*CCAMLR Science*, Vol. 26 No. 1 (2019): 1-4

# A New Discovery of the Asymmetric Subspecies of the Whale Landing Lanceolata (Cephalochordata) in the Izu Islands on the Pacific Coast of Japan May Be a Possible Case of the Stepping Stone Hypothesis

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**Abstract**

In 2016, two mature whale landing swords, *Asymmetron inferum* Nishikawa, were collected in the bottom of the reduced gravel near the hydrothermal vent of the Great Jurassic Cave of the Great Jurassic-Dashii volcano in the Izu Islands on the Pacific coast of Japan. ROV Hyper-Dolphin was used in JAMSTEC's RV *Shinsei-maru* KS-16-6 parade. So far, the species has been known only to the whale drop community at the depth of 219-254 m in the corner of Nommisaki in Kyushu, southwestern Japan. New findings suggest that the stumbling stepping stone hypothesis of the whale drop community is the origin of venting and/or penetrating fauna.

**Key Words**

Hydrothermal vent; *Asymmetron inferum*; Seamount; West Pacific; Stepping-stone hypothesis.

## Introduction

In deep-sea reducing bottoms, ephemeral whale-fall communities may provide dispersal stepping stones for a subset of the vent and seep faunas. This dispersal stepping-stone hypothesis has been suggested by finds of whale-fall species of the phyla Annelida and Mollusca also in the reducing vent and/or seep habitats, and *vice versa*. Here will be given the first such example for the phylum (or subphylum) Cephalochor data (Sharifuzzaman et al., 2017).

The whale-fall lancelet *Asymmetron inferum* Nishikawa, 2004 has so far been known exclusively from reducing bottom sand beneath the bones of the sperm whale *Physeter macrocephalus* Linnaeus, 1758, off Cape Nomamisaki, SW Kyushu, Japan, 219–254 m deep. *Asymmetron inferum* has so far been the only lancelet inhabiting whale-fall communities. The lancelet is not included in Nakajima *et al.*'s species list of the deep-sea benthic macrofauna and megafauna from 42 seeps and vents around the Japanese Archipelago (Tunnicliffe et al., 2010; Uejima et al., 2017). In 2016, however, two lancelet specimens were

collected from reducing gravel bottom near a hydrothermal vent, 196 m deep, at Oomuro Hole of Oomuro-dashi sub-marine volcano in the Izu Islands, Pacific coast of Japan by using the ROV *Hyper-Dolphin* during the RV *Shinseimaru* KS-16-6 cruise of the Japan Agency for Marine-Earth Science and Technology (abbreviated as JAMSTEC hereafter) headed by one of the present authors (KT); the vent was not referred to by Nakajima *et al.* These specimens were safely identified as *A. inferum* in terms of morphology and mitochondrial cytochrome *c* oxidase subunit I (COI) gene sequence data.

The present finding contributes to discussions on the origin of ephemeral populations of the whale-fall lancelet. Further finds of the lancelet from seeps, vents, and whale falls may be highly expected, and will shed new light on the discussions (Metaxas, 2011; Yorisue et al., 2012).

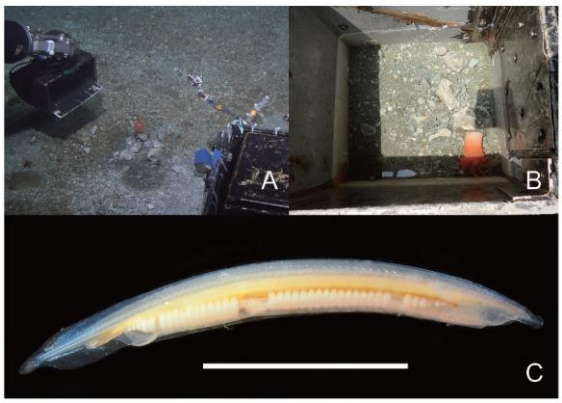


Figure 1: A, sampling site at Oomuro Hole of Oomuro-dashi submarine volcano in the Izu Islands at a depth of 196 m. A sea anemone (center) is raked by a scoop together with its substrate pumiceous gravel, from which one of the lancelets was found. Surface of the site is covered with greyish bacterial mat; B, collected material inside the scoop; C, *Asymmetron inferum* Nishikawa, 2004, male, 26.5 mm, NSMT-Pc 1162. Scale bar=10 mm.

