American Fisheries Society Symposium 72:000–000, 2009

Novel Relationships among Lampreys (Petromyzontiformes) Revealed by a Taxonomically Comprehensive Molecular Data Set

NICHOLAS J. LANG*
Division of Fishes, Department of Zoology, Field Museum of Natural History
1400 South Lake Shore Drive, Chicago, Illinois 60605, USA

KEVIN J. ROE
Natural Resource Ecology and Management, 339 Science II, Iowa State University
Ames, Iowa 50011, USA

CLAUDE B. RENAUD
Research Services Division, Canadian Museum of Nature
Post Office Box 3443, Station D, Ottawa, Ontario, K1P 6P4, Canada

HOWARD S. GILL
School of Biological Sciences and Biotechnology, Murdoch University
Perth, Western Australia 6150, Australia

IAN C. POTTER
School of Biological Sciences and Biotechnology, Murdoch University
Perth, Western Australia 6150, Australia

JÖRG FREYHOF
Leibniz Institute of Freshwater Ecology and Inland Fisheries
Müggelseedamm 310, 12587 Berlin, Germany

ALEXANDER M. NASEKA
Zoological Institute of the Russian Academy of Sciences
Universitetskaya nab. 1, St. Petersburg, 199034, Russia

PHILIP COCHRAN
Saint Mary's University of Minnesota
700 Terrace Heights #10, Winona, Minnesota 55987, USA

HECTOR ESPINOSA PÉREZ
Instituto de Biología, Universidad Nacional Autónoma de México, México, D.F., México

EVELYN M. HABIT
Unidad de Sistemas Acuáticos, Centro de Ciencias Ambientales, EULA-Chile
Universidad de Concepción, Casilla 160-C, Concepción, Chile

---

* Corresponding author: oligocephalus@gmail.com

BERNARD R. KUHAJDA
Department of Biological Sciences, Box 870345, University of Alabama,
Tuscaloosa, Alabama 35487, USA

DAVID A. NEELY
California Academy of Sciences, 875 Howard Street, San Francisco, California 94103, USA

YURI S. RESHETNIKOV
Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences
Leninskii prospect, 33, 119071, Moscow, Russia

VLADIMIR B. SALNIKOV
National Institute of Deserts, Flora and Fauna, Ministry of Nature Protection of Turkmenistan,
Bitarap Turkmenistan Street, 15, 744000, Ashgabat, Turkmenistan

MARIA TH. STOUMBOUDI
Hellenic Centre for Marine Research, Institute of Inland Waters
Post Office Box 712, 190 13, Anavyssos, Greece

RICHARD L. MAYDEN
Department of Biology, 3507 Laclede Ave, Saint Louis University,
St. Louis, Missouri 63103, USA

Abstract.—The systematics of lampreys was investigated using complete mitochondrial cytochrome *b* sequences from all genera and nearly all recognized species. The families Geotriidae and Petromyzontidae are monophyletic, but the family Mordaciidae was resolved as two divergent lineages at the base of the tree. Within Petromyzontidae, the nonparasitic *Lethenteron* sp. S and *Okkelbergia aepyptera* were recognized as distinct lineages, *Lethenteron morii* and *Lampetra zanandreai* were moved to new genera, a sister species relationship was recovered between *Caspiomyzon wagneri* and *Eudontomyzon hellenicus*, and a clade was recovered inclusive of *Entosphenus hubbsi* and western North American *Lampetra* (*L. ayresii* and *L. richardsoni*). The placement of *E. hellenicus* as the sister species to *C. wagneri* reduces the number of genera comprised entirely of parasitic species to two, *Geotria* and *Petromyzon*. The recognition of distinct lineages for *O. aepyptera* and *Lethenteron* sp. S recognizes, for the first time, lineages comprised entirely of nonparasitic species. Apart from the results mentioned above, monophyly was supported for the multispecific genera *Entosphenus*, *Eudontomyzon*, *Ichthyomyzon*, *Lampetra* (restricted to European species), and *Lethenteron*. Intergeneric relationships within Petromyzontidae were poorly resolved, but separate clades inclusive of *Entosphenus* and *Tetrapleurodon* (subfamily Entospheninae) and one comprised of *Eudontomyzon*, *Lampetra*, and *Okkelbergia* were recovered.

Introduction

Lampreys (Order Petromyzontiformes) are not fishes in a taxonomic sense. They are, possibly along with the nonvertebrate hagfishes, surviving members of a lineage that is the sister clade to all jawed vertebrates (sharks and rays, bony fishes, and tetrapods; Meyer and Zardoya 2003; Nelson 2006). Although there are relatively few species (Table 1) in the group, several factors, including a two-stage life cycle, a relative lack of measurable features, and the wide

Table 1. Recognized species of lamprey based on Kottelat and Freyhof (2007), Potter and Gill (2003), and Yamazaki and Goto (1996). Species are listed by family, genus (Potter and Gill 2003), and stem-satellite species groups (Potter 1968; Vladykov and Kott 1979). Parasitic species are labeled with an asterisk.

- Geotriidae
  - *Geotria australis**
- Mordaciidae
  - *Mordacia lapicida**
  - *Mordacia mordax**
  - *Mordacia praecox*
- Petromyzontidae
  - *Caspiomyzon wagneri**
  - *Entosphenus folletti*
  - *Entosphenus hubbsi*
  - *Entosphenus lethophagus*
  - *Entosphenus macrostomus**
  - *Entosphenus minimus**
  - *Entosphenus similis**
  - *Entosphenus tridentatus**
  - *Eudontomyzon danfordi**
  - *Eudontomyzon mariae*
  - *Eudontomyzon vladykovi*
  - *Eudontomyzon hellenicus*
  - *Eudontomyzon morii**
  - *Eudontomyzon stankokaramani*
  - *Ichthyomyzon bdellium**
  - *Ichthyomyzon greeleyi*
  - *Ichthyomyzon castaneus**
  - *Ichthyomyzon gagei*
  - *Ichthyomyzon unicuspis**
  - *Ichthyomyzon fossor*
  - *Lampetra aepyptera*
  - *Lampetra ayresii**
  - *Lampetra pacifica*
  - *Lampetra richardsoni*
  - *Lampetra fluviatilis**
  - *Lampetra planeri*
  - *Lampetra lanceolata*
  - *Lethenteron alaskense*
  - *Lethenteron appendix*
  - *Lethenteron camtschaticum**
  - *Lethenteron reissneri*
  - *Lethenteron kessleri*
  - *Lethenteron* sp. N
  - *Lethenteron* sp. S
  - *Lethenteron zanandreai*
  - *Petromyzon marinus**
  - *Tetrapleurodon geminis*
  - *Tetrapleurodon spadiceus**

geographic distribution of the group, make the study of lamprey systematics problematic. The life cycle of all lampreys includes both an extended larval stage and a relatively brief adult stage. During the larval stage, which lasts several years, the lamprey is referred to as an ammocoete and lacks the characters found in adult specimens that distinguish species and genera of lampreys from one another. During a transformative period of 1 or 2 months, the lamprey develops eyes, fins, and a tooth-bearing oral disk and then usually lives 1 or 2 years before spawning and dying shortly after (Hardisty and Potter 1971a, 1971b). This life cycle results in a relatively short period of time in which specimens exist with fully formed diagnostic adult characteristics, time often spent migrating to and through rivers, lakes, and oceans, making capture potentially difficult.

Once a lamprey has metamorphosed into its adult form, morphological characters are largely limited to three character sets: the number of trunk myomeres; the position of the dorsal fins; and, historically most valuable, the pattern and morphology of teeth in the oral disk. The value of tooth characters in global lamprey systematics is, however, hampered by another aspect of lamprey natural history, the presence of both parasitic and nonparasitic species. Following metamorphosis, parasitic lampreys feed on host fishes for up to 2 years, growing and maturing before spawning and death (Hardisty and Potter 1971b). Nonparasitic lampreys, however, transform with already maturing gonads and within several months spawn and die, having never fed as adults. In general, nonparasitic species do not fully form the tooth patterns that are primarily used to classify parasitic lampreys, and the taxonomic placement of nonparasitic species is based on the hypothesis that such species evolved from parasitic ancestors (Hardisty and Potter 1971c). This hypothesis is supported by the fact that the distributional ranges of nonparasitic species often overlap those of parasitic species with similar numbers of myomeres and/or tooth patterning. Potter (1968) and Vladykov and Kott (1979) identified groupings of lampreys composed of a parasitic (stem) species and one or more nonparasitic (satellite) species (Table 1), putting forth the hypothesis that single parasitic species gave rise to multiple nonparasitic species at different times and/or in different areas.

When faced with a group, such as the lampreys, that possesses so few morphological characters, molecular data represent an incredibly valuable source of information. Herein, we present the first taxonomically comprehensive phylogenetic analysis of lampreys to include both parasitic and nonparasitic species.

Systematic and Taxonomic Review

The three major lamprey lineages are currently recognized as distinct families (Hubbs and Potter 1971; Gill et al. 2003). As a whole, lampreys exhibit an antitropical distribution, with two families endemic to the Southern Hemisphere and the third restricted to the Northern Hemisphere. The family Geotriidae is composed of a single species, *Geotria australis*, which occurs throughout New Zealand, the southern regions of Australia (including Tasmania), and South America. The other Southern Hemisphere family, Mordaciidae, is represented by two species in Australia, *Mordacia mordax* and *M. praecox*, and one in Chile, *M. lapicida* (Potter and Gill 2003).

All lampreys in the Northern Hemisphere belong to the family Petromyzontidae, which contains 38 of the 42 recognized species in the order (Table 1). As currently recognized, the genera *Entosphenus* (*En.*, western North America and eastern Asia), *Eudontomyzon* (*Eu.*, central/eastern Europe and eastern Asia), *Lampetra* (*La.*, western Eurasia and western North America), *Lethenteron* (*Le.*, North America, Asia, and southern Europe), and *Petromyzon* (eastern North America and Europe) are widespread while *Caspiomyzon* (Caspian Sea basin), *Ichthyomyzon* (eastern North America), *Okkelbergia* (eastern North America), and *Tetrapleurodon* (central Mexico) have more restricted distributions (Potter and Gill 2003). Although these nine lineages of petromyzontid lamprey have been recognized since Creaser and Hubbs (1922), relative taxonomic ranks suggested by subsequent studies were highly variable (Figure 1). A 10th lineage, the subgenus *Reighardina* (genus *Ichthyomyzon*), was named by Creaser and Hubbs (1922) but subsequently synonymized by Hubbs and Trautman (1937).

Only *Okkelbergia*, erected as a subgenus for *Lampetra aepyptera* by Creaser and Hubbs (1922), has had its validity challenged over time. *Okkelbergia* is the only lineage of lamprey that does not include a parasitic species, and interpretation of the highly variable dentition of *La. aepyptera* has driven the shifting rank of the group from generic recognition (Hubbs and Potter 1971) to not being recognized at all (Vladykov and Kott 1976). The taxonomic placement of another nonparasitic species, *Lethenteron zanandreai*, has also been controversial. Vladykov (1955) originally described this species in the genus *Lampetra* based on the species' perceived lack of posterior circumoral teeth and possession of tricuspid middle lateral circumoral teeth. Zanandrea (1958) showed, however, that both of these characters are variable, and most subsequent workers have placed this species in *Lethenteron* (Hubbs and Potter 1971; Potter and Gill 2003; but see Kottelat and Freyhof 2007).

There have been two interspecific phylogenetic studies on lampreys. The taxonomically most comprehensive study utilized morphological and chromosomal characters but included only parasitic species (Gill et al. 2003). Gill et al. (2003) recognized three families of lamprey and supported eight genera within Petromyzontidae but were unable to comment on the status of *Okkelbergia* (Figure 2). Although several multi-generic clades were well supported by the analysis of Gill et al. (2003), only two, the subfamily Lampetrinae sensu Vladykov (1972) and the tribe Lampetrini sensu Bailey (1980), had been previously hypothesized. The subfamilies Entospheninae and Petromyzontinae (Petromyzontini sensu Bailey [1980]) sensu Vladykov (1972) were each resolved as grades at the base of Petromyzontidae. The second study utilized mitochondrial DNA (mtDNA) sequences and

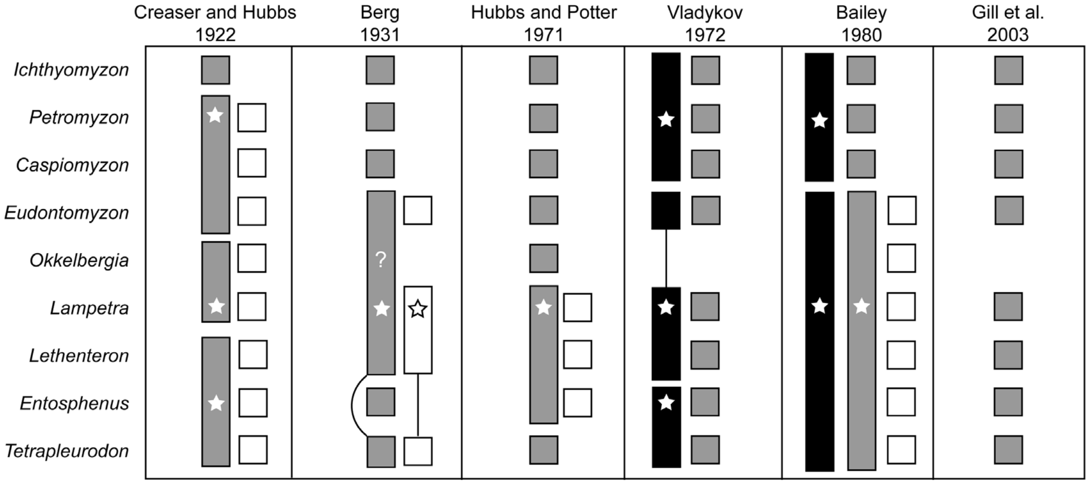

Figure 1. Visual representation of the taxonomic history of petromyzontid lampreys. The *x*-axis indicates major papers in lamprey systematics arranged chronologically while the *y*-axis indicates major lineages that have been proposed within Petromyzontidae. Black boxes indicate the taxonomic breadth of subfamilies (Vladykov 1972) or tribes (Bailey 1980), gray boxes indicate the taxonomic breadth of genera, and white boxes indicate the taxonomic breadth of subgenera. Stars indicate the name-bearing lineage for a given taxonomic group. Lines connect members of a group that could not be situated next to each other. The question mark indicates that Berg (1931) was unsure of his placement of *Okkelbergia* but provisionally synonymized the lineage with *Lampetra*, a move followed by Vladykov (1972). Gill et al. (2003) did not comment on *Okkelbergia* because it was not included in their data set.

