

Evolutionary History of the Extinct Otter Lived in Japanese Islands

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Abstract

Although the river otter (Lutrinae) of Japan was distributed in four main Japanese islands until the 1920s, this animal has not been observed in the wild since 1979. Additionally, the taxonomic status of this otter remains controversial. Previous morphological and molecular genetics studies have suggested that the Japanese otter from the Honshu and Shikoku islands is an independent species of the genus *Lutra*, namely, *Lutra nippon*. However, there are pros and cons about this classification. In a recent study, our group determined the mitochondrial genome sequence of the two Japanese otter museum specimens, using next-generation sequencing technology, and evaluated the phylogenetic status of these specimens in the clade of Lutrinae. We suggested that the Japanese otter is of the genus *Lutra* and that two genetically divergent lineages exist among the Japanese otters living on Honshu and Shikoku. One of the lineages, which diverged from the ancestor of *L. lutra* at 1.27 million years ago, it should be treated either as the independent species *L. nippon* or as an independent subspecies of the Eurasian otter *Lutra lutra nippon*. The other lineage, which diverged from the ancestor of the Chinese population of *L. lutra* at 0.10 million years ago, was identified as *L. lutra*. However, in our previous study, we had analyzed genetic material from only one individual in each lineage. Therefore, our results cannot conclusively illustrate the natural history of the Japanese otter. Hence, future studies should use more than one individual to evaluate the genetic divergence of population of the Japanese otter.

Keywords: Lutrinae; Otter; *Lutra*; Japanese otter; Eurasian otter; Extinct animal; Molecular phylogenetics; Systematics; Natural history of Japanese mammal

Abbreviations: IUCN: International Union for Conservation of Nature; Ma: Mega Annum; mtgenome: Mitochondrial Genome; NGS: High-throughput “next-generation” DNA Sequencing; MIS: Marine Isotope Stage

Introduction

The Japanese otter, one of the semi-aquatic carnivores that lived in the rivers of Japan, was widely distributed in Hokkaido, Honshu, Shikoku and Kyushu until the 1920s (Figure 1) [1]. However, because of overhunting for fur as well as riverine environmental change associated with economic development in the postwar period, the otter population in Japan gradually decreased [2]. Since 1979, when an individual was last documented in Shikoku, no evidence of this species has been reported. In 2012, the Ministry of the Environment declared the Japanese otter to be extinct. The Japanese otter is just nominal designation for otters living in Japanese islands, taxonomic status of this species is now under debate. Gray [3] first classified Japanese otters from Hokkaido as *Lutronektes whiteleyi*. Imaizumi [4] then reclassified the species as *Lutra lutra whiteleyi*, using a new specimen from Hokkaido. In 1989, Imaizumi and Yoshiyuki [5] conducted a comparative morphological study, using 15 Japanese otter specimens (13 individuals from Honshu and Shikoku; 2, from Hokkaido). They concluded that the morphological characteristics of otters from Honshu and Shikoku clearly differed from the species *L. lutra*; therefore, these otters should be classified as a distinct species, namely, *Lutra nippon*. In contrast, they proposed that the otters from Hokkaido should be classified as a distinct subspecies of the Eurasian otter *L. l. whiteleyi*. Additionally, the taxonomic status of the Japanese otters from Honshu and Shikoku as *L. nippon* was supported by the osteometric analysis of seven otter skulls from the Shikoku individuals [6].

Suzuki et al. [7] determined the partial mitochondrial DNA cytochrome *b* (*cytb*) gene sequence (224 bp) from a mummy-like specimen of a Japanese otter from Shikoku. In their study, they

amplified partial *cytb* gene by PCR from total DNA, and sub cloned the products to obtain single amplicons, and sequenced several clones as candidates of *cytb* gene by Sanger method. They reconstructed the molecular phylogenetic tree of Asian otters, using one individual of *L. l. lutra* from Latvia; one of *L. l. chinensis* from the Sichuan province, China; two of *L. lutra* (unknown subspecies and locality) (Waku et al. [8] estimated the locality of these samples of Eurasian otter); and one of the Asian small-clawed otter *Aonyx cinerea*. The phylogenetic tree showed that the Japanese otter diverged genetically from the clade of *L. lutra* and formed a distinct lineage in the clade of *Lutra*. However, the gene sequence they used in their study was only 224 bp long, and their analysis did not include the other two species of Asian otters, namely, the hairy-nosed otter *Lutra sumatrana* and the smooth-coated otter *Lutrogale perspicillata*.