## Materials and Methods

### Sampling

Two lancelets were collected on 16 May 2016 from pumiceous gravel bottom (34°32.8069′N 139°26.5492′E) near a hydrothermal vent at a depth of 196 m, in Oomuro Hole of Oomuro-dashi submarine volcano in the Izu Islands, Pacific coast of Japan by the ROV *Hy-per-Dolphin* #1967 dive during the RV *Shinsei-maru* KS-16-6 cruise of JAMSTEC; the bottom surface of the sampling site was covered with pale greyish bacterial mat (Figure 1A). A lancelet was found in the gravel sampled to collect benthic sea anemones by using a scoop (Figure 1B), while the other in the gravel of a push-core with the diameter of *ca.* 3 cm. After taking photographs with an Olympus OM-DE-M1 digital camera on board, the specimens were fixed with 80% ethanol and preserved in 70% ethanol. They are deposited in the National Museum of Nature and Science, Tsukuba (NSMT).

### Morphological Examination

A stereo microscope was used for morphological observation, measurement of body length, and counting myomeres; specimens were not stained. Body length was measured by using a pair of calipers.

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### DNA Extraction, PCR Amplification and Sequencing

Total genomic DNA was extracted from muscle pieces on the left side of respective specimens by using DNeasy blood and tissue Kit (Qiagen, Hilden, Germany). A partial region of the COI gene (about 729 bp) was amplified by the polymerase chain reaction (PCR) using the following primer pair: AinfCOF214 (5’-CCT GTA ATA ATC GGA GGC TTC-3’) and AinfCOR983 (5’-CCT GCT AGT GTA GCT AAT CAG C-3’). The region failed to be amplified by the primer pairs LCO1490 (5’-GGTCAACAAATCATAAAGATATTGG-3’) and HCO2198 (5’-TAA ACT TCA GGG TGA CCA AAA AAT CA-3’) and AmphL109 (5’-ATT CGN GCN GAA YTN TCN CAG CC-3’) and AmphH1325 (5’-TCN GAA TAY CGN CGW GGT ATN CC-3’). Amplification conditions were as follows: initial denaturation for 4 min at 94°C; 35 cycles of

denaturation for 30 s at 94°C, annealing for 30 s at 55°C, and extension for 1 min at 72°C; and final extension for 7 min at 72°C. All reactions were aided by ExTaq HS polymerase (TaKaRa, Otsu, Japan), and the products were purified by ExoSAP-IT (USB). Sequencing reactions were performed according to the manufacturer's instructions using the BigDye Terminater Cycle Sequencing Reaction Kit ver. 3.1 (Thermo Fisher Scientific, MA, USA). Sequencing reaction products were purified by ethanol precipitation. Labeled fragments were analyzed using an ABI 3500xL Genetic Analyzer (Applied Biosystem). Sequences were obtained from both strands of the gene segments for verification using the same primers. The nucleotide sequences have been submitted to the DNA Data Bank of Japan (DDBJ) under accession numbers LC185086 for a male (NSMT-Pc 1162) and LC185085 for a female (NSMT-Pc 1163).

**Phylogenetic Analysis**

For molecular phylogenetic analysis, obtained from DDBJ were 54 published sequences of *Asymmetron lucayanum* s. str., *A. lucayanum* complex sp. A, *A. lucayanum* complex sp. B, *A. inferum* (AP009352), and further, as an outgroup, *Branchiostome japonicum*. These published sequences and our newly sequenced data from the two Oomuro specimens were analyzed. DNA sequences were aligned using the multiple sequence alignment program CLUSTAL W. The sequences from the COI gene were unambiguously aligned without gap. Phylogenetic relationships were reconstructed by the Neighbor-joining method with MEGA 7.0.14 software. In the analysis, Kimura 2-parameter method of nucleotide substitution was used to estimate genetic distances. To estimate statistical support for branching patterns, 1,000 bootstrap replications were performed.

