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Short communication

Low genetic diversity in *Ozobranchus jantseanus* (Hirudinida: Ozobranchidae) in Japan: Possibility of introduction with their host turtles

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ABSTRACT

Leeches belonging to the genus *Ozobranchus* are ectoparasitic on sea and freshwater turtles. The freshwater species *O. jantseanus* has been recorded from China and Japan. *Ozobranchus jantseanus* inhabiting Japan is considered to be a non-indigenous species, because their primary host, the Reeves' pond turtle, *Mauremys reevesii*, is thought to have been introduced in the last few centuries from adjacent Asian countries. To assess whether the Japanese populations of *O. jantseanus* were likely to have been introduced, their genetic diversity was investigated using mitochondrial cytochrome *c* oxidase subunit I sequences. The very low sequence diversity as well as the historical record of this species from Japan suggest that Japanese populations of *O. jantseanus* may have been artificially introduced along with their host turtles. Molecular phylogenies of COI showed that two marine *Ozobranchus* species formed a clade together with the freshwater *O. jantseanus*.

The proboscidate leech genus *Ozobranchus* de Quatrefages, 1852 includes chelonian-specific ectoparasites, and comprises seven species [1]. Two of them, *O. branchiatus* (Menzies, 1791) and *O. margoi* (Apáthy, 1890), are parasites of sea turtles; four species, *O. jantseanus* Oka, 1912, *O. shipleyi* Harding, 1909, *O. papillatus* Kaburaki, 1921 and *O. polybranchus* Sanjeeva Raj, 1951, are indigenous to the Asian Region, and specific to freshwater turtles [2,3]. *Ozobranchus quatrefagesi* (Poirier and Rochebrune, 1884) from West Africa has been reported to infest crocodiles [4], but its systematic status is unclear.

Ozobranchus jantseanus is a freshwater species, and has been reported only from China and Japan [5,6]. It was originally described based on a specimen collected from Wuchang (Wuhan District), China [7]. Chinese individuals are known to infest Reeves' pond turtle, *Mauremys reevesii* (Gray, 1831) [5]. The primary host for the Japanese populations of *O. jantseanus* is also *M. reevesii*; but it has also rarely been recorded from the endemic Japanese pond turtle, *M. japonica* (Temminck and Schlegel, 1838), and from the red-eared slider, *Trachemys scripta elegans* (Wied, 1839), native to the southern United States [6,8,9].

When discussing *O. margoi*, Nishimura [10] briefly mentioned the possibility that *O. jantseanus* inhabiting Japan might be an introduced species, and this has been repeated in other publications [6,8]. Suzuki et al. [11] showed that Japanese populations of *M. reevesii*, the primary host of *O. jantseanus*, were introduced from China and Korea. Accordingly, if they were introduced, *O. jantseanus* leeches inhabiting Japan would be parasitic primarily on the introduced *M. reevesii*, rather than the native *M. japonica*. To assess whether the Japanese populations of *O. jantseanus* had been introduced with their primary host or not, we analyzed the diversity of cytochrome *c* oxidase subunit I (COI) sequences, from *O. jantseanus* samples collected from various localities in Japan.

Representing the distribution of *O. jantseanus* in Japan, a total of 53 individuals from 11 populations were collected and analyzed in this study (Table 1; Fig. 1). All specimens were parasites of *M. reevesii*, and they were identified as *O. jantseanus* based on the following diagnostic morphological characters [5]: somites XIII–XXV biannulate, (a1 + a2) > a3; and branched-branchiae in 11 pairs, each pair arising from lateral of (a1 + a2) of somites XIII–XXIII. When possible, the turtle species were identified by the last author (Dai Suzuki). Almost all the specimens were relaxed by the gradual addition of absolute ethanol to freshwater for morphological identification. For DNA extraction, a piece comprising less than a quarter of the caudal sucker was removed, and then preserved in 99% ethanol. The remainder of the body was fixed in 10% formalin and preserved in 70% ethanol. Examination and

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Table 1
Samples of *Ozobranchus jantseanus* used for molecular analyses. Locality numbers (see Fig. 1) are accompanied by collection locality, voucher, INSDC accession numbers, and COI haplotype (see Fig. 1). Acronym: KUZ, Zoological Collection of Kyoto University.

Sample #	Locality	Individual #	Voucher	COI INSDC #	Haplotype
1	Ichihara, Chiba Pref.	7	KUZ Z1847–Z1853	LC215673–LC215679	a
2a	Minato-ku, Tokyo	1	KUZ Z1859	LC215685	d
2b		2	KUZ Z1860–Z1861	LC215686, LC215687	a
3	Gifu, Gifu Pref.	1	KUZ Z1814	LC215640	a
4	Joyo, Kyoto Pref.	5	KUZ Z1809–Z1813	LC215688–LC215692	a
5	Naruto, Tokushima Pref.	10	KUZ Z1816–Z1825	LC215642–LC215651	a
6	Manno, Kagawa Pref.	5	KUZ Z1826–Z1830	LC215652–LC215656	a
7	Matsue, Shimane Pref.	5	KUZ Z1831–Z1835	LC215657–LC215661	a
8	Miyoshi, Hiroshima Pref.	6	KUZ Z1836–Z1841	LC215662–LC215667	c
9a	Hatsukaichi, Hiroshima Pref.	4	KUZ Z1842–Z1845	LC215668–LC215671	c
9b		1	KUZ Z1846	LC215672	b
10	Fukuoka, Fukuoka Pref.	1	KUZ Z1815	LC215641	a
11	Iki Island, Nagasaki Pref.	5	KUZ Z1854–Z1858	LC215680–LC215684	a

dissection of the leech samples were performed using a stereoscopic microscope (Leica M125). Specimens analyzed in this study have been deposited in the Zoological Collection of Kyoto University (KUZ).

