Phylogeny of the lamprey genus *Lampetra* inferred from mitochondrial cytochrome *b* and ND3 gene sequences

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Abstract: Mitochrondrial DNA analysis resolved many previously unanswered questions concerning the phylogeny of the lamprey genus *Lampetra* (comprising the subgenera *Entosphenus*, *Lethenteron*, and *Lampetra*). A total of 735 base pairs were sequenced from the cytochrome *b* and NADH dehydrogenase subunit 3 (ND3) genes in 11 lamprey species. With the exception of *L. (E.) hubbsi* from California, species of the *Entosphenus* subgenus formed a tight-knit clade that was very distinct from the other two subgenera. *Lampetra hubbsi* clustered with species of the *Lampetra* subgenus from the west coast of North America (*L. ayresii* and *L. richardsoni*) whereas species of the *Lampetra* subgenus from Atlantic drainages, namely the North American *L. aepyptera* and European *L. fluviatilis*, formed a third cluster. A fourth cluster included two species from the *Lethenteron* subgenus (*L. japonica* and *L. appendix*). Inclusion of published data from a third *Lethenteron* species, *L. zanandreai*, showed it to group with the *L. (L.) fluviatilis* lineage rather than with the other two *Lethenteron* species. Within each subgenus, members of paired and satellite species were closely related to one another (e.g., *L. japonica* and *L. appendix*) or were genetically indistinguishable (e.g., *L. ayresii* and *L. richardsoni*). Using rates of molecular evolution estimated in other fish taxa, these genetically indistinguishable species diverged less than 70 000 years ago.

Résumé : L'analyse de l'ADN mitochrondial a permis de répondre à bon nombre de questions relativement à la phylogénie du genre de lamproie *Lampetra* (englobant les sous-genres *Entosphenus*, *Lethenteron* et *Lampetra*). On a déterminé la séquence d'un total de 735 paires de bases des gènes pour la cytochrome *b* et la sous-unité 3 de la NADH déshydrogénase (ND3) chez 11 espèces de lamproie. À l'exception de *L. (E.) hubbsi* de la Californie, les espèces du sous-genre *Enthosphenus* formaient un clade cohérent très distinct de ceux des deux autres sous-genres. *Lampetra hubbsi* se rapprochait des espèces du sous-genre *Lampetra* de la côte ouest de l'Amérique du Nord (*L. ayresii* et *L. richardsoni*) tandis que les espèces du sous-genre *Lampetra* des bassins de l'Atlantique, à savoir *L. aepyptera* de l'Amérique du Nord et *L. fluviatilis* de l'Europe formaient un troisième groupe. Un quatrième groupe englobait deux espèces du sous-genre *Lethenteron* (*L. japonica* et *L. appendix*). L'ajout de données publiées pour une troisième espèce de *Lethenteron*, *L. zanandreai*, indiquait une parenté avec *L. (L.) fluviatilis* plutôt qu'avec les deux autres espèces de *Lethenteron*. Au sein de chaque sous-genre, les membres d'espèces appariées et satellites étaient fortement apparentés les uns aux autres (p. ex. *L. japonica* et *L. appendix*) ou étaient génétiquement indiscernables (p. ex. *L. ayresii* et *L. richardsoni*). L'utilisation des taux d'évolution moléculaire estimés pour d'autres taxons de poisson montre que ces espèces génétiquement indiscernables ont divergé il y a moins de 70 000 ans.

[Traduit par la Rédaction]

Introduction

Lampreys are extant representatives of the ancient vertebrate group Agnatha and are one of the oldest groups of living fishes in the fossil record, about 300 million years old (Janvier and Lund 1983). There are about 40 extant species of lampreys; four species described from the Southern Hemisphere have been placed in the families Geotriidae and Mordaciidae, while the remaining species inhabit the Northern Hemisphere. The Northern Hemisphere lampreys belong to a single family, Petromyzonidae, and have been assigned to at least six genera (Hubbs and Potter 1971; Potter 1980).

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Lamprey taxonomy is problematic because, compared with bony vertebrates, there are relatively few taxon-distinctive morphological characters. Species identification and generic status have been largely based on adult dentition, as well as on differences in body proportions, number of myomeres, number and morphology of velar tentacles, and pigmentation (Hubbs and Potter 1971; Richards et al. 1982).

Adult body size, life history type, and habitat are also commonly used to distinguish between closely related taxa. All lampreys spend the first years of life as blind larvae that burrow in the silt bottoms of rivers and streams and feed on microscopic organisms. Following metamorphosis, they may remain nontrophic (nonparasitic) in their natal stream and spawn and die within 6–9 months, or they may migrate to freshwater lakes or to seawater and become trophic (parasitic). In most lamprey genera, groups of two or more species exist in which the larvae are morphologically similar but the species adopt different life history types as adults. It is generally believed that these so-called "paired species" (Zanandrea 1959) or multiple "satellite species" (Vladykov and Kott 1979*a*) are very closely related, with the nonparasitic species having evolved from a parasitic ancestor (Zanandrea 1959). Not all authorities, however, recognize life history type as a reliable indication of species differentiation (e.g., McPhail and Lindsey 1970) and consider paired or satellite species to be races or types of a single species. An objective of the current study was to determine the degree of molecular divergence between paired or satellite species.

A second objective was to use molecular data to test the evolutionary relationships among some higher lamprey taxa. A specific goal was to resolve the relationships among the 15 or more species currently assigned to the genus *Lampetra* (Potter 1980), which has controversial morphological characters (Table 1). Potter (1980) named *Entosphenus*, *Lethenteron*, and *Lampetra* as subgenera within the genus *Lampetra*, whereas other authors (e.g., Vladykov and Kott 1979*a*) classified each as distinct genera and further recognized a division between the taxa by placing *Entosphenus* into a separate subfamily from *Lampetra* and *Lethenteron*. Taxonomic questions centre on the degenerate or intermediate characteristics of several so-called relict species, nonparasitic lampreys that occur at or near the extreme southern limits of distribution of the Northern Hemisphere lampreys (Hubbs and Potter 1971). One such relict species is *Lampetra* (*Entosphenus*) *hubbsi* from California (Vladykov and Kott 1976*a*), which appears to demonstrate an intergradation of characters among the three subgenera (Table 1). Similarly, two other nonparasitic species, *Lampetra* (*Lampetra*) *aepyptera* and *Lampetra* (*Lethenteron*) *zanandreai*, possess ambiguous morphological characters (Table 1). The current study examined the evolutionary relationships among the three subgenera of *Lampetra* and evaluated the relationships of the relict species *L. hubbsi* and *L. aepyptera*.

Given the inclusiveness and overlap of lamprey morphological characters, an independent molecular data set from mitochondrial DNA sequences is used to resolve these taxonomic issues. In this study, we sequenced the entire NADH dehydrogenase subunit 3 (ND3) gene and about one third of the cytochrome *b* gene from 11 species of lampreys of the genus *Lampetra* and compared the data with published cytochrome *b* sequences from two additional *Lampetra* species (Tagliavini et al. 1994). Cytochrome *b* sequence has been used to resolve phylogenetic relationships in a variety of fish taxa (e.g., Cantatore et al. 1994), and the ND3 gene, which appears to evolve somewhat faster than cytochrome *b* (Thomas and Beckenbach 1989; McVeigh and Davidson 1991), may be useful in distinguishing among some of the more closely related paired and satellite species.

