Information on the oceanic life-history characteristics and potential homing behaviour of lampreys may provide insight into the mechanisms involved. Little is known about the life cycle of *E. tridentatus*, particularly while in the oceanic environment. This gap includes details such as migratory cues, as well as distribution and time spent in the oceanic environment. For example, oceanic dispersal may be passive with currents and would serve to facilitate mixing among populations, particularly in the absence of strong homing behaviour.

Similar to *E. tridentatus*, anadromous populations of the widely distributed sea lamprey exhibit panmictic genetic structure on both sides of the Atlantic

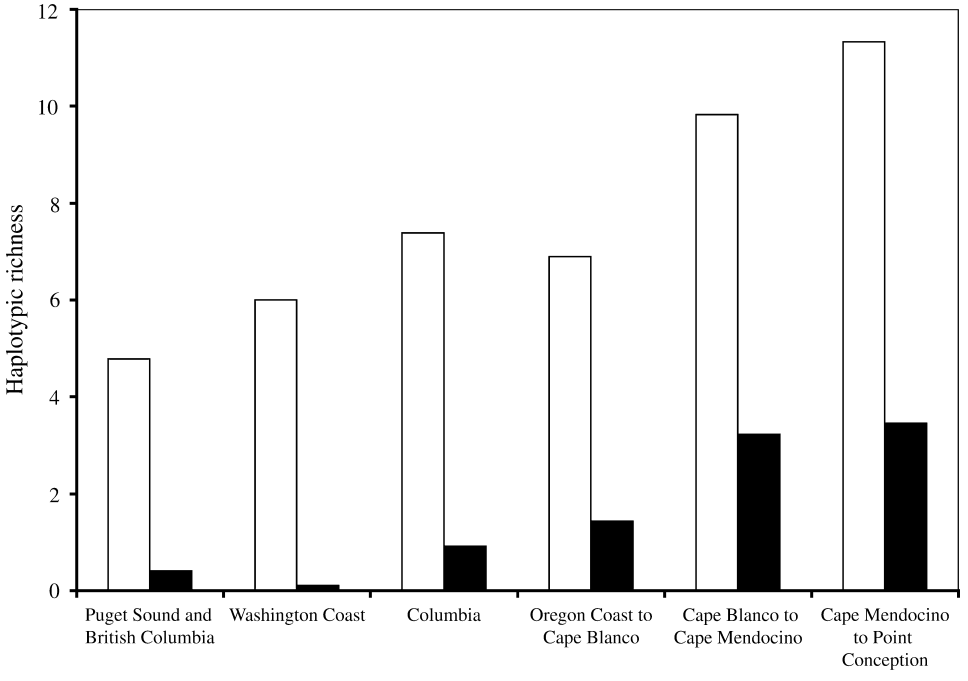

FIG. 2. Haplotypic richness of *Entosphenus tridentatus* haplotypes (□) and region-specific haplotypic richness (■) in six biogeographical regions. Biogeographical regions arranged from north to south, and sample sizes standardized using rarefaction.

Ocean (Bryan *et al.*, 2005; Almada *et al.*, 2007). North American and Spanish populations are fixed for alternative mtDNA control region haplotypes (Rodriguez-Muñoz *et al.*, 2004), but microsatellite markers show no genetic differentiation among North American populations of anadromous sea lampreys (Bryan *et al.*, 2005). However, mtDNA control region sequences failed to reveal genetic structure among European sea lamprey populations (Almada *et al.*, 2007). This apparent lack of differentiation does not appear to be due to insufficient resolution of the markers used since the microsatellite markers identified structure among recently established non-anadromous Great Lakes populations with limited connectivity (Bryan *et al.*, 2005) and since mtDNA control region sequence has also shown differentiation among some Great Lakes populations (Waldman *et al.*, 2004). Low levels of population structure in anadromous populations of the sea lamprey have been attributed to high gene flow between populations as a consequence of low fidelity to natal spawning streams (Rodriguez-Muñoz *et al.*, 2004). Spawning-site fidelity was investigated in non-anadromous sea lamprey by mark-recapture studies in the Great Lakes. Bergstedt & Seelye (1995) tagged 555 juvenile lampreys in the Devil River, Lake Huron. Despite the examination of 47 946 adult sea lampreys throughout Lake Huron, none of the originally tagged lampreys was recovered in the Devil River itself; however, 41 tags were recovered in other streams distributed around the lake, suggesting a lack of natal stream fidelity. Adult migratory behaviour in *P. marinus* is apparently associated with the presence of bile acids

TABLE IV. AMOVA of *Entosphenus tridentatus* haplotype variation among biogeographical regions and drainages. Significance was tested through permutation of the appropriate hierarchical level

Variance component	d.f.	Sum of squares	Variance	% total	*P*	Φ-statistics
Among regions	5	7·215	0·00296	0·46	0·05112	$\Phi_{CT} = 0{\cdot}00462$
Among drainages within regions	53	39·828	0·00621	0·97	0·10642	$\Phi_{SC} = 0{\cdot}00975$
Within drainages	1182	745·502	0·63071	98·57	0·03743	$\Phi_{ST} = 0{\cdot}01433$

produced by ammocoetes (Bjerselius *et al.*, 2000). These apparent attractants, petromyzonol sulfate and allocholic acid, act synergistically with natural stream waters and do not appear to be species-specific (Vrieze & Sorensen, 2001; Sorensen *et al.*, 2003).

The low level of among-population genetic differentiation in Pacific lamprey is similar to that in anadromous North American populations of sea lamprey. Low philopatry of spawners resulting in population heterogeneity has also been reported in an anadromous lamprey from the southern hemisphere, *Geotria australis* Gray (Johnston *et al.*, 1987). There is similarly no evidence of genetic population structure in the anadromous Arctic lamprey, *Lethenteron camtschaticum* (Tilesius) (Docker, 2006). It is possible that migratory adaptations, such as an attraction to ammocoete pheromones, is a trait shared among lamprey species, aiding in the orientation of migrating individuals when dispersed to unfamiliar territory during the feeding stage. The larval pheromone petromyzonol sulfate has been identified in Pacific lamprey, but the importance of these cues for migratory behaviour in Pacific lamprey requires additional investigation (Yun *et al.*, 2003).

Selection of spawning streams in relation to the presence of ammocoetes contrasts markedly with salmonids that home to the unique chemical signature of natal streams (Hasler, 1983; Hasler & Scholz, 1983). The different cues used to identify spawning grounds thus appear to be the factor producing the different population genetic structures observed in Pacific lamprey and anadromous salmonids. The absence of shared haplotypes between North American and Spanish populations of sea lamprey, however, suggests a lack of exchange between the west and south-east Atlantic coasts (Rodriguez-Muñoz *et al.*, 2004). Likewise, North American and Asian Pacific lamprey populations may be genetically distinct, but adult Pacific lamprey migratory distances are unknown (Beamish, 1980).

OTHER PHYLOGEOGRAPHIC PATTERNS

Haplotypic richness, region-specific haplotypic richness and private haplotypes were unevenly distributed throughout the geographical range of the study. No private haplotypes were present north of the Columbia River, and the occurrence of private and rare haplotypes gradually increased in more southern regions. This pattern could be the evidence of historical stability in

southern drainages and recolonizations of northern areas following retreat of glaciers (McPhail & Lindsey, 1986). Heterogeneous distributions of haplotypic richness, region-specific haplotypic richness and private haplotypes have been suggested as evidence of reduced gene flow, particularly when multiple copies are found in a single drainage, suggesting a degree of site fidelity (Slatkin, 1985; Avise, 2000). The significance of this pattern in Pacific lamprey, particularly in relation to the degree of gene flow, is difficult to interpret because of extremely low frequencies in the sample set (17 individuals or 1·4% of the total sample).

Another interesting phylogeographic pattern is the high frequency of a rare haplotype in the Fraser River Drainage, B.C. Haplotype 22 is the second most abundant haplotype in the Fraser drainage, making up *c.* 30% of the sample, while it appears only in scattered individuals from the Columbia River, WA to Point Conception, CA. Haplotype 22 is the only rare haplotype encountered, which shows high abundance in a single drainage. Interestingly, in other anadromous and non-anadromous species in several diverse taxa, the Fraser River fauna is dominated by fishes from the Columbia River, both in terms of species composition (McPhail & Lindsey, 1986), as well as specific genetic and morphometric variants within species (McPhail & Lindsey, 1970, 1986; Smith, 1978; Haas, 1998; Haas & McPhail, 2001; Smith *et al.*, 2001). In the current study, however, only one of 184 Pacific lamprey (0·5%) from the Columbia River drainage carried haplotype 22, which is inconsistent with the purported shared history between these rivers. The factors influencing the unique haplotype pattern in the Fraser River Pacific lamprey are unclear from the current data set and require additional investigation.

In conclusion, this first genetic survey of Pacific lamprey, a notable lack of genetic population structure was identified, which is most likely to be the product of a life-history strategy facilitating gene flow among populations. This pattern is consistent with a population structure identified in the anadromous form of another widely distributed lamprey in the Atlantic and is in contrast to patterns in anadromous salmonids, which are apparently driven by high fidelity to natal streams. Spawning-site fidelity of Pacific lampreys could be investigated through a mark-recapture study. However, identifying the behaviours or mechanisms facilitating gene flow is hampered by the lack of information on oceanic life-history and migratory patterns in *E. tridentatus*. Several additional phylogeographic patterns are apparent in the data but are difficult to interpret due to low haplotype frequencies. These tantalizing indications of underlying phylogeographic complexity require further exploration.

We thank the USFWS King Salmon Field Office, B. Chesney, T. Steelman (California Department of Fish and Game), D. Therry (USFWS), A. Brumo and D. Markle (Oregon State University), C. Garza (National Marine Fisheries Service), C. Swift (Entrix Environmental Consultants) and S. Putnam (Idaho Department of Fish and Game) for providing lamprey samples. Additional support was provided by P. Pister and the Desert Fishes Council, S. Gunckel and S. Jacobs (Oregon Department of Fish and Wildlife), D. Heath and R. Provost (University of Windsor), C. Chamberlain, D. Campton, J. B. Baumsteiger and N. Hetrick (USFWS). Information on collection localities were provided by R. Dagit, S. B. Engblom (Cachuma Operation and Maintenance Board), D. Salsberry and M. Moore (Santa Clara Valley Water District), S. D. Chase

(Sonoma County Water Agency), R. Topinka (University of California, Davis), L. Lentsch (Casitas Water Board) and M. Capelli (Friends of the Ventura River). Collection permits were generously provided by California Department of Fish and Game, Oregon Department of Fish and Wildlife and Washington Department of Fish and Wildlife. We thank the United States Fish and Wildlife Service (D.H.G., S.B.R., M.F.D.) and Habitat Conservation Trust Fund of British Columbia (M.F.D., G.R.H.) for funding and other support that made this project possible.

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