## Results

**Morphological Examination**

The material consists of a 26.5 mm long male (NSMT-Pc 1162; Figure 1C) and a 25.0 mm long female (NSMT-Pc 1163), each with matured gonads only on the right side; approximate number of myomeres is 85 in the former, while 87 in the latter. Each was provided with a remarkable urostyloid process. Further detailed examination was impossible due to ill shrinkage. However, the asymmetrical arrangement of gonads and the existence of urostyloid process in these specimens may safely justify their affiliation to the genus *Asymmetron*. And their myomere numbers (85 and 87) are similar to that of the original description of *A. inferum* (83) by Nishikawa, quite distinct from the other known congeners. Thus, the specimen could be safely identified as *A. inferum*.

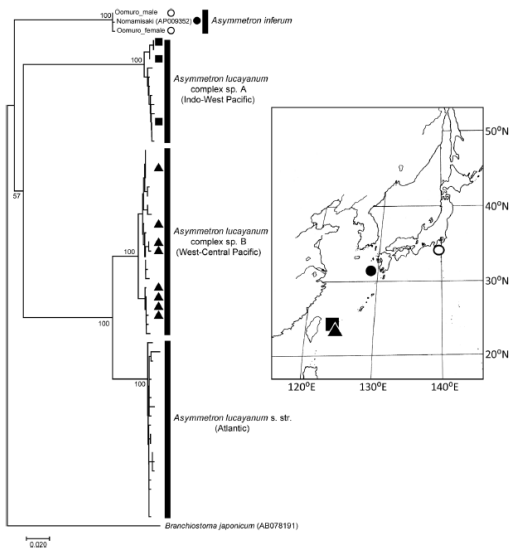


Figure 2: Neighbor-joining tree of the lancelets of the genus *Asymmetron*, and map showing their Japanese localities with A. *inferum* shown by open and solid circles for new and known localities, respectively, and A. *lucayanym* complex spp. A and B by solid squares and triangles, respectively. The tree was constructed on the basis of the cytochrome c oxidase subunit I gene (668 bp), using Kimura 2-parameter method; numbers beside major internal branches indicate bootstrap probabilities based on 1,000 replicates.

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**DNA Barcording and Phylogenetic Analysis**

The complete mitochondrial genome sequence of *A. inferum* is registered in the DDBJ, representing so far the only published genome data for this species, based on the specimen from the type locality (off Cape Nomamisaki). In the COI gene, the present sequences differ from the registered one by only 1 (out of 671 nucleotides in the male: LC185086; NSMT-Pc 1162) or 2 (of 668 in the female: LC185085; NSMT-Pc 1163) nucleotides, representing at most 0.3% variation. The male and female sequences differ from each other by only a single nucleotide. On the other hand, the present sequences differ from the registered ones of congeneric three species of tropical shallow-water *A. lucayanum* species complex by 16–18%. Further, our Oomuro specimens are markedly included in a distinct clade with that of *A. inferum* collected from the type locality (Figure 2). Therefore, the molecular analyses also support the present Oomuro specimens to be identified as *A. inferum*.

## Discussion

The two specimens were found in a very limited volume of bottom gravel, and they are matured. Therefore, the present Oomuro population of *Asymmetron inferum* can be supposed stable, possibly with a rather high density. Therefore, it may be said that the newly found vent population probably represents a new stable habitat for the whale-fall lancelet, and that the present find suggests the dispersal stepping-stone hypothesis. Future detailed comparisons must be highly expected between the Kagoshima and Oomuro populations of this species in terms of molecular population genetics, which will reveal their history of colonization. Further finds of this lancelet may be highly expected from seeps and vents, as well as whale falls.

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