Materials and methods

Eleven lamprey species of the genus *Lampetra* were collected in Canada (British Columbia and Ontario), the United States (Oregon, California, and Tennessee), Germany, and Russia (Table 2). Species identification was confirmed morphologically in adults; ammocoetes were used only where a single species was present or where adequate larval characteristics exist to distinguish between species (Richards et al. 1982). DNA analysis was performed on two to four individuals per species or collection site. A single landlocked sea lamprey (*Petromyzon marinus*) from Ontario was collected for use as an outgroup, and cytochrome *b* and ND3 sequences were compared with those from an anadromous sea lamprey from New Hampshire (Lee and Kocher 1995). All samples were either frozen at –70°C or preserved in 95% ethanol.

In *L. japonica*, DNA was extracted from liver tissue by digesting overnight at 37°C with proteinase K in proteinase K buffer (10 mM Tris (pH 8.0), 10 mM EDTA, 1% SDS, 200 µg proteinase K/mL), cleaning with phenol–chloroform (1:1), and precipitating with NaOAc and isopropanol. In the other specimens, DNA was extracted from fin or tail clippings, which were digested for 1 h at 55°C with proteinase K and used as crude homogenates.

Polymerase chain reaction (PCR) amplification of 414 base pairs (bp) at the 5′ end of the cytochrome *b* gene used primers located in the tRNA-Glu and cytochrome *b* genes: GLUDG-L (5′-TGACTTGAARAACCAYCGTTG-3′) and CB2-H (5′-CCCTCAGAATGATATTTGTCCTCA-3′), respectively (Palumbi 1996). Since lampreys possess a large noncoding region between these two genes (Lee and Kocher 1995), the PCR product exceeded 1000 bp. Two internal primers (5′-CCATCCAACATCTCAGCWTGATGAAA-3′ (L) from Palumbi (1996) and 5′-CAAGGCTAGTTCAGTRTTAGC-3′ (H) designed from conserved regions of lamprey sequence) were used to amplify subsections of this larger fragment. The entire 351 bp of the lamprey ND3 gene were amplified with primers designed from conserved regions in the tRNA-Arg gene of other vertebrates (e.g., Thomas and Beckenbach 1989) and the tRNA-Gly gene of sea lamprey (Lee and Kocher 1995): 5′-ATGCGGATCCTTTTGAGCCGAAATCA-3′ and 5′-ACGTGAATTCTATAGTTGGGTTCCAACCA-3′, respectively.

Each 50-µL reaction contained standard PCR buffer (10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin), 0.2 mM dNTP, 50 pmol of each primer, and 1.25 units of *Taq* DNA polymerase (Gibco BRL, Burlington, Ont.). The reactions were run in a Perkin Elmer thermocycler (Perkin Elmer Cetus, Norwalk, Conn.) for 30 cycles consisting of denaturation at 94°C for 1 min, primer annealing at 60°C for 1 min, and extension at 72°C for 2 min; the 30 cycles were preceded by an initial denaturation for 3 min at 94°C and followed by a final 10-min 72°C extension. Negative controls, which used distilled water instead of template DNA, were included in each series of reactions to screen for possible contamination.

The PCR products were sequenced using the Cyclist DNA Sequencing Kit (Stratagene, La Jolla, Calif.) or the Sequenase PCR Product Sequencing Kit (United States Biochemical, Cleveland, Ohio); the manufacturer's instructions were followed. Given the heteroplasmic nature of the noncoding insert located at the 5′ end of cytochrome *b* (M.F. Docker, J.H. Youson, R.J. Beamish, J.M.P. Joss, T.D. Kocher, and R.H. Devlin, unpublished data), all cytochrome *b* sequencing was accomplished from the 3′ end of the

Table 1. Summary of the taxonomy of the lamprey genus *Lampetra*.

Species	Comments regarding classification	Relevant morphological characteristics[a]	Life history type and distribution
Subgenus *Lampetra*	Considered by Vladykov and co-workers as distinct genus and included in subfamily Lampetrinae with genera *Lethenteron* and *Eudontomyzon*[1]	Supraoral (SO) lamina 2 cusps; 3 inner laterals[b] with 2–3–2 cusps; infraoral (IO) lamina 7–8 cusps; posterial circumorals (posterials) absent[2]	
ayresii (river lamprey)	Initially considered synonymous with *L. fluviatilis*; described as distinct species (Vladykov and Follett 1958)	Velar tentacles 4; larger eye, longer prebrancial, and shorter branchial region relative to *L. fluviatilis*	Parasitic, anadromous; Pacific coast of North America from Skeena River, B.C., to San Francisco Bay, Calif.
richardsoni (western brook lamprey)	Derivative of *L. ayresii* (Vladykov and Follett 1965)		Nonparasitic, freshwater; Pacific drainages from B.C. to Oreg.
pacifica (Pacific brook lamprey)	Described as second nonparasitic derivative of *L. ayresii* (Vladykov 1973); now considered by American Fisheries Society to be synonymous with *L. richardsoni*[3]	Fewer myomeres and less well-developed teeth than typical *L. richardsoni*	Nonparasitic, freshwater; Columbia River basin, Oreg.; San Joachin – Sacramento River system, Calif.
fluviatilis (European river lamprey)			Parasitic, anadromous: western European watersheds of the Atlantic, Baltic, and Mediterranean; some landlocked[4]
planeri (European brook lamprey)	Derivative of *L. fluviatilis*		Nonparasitic, freshwater; similar distribution to *fluviatilis* but further inland in central and northern Europe[5]
aepyptera (least brook lamprey)	Occurs far from any parasitic *Lampetra*; perhaps derived from *L. ayresii*,[1] from *Eudontomyzon–Tetrapleurodon* type,[6] or *L. japonica*[4,7]; may bridge gap between *Lampetra* and *Lethenteron*[7]	Dentition extremely degenerate and variable; posterials absent; reduced number of myomeres[8]	Nonparasitic, freshwater; southeastern U.S. in Ohio River basin, Gulf and Atlantic plains
Subgenus *Entosphenus*	Generic status in subfamily Entosphenidae with genus *Tetrapleurodon*[1]	SO lamina 3 cusps; 4 inner laterals with 2–3–3–2 cusps; IO lamina with 5 cusps; posterials present[2]	
tridentata (Pacific lamprey)		Posterials 18 (2 bicuspid); velar tentacles 13	Parasitic, anadromous; spawns in rivers of Pacific basin from Alaska to southern Calif.
macrostoma (lake lamprey)	Derivative of *L. tridentata* (Beamish 1982); recognized as distinct species by American Fisheries Society[3]	Posterials 18 (3 bicuspid); velar tentacles 13	Parasitic, freshwater; reported only in 2 lakes on Vancouver Island, B.C.
similis (Klamath River lamprey)	Derivative of *L. tridentata* (Vladykov and Kott 1979*b*); once regarded as subspecies of *tridentata*,[9] now recognized as distinct[3]	Posterials 18 (6 bicuspid); velar tentacles 8	Parasitic, freshwater; Klamath River drainage, Calif. and Oreg.
lethophaga (Pit–Klamath brook lamprey)	Derivative of *L. tridentata* (Hubbs 1971)	Dentition more variable and degenerate than other *Entosphenus*; velar tentacles 8; adult size about 140 mm[10]	Nonparasitic, freshwater; Klamath and Pit River systems in Oreg. and Calif.
folletti	Described as second nonparasitic derivative of *L. tridentata* (Vladykov and Kott 1976*c*); considered by American Fisheries Society as synonymous with *lethophaga*[9]	Dentition more robust than typical *lethophaga*; adult size about 200 mm	Nonparasitic, freshwater; restricted to Klamath River drainage of northern Calif.