All these studies suggest that the Japanese otter from Honshu and Shikoku could be an independent species, *L. nippon*, because it diverged genetically and morphologically from *L. lutra*, and the book Mammal Species of the World introduced this classification for the Japanese otter [9]. However, the International Unions for Conservation of Nature (IUCN) Red List of Threatened Species treats this species name (*L. nippon*) as a synonym for the Eurasian otter (*L. lutra*) pending further review [2]. In this review, we focus on the subject of systematics of the Japanese otter from the view point of phylogenetic status and natural history of this species.

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Figure 1: Map showing the Japanese islands (Hokkaido, Honshu, Shikoku and Kyushu), Sakhalin and the Eurasian continent.



Figure 2: Stuffed specimen of Japanese otter captured in the Kochi prefecture in 1977.

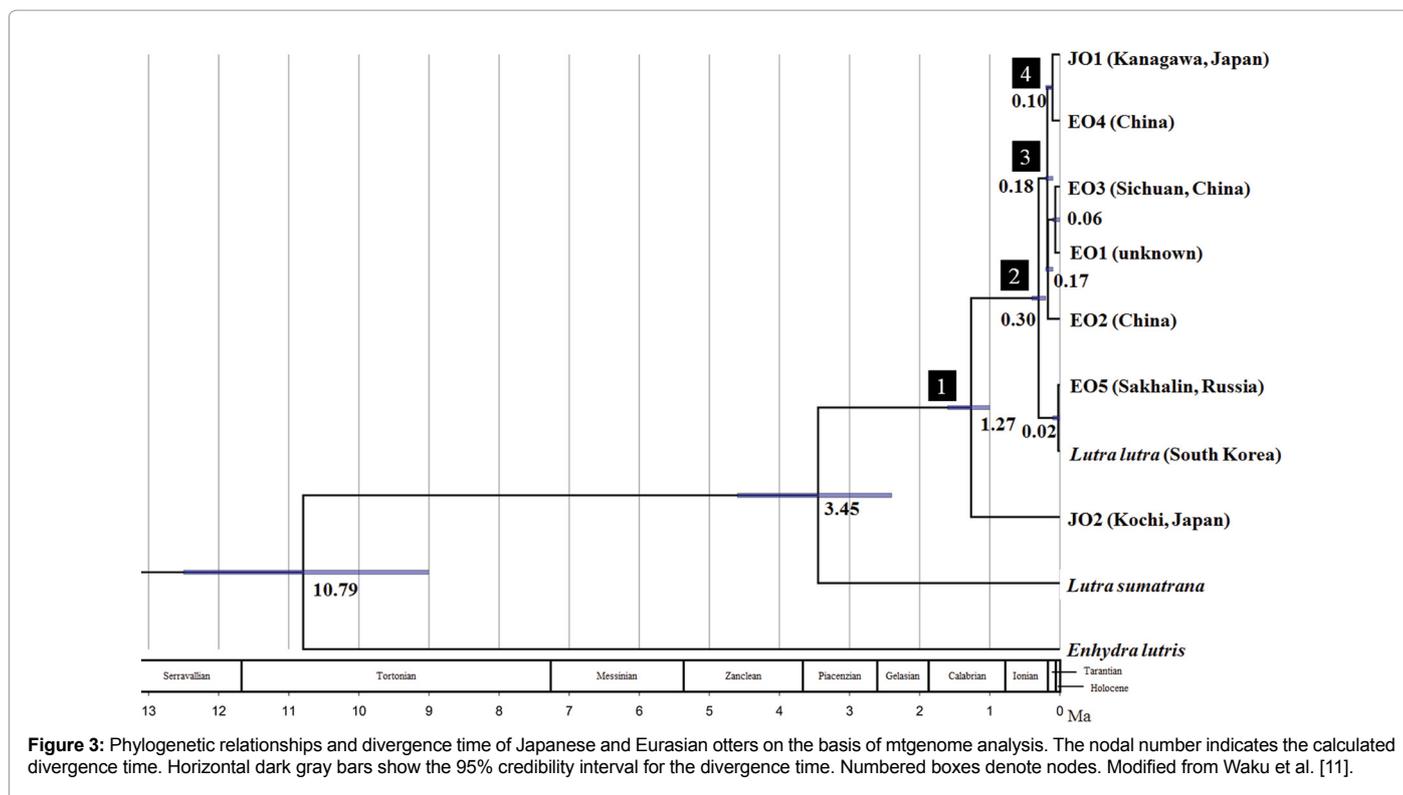
Technological Innovation Paved the Way for New Research

