

DNA Barcoding of *Nereiphylla hera* (Annelida: Polychaeta: Phyllodocidae) from South Korea

Hana Kim^{1,2}, Hyun Ki Choi^{1,*}

¹National Marine Biodiversity Institute of Korea, Seocheon 33662, Korea
²Department of Biological Sciences, Inha University, Incheon 22212, Korea

ABSTRACT

The phyllodid polychaete species, *Nereiphylla hera* Kato and Mawatari, 1999 is reported from the intertidal habitats of the eastern coast of South Korea. We determined the DNA barcoding region of the mitochondrial cytochrome c oxidase subunit I (COI) of *N. hera* and compared nucleotide variation with its congeners. The intra-specific genetic distance between the three COI sequences of *N. hera* was ranged from 0 to 0.4%. The inter-specific distances between *N. hera* and other *Nereiphylla* species ranged from 18.8 to 22.3%. In this study, we reported the first COI barcodes of *N. hera* with the morphological diagnosis and the photographs. These results would be helpful to understand taxonomy of *Nereiphylla*.

Keywords: *Nereiphylla hera*, DNA barcode, COI, Korea

INTRODUCTION

The genus *Nereiphylla* Blainville, 1828 is one of 18 genera in the family Phyllodocidae Örsted, 1843. The genus is widely distributed from intertidal to deep sea and it is known to inhabit both soft and rocky bottoms (Kato and Mawatari, 1999; Eklöf et al., 2007). To date, 11 valid species of this genus have been classified worldwide (WoRMS, 2019). Among them, three *Nereiphylla* species, *N. castanea* (Marenzeller, 1879), *N. crassa* Imajima, 2003, and *N. hera* Kato and Mawatari, 1999 have been recorded from the East Asia (Izuka, 1912; Imajima and Hartman, 1964; Paik, 1982, 1989; Kato and Mawatari, 1999; Imajima, 2003). *Nereiphylla hera*, originally described from Hokkaido in northern Japan, has been found in the eastern coast of South Korea (Choi et al., 2015). This species has been distinguished easily from its relatives through the following morphological features: the tentacular cirri are flat, spatulate with narrowly distinct tips that are broader than their cirrophores, the parapodia possess elongated dorsal cirri (Kato and Mawatari, 1999; Choi et al., 2015).

Recently, in the polychaete taxonomy, the cytochrome c oxidase subunit I (COI) DNA barcode has been in common use for the identification of the species and the revision of the traditional taxonomy based on the morphology (Carr et al., 2011; Choi et al., 2017; Park and Kim, 2017). Neverthe-

less, as of 16 July 2019, GenBank contained only three COI sequences of two *Nereiphylla* species, *N. lutea* (Malmgren, 1865) from Norway and *N. castanea* (Marenzeller, 1879) from Canada (Eklöf et al., 2007; Carr et al., 2011).

In this study, we determined the first COI sequences of *N. hera* and attempted to verify the effectiveness of the DNA barcoding for *Nereiphylla* in comparison with closely related species.

The specimens of *N. hera* were collected from Goseong-gun (38°10'17"N, 128°37'40"E), South Korea. Their morphological characterization was carried out under a light microscope (Carl Zeiss Axioskop II, Göttingen, Germany) on the basis of Choi et al. (2015). Photographs of specimen were captured using an image system (LAS V4.7, Leica Microsystems, Heerbrugg, Switzerland) and presented in Fig 1. The voucher specimens were deposited in the National Marine Biodiversity Institute of Korea (MABIKNA00146571-00146572, 146575). Genomic DNA extraction and sequencing followed the methods of Maturana et al. (2011). The newly obtained sequences were deposited in GenBank under accession numbers MN126596-MN126598 and aligned with the congeners (*N. lutea* and *N. castanea*) using Geneious Pro v.9.1.8 (Biomatters, Auckland, New Zealand). The genetic distances calculated using the Kimura-2-parameter (K2P) model by MEGA X software (Kumar et al., 2018).