Table 1 (*concluded*).

Species	Comments regarding classification	Relevant morphological characteristics[a]	Life history type and distribution
hubbsi (Kern brook lamprey)	Presumed derivative of *L. tridentata* (Vladykov and Kott 1976*a*), although prior to canal, Kern River basin lacked connection with other *Entosphenus*; *L.* (*L.*) *pacifica* (*L. richardsoni*) only other species in canal	SO lamina 2 cusps; 4 inner laterals unicuspid; IO lamina 5 cusps; posterials about 10 (unicuspid); velar tentacles 3; reduced number of myomeres	Nonparasitic, freshwater; limited distribution in Calif. in San Joachin basin[1]; probably originated in Kern River, which now connects with San Joachin River through artificial Millerton Lake
Subgenus *Lethenteron*	Considered distinct genus in subfamily Lampetrinae with *Lampetra* and *Eudontomyzon*[1]	SO lamina 2 cusps; 3 inner laterals with 2–2–2 cusp pattern; IO lamina with >5 cusps; unicuspid posterials[6]	
japonica (Arctic lamprey)			Parasitic, anadromous; from Varangar Fjord in Europe to Pacific coast of Japan; in North America, in Alaska and northern Canada
appendix (American brook lamprey)	Synonymous with *L.* (*Le.*) *lamottenii*; designated *L. appendix* by American Fisheries Society[9]; current distribution differs from *L. japonica*, but almost certainly one of its many derivatives; considered indistinguishable from *L.* (*Le.*) *alaskense*[9]		Nonparasitic, freshwater; tributarites of Great Lakes, St. Lawrence and Mississippi River systems, and rivers tributary to the Atlantic from N.H. to N.C.
zanandreai (Po brook lamprey)	Originally *Lampetra* (Vladykov 1955), but posterials and inner lateral cusp formula suggest *Lethenteron*[4]; ancient southern relict of *L.* (*L.*) *fluviatilis*[1] or *L.* (*Le.*) *japonica*[6]	Inner laterals 2–2–2 cusp pattern; single row of minute posterials, which may sometimes be lacking or deeply embedded[11]; velar tentacles 3–5; reduced number of myomeres	Nonparasitic, freshwater; restricted to Po River drainage in northern Italy

Note: References other than original species descriptions: 1, Vladykov and Kott (1979*a*); 2, Vladykov and Kott (1976*a*); 3, Robins et al. (1991); 4, Potter (1980); 5, Hardisty (1986); 6, Hubbs and Potter (1971); 7, Bailey (1980); 8, Vladykov and Kott (1976*b*); 9, Robins et al. (1980); 10, Vladykov and Kott (1976*c*); 11, Bianco (1986).

[a]Mean or mode given.

[b]Inner laterals equivalent to lateral circumorals.

Table 2. Collection data for the lamprey species analyzed in this study.

Species	Collection site	*N*	Stage
Genus *Lampetra*			
Subgenus *Lampetra*			
ayresii	Strait of Georgia, Fraser River discharge, B.C.	2 (fr)	Juvenile
richardsoni	Cowichan River, Vancouver Island, B.C.	2 (fr)	Ammocoete
	Chemainus River, Vancouver Island, B.C.	2 (fr)	Ammocoete
fluviatilis	Elba River, Germany	2 (EtOH)	Adult
aepyptera	Cane Creek, Tenn.	2 (EtOH)	Ammocoete
Subgenus *Entosphenus*			
tridentata	Cowichan River, Vancouver Island, B.C.	2 (fr)	Ammocoete
	Chemainus River, Vancouver Island, B.C.	2 (fr)	Ammocoete
	Elk River, coastal Oreg.	4 (EtOH)	Ammocoete
macrostoma	Cowichan Lake, Vancouver Island, B.C.	2 (fr)	Juvenile
lethophaga	Pit River, Calif.	2 (fr)	Adult
	Hat Creek, Calif.	2 (fr)	Ammocoete
similis 1–3	Agency Lake, Klamath Basin, Oreg.	3 (fr)	Adult
similis 4–7	Sprague River, Klamath Basin, Oreg.	4 (fr)	Adult
similis	Merced River, Calif.	2 (fr)	Ammocoete
hubbsi	Merced River, Calif.	2 (fr)	Adult
	Kings River, Calif.	2 (fr)	Adult
Subgenus *Lethenteron*			
japonica	Kamchatka Channel, Russia	2 (EtOH)	Adult
appendix	Nolachucky River, Ont.	2 (EtOH)	Ammocoete
Genus *Petromyzon*			
marinus	Bronte Creek, Ont. (landlocked)	1 (EtOH)	Ammocoete

Note: Sample size (*N*), method of preservation (fr, frozen at –70°C; EtOH, preserved in 95% ethanol), and life cycle stage are indicated.

fragment or with the internal primers. The sequences have been deposited in GenBank with accession Nos. AF177947–AF177976.

Sequence comparisons were made on 384 bp at the 5′ end of the cytochrome *b* gene, on the entire ND3 gene, and on the combined sequences (735 bp). Using the Molecular Evolutionary Genetics Analysis (MEGA) program (version 1.01) developed by Kumar et al. (1993), genetic distances were estimated with Kimura's (1980) two-parameter distances and phylogenetic relationships were inferred from the neighbour-joining (NJ) method; support of the data set for each branch point was tested by 500 bootstrap replications. For comparison, we also carried out the maximum parsimony procedure as implemented in MEGA (branch and bound method). Published cytochrome *b* sequences from two European species, *L.* (*L.*) *planeri* and *L.* (*Le.*) *zanandreai* (Tagliavini et al. 1994), were added to our data set, and the phylogenetic analyses above were repeated on the 267 bp of sequence (positions 118–384) common to all 13 species.

Results

Phylogenetic analysis

The portion of the cytochrome *b* gene sequenced had a 12-bp insert (positions 10–21) in lampreys relative to most teleosts (e.g., Cantatore et al. 1994), but there were no insertions or deletions among the lamprey species examined. The ND3 gene likewise showed no interspecific differences in length.

