

First Record of the Monotypic Species, *Nonparahalosydna pleiolepis* (Polychaeta: Polynoidae) from Korean Waters, with Its DNA Barcoding Information

Kwang-Soo Kim^{1,2}, Hyun Ki Choi¹, Wonchoel Lee², Taeseo Park^{1,*}

¹National Institute of Biological Resources, Incheon 22689, Korea

²Department of Environmental Science, College of Natural Science, Hanyang University, Seoul 04763, Korea

ABSTRACT

The aim of this study is to report monotypic species, *Nonparahalosydna pleiolepis* (Marenzeller, 1879) for the first time from Korean waters with its DNA barcoding data. We collected individuals of the species from the subtidal zone of southern coast of Korea through scuba diving. To estimate DNA barcoding gap, the pairwise genetic distances were calculated between *N. pleiolepis* and its congeners (*Halosydna brevisetosa* Kinberg, 1856 and *Lepidonotus squamatus* (Linnaeus, 1758)) based on the cytochrome *c* oxidase subunit I gene (*COI*). Inter-specific genetic distances ranged from 18.7% to 24.6%, while intra-specific genetic distance within *N. pleiolepis* ranged from 0.3% to 0.5%. The maximum intra-specific genetic distance among the three species was 1.4%. The morphological diagnosis of *N. pleiolepis* with a taxonomic note on the species were also provided.

Keywords: Lepidonotinae, monotypic species, taxonomy, *COI*, DNA barcode

INTRODUCTION

The genus *Nonparahalosydna* Uschakov, 1982, one of the genera belongs to the family Polynoidae Kinberg, 1856, is represented by a monotypic species, *N. pleiolepis* (Marenzeller, 1879) (Uschakov, 1982; Imajima, 1997). This species was originally described as *Polynoe (Lepidonotus) pleiolepis* Marenzeller, 1879, and was assigned to the genus *Parahalosydna* Horst, 1915 (Uschakov and Wu, 1959, 1965) which is distinguished from genus *Polynoe* by having 15 pairs of elytra covering entire dorsum, and lateral antennae present terminally in prostomium. However, Uschakov (1982) found out the distinguished characteristic of *P. pleiolepis* from other *Parahalosydna* species, i.e. the absence of ceratophores in lateral antennae, and established a new genus of *Nonparahalosydna* for *P. pleiolepis*.

Nonparahalosydna pleiolepis was widely reported from East Asia, including in Chinese waters (Monro, 1928; Uschakov and Wu, 1959; Uschakov, 1982; Yan et al., 2006; Liu, 2008; Cai et al., 2013; Salazar-Vallejo et al., 2014) and Japanese waters (Izuka, 1912; Uschakov, 1982; Imajima, 1997; Jimi et al., 2018) (Fig. 1). However, taxonomic work for this

species has not yet been conducted in Korean waters.

DNA barcoding based on the mitochondrial cytochrome *c* oxidase subunit I (*COI*) has been used frequently as a powerful tool for reliable species identification among polychaetes (Park and Kim, 2007; Carr et al., 2011; Choi et al., 2017; Park and Kim, 2017; Park et al., 2019). However, the DNA barcoding information of polynoid species, including *N. pleiolepis*, is still very poor.

In this study, we report *N. pleiolepis* for the first time from Korean waters with its *COI* DNA barcoding sequence. The morphological diagnosis of *N. pleiolepis* was also provided with a taxonomic note based on specimens collected from Korean waters.

RESULTS AND DISCUSSION

Specimens of *N. pleiolepis* were collected from the subtidal zone of Chuja-myeon, Jeju-do, and Yeosu-si, Jeollanam-do (Fig. 1). All voucher specimens were deposited at the National Institute of Biological Resources. Specimen observation was conducted using a stereomicroscope (SZX10; Olympus,

© 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: 82-32-590-7108, Fax: 82-32-590-7223
E-mail: polychaeta@gmail.com

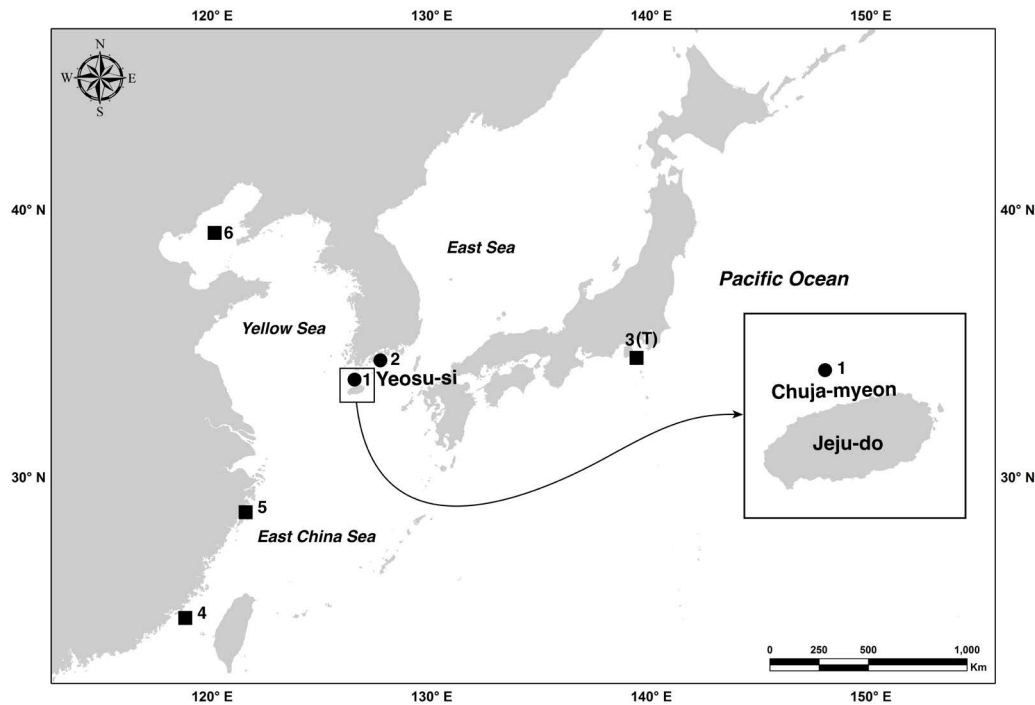


Fig. 1. Distribution of *Nonparahalosydna pleiolepis* (Marenzeller, 1879) in East Asia. 1, Chuja-myeon, Korea; 2, Yeosu-si, Korea; 3, Sagami Bay, Japan (Marenzeller, 1879); 4, Amoy Bay (Xiamen Bay), China (Monro, 1928); 5, Kiaochow Bay (Qingdao), China (Uschakov, 1982); 6, Gulf of Chihli (Bohai Sea) and Liaotung, China (Uschakov, 1982). (●), present study; (■), previous study; (T), type locality.

Tokyo, Japan) and a scanning electron microscope (JSM-6390, Jeol, Tokyo, Japan). The 658 bp of partial *COI* sequences from three individuals were obtained using a set of specific primers polyLCO (F) 5'-GAYTATWTTCAACAAATCATAAAGATATTGG-3' and polyHCO (R) 5'-TAMACTTCWGGGTGACCAAARAATCA-3' (Carr et al., 2011). Sequences were aligned by Geneious Prime (Biomatters, Auckland, New Zealand) program, and genetic distances were calculated by MEGA version X (Stecher et al., 2020), based on the Kimura-2-parameter model. The newly obtained sequences were deposited in GenBank under accession numbers MT445718–MT446720.

The pairwise genetic distances were calculated between *N. pleiolepis* and two relative species of *Halosydna brevisetosa* Kinberg, 1856 and *Lepidonotus squamatus* (Linnaeus, 1758), belong to the same family of Polynoidae based on the *COI* sequences mined from GenBank (Carr et al., 2011; Hardy et al., 2011). Inter-specific genetic distances between *N. pleiolepis* and the latter two species ranged from 18.7% to 24.6%, while intra-specific genetic distance within *N. pleiolepis* ranged from 0.3% to 0.5% (Table 1). The maximum intra-specific distance among the three species was 1.4%. All

polynoid species in this analysis showed specific validity, and the barcoding gap should exist between 1.4% and 18.7%. The *COI* sequences of *N. pleiolepis* reported in this study will be useful in future studies of polynoid taxonomy.

Family Polynoidae Kinberg, 1856

Genus ^{1*}*Nonparahalosydna* (Uschakov, 1982)

^{2*}*Nonparahalosydna pleiolepis* (Marenzeller, 1879)

Polynoe (Lepidonotus) pleiolepis Marenzeller, 1879: 114, fig. 4.

Polynoe pleiolepis: Izuka, 1912: 25, figs. 12–14.

Parahalosydna chinensis: Monro, 1928: 314–316, figs. 3, 4.

Parahalosydna pleiolepis: Uschakov & Wu, 1959: 30; 1965: 160 (trans. 1979: 19).

Nonparahalosydna pleiolepis: Uschakov, 1982: 101, figs. 1–5; Imajima, 1997: 85, figs. 40–42.

Material examined. Korea: 6 inds., Jeju-do: Jeju-si, Chuja-myeon, 31 Mar 2009, depth 25 m, Park T, Kil HJ (NIBRIV 0000164155, NIBRIV0000164180–81, NIBRIV0000165829, NIBRIV0000165831, NIBRIV0000169137); 1 ind., Jeolla-

Korean name: ^{1*}이형미룩비늘갯지렁이속 (신칭), ^{2*}이형미룩비늘갯지렁이 (신칭)

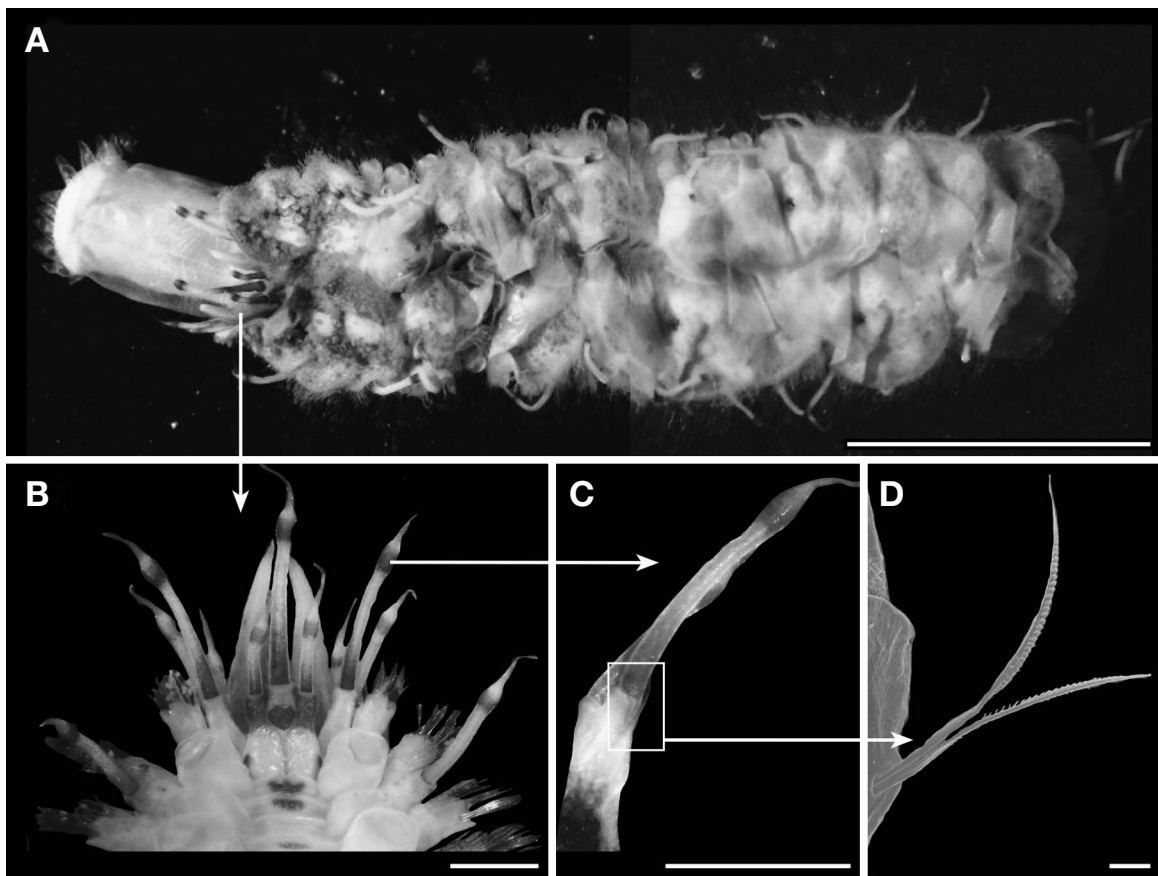


Fig. 2. *Nonparahalosydna pleiolepis* (Marenzeller, 1879). A, Dorsal view of the body; B, Dorsal view of the prostomium; C, Tentacular cirri (right); D, Two chaetae on the tentaculophore. Scale bars: A=2 mm, B, C=1 mm, D=0.05 mm.

nam-do: Yeosu-si, 24 Jun 2009, Kim YH, Hong SS (NIBR IV0000829796).

Diagnosis. Body elongated, flattened with 31 segments (Fig. 2A). Prostomium bilobed without cephalic peak with two pairs of eyes, palps, and three antennae. Median antenna with cylindrical ceratophore inserted in anterior notch, lateral antennae attached on anterior prolongations of prostomium (Fig. 2B). Tentacularphores with two chaetae respectively (Fig. 2C, D). Proboscis with nine pairs of distal papillae. Elytra 15 pairs attached on segments 2, 4, 5, 7, alternate segments to 29, covering dorsum entirely, overlapping medially and posteriorly with marginal fringes and micro- and/or macro-tubercles on surface (Fig. 3A–D). Parapodia sub-biramous. Notopodia smaller than neuropodia, anterodorsal face of neuropodia. Neuropodia more than 3 times wider and longer than notopodia, with similar length of pre-, post-chaetal lobes. Dorsal cirri with cirrophores; styles long and slender, cylindrical shape with tapered tips. Ventral cirri short, subulate. All chaetae unidentate with many rows of spines. Two kinds of notochoetae present; upper notochoetae

shorter and thicker, with tapered tip, and lower notochoetae longer and thinner, with fine tip (Fig. 3E). Two kinds of neurochaetae present; upper neurochaetae, longer, capillary shape with pointed tip, and lower neurochaetae shorter and thicker, with slightly hooked tip (Fig. 3F, G). Pygidium with one pair of anal cirri.

Remarks. *Nonparahalosydna pleiolepis* is characterized by a combination of the following characteristics: (1) lateral antennae are attached to anterior prolongations of the prostomium; (2) 31 segments and 15 pairs of elytra cover the dorsum entirely and posteriorly; and (3) neurochaetae are unidentate (Marenzeller, 1879; Uschakov, 1982; Imajima, 1997). These features were clearly apparent in the Korean specimens examined in this study. The number of elytra and body segments, which are regarded as important taxonomic features in polynoids, was incorrectly reported in some previous records of this species (Seidler, 1924; Imajima and Hartman, 1964). They mistakenly reported that the species has a body with 27 segments and 12 pairs of elytra, rather than 31 body segments and 15 pairs of elytra in the original description (Marenzeller,

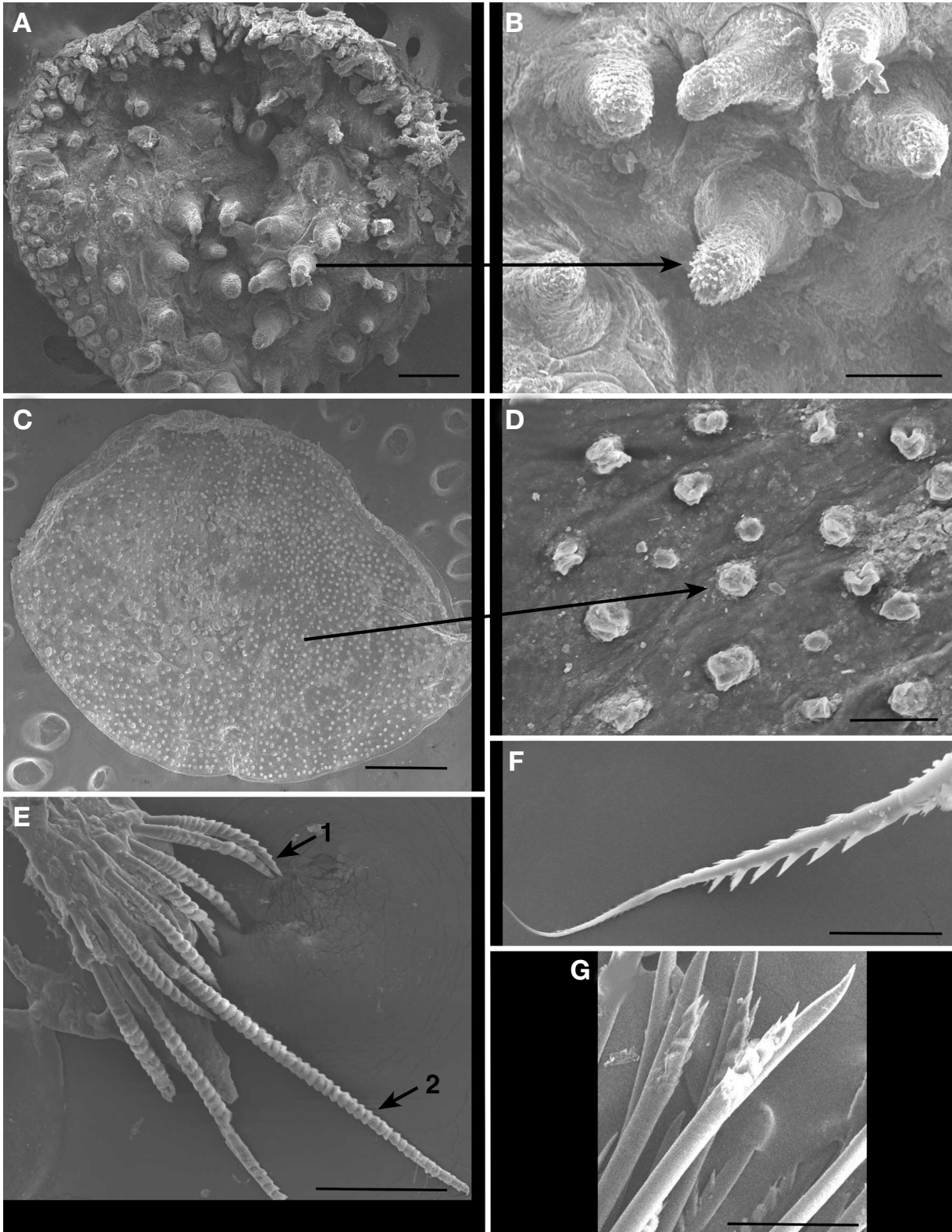


Fig. 3. Scanning electron microscope images of *Nonparahalosydna pleiolepis* (Marenzeller, 1879) (NIBRIV0000165831). A, The first elytron on segment 2; B, Macrotrubercles on the first elytron; C, The 8th elytron from segment 15; D, Microtubercles on the 8th elytron; E, Two kinds of notochaetae, upper (1) and lower (2) notochaetae; F, Upper chaeta of neurochaetal fascicle; G, Lower chaeta of neurochaetal fascicle. Scale bars: A=200 μ m, B, E, G=100 μ m, C=500 μ m, D=40 μ m, F=20 μ m.

Table 1. Pairwise genetic distance (K2P) based on COI sequences from three polynoid species

No.	Species	Accession No. (voucher No.)	1	2	3	4	5	6	7	8	9	References
1	<i>Nonparahalosydna pleiolepis</i>	MT445718 (NIBRIV0000164180)	-	0.005	0.003	0.189	0.193	0.187	0.246	0.246	0.246	Present study
2		MT445719 (NIBRIV0000829796)	0.005	-	0.005	0.193	0.198	0.191	0.246	0.246	0.246	
3		MT445720 (NIBRIV0000164181)	0.003	0.005	-	0.191	0.196	0.189	0.241	0.241	0.241	
4	<i>Halosydna brevisetosa</i>	HM473404	0.189	0.193	0.191	-	0.005	0.014	0.214	0.214	0.214	Carr et al. (2011)
5		HM473403	0.193	0.198	0.196	0.005	-	0.012	0.214	0.214	0.214	
6		HM473402	0.187	0.191	0.189	0.014	0.012	-	0.210	0.210	0.210	
7	<i>Lepidonotus squamatus</i>	GU672519	0.246	0.246	0.241	0.214	0.214	0.210	-	0.000	0.000	Hardy et al. (2011)
8		GU672518	0.246	0.246	0.241	0.214	0.214	0.210	0.000	-	0.000	
9		GU672517	0.246	0.246	0.241	0.214	0.214	0.210	0.000	0.000	-	

1879; Uschakov, 1982). In this respect, we presumed that their records were not about *N. pleiolepis* but about another species.

Habitats. The species occupies a variety of habitats, such as soft (Cai et al., 2013) and hard bottomed substrates (Yan et al., 2006; Jimi et al., 2018).

ORCID

Kwang-Soo Kim: <http://orcid.org/0000-0001-6132-8745>

Hyun Ki Choi: <http://orcid.org/0000-0001-5877-6256>

Wonchoel Lee: <http://orcid.org/0000-0002-9873-1033>

Taeseo Park: <https://orcid.org/0000-0003-2057-9411>

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENTS

This work was supported by a grant (NIBR201601111) from the National Institute of Biological Resources (NIBR), Ministry of Environment of Republic of Korea.

REFERENCES

- Cai W, