or at least the identification of a subspecies.

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A Comparative analysis of two phoronid species: *Phoronopsis viridis* and *Phoronopsis harmeri* and the problem of phoronid taxnomy

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INTRODUCTION & AIM

Phoronids are a small phylum of marine invertebrates consisting of only 15 species, most of which have a worldwide distribution. In the majority of marine ecosystems, phoronids are the dominant species, forming high-density aggregations and thus playing an important edificatory role. The external morphology of phoronids is poor, and for an exact identification of the species it is necessary to obtain a series of histological sections. The problem in phoronid taxonomy is the synonymization of species, which in many cases is very controversial. One important issue in phoronid taxonomy is the description of species by their larval stages — actinotrochs. More than 50 so-called "larval species" have been described, which indicates the existence of undescribed species of adult phoronids.
The present work is devoted to the anatomy and phylogenetic analysis of two populations of *Phoronopsis harmeri*: from Vostok Bay, the Sea of Japan, and from Friday Harbor, on the Pacific coast of North America.

METHOD

The external morphology of the body and tubes was studied using an Olympus SZX-7 stereomicroscope. Photographs were taken using a Toupcam digital camera. For histology methods, specimens were dissected into head ends and upper region of the body and dehydrated in alcohol series and then embedded into paraplast. Sections were cut at a thickness of 5 µm with Leica RM2125 microtome and stained with haematoxylin. Sections were observed with an Olympus BX-51 microscope and photographed with a Toupcam digital camera.
For molecular genetic analysis, we studied the sequences of the 28S rRNA and COX1 genes obtained by us from *Phoronopsis harmeri / viridis* samples from the indicated locations, as well as those taken from the GenBank and belonging to individuals of these two species from different waters of the World Ocean. Evolutionary analyses were conducted in MEGA11. The evolutionary history was inferred using the Maximum Parsimony method. The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm.

RESULTS & DISCUSSION

Fixed samples both have a pale yellow color (Fig. 1). The body consists of a head end with a lophophore, an anterior trunk region with dense integuments, a posterior trunk region with thin integuments, and an ampule. The lophophore is spiral, with 1.5 turns (Fig. 2). Tentacles in a straightened state are up to 1.5 mm in length. *P. harmeri* has approximately 160 tentacles in the lophophore, while *P. viridis* has 180 tentacles.
The coelom of the anterior trunk region is divided by the mesenteries into four chambers: left and right oral and left and right anal (Fig. 3). The left lateral blood vessel passes through the left oral chamber, and the medial blood vessel passes through the right anal chamber., The single giant nerve fiber lies under the epithelium at the base of the left lateral mesentery. The longitudinal muscles in both studied samples are feather-like type.