Intraspecific variation in the nucleotide sequence of both genes was relatively low in the current study. Cytochrome *b* and ND3 sequences were identical in eight Pacific lampreys (*L. tridentata*) from British Columbia and Oregon, and four *L. richardsoni*, *L. lethophaga*, and *L. hubbsi* likewise showed no intraspecific differences, even among sites. Both gene sequences were identical in the landlocked sea lamprey of the current study and the anadromous sea lamprey from Lee and Kocher (1995). Intraspecific sequence differences were noted, however, in two species: *L. aepyptera* (cytochrome *b*) and *L. similis* (both genes) (Table 3). *Lampetra similis* from the Merced River in California varied consistently from the Oregon specimens by a total of two or three nucleotides, but the differences among the Oregon specimens (one or two nucleotides) were not population specific. All intraspecific nucleotide substitutions were third-position transitions.

Within the *Lampetra* genus, interspecific sequence variation ranged from 0 to 9.1% in cytochrome *b* and from 0 to 12.5% in ND3 (Table 3). In both genes, there were no differences between *L. ayresii* and *L. richardsoni* and among *L. tridentata*, *L. macrostoma*, *L. lethophaga*, and *L. similis* from California. The greatest differences were observed between a haplotype of *L. similis* and *L. appendix* (cytochrome *b*) and *L. similis* and *L. hubbsi* (ND3). Comparisons between the *Lampetra* species and *P. marinus* showed variation between genera to range from 13.0 to 14.3% in cytochrome *b* and from 16.0 to 17.4% in ND3.

The cytochrome *b* and ND3 nucleotide sequences were used to estimate the phylogeny of the 11 *Lampetra* species using sea lamprey as an outgroup. Since the tree topologies obtained from each gene separately or together and those by the NJ or maximum parsimony method were almost consensus, just the phylogenetic analysis of the NJ method using the combined data is shown (Fig. 1). Only the topology of the NJ tree using the ND3 data differed slightly from Fig. 1

Table 3. Pairwise sequence differences (expressed as first/second/third codon position substitutions) in 384 bp of the lamprey cytochrome *b* gene (above the diagonal) and in the 351-bp ND3 gene (below the diagonal).

	1	2	3	4	5	6	7	8	9	10	11	12	13
Genus *Lampetra*													
Subgenus *Lampetra*													
1. *ayresii, richardsoni*	—	2/0/22	4/0/21	4/0/22	2/0/26	2/0/25	2/0/25	2/0/25	2/0/26	3/0/8	1/1/24	1/2/23	7/1/42
2. *fluviatilis*	5/1/27	—	4/0/11	4/0/12	4/0/20	4/0/21	4/0/21	4/0/21	4/0/22	5/0/26	1/1/22	1/2/23	7/1/44
3. *aepyptera* 1	9/2/24	6/1/18	—	0/0/1	6/0/23	6/0/24	6/0/24	6/0/24	6/0/25	7/0/25	5/1/25	5/2/24	11/1/39
4. *aepyptera* 2	9/2/24	6/1/18	0/0/0	—	6/0/24	6/0/25	6/0/25	6/0/25	6/0/26	7/0/26	5/1/26	5/2/25	11/1/40
Subgenus *Entosphenus*													
5. *tridentata, macrostoma, lethophaga, similis* (Calif.)	5/2/24	8/3/29	8/4/28	8/4/28	—	0/0/1	0/0/1	0/0/1	0/0/2	5/0/28	3/1/27	3/2/28	9/1/42
6. *similis* 2, 4–6	5/2/29	8/3/30	8/4/29	8/4/29	0/0/1	—	0/0/0	0/0/0	0/0/1	5/0/27	3/1/28	3/2/29	9/1/41
7. *similis* 1	5/2/30	8/3/31	8/4/30	8/4/30	0/0/2	0/0/1	—	0/0/0	0/0/1	5/0/27	3/1/28	3/2/29	9/1/41
8. *similis* 3	5/2/28	8/3/31	8/4/29	8/4/29	0/0/2	0/0/1	0/0/2	—	0/0/1	5/0/27	3/1/28	3/2/29	9/1/41
9. *similis* 7	5/2/29	8/3/30	8/4/29	8/4/29	0/0/1	0/0/0	0/0/1	0/0/1	—	5/0/28	3/1/29	3/2/30	9/1/40
10. *hubbsi*	5/2/15	6/1/28	10/2/26	10/2/26	8/4/30	8/4/31	8/4/32	8/4/32	8/4/31	—	4/1/28	4/2/27	10/1/41
Subgenus *Lethenteron*													
11. *japonica*	9/1/28	6/0/27	7/1/22	7/1/22	9/3/28	9/3/29	9/3/30	9/3/30	9/3/29	8/1/26	—	0/1/1	6/2/47
12. *appendix*	9/1/28	6/0/27	8/1/22	8/1/22	10/3/28	10/3/29	10/3/30	10/3/30	10/3/29	8/1/26	2/0/0	—	6/3/46
Genus *Petromyzon*													
13. *marinus*	10/1/48	9/2/45	15/3/43	15/3/43	13/3/45	13/3/45	13/3/46	13/3/44	13/3/45	10/3/46	12/2/42	12/2/42	—

Note: Sequences of both genes were identical in *L. ayresii* and *L. richardsoni*, as were those in *L. tridentata, L. macrostoma, L. lethophaga*, and *L. similis* from the Merced River, Calif. *Lampetra similis* 1–7 were collected in Oregon; sample sizes and collection sites are given in Table 2.

in that the *L. ayresii* and *L. hubbsi* clade clustered with the *Entosphenus* species rather than with the other *Lampetra* or *Lethenteron* species, but this difference was not reproducible at more than 50% bootstrapping. In all cases, four distinct clades were apparent: (*i*) the *Entosphenus* subgenus without *L.* (*E.*) *hubbsi*, (*ii*) *L.* (*E.*) *hubbsi* with the westcoast North American *L.* (*L.*) *ayresii* and *L.* (*L.*) *richardsoni*, (*iii*) species of the *Lampetra* subgenus from Atlantic drainages, i.e., *L. fluviatilis* and *L. aepyptera*, and (*iv*) the *Lethenteron* species *L. japonica* and *L. appendix*.

A second consensus tree (not shown) was constructed to include 267 bp of cytochrome *b* sequence from *L.* (*L.*) *planeri* and *L.* (*Le.*) *zanandreai* from Italy (Tagliavini et al. 1994). Cytochrome *b* sequence in *L. planeri* was identical to that of *L. fluviatilis* from Germany and the three European species grouped together; *L. zanandreai* did not form a clade with the other *Lethenteron* species. *Lampetra aepyptera* clustered with the three European species but with a lower bootstrap confidence limit (57%) than it did with *L. fluviatilis* in Fig. 1.