focused on the subfamilies Lampetrinae and Entospheninae sensu Vladykov (1972). Docker et al. (1999), expanded upon by Docker (2006), hypothesized that both Lampetra and Entosphenus are nonmonophyletic due to recovery of a clade inclusive of *La. ayresii*, *La. richardsoni*, and *En. hubbsi* that was not closely related to clades comprising the remaining species in those genera. Docker et al. (1999) and Docker (2006) also hypothesized that *Okkelbergia* should not be recognized at any taxonomic level, that *Lethenteron zanandreai* should be moved to *Lampetra*, and that *Le.* sp. S of Yamazaki and Goto (1996) may be more closely related to species of *Entosphenus* than species of *Lethenteron*. Species of *Eudontomyzon* and *Tetrapleurodon* were not available for inclusion in the mtDNA studies, however, and several species, such as *Le. zanandreai* and *Le.* sp. S, were represented by very short, partial sequences (267 base pairs [bp] of cytochrome *b* [cyt *b*]). Finally, although not specifically a phylogenetic study, Silver et al. (2004) presented a tree of a nuclear coding gene in which they recovered a clade uniting Petromyzontidae and Geotriidae, which was in turn sister to Mordaciidae.

Methods

Specimens were collected using a variety of methods (hand, nets, traps, and/or electroshocking equipment) and were either frozen or fixed in 95% ethanol. When only a portion of the specimen was fixed in ethanol, generally a fin or a small muscle plug from the dorsolateral surface, the remaining specimen was fixed in formalin to serve as voucher material. Whenever possible, fully metamorphosed adult specimens, which were identified by ichthyologists expert in both the local fauna and the global diversity of lampreys, were utilized during this study. The only species represented solely by ammocoetes are *Entosphenus hubbsi*, which was represented by adult specimens in Docker

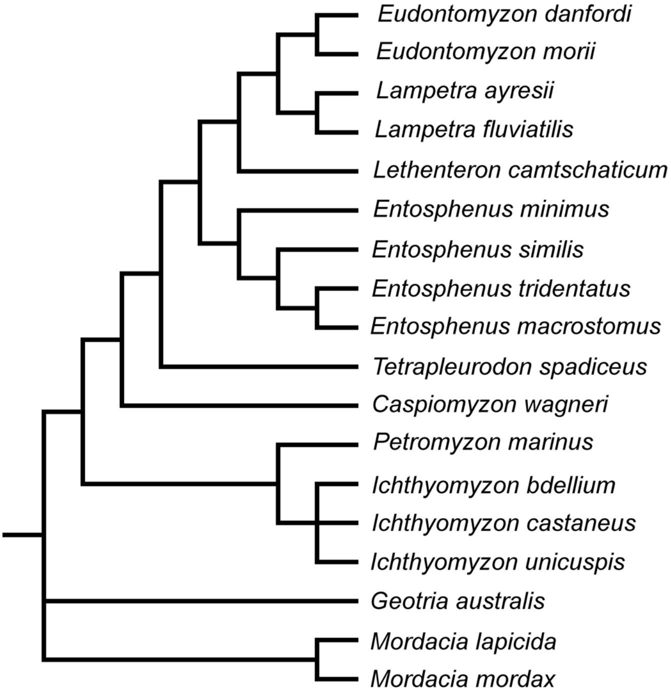

Figure 2. Phylogenetic relationships of lampreys recovered by Gill et al. (2003) based on morphology of parasitic species.

et al. (1999), and *Eudontomyzon morii*. The data set includes all species recognized by Potter and Gill (2003) except for *En. folletti*, *Lampetra pacifica*, and *Tetrapleurodon spadiceus*, from which tissues were unavailable. Several species that were either identified (*Lethenteron* sp. N and *Le.* sp. S of Yamazaki and Goto (1996)) or elevated (*Eudontomyzon stankokaramani* [Holčík and Šorić 2004] and *Eu. vladykovi* [Kottelat and Freyhof 2007]) recently, but were not included in Potter and Gill (2003), were also included. Although most species are represented by a single specimen, two specimens each were included of *Eu. hellenicus* and species in monotypic genera, except for *Petromyzon marinus*, which has been shown to possess extremely little divergence across its range (Rodríguez-Muñoz et al. 2004). The only species represented exclusively by data from GenBank is *Le.* sp. S (Yamazaki et al. 2006). Materials are listed in Appendix 1.

Whole DNA was extracted using either a standard phenol-chloroform method or the DNEasy Kit (QIAGEN, Valencia, California). The use of cyt *b* in lamprey systematics presents a series of problems due to the facts that not only is the gene flanked on the 3’ end by a large noncoding region of DNA (Lee and Kocher 1995; Delarbre et al. 2000), but the three petromyzontiform families are also sufficiently divergent to preclude the use of a single set of conserved primers. Preliminary cyt *b* amplifications utilized the conserved primers “cytbL” and “H15149,” and lineage-specific primers were developed from the preliminary sequence data and available mitogenomic information (Table 2). Amplification products of the expected size were purified using a gel extraction kit (QIAGEN, Valencia, California) and sequenced utilizing the same primers as the amplifications, except for “cytbL,” which would have sequenced the noncoding region to the 3’ end of cyt *b*, resulting in single stranded data for the first ~300 bp. Sequence reaction products were visualized using the CEQ 8000 Genetic Analysis System

Table 2. Primer combinations used to generate sequence data.

- Petromyzontiformes
  - CytbL: GTGACTTGAAAAACCACCGTTG
  - H15149: AAACTGCAGCCCCTCAGAATGATATTTGTCCT
- Geotriidae
  - Geotria496L: CTTTTTCCTCTGTAATCCACATCTGCCG
  - Phe1612H: CTTCAGTGCTCTGCTTTAATG
- Mordacia lapicida
  - Mlap348L: ATGAAACGTAGGAGTAATCT
  - 12SLamp: TCACGGGAGTGCGGAAACTTGC
- Mordacia mordax and M. praecox
  - Mord540L: TCGATTCTTTACCTTTCATTTTATTCTTCC
  - 12SLamp: TCACGGGAGTGCGGAAACTTGC
- Petromyzontidae
  - Cytb494L: AGCCTTCTCTTCAGTTATACACATTTGTCG
  - Phe1612H: CTTCAGTGCTCTGCTTTAATG

(Saint Louis University, St. Louis, Missouri) or an ABI 3700 (Auburn University Genomics Laboratory, Auburn, Alabama or Macrogen Inc., Seoul, Republic of Korea).

Sequence files were edited, contigs were assembled, and sequences were aligned by eye using BioEdit. Maximum parsimony analyses were implemented in PAUP*4.0b10 (Swofford 2002), with 100 repetitions of random stepwise addition and TBR branch swapping. *Branchiostoma belcheri* (AJ404477), the Japanese lancelet, and *Myxine glutinosa* (AJ278504), a hagfish, were designated as outgroups for all parsimony analyses, and gaps were treated as a fifth base. Support for nodes was estimated using 1,000 "fast-stepwise" addition replicates of bootstrapping.

MrModelTest 2.2.1 for Classic (Nylander 2004) was used to hypothesize the best-fit model for position-defined partitions of the data set. These models were used in mixed model Bayesian analyses in MrBayes3.1 (Ronquist and Huelsenbeck 2003) that ran for 5,000,000 generations. Priors for these analyses were flat, four chains were utilized, and trees were sampled every 5,000 generations. *Branchiostoma belcheri* was designated as the outgroup in Bayesian analyses. Stationarity of negative log-likelihood values was evaluated by plotting these values against generation, and all trees before the value stabilized were discarded.

Results

The data matrix was truncated to 1,133 characters due to alignment ambiguity past that point. Although minor length variation exists among the outgroups, *Geotria australis*, and all remaining sampled taxa, the sequences are easily aligned by eye and gaps were placed that do not interrupt the reading frame of cyt *b* (positions 7–15 in the outgroups and 13–15 in *G. australis*). All included sequences are complete except that of *Lethenteron* sp. S from GenBank (missing bp 385–1,133). There are 718 variable characters of which 610 are parsimony informative. Parsimony analysis recovered a set of eight trees with a length of 2,393 steps.

MrModelTest suggested a mixture of models for each codon position. The third position approached complete agreement, with all hierarchical likelihood ratio tests (hLRTs) and the Akaike information criterion (AIC), suggesting the general time-reversible model with the gamma shape parameter (GTR+Γ) and all but one LRT adding the invariant sites parameter (GTR+I+Γ), which was used in all Bayesian analyses. The models suggested for the first and second positions, however, were a mixture of the GTR+Γ and the Hasegawa-Kishino-Yano (HKY+G) model. For the first position, GTR+I+Γ was used based on it being suggested by two of four LRTs and the AIC. Although the AIC suggested GTR+Γ for the second position, only a single LRT agreed while the other three suggested HKY+Γ. Initial analyses were run using both GTR+Γ and HKY+Γ as the model for the second position, and because no substantive differences were found between the approaches, HKY+Γ was used in the analysis reported herein. Bayesian analyses resulted in two sets of 1,001 trees, both of which conservatively reached stationarity at 100,000 generations. Fifty-percent majority rule consensus trees were generated for each set using all but the first 21 trees. Support values of at least 0.95/1.0 for Bayesian posterior probability (BPP; the average of two runs, rounded to two decimal points) and 90/100 for parsimony bootstrap (PB) are considered well supported in the following discussion.

Interfamilial Relationships

All analyses recovered the Petromyzontiformes (PB: 95, BPP: 1.0), the Geotriidae (PB: 100, BPP: 1.0), and the Petromyzontidae (PB: 100, BPP: 1.0) as monophyletic clades (Figures 3 and 4). Mordaciidae was recovered in all analyses as two distinct lineages at the base of the tree. Although the node uniting the Australian *Mordacia* (*M. mordax* and *M. praecox*) with Geotriidae and Petromyzontidae is present in all consensus trees, it receives no bootstrap and very low (0.76) posterior probability support, effectively creating an unresolved trichotomy among the Chilean *M. lapicida*, the Australian *Mordacia* (PB: 100, BPP: 1.0), and Geotriidae plus Petromyzontidae (no bootstrap support; BPP: 1.0).

Relationships within Petromyzontidae

Several hypothesized relationships require early mention in order to facilitate further discussion. First, we recover the previously recognized *Eudontomyzon hellenicus* (PB: 100. BPP: 1.0) as the sister species to *Caspiomyzon wagneri* (PB: 100, BPP: 1.0) in all analyses (PB: 97, BPP: 1.0). Second, all analyses recover species of *Lampetra*

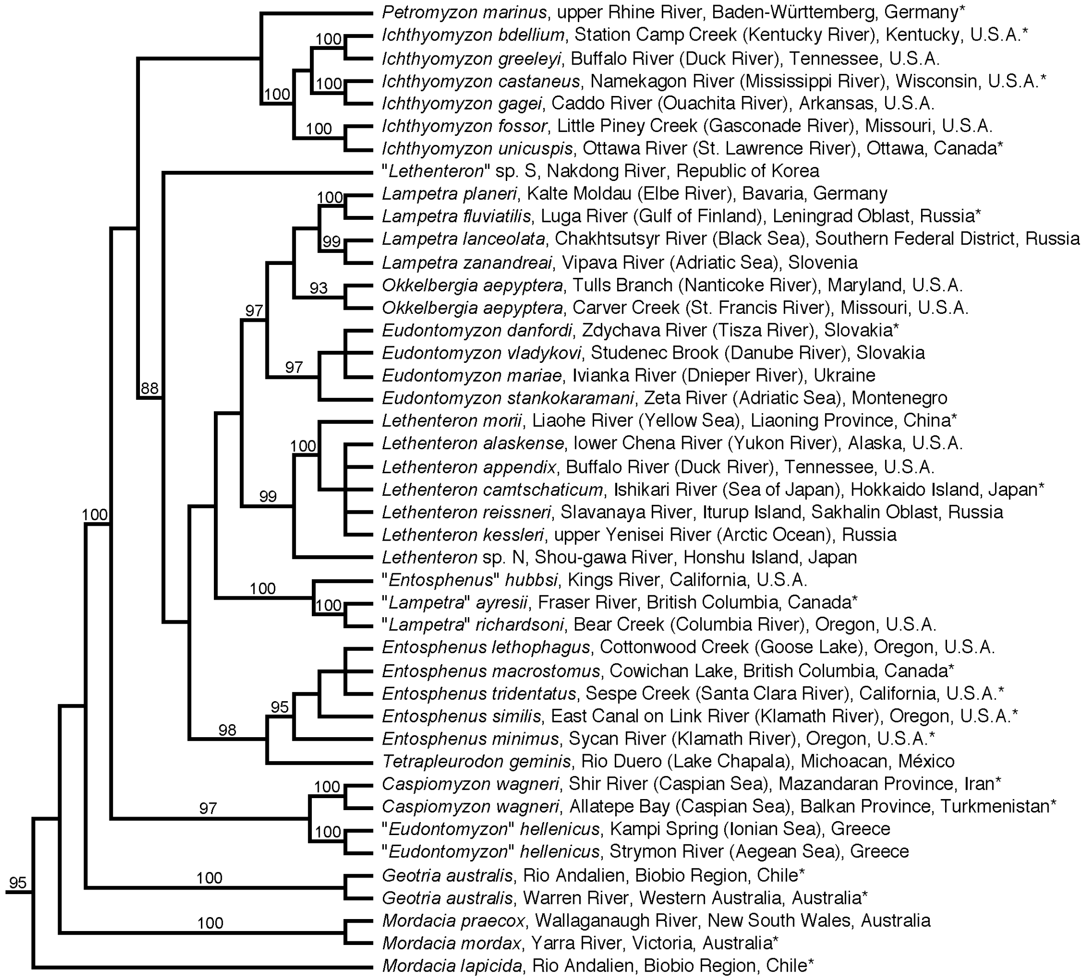

Figure 3. Strict consensus of all equally parsimonious trees recovered in PAUP*. Nodes are labeled with bootstrap values greater than 85. Parasitic species are labeled with an asterisk.

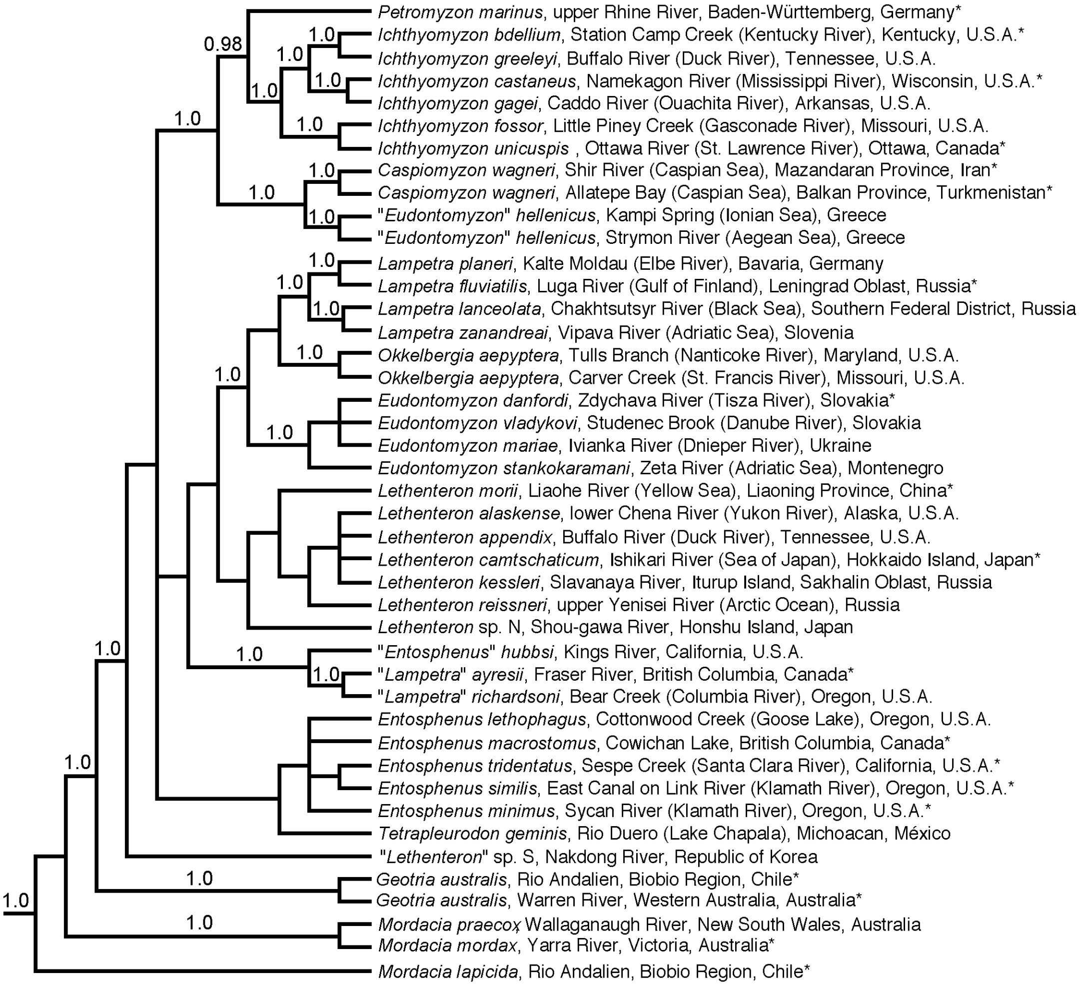

Figure 4. **Majority-rule consensus of postburnin trees resulting from one of the Bayesian analyses. Nodes are labeled with Bayesian posterior probabilities greater than 0.95. Parasitic species are labeled with an asterisk.**

from western North America, *La. ayresii* and *La. richardsoni*, as a clade (PB: 100, BPP: 1.0) that is not only distinct from species of *Lampetra* from both eastern North America and Eurasia, but is also sister to *Entosphenus* hubbsi (PB: 100, BPP: 1.0). Also, *Lethenteron* sp. S of Yamazaki and Goto (1996), although clearly placed within the Petromyzontidae in our analyses, is not well supported as a member of any hypothesized clade. Finally, two species, *Eu. morii* and *Le. zanandreai*, were recovered in genera *Lethenteron* and *Lampetra*, respectively, other than those in which they were placed by Potter and Gill (2003). In cases where a species was recovered within a well-supported clade with an available generic name (e.g., *Eu. morii* in *Lethenteron*), the generic designation is simply changed. For those clades that have no available name (e.g., western North American *Lampetra* plus *En. hubbsi*), previous generic designation are retained in quotation marks.

The relationships within Petromyzontidae differ significantly between the Bayesian and Parsimony analyses due to the collapse of several parsimony-supported nodes in the Bayesian analysis. Those genera represented by multiple samples for which monophyly is supported by all analyses are *Ichthyomyzon* (PB: 100, BPP: 1.0), *Caspiomyzon* (PB: 100, BPP: 1.0), *Eudontomyzon* (PB: 97, BPP: 1.0), and *Okkelbergia* (PB:93, BPP: 1.0). Clades comprised of *Lampetra fluviatilis* plus *La. planeri* (PB: 100, BPP: 1.0) and *La. lanceolata* plus *La. zanandreai* (PB: 99, BPP: 1.0) are recovered as sister clades in all analyses, but only supported as such by the Bayesian analyses (BPP: 1.0). The genera *Entosphenus* (PB: 95) and *Lethenteron* (PB: 99) both fail to find support in the Bayesian analysis. Support for multi-generic clades also differs between analyses, with only a clade inclusive of *Eudontomyzon*, *Lampetra*, and *Okkelbergia* receiving both parsimony and Bayesian support (PB: 97, BPP: 1.0). The parsimony analysis recovers a well-supported clade uniting *Entosphenus* and *Tetrapleurodon* (Entospheninae sensu Vladykov (1972), PB: 98) while the Bayesian analysis supports not only a clade uniting *Ichthyomyzon* and *Petromyzon* (BPP: 0.98), but also a sister relationship between this clade and one composed of *Caspiomyzon* and "*Eu.*" *hellenicus* (Petromyzontinae sensu Vladykov (1972), BPP: 1.0). The only well-supported intrageneric relationships were among species of *Ichthyomyzon* wherein not only were the satellite species pairs of Vladykov and Kott (1979) (*I. bdellium* plus *I. greeleyi* [PB: 100, BPP: 1.0], *I. castaneus* plus *I. gaugei* [PB: 100, BPP: 1.0], and *I. unicuspis* plus *I. fossor* [PB: 100, BPP: 1.0]) all well supported, but a sister clade relationship between the *I. bdellium–I. greeleyi* and *I. castaneus–I. gaugei* pairs was also recovered in both analyses, although only supported by the Bayesian analysis (BPP: 1.0).

Discussion

Our study recovered a reassuringly high number of well-supported clades that correspond to previously hypothesized groupings. All nine of the previously hypothesized petromyzontid lineages were recovered as distinct clades by at least one analytical method and are herein treated as genera. Except for the placement of *Mordacia lapicida*, for which molecular data have not been previously presented, the interfamilial relationships we recovered agree with those of Silver et al. (2004). Although the generic name *Caragola* Gray is available for *M. lapicida*, we choose to retain the species in *Mordacia* based on available morphological data (Gill et al. 2003) and await additional data, perhaps from more slowly evolving genes, before rejecting the monophyly of Mordaciidae.

Given the similarities in their general distribution, the difference in the level of sequence divergence exhibited across the Southern Hemisphere in *Geotria* (<2%) and *Mordacia* (~21%) is intriguing. Differences in life history and habitat, or that of their host species, may have restricted *Mordacia* to a nearshore lifestyle, leaving them intimately tied to the drifting continents while *Geotria* was able to traverse long distances at sea. Indeed, *Geotria* breeds in widespread areas, including Tasmania, the entire southern tip of South America (Chile and Argentina), New Zealand, the Falkland Islands, and both southeastern and southwestern Australia, while breeding Mordacia are restricted to relatively small regions of the southern continents (southeastern Australia, Tasmania, and Chile). *Geotria australis* is also known to make up a large proportion of the diet of nesting albatrosses on South Georgia Island, ~1,400 km ESE of the nearest known breeding grounds in the Falkland Islands, although it is unclear how much of that distance is covered by either prey or predator (Potter et al. 1979).

We cannot explain the failure of the Bayesian analyses to recover *Lethenteron*, *Entosphenus*, and *Entosphenus* plus *Tetrapleurodon* as monophyletic groups at this time. In order to test the effect of outgroup choice on relationships within Petromyzontidae, we removed *Branchiostoma belcheri*, *Myxine glutinosa*, and the species of *Mordacia* from the data set and performed additional analyses using *Geotria* australis as the sole outgroup. These analyses also failed to recover the problematic clades mentioned above. Although there is reason to expect the results of a parsimony analysis to be problematic when using a data set composed of highly divergent lineages (i.e., Long Branch Attraction, Bergsten [2005]), we are aware of no reason to expect a Bayesian analysis to fail to support clades composed of sequences that are less than 0.9% divergent (e.g., *Entosphenus*). While this is an issue that requires further investigation with a larger data set, the number of clades on which the methods agree and the fact that those clades recovered by parsimony analysis alone correspond to previously hypothesized clades make us confident in our results.

Given that all three studies rely on mtDNA, it is not surprising that our results largely agree with those of Docker et al. (1999) and Docker (2006). We also recover a clade comprised of western North American "*Lampetra*" plus "*Entosphenus*" *hubbsi*, remove *Lampetra zanandreai* from *Lethenteron*, and hypothesize that "*Le.*" sp. S is a distinct lineage within Petromyzontidae. These hypotheses are strengthened in our analysis by the inclusion of not only species of *Eudontomyzon* and *Tetrapleurodon*, but also a more complete sequence for *La. zanandreai*. The only major change facilitated by the inclusion of additional species is our recognition of *Okkelbergia* as a distinct genus. Inclusion of species of *Eudontomyzon* in our data set revealed that the well-supported node uniting *O. aepyptera* and *La. fluviatilis* in Docker et al. (1999) actually defines a trichotomy among *Eudontomyzon*, *Lampetra*, and *Okkelbergia*. Although additional data may resolve this node by placing *O. aepyptera* in the genus *Lampetra*, we refute the hypothesis of Vladykov and Kott (1979) that the closest relatives of *O. aepyptera* are found in western North America rather than Europe.

Apart from the sister clade relationship between *Entosphenus* and *Tetrapleurodon* (Entospheninae sensu Vladykov [1972]), and the trichotomous clade composed of *Eudontomyzon*, *Lampetra*, and *Okkelbergia*, there are no intergeneric relationships that are well supported by all of our analyses. Although the Bayesian analyses strongly support recognition of the subfamily Petromyzontinae sensu Vladykov (1972), parsimony analysis does not. Hypotheses regarding relationships among genera and subfamilial designation will require additional data, and current hypotheses should be further tested using data from the nuclear genome.

The changes to the generic designation of *Lampetra zanandreai* and *Lethenteron morii* place both species into genera that seem more plausible than previous designations, given their geographic distribution. For instance, *La. zanandreai* is distributed in drainages of the northern Mediterranean Sea. These and neighboring drainages are home to other species of *Lampetra*, but the nearest species of *Lethenteron* occupy the Arctic Ocean drainage. Similarly, when *Le. morii* was placed in the genus *Eudontomyzon*, it was the only species in the genus east of the Caspian Sea drainage. Other species of *Lethenteron*, however, are widespread across northeast Asia, in drainages surrounding the distribution of *Le. morii*. In the original description of *Lethenteron morii*, Berg (1931) commented on the remarkable nature of such a disjunct distribution for the subgenus *Eudontomyzon* and raised the possibility that the subgenus, as he constructed it, was not monophyletic. The specimen of *Le. morii* in our data set is a metamorphosing individual with developing diagnostic teeth in the lateral fields of the oral disk (exolaterals). It is also from the Liaohe River drainage, immediately west of the Yalu River drainage, which contains the type locality. Although the haplotype sampled from our specimen is distinct within *Lethenteron*, and Berg (1931) commented that ammocoetes he examined from the Liaohe drainage most likely represented *Le. morii*, exolateral teeth are not unknown in other species of *Lethenteron*, the distribution of which in the northern Korean Peninsula and neighboring regions of the People's Republic of China is poorly known. It is therefore possible that our specimen is an individual of a *Le. reissneri*-like species, and the collection of adult *Le. morii* must be a primary goal of future efforts.

It is clear from the results of our study that molecular characters will play an important role in shaping our future views of lamprey systematics. This source of data not only allowed us to include nonparasitic species in our analyses, leading to several novel hypotheses, but also revealed a deep divergence between parasitic species that are indistinguishable using tooth characteristics. Until Vladykov and Follett (1958) separated them based on pigmentation and morphometrics, the western North American "*Lampetra*" *ayresii* and the European *Lampetra fluviatilis* were considered conspecific (Creaser and Hubbs 1922). In general, the satellite species groups of Potter (1968) and Vladykov and Kott (1979) are herein supported. Our analyses indicate, however, that two lineages, *Okkelbergia* and "*Lethenteron*" sp. S, are composed solely of nonparasitic species, and it is possible that better sampled studies of other groups will reveal additional cryptic lineages within nonparasitic species complexes. Although "*Le.*" sp. S is morphologically indistinguishable from *Lethenteron* sp. N, it has been shown to be genetically distinct using both allozyme and mitochondrial sequence data (Yamazaki and Goto 1998; Yamazaki et al. 2006). Unfortunately, we were unable to obtain tissue of this species, and its inclusion in our data set is based on a partial sequence. The placement of this species within Petromyzontidae must be explored using additional sources of data in the future. Finally, the well-supported sister species relationship hypothesized between *Caspiomyzon wagneri* and "*Eudontomyzon*" *hellenicus* is a very exciting result of our analyses. This hypothesis not only aligns a nonparasitic species with *C. wagneri* for the first time, leaving *Geotria* and *Petromyzon* as the only strictly parasitic lineages, but also moves "*Eu.*" hellenicus across the subfamilial/tribal divide proposed by Vladykov (1972) and Bailey (1980). Although it is possible to collect samples of *C. wagneri* from additional portions of its range (e.g., the Volga River drainage), there are no additional known localities for "*Eu.*" *hellenicus*. Testing of this hypothesis will have to rely on characters from the nuclear genome and/or a reappraisal of morphological characters in these two species.

While our study suggests that more morphological characters may be homoplasious within lampreys than previously hypothesized, our failure to resolve well-supported intergeneric clades precludes us from truly testing the hypothesis of Gill et al. (2003). It is our intention for this, the most comprehensive study on lamprey systematics to date, to be a starting point from which future questions of lamprey systematics can be effectively pursued.

Acknowledgments

This study was funded in part by NSF 89507 and 296162 awarded to RLM and KJR (CO-PI's) and ICP, HSG, and CBR (International Collaborators). Specimens, tissues and/or field assistance were provided by the following people: A. Abdoli, J. A. Anderson, S. A. Barndt, R. E. Blanton, H. T. Boschung, J. S. Boyce, B. M. Burr, B. W. Coad, A. L. George, J. E. Gersmehl, F. B. Gutermuth, S. He, A. J. Henry, D. B. Henry, J. Holčík, A. P. Kinziger, T. Lambert, T. J. Landewe, B. A. Levin, J. Lyons, E. L. Macdonald, D. F. Markle, D. L. Morgan, L. Pekárik, J. S. Peyton, T. W. Pietsch, S. L. Powers, J. M. Ray, S. R. Reid, R. Šanda, J. M. Serb, A. M. Simons, D. E. Stevenson, J. F. Switzer, L. E. Thompson, J. R. Tomelleri, D. Turan, R. M. Wood, X-C. Wu, Y. Yamazaki, and M. Zontág.

References

Bailey, R. M. 1980. Comments on the classification and nomenclature of lampreys: an alternative view. Canadian Journal of Fisheries and Aquatic Sciences 37:1626–1629.

Berg, L. S. 1931. A review of the lampreys of the northern hemisphere. Annuaire du Musée Zoologique de l'Académie des Sciences de l'URSS 32:87–116.

Bergsten, J. 2005. A review of long-branch attraction. Cladistics 21:163–193.

Creaser, C. W. and C. L. Hubbs. 1922. A revision of the holarctic lampreys. Occasional Papers of the Museum of Zoology University of Michigan 120:1–14.

Delarbre, C., H. Escriva, C. Gallut, V. Barriel, P. Kourilsky, P. Janvier, V. Laudet, and G. Gachelin. 2000. The complete nucleotide sequence of the mitochondrial DNA of the agnathan *Lam-*

petra fluviatilis: bearings on the phylogeny of cyclostomes. Molecular Biology and Evolution 17:519–529.

Docker, M. F. 2006. Bill Beamish's contributions to lamprey research and recent advances in the field. Guelph Ichthyology Reviews 7:1–52.

Docker, M. F., J. H. Youson, R. J. Beamish, and R. H. Devlin. 1999. Phylogeny of the lamprey genus *Lampetra* inferred from mitochondrial cytochrome *b* and ND3 gene sequences. Canadian Journal of Fisheries and Aquatic Sciences 56:2340–2349.

Gill, H. S., C. B. Renaud, F. Chapleau, R. L. Mayden, and I. C. Potter. 2003. Phylogeny of living parasitic lampreys (Petromyzontiformes) based on morphological data. Copeia 2003:687–703.

Hardisty, M. W., and I. C. Potter. 1971a. The behaviour, ecology and growth of larval lampreys. Pages 85–125 *in* M. W. Hardisty and I. C. Potter, editors. The biology of lampreys, volume 1. Academic Press, New York.

Hardisty, M. W., and I. C. Potter. 1971b. The general biology of adult lampreys. Pages 127–206 *in* M. W. Hardisty and I. C. Potter, editors. The biology of lampreys, volume 1. Academic Press, New York.

Hardisty, M. W., and I. C. Potter. 1971c. Paired species. Pages 249–277 *in* M. W. Hardisty and I. C. Potter, editors. The biology of lampreys, volume 1. Academic Press, New York.

Holčík, J., and V. Šorić. 2004. Redescription of *Eudontomyzon stankokaramani* (Petromyzontes, Petromyzontidae): a little known lamprey from the Drin River drainage, Adriatic Sea basin. Folia Zoologica 53:399–410.

Hubbs, C. L., and I. C. Potter. 1971. Distribution, phylogeny and taxonomy. Pages 1–65 *in* M. W. Hardisty and I. C. Potter, editors. The biology of lampreys, volume 1. Academic Press, New York.

Hubbs, C. L., and M. B. Trautman. 1937. A revision of the lamprey genus *Ichthyomyzon*. Miscellaneous Publications of the Museum of Zoology University of Michigan 35:7–109.

Kottelat, M., and J. Freyhof. 2007. Handbook of European freshwater fishes. Kottelat, Cornol, Switzerland and Freyhof, Berlin, Germany.

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

Meyer, A., and R. Zardoya. 2003. Recent advances in the (molecular) phylogeny of vertebrates. Annual Review of Ecology Evolution and Systematics 34:311–338.

Nelson, J. S. 2006. Fishes of the world, 4th edition. Wiley, Hoboken, New Jersey.

Nylander, J. A. A., 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Norbyvägen 18, SE-752 36 Uppsala, Sweden.

Potter, I. C. 1968. Mordacia praecox n. sp., a nonparasitic lamprey (Petromyzonidae), from New South Wales, Australia. Proceedings of the Linnean Society of New South Wales 92:254–261.

Potter, I. C., and H. S. Gill. 2003. Adaptive radiation of lampreys. Journal of Great Lakes Research 29 (Supplement 1):95–112.

Potter, I. C., P. A. Prince, and J. P. Croxall. 1979. Data on the adult marine and migratory phase in the life of the Southern Hemisphere lamprey, *Geotria australis* Gray Environmental Biology of Fishes 4:65–69.

Rodríguez-Muñoz, R., J. R. Waldman, C. Grunwald, N. K. Roy, and I. Wirgin. 2004. Absence of shared mitochondrial DNA haplotypes between sea lamprey from North American and Spanish rivers. Journal of Fish Biology 64:783–787.

Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Silver, M. R., H. Kawauchi, M. Nozaki, and S. A. Sower. 2004. Cloning and analysis of the lamprey GnRH-III cDNA from eight species of lamprey representing the three families of Petromyzontiformes. General and Comparative Endocrinology 139:85–94.

Swofford, D. L. 2002. PAUP*. phylogenetic analysis using parsimony (* and other methods) 4.0. Sinauer Associates, Sunderland, Massachusetts.

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

Vladykov, V. D. 1972. Sous-division en trois sous-familles des lamproies de l`hémisphère nord de la famille Petromyzonidae. Association canadienne-française pour l`avancement des sciences 39:148.

Vladykov, V. D., and W. I. Follett. 1958. Redescription of Lampetra ayresii (Günther) of western North America, a species of lamprey (Petromyzontidae) distinct from *Lampetra fluviatilis* (Linnaeus) of Europe. The Journal of the Fisheries Research Board of Canada 15:47–77.

Vladykov, V. D., and E. Kott. 1976. Is Okkelbergia Creaser and Hubbs, 1922 (Petromyzonidae) a distinct taxon? Canadian Journal of Zoology 54:421–425.

Vladykov, V. D., and E. Kott. 1979. Satellite species among the holarctic lampreys (Petromyzonidae). Canadian Journal of Zoology 57:860–867.

Yamazaki, Y., and A. Goto. 1996. Genetic differentiation of *Lethenteron reissneri* populations, with reference to the existence of discrete taxonomic entities. Ichthyological Research 43:283–299.

Yamazaki, Y., and A. Goto. 1998. Genetic structure and differentiation of four *Lethenteron* taxa from the Far East, deduced from allozyme analysis. Environmental Biology of Fishes 52:149–161.

Yamazaki, Y., R. Yokoyama, M. Nishida, and A. Goto. 2006. Taxonomy and molecular phylogeny of *Lethenteron* lampreys in eastern Eurasia. Journal of Fish Biology 68 (Supplement B):251–269.

Zanandrea, G. 1958. Posizione sistematica e distribuzione geografica di *Lampetra zanandreai* Vladykov. Memorie del Museo Civico di Storia Naturale di Verona 6:207–237.

Appendix 1

Materials examined. Institutional abbreviations are as follows: CMNFI—Canadian Museum of Nature Fish Collection, Ottawa, Ontario, Canada; OSU—Oregon State University Fish Collection, Corvallis, Oregon, USA; STL—Tissue Collection of Saint Louis University, St. Louis, Missouri, USA; UAIC—University of Alabama Ichthyological Collection, Tuscaloosa, Alabama, USA. Genbank accession numbers are listed in parentheses.

Geotriidae

Geotria australis: STL 1260.01, Rio Andalien, Biobio Region, Chile (GQ206164); UAIC 11977.01, Warren River, Western Australia, Australia (GQ206165).

Mordaciidae

Mordacia lapicida: STL 1260.01, Rio Andalien, Biobio Region, Chile (GQ206185)

Mordacia mordax: UAIC 15569.01, Yarra River, Victoria, Australia (GQ206188).

Mordacia praecox: UAIC 15570.01, Wallaganaugh River, New South Wales, Australia (GQ206186).

Petromyzontidae

Caspiomyzon wagneri: STL 801.01, Allatepe Bay (Caspian Sea), Balkan Province, Turkmenistan (GQ206152); UAIC 12933.01, Shir River, Mazandaran Province, Iran (GQ206151).

“*Entosphenus*” *hubbsi*: UAIC 11547.01, Kings River, California, USA (GQ206150).

Entosphenus lethophagus: UAIC 15571.01, Cottonwood Creek, Oregon, USA (GQ206153).

Entosphenus macrostomus: UAIC 15572.01, Cowichan Lake, Vancouver Island, British Columbia, Canada (GQ206154).

Enstosphenus minimus: UAIC 12934.01, Sycan River, Oregon, USA (GQ206155).

Entosphenus similis: UAIC 12935.01, East Canal on Link River, Oregon, USA (GQ206156).

Entosphenus tridentatus: UAIC 11044.01, Sespe Creek, California, USA (GQ206157).

“*Eudontomyzon*” *hellenicus*: STL 1253.01, Kampi Spring, Greece (GQ206159);UAIC 15573.01, Strymon River, Greece (GQ206160).

Eudontomyzon danfordi: CMNFI 2008-0002, Zdychava River, Slovakia (GQ206158).

Eudontomyzon mariae: STL 957.01, Ivianka River, Ukraine (GQ206162).

Eudontomyzon stankokaramani: STL 1331.01, Zeta River, Montenegro (GQ206189).

Eudontomyzon vladykovi: CMNFI 2002-0009, Studenec Brook, Slovakia (GQ206161).

Ichthyomyzon bdellium: UAIC 12459.01, Station Camp Creek, Kentucky, USA (GQ206166).

Ichthyomyzon castaneus: UAIC 11978.01, Namekagon River, Wisconsin, USA (GQ206168).

Ichthyomyzon fossor: UAIC 11156.01, Little Piney Creek, Missouri, USA (GQ206170).

Ichthyomyzon gagei: UAIC 10065.01, Caddo River, Arkansas, USA (GQ206169).

Ichthyomyzon greeleyi: UAIC 10601.06, Buffalo River, Tennessee, USA (GQ206167).

Ichthyomyzon unicuspis: CMNFI 2008-0004, Ottawa River, Ontario, Canada (GQ206171).

“*Lampetra*” *ayresii*: UAIC 15574.01, Fraser River, British Columbia, Canada (GQ206174).

“*Lampetra*” *richardsoni*: OSU 15388, Bear Creek, Oregon, USA (GQ206177).

Lampetra fluviatilis: STL 982.01, Luga River, Leningrad Oblast, Russia (GQ206175).

Lampetra lanceolata: STL 958.01, Chakhtsutsyr River, Southern Federal District, Russia (GQ206176).

Lampetra planeri: STL 1118.01, Kalte Moldau, Bavaria, Germany (GQ206149).

Lampetra zanandreai: STL 1252.01, Vipava River, Slovenia (GQ206184).

“*Lethenteron*” sp. S: Naktong River, Republic of Korea (AB220183).

Lethenteron alaskense: CMNFI 1999-0046, lower Chena River, Alaska, USA (GQ206178).

Lethenteron appendix: UAIC 10655.01, Buffalo River, Tennessee, USA (GQ206179).

Lethenteron camtschaticum: UAIC 11987.01, Ishikari River, Hokkaido Island, Japan (GQ206180).

Lethenteron kessleri: STL 965.01, upper Yenisei River, Russia (GQ206183).

Lethenteron morii: CMNFI 2001-0042, Liaohe River, Liaoning Province, China (GQ206163).

Lethenteron reissneri: UAIC 12326.01, Slavanaya River, Iturup Island, Russia (GQ206181).

Lethenteron sp. N: STL 1333.01, Shou-gawa River, Honshu Island, Japan (GQ206182).

Okkelbergia aepyptera: STL 613.01, Tulls Branch, Maryland, USA (GQ206172); uncatalogued, Carver Creek, Missouri, USA (GQ206173).

Petromyzon marinus: STL 1116.01, upper Rhine River, Baden-Württemberg, Germany (GQ206148).

Tetrapleurodon geminis: UAIC 15575.01, Rio Duero, Michoacan, México (GQ206187).