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MITOGENOME ANNOUNCEMENT

Complete mitochondrial genomes of the anadromous and resident forms of the lamprey *Lethenteron camtschaticum*Evgeniy S. Balakirev^{1,2}, Valery A. Parensky², and Francisco J. Ayala¹¹Department of Ecology and Evolutionary Biology, University of California, Irvine, CA, USA and ²A. V. Zhirmunsky Institute of Marine Biology, Far Eastern Branch, Russian Academy of Science, Vladivostok, Russia

Abstract

The complete mitochondrial genomes were sequenced in anadromous and resident forms of the lamprey *Lethenteron camtschaticum*. The sizes of the genomes in the two isolates are 16,245 and 16,295 bp. The gene arrangement, base composition, and size of the two sequenced genomes are similar to the lamprey genomes previously published. The total sequence divergence between the two genomes is very low (0.14%), supporting conspecificity of the anadromous and resident forms of *L. camtschaticum*. Comparison of the genomes sequenced in the present work with other genomes of lampreys available in GenBank, reveals two distinct evolutionary lineages with a genera level of divergence among the lampreys of eastern Eurasia.

Keywords

Anadromous and resident forms, complete mitochondrial genome, *Lampetra*, *lethenteron*, *lethenteron camtschaticum*, northern hemisphere lampreys

History

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Using a mitochondrial (mt) DNA marker (the *COI* gene), Artamonova et al. (2011) showed that the parasitic anadromous lamprey *Lethenteron camtschaticum* Tilesius and the non-parasitic resident lamprey *L. reissneri* Dybowski belong to the same species (*L. camtschaticum*). Similar conclusions were reached previously with two other sets of “paired” (Zanandrea, 1959) or “satellite” (Vladykov & Kott, 1979) species, European river (*Lampetra fluviatilis*) and brook (*Lampetra planeri*) lampreys (Espanhol et al., 2007) and American parasitic silver lamprey, *Ichthyomyzon unicuspis* and non-parasitic northern brook lamprey, *I. fossor* (Docker et al., 2012).

We have sequenced two complete mitochondrial genomes of the parasitic, anadromous and non-parasitic, freshwater resident forms of *Lethenteron camtschaticum*, collected from the Tugur river (Amur river estuary, Russia; parasitic anadromous form; isolate LC3; GenBank accession number KJ866208) and Azabachye lake creek (Kamchatka, Russia; freshwater resident form; isolate MA1; GenBank accession number KJ866209). The primers were designed with the program mitoPrimer_V1 (Yang et al., 2011). The sizes of the mt genomes were slightly different, 16,245 and 16,295, in the two isolates (LC3 and MA1), due to a variable number of repeat sequences within the control region (Okada et al., 2010). The A+T base composition, 61.5% was higher than G+C, 38.5%. There were 23 single nucleotide and four length differences (three of them are within the control region) between the LC3 and MA1 haplotypes; total sequence divergence (D_{xy}) was very low, 0.0014 ± 0.0003 , consistent with the intraspecific level of divergence in fishes (e.g. Ward et al., 2005). We have previously detected very similar levels of intraspecific divergence between complete mt genomes in

Siberian taimen *Hucho taimen* ($D_{xy} = 0.0015 \pm 0.0003$; Balakirev et al., 2014c) and Sakhalin taimen *Parahucho perryi* ($D_{xy} = 0.0015 \pm 0.0003$; Balakirev et al., 2014a). The level of intraspecific difference was somewhat higher for the blunt-snouted lenok *Brachymystax tumensis* ($D_{xy} = 0.0051 \pm 0.0006$; Balakirev et al., 2014b). Thus, our results support the conclusion of Artamonova et al. (2011) that the anadromous *L. camtschaticum* and resident *L. reissneri* belong to the same species.

The comparison of mt genomes now obtained with other complete lamprey mt genomes available in GenBank reveals close affinity between our specimens (LC3 and MA1) and the genome of *L. japonicum* (KF701113; Kawai et al., 2014; Figure 1). The level of divergence between the genomes inferred from 12 protein-coding genes is low, 0.14%. Consequently, this group (“Lineage 1”, Figure 1) of three genomes belongs to the same lamprey species. According to Kottelat (1997), *L. japonicum* (Martens) is a junior synonym of *L. camtschaticum*. Another group of sequences (“Lineage 2”, Figure 1) includes genomes AB565771 (Okada et al., 2010), KC353466 (Hwang et al., 2013a), and KC353468 (Hwang et al., 2013b). The level of divergence between the sequences within lineage 2 is higher than within lineage 1, but still relatively low, 1.92–2.02%, indicating conspecificity within the lineage. The level of divergence between lineages 1 and 2 is much higher, 11.17 ± 0.33 , than the level of divergence within the lineages.

We have calculated the average level of divergence (D_{xy}) between species from different lamprey genera within the family Petromyzonidae (the Northern Hemisphere lampreys) showing no sign of paraphyly or polyphyly (*Ichthyomyzon*, *Petromyzon*, *Entosphenus*, *Lampetra*, *Caspiomyzon*, and *Eudontomyzon*). For the *COI* gene the average distance (D_{xy}) is 9.64 ± 0.71 and for the *CytB* gene the distance is 9.71 ± 0.48 . Thus, the level of divergence between lineages 1 and 2 (11.17 ± 0.33) fits the value of divergence between different genera within the family Petromyzonidae. The difference between lineage 1 and

Figure 1. Neighbor-joining tree of lampreys based on 12 protein coding genes (10,886 bp). For the tree reconstruction we used Tamura-Nei + gamma model of substitution. Numbers at the nodes are bootstrap percent support values based on 10,000 replications. The GenBank species (or genera) names are in parentheses.

Lampetra fluviatilis (Y18683; Delarbre et al., 2000) (7.95 ± 0.27) and difference between lineage 2 and *L. fluviatilis* (10.60 ± 0.31) is less than the difference between the lineages 1 and 2 (11.17 ± 0.33). Thus, there is less divergence between European and eastern Eurasian lampreys than between two lineages of eastern Eurasian lampreys. The lineages 1 and 2 are closer to *Lampetra fluviatilis* than to each other. This affinity could not be explained by introgression, which is not revealed in any of the genomes analyzed here (data not shown). This pattern could suggest three separate evolutionary lineages of supraspecific (genera) level. The results are surprising because both lineages (1 and 2) include the eastern Eurasian lampreys and they are usually considered as members of the genus *Lethenteron*. Hwang et al. (2013a) and Hwang et al. (2013b) use the genera name “*Lampetra*” in the publications, but “*Lethenteron*” in GenBank (KC353466 and KC353468; Figure 1).

Thus, the data obtained clearly show some ambiguities in the relationships among the lampreys from eastern Eurasia belonging to the *Lethenteron* and *Lampetra* genera; a highly debated and long lasting question (Potter, 1980; Vladykov & Kott, 1979; Yamazaki et al., 2006). The mt genome data revealed two distinct evolutionary lineages a genera level of divergence among the lampreys of eastern Eurasia. The described scenario is supported by the *COI* and *CytB* genes from GenBank (data not shown) where the lineage 1 and 2 are separated by other lampreys with the rank of genera (*Entosphenus*). However, more data from the nuclear genomes are necessary to draw a definite picture of the composition and relationships between the lampreys in eastern Eurasia.

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Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper. This study was supported by Bren Professor Funds at the University of California Irvine to Francisco J. Ayala and Evgeniy S. Balakirev.

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