Patterns of variation in cytochrome *b* and ND3

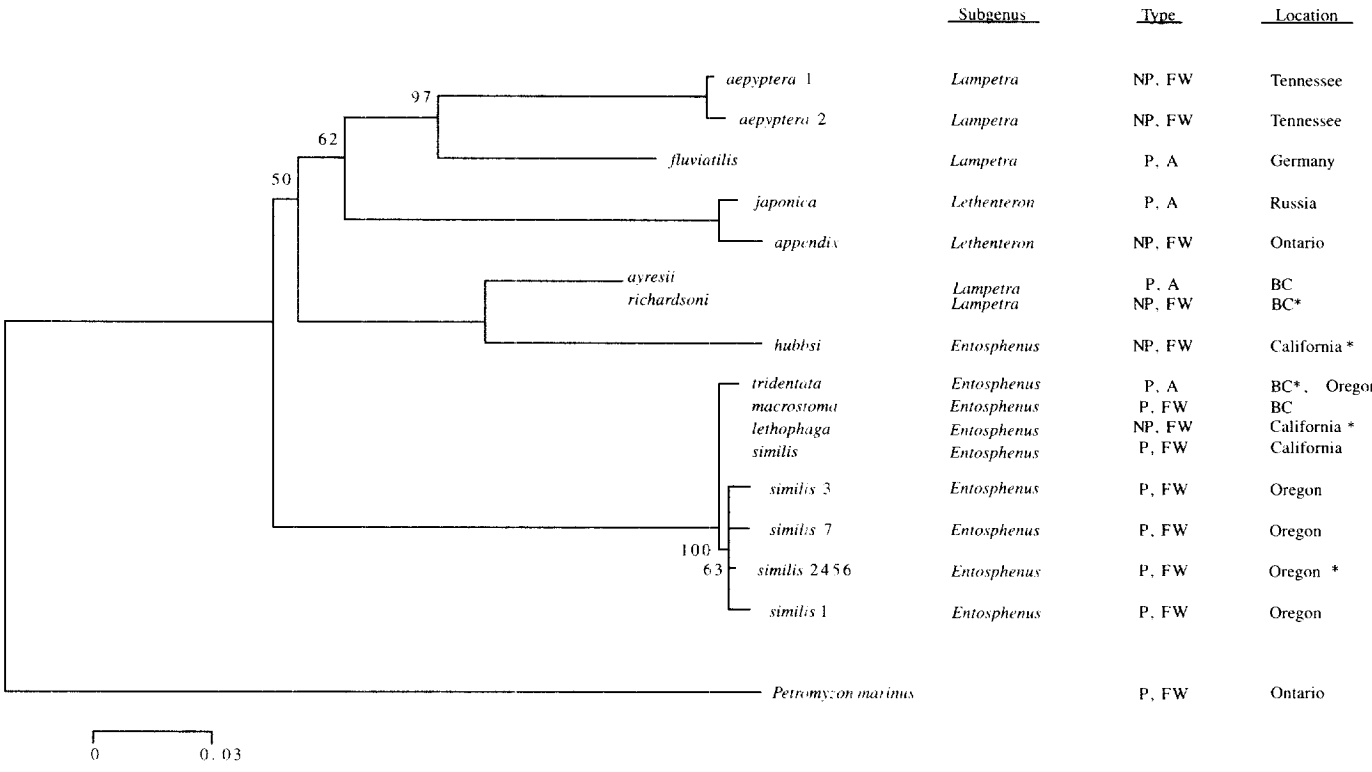
Overall nucleotide sequence variation among lamprey species was, on average, 1.35 times greater in the ND3 gene than in the cytochrome *b* gene (Table 3). In both genes, third-position substitutions were more common than variations at first- and second-codon positions, and transition substitutions outnumbered transversions. The fraction of substitutions observed in first-, second-, and third-position sites was 13.8, 2.6, and 83.4%, respectively, in cytochrome *b* and 20.8, 5.2, and 74.1%, respectively, in ND3. In cytochrome *b*, transitions accounted for 85.2% of all substitutions but only 77.1% of substitutions in ND3. Accordingly, the frequency of nonsilent nucleotide substitutions was two to three times higher in ND3 than in cytochrome *b*, and amino acid substitutions averaged 5.1% in ND3 but only 2.0% in cytochrome *b*. However, whereas the frequency of nonsilent substitutions for a given genetic distance was relatively constant among species pairs in ND3, this value was not uniform in cytochrome *b* (Fig. 2). For example, despite a genetic distance between *L. ayresii* and *L. tridentata* nucleotide sequences of 0.079, there were no amino acid differences. Similarly, there were only two amino acid differences between either *L. ayresii* or *L. tridentata* and *P. marinus*. In contrast, all pairwise comparisons with the relict nonparasitic species (*L. aepyptera* or *L. hubbsi*) showed comparatively high rates of nonsilent substitutions, and intermediate rates were observed in pairwise comparisons with *L. appendix* (Fig. 2).

Discussion

Relict species and subgenera

Analysis of partial mitochrondrial DNA sequence permitted resolution of a number of questions concerning the phylogeny of the lamprey genus *Lampetra*. One of the clearest and most notable findings was that *L. hubbsi*, assigned to the genus *Entosphenus* by Vladykov and Kott (1976*a*) based largely on adult dentition (Table 1), appears more closely related to *L.* (*L.*) *ayresii* and *L.* (*L.*) *richardsoni* than it is to any other *Entosphenus* species. On the strength of the mito-

Fig. 1. Neighbour-joining analysis comparing species of the genus *Lampetra* using 735 bp of cytochrome *b* and ND3 sequence; *P. marinus* is the outgroup. Numbers at nodes indicate bootstrap confidence levels; life history type is indicated: P, parasitic; NP, nonparasitic; A, anadromous; FW, freshwater. Collection data are provided in Table 2; locations in which more than one site was sampled are indicated by an asterisk.