High-throughput, “next-generation” DNA sequencing (NGS) was developed after 2000, making it possible for researchers to determine DNA sequences from old specimens (i.e., ancient DNA). NGS technology provided a new avenue for the genetic analysis of extinct organisms. Recently, our group extracted the DNA samples from small amounts of sample tissue, which remained on the museum specimens of two Japanese otter individuals. One of the individuals was captured in the Kanagawa prefecture in 1915 or 1916 (JO1) and the other was captured in the Kochi prefecture in 1977 (JO2; Figure 2). Subsequently, we used NGS to determine the mitochondrial genome (mtgenome) sequence (approximately 16,400 bp) of our samples [10]. Recently, various genetic studies for estimating the evolution of extinct organisms have been reported; however, these results were obtained from a limited number of study groups [11-13]. It suggests that such study requires a high level of experimental skill.

Molecular Phylogenetic Status of the Japanese Otter

JO1 formed a monophyletic group with six individuals of *L. lutra*, indicating that the JO1 lineage evolved from a common ancestor of *L. lutra* (Figure 3, node 2) [10]. In particular, the phylogenetic tree suggests

that JO1 lineage has a close genetic relationship to Chinese populations of *L. lutra*, and JO1 was identical to the species of *L. lutra*. In contrast, JO2 showed a distinct and independent lineage, which diverged from the monophyletic group of *L. lutra* and JO1 (Figure 3, node 1) [10]. This result suggests that the JO2 lineage should be treated as an independent species (*L. nippon*) or subspecies (*L. l. nippon*). Waku et al. [10] suggested that the Japanese otter was divided into Honshu and Shikoku lineages based on a molecular phylogenetic analysis. The molecular divergence time, which is estimated based on mtgenome sequences, indicates the start of a unique evolutionary history of each lineage. These unique evolutionary histories mark the end of gene flow between one lineage and another. In the course of time, each lineage evolves to a different species. JO2 diverged from the ancestral lineage of *L. lutra*, which includes an ancestor of JO1 and the divergence time was calculated at approximately 1.27 Mega annum (Ma) during the Calabrian stage (1.80-0.78 Ma) of the Early Pleistocene (Figure 3, node1) [10]. During this stage, Japanese islands and the Eurasian continent were connected by a land bridge, the presence of which is supported by geological study [14]. Fossil records suggest that the steppe mammoth (*Mammuthus trogontherii*) migrated from the continent to the Japanese islands via the land bridge at approximately 1.2 Ma [15]. Therefore, the ancestral population of the JO2 lineage could also have migrated to the Japanese islands via the land bridge, and then evolved into a unique species. The divergence time of JO1, however, was calculated at approximately 0.10 Ma (Figure 3, node 4), at which point the ancestor of JO1 diverged from the ancestor of the Chinese otter (EO4) and migrated to the Japanese islands [10]. The moose (*Alces alces*) migrated from the continent to Honshu island via Hokkaido island during the glacial Marine Isotope Stage (MIS) 4 (approximately 71 thousand years ago) [16]. However, there is no evidence that a land bridge was present between the continent and the Japanese islands during this stage. Therefore, we also considered the possibility that the JO1 individual was artificially transported from the continent to Japan in modern times, because JO1 was captured in Jogashima, Kanagawa prefecture, where a flourishing



base of deep-sea fishing had existed. It is an undeniable possibility, therefore, that humans imported the JO1 individual from the continent via deep-sea fishing vessels.

Future Prospects for the Study of the Japanese Otter

Currently, the mtgenome analysis suggests the presence of two lineages in the population of Japanese otters from Honshu and Shikoku [10]. However, given that our previous study analyzed only one individual from each lineage, there remains room for verification. It is hoped that the evolutionary history of this animal will be resolved by molecular phylogenetic studies that analyze more than one individual from each population (Hokkaido, Honshu, Shikoku, and Kyushu), and evaluate the genetic divergence of the Japanese otter at the population level.

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