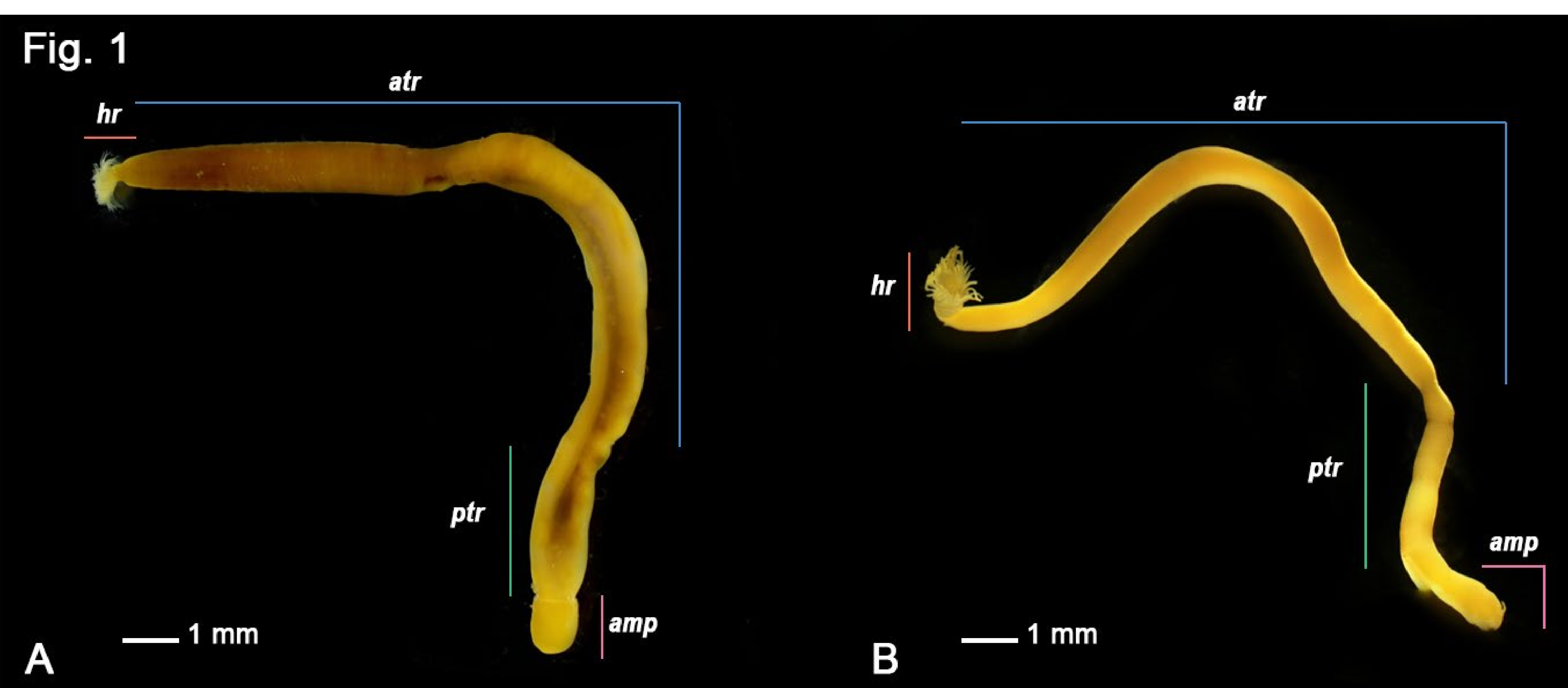

Figure 1. External morphology of two species.
A – *P. harmeri*, **B** – *P. viridis; **amp** – ampule, **atr** – anterior body region, **hr** – head region with lophophore, **ptr** – posterior body region,*

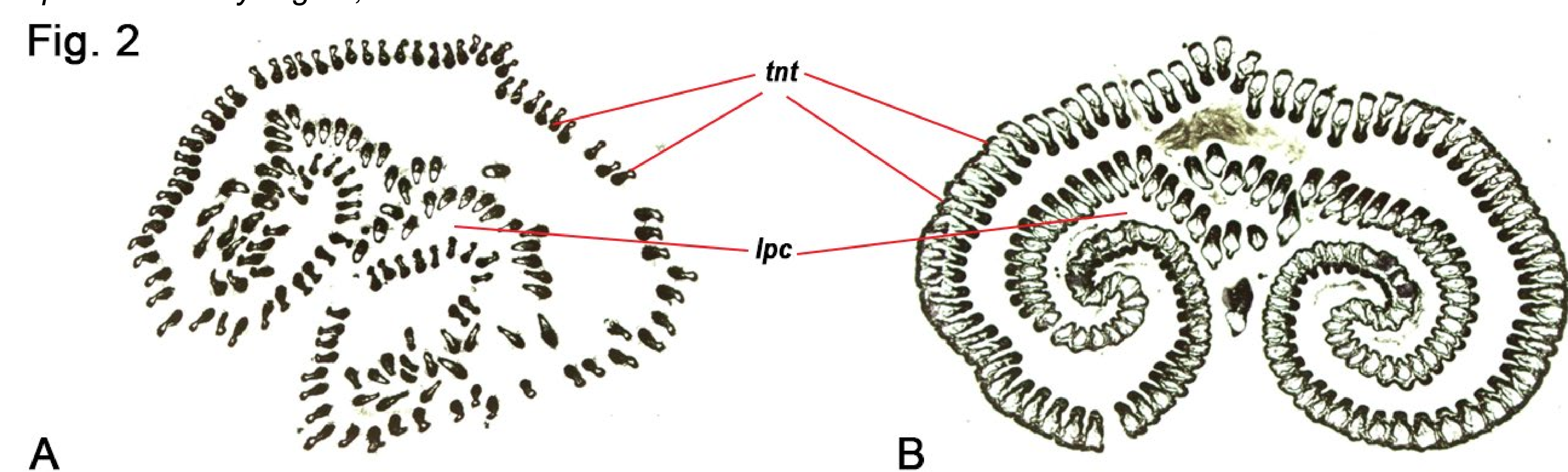

Figure 2. Cross section through the lophophore.
A – *P. harmeri*, **B** – *P. viridis*; ***lpc*** – lophophore cavity, ***tnt*** – tentacles