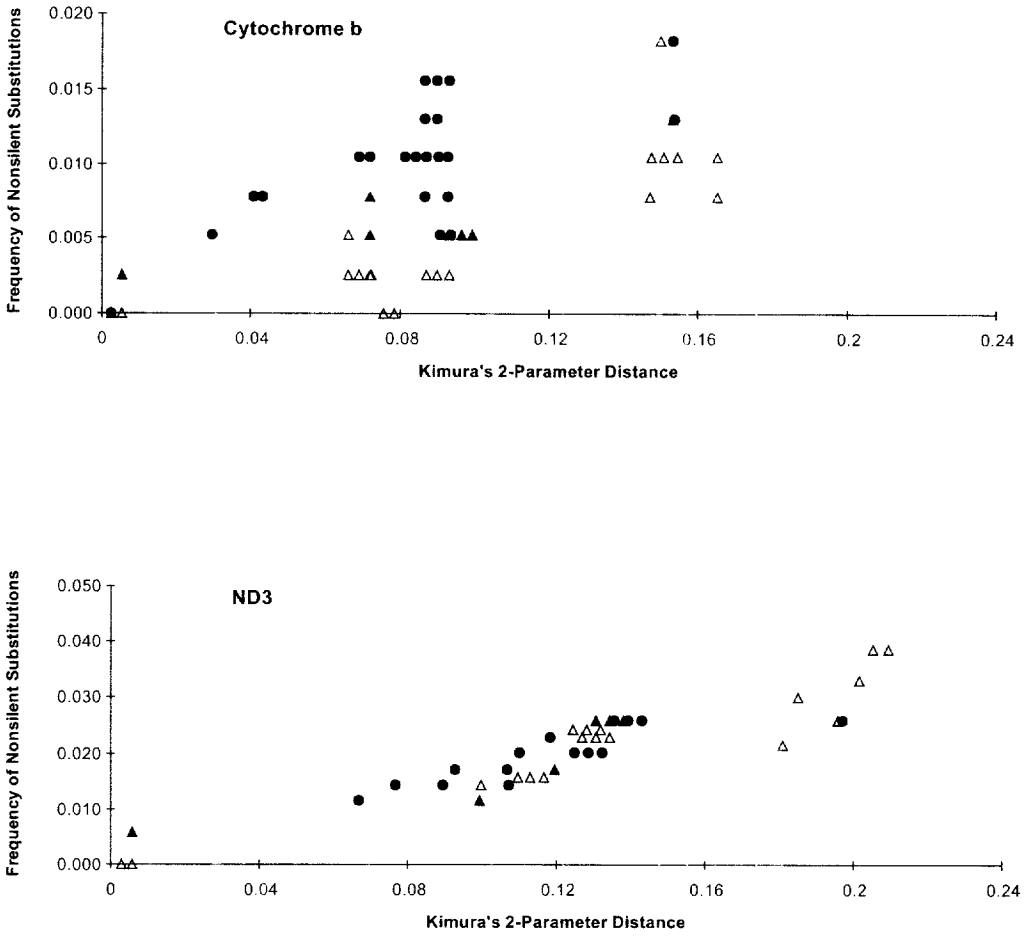
chondrial DNA data, it appears that *L. hubbsi* is a southern relict species of the *L. ayresii* lineage and should be placed in the subgenus *Lampetra*.

Also notable were the findings that two other relict species of the *Lampetra* genus, *L.* (*Le.*) *zanandreai* and *L.* (*L.*) *aepyptera*, both appear to have descended from the *L.* (*L.*) *fluviatilis* lineage. The molecular data show *L. zanandreai* to be more closely related to *L.* (*L.*) *fluviatilis* than to the two *Lethenteron* species and support its original designation as a species of *Lampetra* (Vladykov 1955). As was suggested by Vladykov and Kott (1979*a*), it appears that *L. zanandreai* descended from an *L. fluviatilis*-type ancestor, even though it is no longer sympatric with the present-day *L. fluviatilis* (Bianco 1986). *Lampetra* (*L.*) *aepyptera* likewise is no longer sympatric with any likely parasitic ancestor, and its phylogenetic position within the *Lampetra* genus has been the subject of considerable debate. Its morphological characteristics "are so poorly developed that they render its position and generic placement difficult and somewhat dubious" (Hubbs and Potter 1971), and at one time, it was placed in the "provisional and noncommittal" genus *Okkelbergia* (Hubbs and Potter 1971). Vladykov and Kott (1976*b*), however, concluded that this species displays no characteristics not found in *Lampetra*, and descent from either an *L. ayresii*-type ancestor (Vladykov and Kott 1979*a*) or an *L.* (*Le.*) *japonica*-type ancestor (Bailey 1980; Potter 1980) has been suggested, since both occur in North America. The mitochondrial DNA data are consistent with its present placement in the *Lampetra* subgenus as a nonparasitic species of considerable antiquity (Potter 1980) but suggest descent from an *L.* (*L.*) *fluviatilis*-type ancestor rather than from either North American species.

In addition to resolving these relationships within each of the *Lampetra* subgenera, the current molecular study has resolved some of the relationships among the subgenera. Potter (1980) grouped *Entosphenus*, *Lethenteron*, and *Lampetra* within a single genus largely due to the intergradation of characteristics found in *L. hubbsi*. Its bicuspid supraoral lamina and few velar tentacles are typical of *Lampetra* and *Lethenteron*, but its posterial circumorals and other features of its dentition (Table 1) resulted in its placement in *Entosphenus* (Vladykov and Kott 1976*a*). If *L. hubbsi* is removed from the *Entosphenus* taxon because of its closer genetic similarity to *L.* (*L.*) *ayresii*, however, the molecular data support the previous classifications which have held *Entosphenus* distinct from *Lampetra* and *Lethenteron*.

In contrast, the *Lampetra* and *Lethenteron* subgenera were not clearly delineated. Rather, the species assigned to these two taxa fell into three general groups and there was considerable overlap between the putative subgenera. The three groups were (*i*) the westcoast North American *Lampetra* species (including *L.* (*E.*) *hubbsi*), (*ii*) Atlantic *Lampetra* species from both North America and Europe and *L.* (*Le.*) *zanandreai*, and (*iii*) the two other *Lethenteron* species examined in the current study. It was surprising that the North American and European species of the *Lampetra* subgenus had little more genetic affinity with one another than either did with the other subgenera, since *L. ayresii* and *L. richardsoni*, respectively, were once considered North American races of *L. fluviatilis* and *L. planeri* (Hubbs and

Fig. 2. Frequency of nonsilent substitutions in the cytochrome *b* and ND3 genes of lampreys versus genetic distance for 55 intra- and inter-specific pairwise comparisons. Solid circles represent all comparisons involving the relict nonparasitic species *L. hubbsi* and *L. aepyptera*, closed triangles represent the remaining comparisons involving the nonparasitic *L. appendix*, and open triangles represent all other species comparisons.



Potter 1971). The distinction between *L. ayresii* and *L. fluviatilis* was based on relatively small differences in body proportions (Table 1) whereas other morphological differences among other species seem not to be taxon distinctive. In particular, the presence of posterial circumorals does not seem to reflect a common evolutionary origin. As was discussed above, *L.* (*E.*) *hubbsi* is genetically most similar to *L.* (*L.*) *ayresii* despite the absence of posterials in the latter species. Posterials are present in most *Lethenteron* species, and like *L. hubbsi*, they are all unicuspid, but *L. hubbsi* did not cluster with these *Lethenteron* species. Likewise, *L. zanandreai* appears to be the only species with posterials in the *L. fluviatilis* lineage, and posterials may be absent in some *L. zanandreai* (Hubbs and Potter 1971; Bianco 1986). As was suggested by Bailey (1980), the presence of posterials appears to be inconsistent and unreliable in the separation of the subgenera, and given the substantial overlap between *Lampetra* and *Lethenteron*, morphologically and genetically, there appears to be little justification for the continued division. However, further clarification is needed; longer gene sequences are required from *L. zanandreai*, and more of the European and Asian representatives of the genus need to be examined. Similarly, complete resolution of the *Lampetra–Lethenteron* relationship will require examination of the *Eudontomyzon* and *Tetrapleurodon* genera. Bailey (1980) included these two taxa from Eurasia and Mexico, respectively, in the *Lampetra* genus, and the subfamily classification of Vladykov and Kott (1979*a*) that groups *Eudontomyzon* with *Lampetra* and *Lethenteron* and *Tetrapleurodon* with *Entosphenus* also implies interrelatedness of these taxa with the three subgenera of *Lampetra*. Molecular data should go a long way toward resolving these taxonomic questions.