The COI sequences (1267 bp) were determined using the modified methods described in Nakano and Lai [12]; one of the primers for COI, LCO-in, was replaced by LCO-inOzo (5′-GCTGCAGCAATTACAATACTT-3′; this study). In total 53 COI sequences were newly obtained and deposited with the International DNA Database Collaboration (INSDC) through DNA Data Bank of Japan (Table 1). Additionally, a partial COI sequence (1267 bp) concordant with our data set was obtained from the complete mitogenome (KY861060) of a Chinese *O. jantseanus* specimen, which was collected from Jieyang, southern China, deposited with INSDC [13]. The alignment of COI was trivial, as no indels were observed. Relationships between the obtained COI haplotypes were estimated by statistical parsimony network using PopART v. 1.7 [14].

The phylogenetic position of *O. jantseanus* was estimated based on the obtained COI sequences with the aid of the COI sequences of *O. branchiatus* (16 sequences: GU985465, GU985466, KF728206–KF728213, and KJ451399–KJ451404) and *O. margoi* (six sequences: AF003268, GU985467, HM590711, and KJ451405–KJ452407) obtained from INSDC. Two proboscidate piscicolids, *Pontobdella muricata* (Linnaeus, 1758) (AY336029) and *Stibarobdella tasmanica* (Hickman, 1942) (DQ414343) and one proboscidate glossiphoniid, *Theromyzon tessulatum* (O.F. Müller, 1774) (AY047318), OTUs were included as outgroup taxa according to the results of previous molecular phylogenetic analyses [15–17]. Phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian inference (BI). Details for the reconstruction procedures are provided in Nakano and Lai [12]. The ML analysis with nonparametric bootstrapping (BS) with 1000 replicates was conducted based on the non-partitioned dataset. The best-fit partition scheme and models for each partition for the BI and Bayesian posterior probabilities (PPs) were selected as follows: for the 1st position of COI, GTR + I; for the 2nd position, F81 + I; and for the 3rd position, HKY + G; conducted for 1 million generations, and the tree was sampled every 100 generations; the first 3001 trees were discarded based on the results of the parameter estimates and convergence.

The COI data obtained from *O. jantseanus* samples showed low

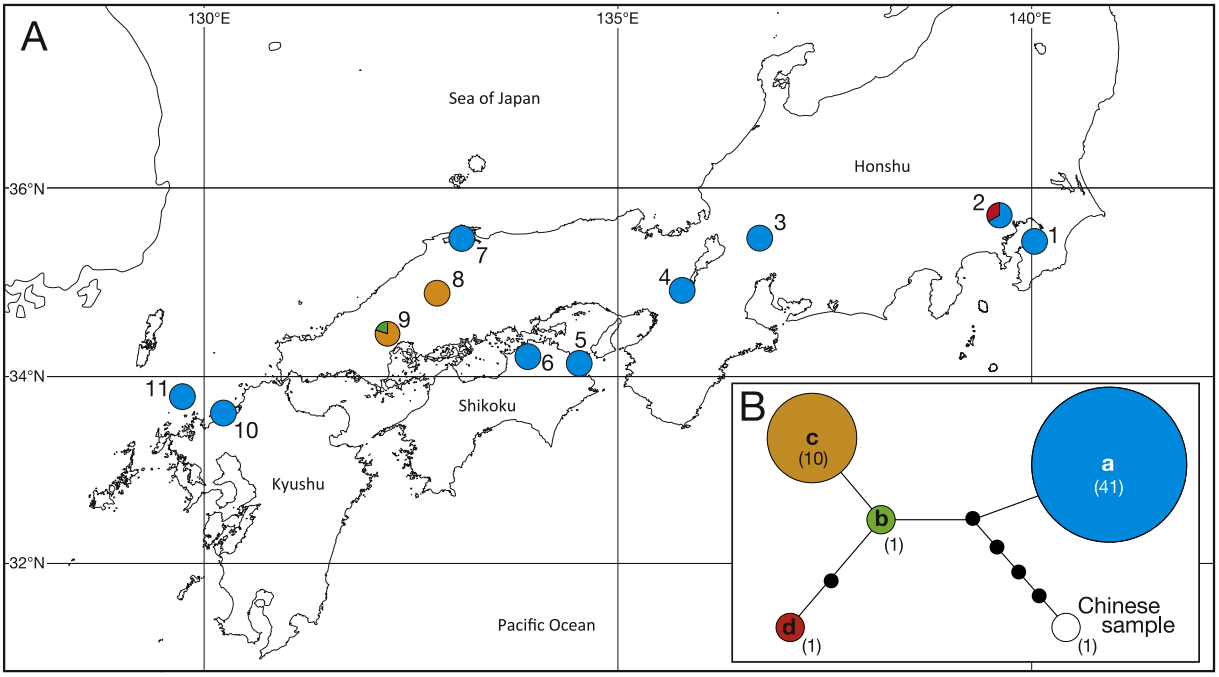

Fig. 1. Map showing sampling localities, and haplotype network of *Ozobranchus jantseanus*. A) Map showing the 11 sampling localities in the present study (see Table 1). Each colored circle indicates respective haplotype of mitochondrial cytochrome *c* oxidase subunit I (COI). B) Statistical parsimony network of COI haplotypes. Filled circles indicate missing haplotypes. Each numeral in parentheses denotes the sample size of the respective haplotype.