

First Record of *Orobdella tsushimensis* (Hirudinida: Arhynchobdellida: Orobdellidae) from Korea (Gageodo Island) and its Molecular Phylogenetic Position within the Genus

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Specimens of the genus *Orobdella* Oka, 1895 from Gageodo Island, Korea, are identified as *Orobdella tsushimensis* Nakano, 2011 based on the following characteristics: IV uniannulate; 1/2+5 annuli between the gonopores; a bottle-shaped gastroporal duct; and epididymides in XVII–XIX. The diagnosis of *O. tsushimensis* is amended since the Korean specimens have a reddish dorsal surface and ovate atrial cornua. Phylogenetic analyses using mitochondrial COI, tRNA^{Cys}, tRNA^{Met}, 12S rRNA, tRNA^{Val}, and 16S rRNA markers show that the specimen from Gageodo Island and those identified as *O. tsushimensis* from Tsushima Island, Japan, form a monophyletic clade, confirming the species identification of the former. This is the first record of the genus *Orobdella*, and of the monotypic family Orobdellidae, from Korea.

Key Words: Hirudinida, Orobdellidae, *Orobdella tsushimensis*, first record, molecular phylogeny, Korea.

Introduction

The terrestrial macrophagous leech genus *Orobdella* Oka, 1895 is distributed in the Far East (Sawyer 1986). Ten species have been described from Japan (Oka 1895; Richardson 1975; Nakano 2010, 2011a, b, 2012a, b). Outside Japan, in contrast, just *O. whitmani* Oka, 1895 has been recorded from Primorsky Krai, Russia (Gilyarov *et al.* 1969), but the identification of the Russian specimens is doubtful (Nakano 2011a, 2012a). Several undescribed species of *Orobdella* are also recognized in Taiwan (T. Nakano and Y.-T. Lai, unpublished data), but on the whole, inventory surveys of this genus outside Japan appear to be critically in arrears.

Recently, specimens of quadrannulate *Orobdella* were collected from Gageodo Island, South Korea. This is the first record of this species and of the genus *Orobdella* in Korea (see Yun (1997) for the most recent Korean leech list). Based on a morphological examination of these specimens, the identification and a brief description of the Korean *Orobdella* are here presented. In addition, its phylogenetic position is estimated using mitochondrial COI, tRNA^{Cys}, tRNA^{Met}, 12S rRNA, tRNA^{Val}, and 16S rRNA (tRNA^{Cys}-16S) sequence data.

Material and Methods

Leeches were newly collected from Gageodo Island, Korea (Fig. 1) along a mountain trail. Altitudes and

coordinates for localities were obtained using a Garmin eTrex® GPS unit.

For DNA extraction, botryoidal tissue was taken from the posterior part of the body around the caudal sucker of one specimen, which had been fixed and preserved in 99% ethanol. All of the other specimens were preserved in 70% ethanol. Two measurements were taken: body length (BL) from the anterior margin of the oral sucker to the posterior margin of the caudal sucker, and maximum body width (BW). Examination, dissection, and drawings of the specimens were accomplished under a stereoscopic microscope equipped with a drawing tube (Leica M125). Specimens used in this study have been deposited in the Zoological Collection of Kyoto University (KUZ).

The numbering convention is based on Moore (1927): body somites are denoted by Roman numerals and the annuli in each somite are given alphanumeric designations.

The extraction of genomic DNA and DNA sequencing methods for COI and tRNA^{Cys}-16S followed Nakano (2012a). DNA sequences of *O. tsushimensis* from Gageodo Island were obtained and deposited in GenBank. In addition to these, DNA sequences of ten species of *Orobdella*, and those of three other species of Erpobdelliformes were obtained from GenBank (Table 1).

Sequences of mitochondrial COI were aligned by eye because there were no indels. Mitochondrial tRNA^{Cys}-16S sequences were aligned using MAFFT L-INS-i (Katoh *et al.* 2005) and then refined with GBLOCKS (Castresana 2000). The length of the aligned COI sequences was 1266 bp, and that of the tRNA^{Cys}-16S was 814 bp (279 characters were