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

***To whom correspondence should be addressed**
Tel: 82-41-950-0880, Fax: 82-41-950-0880
E-mail: choi3112@gmail.com

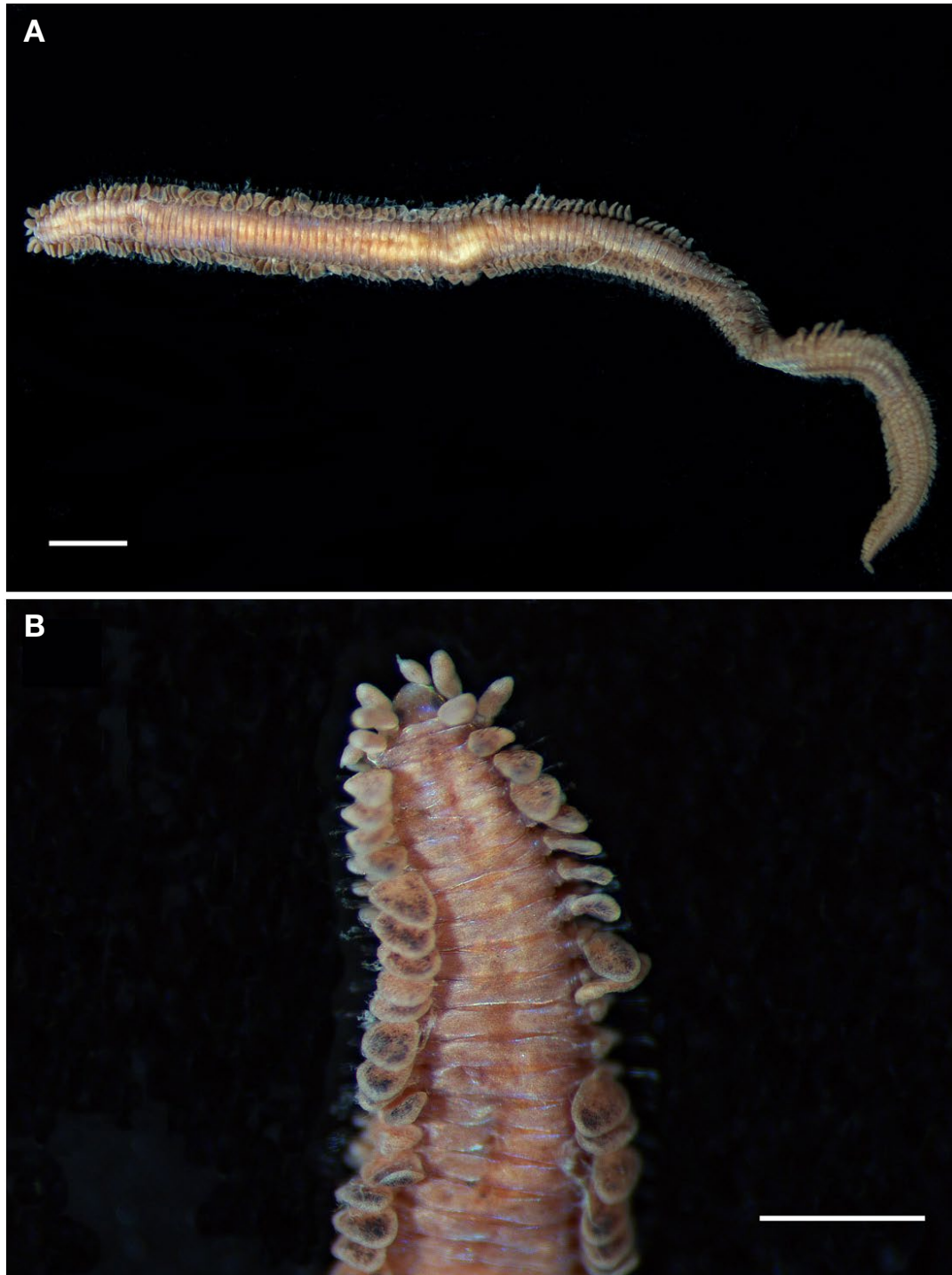


Fig. 1. Photographs of *Nereiphylla hera* Kato & Mawatari, 1999 in the eastern coast of South Korea, lateral view (A) and anterior region (B). Scale bars: A=1.0 mm, B=0.5 mm.

RESULTS AND DISCUSSION

The length of COI sequence alignment was 658 bp for three *Nereiphylla* species. The intra-specific variations of *N. hera*

were 0–0.4% (Table 1). The inter-specific variations among *Nereiphylla* species ranged from 18.8 to 22.3%. Consequently, the gap between intra- and inter-species variations was consistent with those obtained in the previous study for Southern

Table 1. Genetic distance (K2P) based on 658 bp size of COI sequence among three *Nereiphylla* species

No	Species	Accession No.	1	2	3	4	5	6	Data source
1	<i>Nereiphylla hera</i>	MN126596	–						Present study
2	<i>Nereiphylla hera</i>	MN126597	0.000	–					"
3	<i>Nereiphylla hera</i>	MN126598	0.004	0.004	–				"
4	<i>Nereiphylla lutea</i>	AY996118	0.188	0.188	0.193	–			Eklöf et al. (2007)
5	<i>Nereiphylla castanea</i>	HM473497	0.221	0.221	0.221	0.223	–		Carr et al. (2011)
6	<i>Nereiphylla castanea</i>	HM473498	0.218	0.218	0.218	0.220	0.005	–	"

K2P, Kimura-2-parameter; COI, cytochrome c oxidase subunit I.

European Atlantic polychaetes (Lobo et al., 2016).

In this study, we reported the first COI barcodes for *N. hera* with the morphological diagnosis and the photographs. These results would be helpful to understand taxonomy of phyllodid polychaetes, although further analysis of undetermined taxa is considered for the COI DNA barcoding and its application.

