

First Record of the Interstitial Annelid *Pharyngocirrus uchidai* (Annelida: Saccocirridae) from Korea, Confirmed by Topotypic DNA Barcoding Data from Japan

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ABSTRACT

The marine interstitial annelid *Pharyngocirrus uchidai* (Sasaki, 1981) has been only known from Japan. In this study, we report the occurrence of *P. uchidai* for the first time in four localities along the eastern coast of Korea: Bukmyeon, Gamchu, Gase, and Oeongchi. Species identification was confirmed by comparison of DNA barcoding sequences with morphological examination from the type locality, Oshoro, Japan. We generated a total of 25 sequences of a partial segment (580 bp) of the cytochrome *c* oxidase subunit I gene (*COI*), representing five specimens from each locality. Maximum intra-specific variation was 1.2% in terms of Kimura two-parameter (K2P) distance, observed between two individuals each from Gamchu (i.e., between two specimens from the single locality), Gamchu and Oeongchi, Gamchu and Oshoro, and Oeongchi and Oshoro. On the other hand, an identical haplotype was found in all the five localities, substantiating our species identification for the Korean populations. Inter-specific K2P distance between *P. uchidai* and an unidentified *Saccocirrus* sp. from Canada (based on public database entries) was 22.4–23.4%.

Keywords: Archiannelida, *COI*, DNA barcode, mesopsammon, marine invertebrates

INTRODUCTION

The annelid family Saccocirridae is a group of marine polychaetes that live in the interstices among particles of coarse sand and gravel on surf beaches. Of the 22 species known worldwide (Di Domenico et al., 2014), two have been recorded from the Northwest Pacific: *Pharyngocirrus labilis* (Yamanishi, 1973) and *P. uchidai* (Sasaki, 1981) (Fig. 1). Both species have hitherto been known only in Japan (Uchida, 1933; Uchida and Okuda, 1953; Yamanishi, 1973; Sasaki, 1981; Sasaki and Brown, 1983).

Interstitial annelids generally have a simple body plan, often rendering species identification difficult solely based on morphological characters, whereas subtle inter-specific differences can be found by detailed light and scanning electron microscopy (e.g., Di Domenico et al., 2013; Kajihara et al., 2015). A ~650 base-pair segment of the mitochondrial cyto-

chrome *c* oxidase subunit I (*COI*) gene is commonly used as a DNA barcoding region among Metazoa including Annelida and is a powerful tool for reliable species identification (e.g., Carr et al., 2011). However, as of writing, there is no barcoding sequence in public databases pertaining to any of the 22 named species of saccocirrids.

In this paper, we report *P. uchidai* for the first time from Korea. To ensure species identification, we compare barcoding sequences of the Korean specimens with those from the type locality in Japan and provide diagnosis of *P. uchidai*.

RESULTS AND DISCUSSION

Specimens were collected from four localities in South Korea and one in Japan: Oeongchi Beach, Sokcho, Korea (38°11' 00"N, 128°36'34"E); Gase Beach, Donghae, Korea (37°31'

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Table 1. Pairwise genetic distances and accession number of DDBJ

Specific name	Location	DDBJ No.
<i>Pharyngocirrus uchidai</i>	Oeongchi	LC440427
<i>Pharyngocirrus uchidai</i>	Oeongchi	LC440428
<i>Pharyngocirrus uchidai</i>	Oeongchi	LC440429
<i>Pharyngocirrus uchidai</i>	Oeongchi	LC440430
<i>Pharyngocirrus uchidai</i>	Oeongchi	LC440431
<i>Pharyngocirrus uchidai</i>	Gamchu	LC440432
<i>Pharyngocirrus uchidai</i>	Gamchu	LC440433
<i>Pharyngocirrus uchidai</i>	Gamchu	LC440434
<i>Pharyngocirrus uchidai</i>	Gamchu	LC440435
<i>Pharyngocirrus uchidai</i>	Gamchu	LC440436
<i>Pharyngocirrus uchidai</i>	Gase	LC440437
<i>Pharyngocirrus uchidai</i>	Gase	LC440438
<i>Pharyngocirrus uchidai</i>	Gase	LC440439
<i>Pharyngocirrus uchidai</i>	Gase	LC440440
<i>Pharyngocirrus uchidai</i>	Gase	LC440441
<i>Pharyngocirrus uchidai</i>	Bukmyeon	LC440442
<i>Pharyngocirrus uchidai</i>	Bukmyeon	LC440443
<i>Pharyngocirrus uchidai</i>	Bukmyeon	LC440444
<i>Pharyngocirrus uchidai</i>	Bukmyeon	LC440445
<i>Pharyngocirrus uchidai</i>	Bukmyeon	LC440446
<i>Pharyngocirrus uchidai</i>	Oshoro	LC440447
<i>Pharyngocirrus uchidai</i>	Oshoro	LC440448
<i>Pharyngocirrus uchidai</i>	Oshoro	LC440449
<i>Pharyngocirrus uchidai</i>	Oshoro	LC440450
<i>Pharyngocirrus uchidai</i>	Oshoro	LC440451
<i>Saccocirrus</i> sp.	Canada	MF121674.1
<i>Saccocirrus</i> sp.	Canada	HM473660.1
<i>Protodrilus pythionius</i> (Outgroup)	Brazil	KC763840.1