RESULTS & DISCUSSION

Muscular formula is distribution of strands of longitudinal muscles between the coelom chambers in the anterior trunk region. Upper numbers is for oral side, and lower is for anal side.
The muscular formula of *P. harmeri* is as follows:

$$\frac{34|40}{21|13}$$

The muscular formula of *P. viridis* is different; there are more strands on the oral side and they are distributed less evenly between the right and left sides:

$$\frac{31|31}{21|15}$$

Most of the 28S rRNA data are regions of about 300 bp in length. Such sequences are not suitable for studying intraspecific or interspecific variation. Intraspecific variability of mitochondrial COX1 is much higher than that of nuclear rRNA genes. Specimens cluster into three clades correlated with geographic distribution (Fig. 4).
One of the problems we have encountered in analyzing genetic data is the inaccurate species identification of specimens which nucleotide sequences are uploaded to the GenBank. For example, OC0003C (AY428841.1) is identified as *P. viridis*, and grouped with it OC0006C (EU484465.1) as *P. harmeri*.

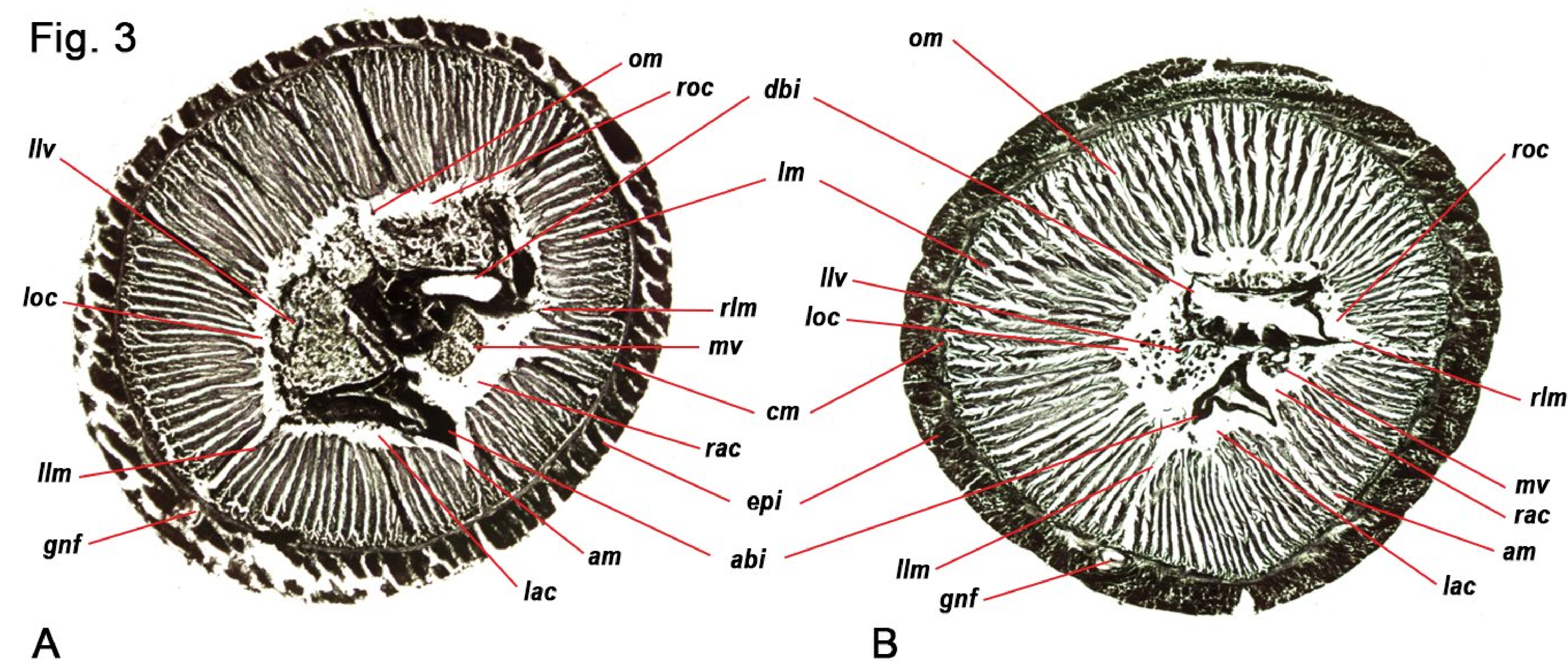

Figure 3. Cross section through the anterior trunk region.
A – *P. harmeri*, **B** – *P. viridis*; ***abi*** – ascending branch of the intestine, ***am*** – anal mesentery, ***cm*** – circular muscles, ***dbi*** – descending branch of the intestine, ***epi*** – epithelium, ***gnf*** – giant nerve fiber, ***lac*** – left anal coelom chamber, ***llm*** – left lateral mesentery, ***llv*** – left lateral blood vessel, ***lm*** – longitudinal muscles, ***loc*** – left oral coelom chamber, ***mv*** – median blood vessel, ***om*** – oral mesentery, ***rac*** – right anal coelom chamber, ***rlm*** – right lateral mesentery, ***roc*** – right oral coelom chamber