¹*Order Phyllodocida Örsted, 1843

²*Family Phyllodocidae Örsted, 1843

³*Genus *Nereiphylla* Blainville, 1828

⁴**Nereiphylla hera* Kato & Mawatari, 1999 (Fig. 1)

Nereiphylla hera Kato and Mawatari, 1999: 353, figs. 1–3;

Choi et al., 2015: 279, fig. 1.

Diagnosis. Prostomium with 4 antennae and paired eye and median antenna absent. Proboscis with numerous minute papillae irregularly distributed. Nuchal papilla absent. Tentacular cirri flattened, 4 pairs, with narrowly distinct tip. Tentacular formula: (1 + S1/1) + S1/N. Segments 1 and 2 fused dorsally. Segment 2 without neuropodia and with setae only. Parapodia uniramous, with neuropodia and well developed dorsal and ventral cirri. Dorsal cirri heart-shaped, elongated, partially covered dorsum. Ventral cirri expanded, boarder than neuropodia. Chaetae composite with rostrum of chaetal shaft with distal crown bearing numerous teeth. Pygidium with two cirri slightly flattened.

ACKNOWLEDGMENTS

This work was supported by the grants of National Marine Biodiversity Institute of Korea (2019M00100).

REFERENCES

Carr CM, Hardy SM, Brown TM, Macdonald TA, Hebert PDN,

2011. A tri-oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. *PLoS ONE*, 6:e22232. <https://doi.org/10.1371/journal.pone.0022232>

Choi HK, Jung TW, Yoon SM, 2015. First report of *Nereiphylla hera* (Polychaeta: Phyllodocidae) from Korea. *Korean Journal of Environmental Biology*, 33:279–282. <https://doi.org/10.11626/KJEB.2015.33.3.279>

Choi HK, Kim JG, Kang DW, Yoon SM, 2017. A new species of *Leodice* from Korean waters (Annelida, Polychaeta, Eunicidae). *ZooKeys*, 715:53–67. <https://doi.org/10.3897/zookeys.715.20448>

Eklöf J, Pleijel F, Sundberg P, 2007. Phylogeny of benthic Phyllodocidae (Polychaeta) based on morphological and molecular data. *Molecular Phylogenetics and Evolution*, 45:261–271. <https://doi.org/10.1016/j.ympev.2007.04.015>

Imajima M, 2003. Polychaetous annelids from Sagami Bay and Sagami Sea collected by the Emperor Showa of Japan and deposited at the Showa Memorial Institute. *National Science Museum Monographs*, 23:1–221.

Imajima M, Hartman O, 1964. The polychaetous annelids of Japan, Part II. Occasional Papers of the Allan Hancock Foundation, 26:239–452.

Izuka A, 1912. The Errantiate Polychaeta of Japan. *Journal of the College of Science, Imperial University of Tokyo*, 30:1–262.

Kato T, Mawatari SF, 1999. A new species of *Nereiphylla* (Polychaeta, Phyllodocidae) from Hokkaido, Northern Japan. *Species Diversity*, 4:353–360. <https://doi.org/10.12782/specdiv.4.353>

Kumar S, Stecher G, Li M, Knyaz C, Tamura K, 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35:1547–1549. <https://doi.org/10.1093/molbev/msy096>

Lobo J, Teixeira MA, Borges LM, Ferreira MS, Hollatz C, Gomes PT, Sousa R, Ravara A, Costa MH, Costa FO, 2016. Starting a DNA barcode reference library for shallow water polychaetes from the southern European Atlantic coast. *Molecular Ecology Resources*, 16:298–313. <https://doi.org/10.1111/1755-0998.12441>

Maturana CS, Moreno RA, Labra FA, González-Wevar CA, Rozbaczylo N, Carrasco FD, Poulin E, 2011. DNA barcoding of marine polychaetes species of southern Patagonian fjords.

Korean name: ¹*부채발갯지렁이목, ²*부채발갯지렁이과, ³*납작수염부채발갯지렁이속, ⁴*뽕족납작수염부채발갯지렁이

- Revista de Biología Marina y Oceanografía, 46:35-42.
- Paik EI, 1982. Taxonomic studies on polychaetous annelids in Korea. Research Bulletin of the Hyosung Women's University, 24:745-913.
- Paik EI, 1989. Illustrated encyclopedia of fauna and flora of Korea. Vol. 32. Polychaeta. Ministry of Education Press, Seoul, pp. 1-764.
- Park T, Kim W, 2017. Description of a new species for Asian populations of the "Cosmopolitan" *Perinereis cultrifera* (Annelida: Nereididae). Zoological Science, 34:252-260. <https://doi.org/10.2108/zs160154>
- WoRMS, 2019. World Register of Marine Species [Internet]. World Register of Marine Species, Accessed 21 May 2019, <<http://www.marinespecies.org/>>.
- Received July 8, 2019
Revised July 16, 2019
Accepted July 16, 2019