Assuming an overall rate of mitochondrial DNA sequence divergence of 2% per million years calculated for several mammalian species (Brown et al. 1979) and used to estimate divergence times in a variety of fish taxa (see review by Billington and Hebert 1991), the three relict species are estimated to have diverged from the *L. fluviatilis* or *L. ayresii* lineages about 0.9–2.7 million years ago. Divergences among the four major clades within the *Lampetra* genus occurred about 3–5 million years ago, and the *Lampetra* and *Petromyzon* genera diverged about 9–13 million years ago, maybe more if a general trend for slower substitution rates in many fishes and other ectotherms (see Billington and Hebert 1991) is also true for lampreys. In particular, suggestions of a slower rate of nonsilent substitutions in fishes relative to birds and mammals (Thomas and Beckenbach 1989) may be true of lampreys. For example, although 28 nucleotide differences were observed in the 384 bp of cytochrome

b sequences from *L. ayresii* and *L. tridentata*, all were silent, and even relative to *P. marinus*, *L. ayresii* or *L. tridentata* cytochrome *b* showed only two amino acid substitutions. It was interesting to note, however, that cytochrome *b* amino acid sequences were less conserved in the relict species (Fig. 2). There were two amino acid differences between the closely related *L. hubbsi* and *L. ayresii* and three between *L. aepyptera* and *L. fluviatilis*. A similarly high rate of replacement substitutions in cytochrome *b* was observed in *L. zanandreai* relative to *L. planeri* (Tagliavini et al. 1994) and suggests either a relaxation of certain functional constraints in these southerly nonparasitic lampreys or directional mutation. This same trend was not seen in the ND3 gene.

Paired and satellite species

In addition to addressing the ambiguous evolutionary relationships of some of the relict nonparasitic species, molecular analysis confirmed that the hypothesized paired species are closely related and diverged from one another more recently than the relict species diverged from their parasitic ancestors. For example, the molecular data agree with the present-day consensus of morphological studies that consider *L.* (*Le.*) *appendix* to be a derivative of *L.* (*Le.*) *japonica*. The paired nature of these two species, however, was not always considered obvious: the two species are no longer sympatric (McPhail and Lindsey 1970), and Zanandrea (1961) listed *L. appendix* as one of three nonparasitic species without a paired species. The molecular data are thus consistent with this hypothesis that *L. appendix* is part of the *L. japonica* assemblage yet more divergent from *L. japonica* than most of the other nonparasitic *Lethenteron* species (e.g., *L. reissneri*; Potter 1980). Applying the above rate of 2% sequence divergence per million years (Brown et al. 1979), *L. japonica* and *L. appendix* are estimated to have diverged about 270 000 years ago. Furthermore, like the above relict species, *L. appendix* showed a relatively high rate of replacement substitutions relative to other species; of the four substitutions observed in both genes between *L. japonica* and *L. appendix*, three were nonsilent.

In contrast, the close genetic relationship between *L. planeri* and the parasitic *L. fluviatilis* was not surprising, since they have long been recognized as paired species (Hubbs and Potter 1971). Similarly, close relationships between *L. richardsoni* and *L. ayresii* and among *L. tridentata* and its presumed freshwater derivatives were expected from morphology-based taxonomies. Most members of paired and satellite species, in fact, were genetically indistinguishable from each other. Given the 735 bp sequenced in the current study, this implies divergences within the past 70 000 years. This rapid speciation is consistent with the distribution of these species and hypothesized changes in drainage patterns and river connections with glaciation. Lake Cowichan drainage patterns, for example, changed about 10 000 years ago, resulting in isolation of the *L. macrostoma* lineage from sea-run *L. tridentata* in the Strait of Georgia (Beamish 1982). In rivers east of the Baltic, *L. planeri* is also believed to have originated within the last 10 000 years (Hubbs and Potter 1971). To detect divergence times of 10 000 years or less, however, it is estimated that more than 5000 bp of the mitochondrial genome would have to be sequenced, or perhaps a more variable region such as the control region (Lee et al. 1995) would be useful.

The lack of observed genetic divergence between members of paired and satellite species is not necessarily inconsistent with each being given species designation. Meyer et al. (1990) found a 363-bp fragment of cytochrome *b* to be identical among seven species of Lake Victoria cichlids, and species designation cannot be decided on sequence differences alone, since rapid morphological differentiation resulting in distinct species may be decoupled from molecular divergence. Most lamprey taxonomies recognize life history type as a significant characteristic distinguishing between closely related species, and the different life history types appear to result in reproductive isolation. Although artificial hybridization between paired species has succeeded in producing viable larvae (e.g., Beamish and Neville 1992), interbreeding appears to be impaired by size differences between the life history types (Beamish and Neville 1992) and temporal and spatial differences in spawning (Beamish 1982).

Despite the lack of detectable differences among several species, intraspecific sequence differences were observed in *L. aepyptera* and *L. similis*. These were within the range found in other fish species (Billington and Hebert 1991), but the consistent genetic differences between populations of *L. similis* were notable. Although *L. similis* from the Merced River in California was genetically indistinguishable from *L. tridentata*, *L. similis* from the Klamath Basin of Oregon differed from them by two or three third-position transitions. This may indicate that the Merced River *L. similis* has arisen from *L. tridentata* more recently than the Oregon populations, and preliminary data suggesting that Merced River *L. similis* exhibits significant morphological and life history differences from the Oregon specimens (R.J. Beamish and J.H. Youson, unpublished data) support this hypothesis. Wide-ranging anadromous lampreys may have given rise to multiple freshwater parasitic or nonparasitic derivatives at different times, and the morphological differences observed among populations in other species (e.g., between *L. pacifica* and the now-synonymous *L. richardsoni* (Table 1), or *L. folletti* and *L. lethophaga*) may also reflect separate origins. More extensive molecular surveys will no doubt uncover more intraspecific differences and reveal the polyphyletic origins of a number of derived species.

In summary, mitochrondrial DNA sequence analysis answered several questions concerning the phylogeny of the lamprey genus *Lampetra*. *Lampetra hubbsi*, assigned to the genus *Entosphenus* largely on the basis of adult dentition, is most closely related to *L. ayresii* and should be placed in the subgenus *Lampetra*. *Lampetra* (*Le.*) *zanandreai* is genetically more similar to *L.* (*L.*) *fluviatilis* than to the other two *Lethenteron* species, and *L.* (*L.*). *aepyptera* is more closely related to *L.* (*L.*) *fluviatilis* than to the other North American species. Although there was no distinct division between the subgenera *Lampetra* and *Lethenteron*, *Entosphenus* (without *L. hubbsi*) formed a genetically separable taxon.

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References

Bailey, R.M. 1980. Comments on the classification and nomenclature of lampreys — an alternative view. Can. J. Fish. Aquat. Sci. **37**: 1626–1629.

Beamish, R.J. 1982. *Lampetra macrostoma*, a new species of freshwater parasitic lamprey from the west coast of Canada. Can. J. Fish. Aquat. Sci. **39**: 736–747.

Beamish, R.J., and Neville, C-M.E. 1992. The importance of size as an isolating mechanism in lampreys. Copeia, **1992**: 191–196.

Bianco, P.G. 1986. *Lethenteron zanandreai* (Vladykov, 1955). *In* The freshwater fishes of Europe. Vol. 1. Part I. Petromyzoniformes. *Edited by* J. Holčík. AULA-Verlag. Weisbaden. pp. 237–246.

Billington, N., and Hebert, P.D.N. 1991. Mitochondrial DNA diversity in fishes and its implications for introductions. Can. J. Fish. Aquat. Sci. **48**(Suppl. 1): 80–94.

Brown, W.M., George, M., Jr., and Wilson, A.C. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. U.S.A. **76**: 1967–1971.

Cantatore, P., Roberti, M., Pesole, G., Ludovico, A., Milella, F., Gadaleta, M.N., and Saccone, C. 1994. Evolutionary analysis of cytochrome *b* sequences in some Perciformes: evidence for a slower rate of evolution than in mammals. J. Mol. Evol. **39**: 589–597.

Hardisty, M.W. 1986. General introduction. *In* The freshwater fishes of Europe. Vol. 1. Part I. Petromyzoniformes. *Edited by* J. Holčík. AULA-Verlag, Weisbaden. pp. 19–83.

Hubbs, C.L. 1971. *Lampetra (Entosphenus) lethophaga*, new species, the nonparasitic derivative of the Pacific lamprey. Trans. San Diego Soc. Nat. Hist. **16**: 125–164.

Hubbs, C.L., and Potter, I.C. 1971. Distribution, phylogeny and taxonomy. *In* The biology of lampreys. Vol. 1. *Edited by* M.W. Hardisty and I.C. Potter. Academic Press, London, U.K. pp. 1–65.

Janvier, P., and Lund, R. 1983. *Hardistiella montanensis* n. gen. et sp. (Petromyzontida) from the Lower Carboniferous of Montana, with remarks on the affinities of lampreys. J. Vertebr. Paleontol. **2**: 407–413.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. **16**: 111–120.

Kumar, S., Tamura, K., and Nei, M. 1993. MEGA: Molecular Evolutionary Genetics Analysis, version 1.01. Pennsylvania State University Press, University Park, Pa.

Lee, W-J., and Kocher, T.D. 1995. Complete sequence of a sea lamprey (*Petromyzon marinus*) mitochondrial genome: early establishment of the vertebrate genome organization. Genetics, **139**: 873–887.

Lee, W-J., Conroy, J., Howell, W.H., and Kocher, T.D. 1995. Structure and evolution of teleost mitochondrial control regions. J. Mol. Evol. **41**: 54–66.

McPhail, J.D., and Lindsey, C.C. 1970. Freshwater fishes of northwestern Canada and Alaska. Bull. Fish. Res. Board Can. No. 173.

McVeigh, H.P., and Davidson, W.S. 1991. A salmonid phylogeny inferred from mitochondrial cytochrome *b* gene sequences. J. Fish Biol. **39A**: 277–282.

Meyer, A., Kocher, T.D., Basasibwaki, P., and Wilson, A.C. 1990. Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. Nature (Lond.), **347**: 550–553.

Palumbi, S.R. 1996. Nucleic acids II: the polymerase chain reaction. *In* Molecular systematics. 2nd ed. *Edited by* D.M. Hillis, C. Moritz, and B.K. Mable. Sinauer Associates, Inc., Sunderland, Mass. pp. 205–247.

Potter, I.C. 1980. The Petromyzoniformes with particular reference to paired species. Can. J. Fish. Aquat. Sci. **37**: 1595–1615.

Richards, J.E., Beamish, R.J., and Beamish, F.W.H. 1982. Descriptions and keys for ammocoetes of lampreys from British Columbia, Canada. Can. J. Fish. Aquat. Sci. **39**: 1484–1495.

Robins, C.R., Bailey, R.M., Bond, C.E., Brooker, J.R., Lachner, E.A., Lea, R.N., and Scott, W.B. 1980. A list of common and scientific names of fishes from the United States and Canada. Am. Fish. Soc. Spec. Publ. No. 12.

Robins, C.R., Bailey, R.M., Bond, C.E., Brooker, J.R., Lachner, E.A., Lea, R.N., and Scott, W.B. 1991. Common and scientific names of fishes from the United States and Canada. Am. Fish. Soc. Spec. Publ. No. 20.

Tagliavini, J., Tizzi, R., Conterio, F., Mariottini, P., and Gandolfi, G. 1994. Mitochondrial DNA sequences in three genera of Italian lampreys. Boll. Zool. **61**: 331–333.

Thomas, W.K., and Beckenbach, A.T. 1989. Variation in salmonid mitochondrial DNA: evolutionary constraints and mechanisms of substitution. J. Mol. Evol. **29**: 233–245.

Vladykov, V.D. 1955. *Lampetra zanandreai*, a new species of lamprey from northern Italy. Copeia, **1955**: 215–223.

Vladykov, V.D. 1973. *Lampetra pacifica*, a new nonparasitic species of lamprey (Petromyzontidae) from Oregon and California. J. Fish. Res. Board Can. **30**: 205–213.

Vladykov, V.D., and Follett, W.I. 1958. Redescription of *Lampetra ayresii* (Günther) of western North America, a species of lamprey (Petromyzonidae) distinct from *Lampetra fluviatilis* (Linnaeus) of Europe. J. Fish. Res. Board Can. **15**: 47–77.

Vladykov, V.D. and Follett, W.I. 1965. *Lampetra richardsoni*, a new nonparasitic species of lamprey (Petromyzonidae) from western North America. J. Fish. Res. Board Can. **22**: 139–158.

Vladykov, V.D., and Kott, E. 1976*a*. A new nonparasitic species of lamprey of the genus *Entosphenus* Gill, 1862 (Petromyzonidae) from south central California. Bull. South. Calif. Acad. Sci. **75**: 60–67.

Vladykov, V.D., and Kott, E. 1976*b*. Is *Okkelbergia* Creaser and Hubbs, 1922 (Petromyzonidae) a distinct taxon? Can. J. Zool. **54**: 421–425.

Vladykov, V.D., and Kott, E. 1976*c*. A second nonparasitic species of *Entosphenus* Gill, 1862 (Petromyzonidae) from Klamath River system, California. Can. J. Zool. **54**: 974–989.

Vladykov, V.D., and Kott, E. 1979*a*. Satellite species among the holarctic lampreys (Petromyzonidae). Can. J. Zool. **57**: 860–867.

Vladykov, V.D., and Kott, E. 1979*b*. A new parasitic species of the holarctic lamprey genus *Entosphenus* Gill, 1862 (Petromyzonidae) from Klamath River, in California and Oregon. Can. J. Zool. **57**: 808–823.

Zanandrea, G. 1959. Speciation among lampreys. Nature (Lond.), **184**: 380.

Zanandrea, G. 1961. Studies on European lampreys. Evolution, **15**: 523–534.