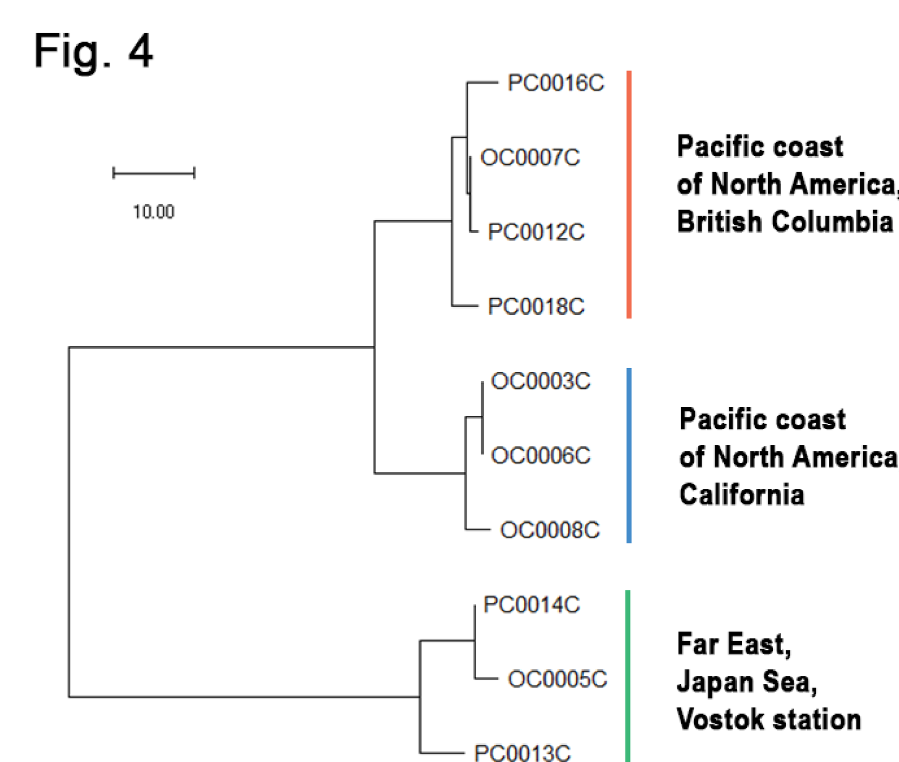

Figure 4. Phylogenetic tree of *Phoronopsis harmeri / viridis.*
The reconstruction is based on 10 COX1 sequences. PC is from a personal collection, and OC is from GenBank.
The length of parsimony equals 135. The consistency index is 0.955556 (0.946903), the retention index is 0.969543 (0.969543), and the composite index is 0.926452 (0.918063) for all sites and parsimony-informative sites
The tree is drawn to scale , with branch lengths calculated using the average pathway and are in the units of the number of changes over the whole sequence. There were a total of 1540 positions in the final dataset.
Full genetic data set can be sent upon request.

CONCLUSION

Morphological and anatomical differences between samples from the two populations indicate at least widespread intraspecific polymorphism. The large extent of the habitat of *Phoropsis harmeri / viridis* leads to genetic heterogeneity. The obtained data allow us to raise the question of the possible revalidation of the species *Phoronopsis viridis*, or at least the identification of a subspecies.

FUTURE WORK

Further research and improvement of both morphological-anatomical and molecular methods for analyzing the taxonomy of phoronids are necessary to resolve the issue of the status of *Phoropsis harmeri / viridis*. We are planning more detailed studies of the anatomy and morphology of samples from various locations, as well as obtaining complete sequences of mitochondrial and nuclear genes from the available material.