DDBJ, DNA Data Bank of Japan.

46°N, 129°07'15"E); Gamchu Beach, Donghae, Korea (37°30'56"N, 129°07'33"E); Bugu Beach, Uljin, Korea (37°06'22"N, 129°22'35"E); Oshoro Bay, Otaru, Japan (43°12'37"N, 140°51'25"E). Morphologically, the Korean specimens generally accorded with the original description of the species by Sasaki (1981). Sequences were amplified with the specific *COI* primers LCO_Su 5'-CCT-TTT-AGG-CAC-TTC-TAT-AAG-AC-3' and HCO_Su 5'-CAA-AAC-AAA-TGC-TGG-TAG-AGA-ACG-3' (Hiruta unpublished) and aligned by MEGA 7 (Kumar et al., 2016). The 25 sequences generated in this study consist of 580 base pairs, which have been deposited in DNA Data Bank of Japan (DDBJ), with accession numbers LC440427–LC440451. Pairwise genetic distances were calculated by the Kimura two-parameter distance (K2P) (Kimura, 1980). To estimate potential barcode gap, we compared our sequences with those of *Saccocirrus* sp. from Canada (HM473661, Carr et al., 2011; MF121674 and MF121389, Dewaard, unpublished).

Maximum intra-specific variation in terms of K2P was 1.2% (Table 1), which was observed between two individuals each from (1) Gamchu (i.e., two specimens in a single locality), (2) Gamchu and Oeongchi, (3) Gamchu and Oshoro, and (4) Oeongchi and Oshoro. An identical haplotype was found in all the five localities, supporting the species identification for the Korean populations. Inter-specific K2P distance between *P. uchidai* and an unidentified *Saccocirrus* sp. from Canada was 22.4–23.4% (Table 1). Therefore, a barcoding gap among saccocirrids should exist somewhere between 1.2% and 22.4% in terms of K2P. The intra- and inter-specific distances observed in this study are comparable to, or slightly greater than, those for other annelids; e.g., 0.10% to 0.14% within-species and 19.3% to 22.9% between-species distances for marine tubificid genus *Tubificoides* (Erséus and Kvist, 2007).

***Pharyngocirrus uchidai* (Sasaki, 1981) (Fig. 1)**

Saccocirrus major Pierantoni, 1907: 1; Uchida, 1933: 128.

Saccocirrus uchidai Sasaki, 1981: 259.

Pharyngocirrus uchidai (Sasaki, 1981); Di Domenico et al., 2014: 202.

Diagnosis. Body 5.53–13.64 mm long, 0.13–0.32 mm wide. Number of segment 49–106. All segments setigerous except prostomium, peristomium and last one to 18 segments before pygidium depending on the specimen.

Cylindrical tentacle, 0.43–0.98 mm long. Pair of dark brown eyes. Ciliary tuft on tentacle, between prostomium and tentacle, head of prostomium and on both side of peristomium. Nuchal organs on dorsal side between prostomium and peristomium. Three lips of mouth with ciliary patch on the peristomium. Muscular pharynx.



Fig. 1. *Pharyngocirrus uchidai* (Sasaki, 1981) from Oshoro bay, Japan. Scale bar=5 mm.

Three types of setae on parapodium; long hair setae symmetrically bifurcated; medium setae thick, flat and symmetrically bifurcated, outer margin of apical end of medium setae bordered by big blunt teeth, between which small denticles lie; third setae the shortest, forked and stumpy shape.

Bifurcated pygidium; pygidial lobe 0.09–0.24 mm long; ciliary tuft; number of papillate edge 7–11.

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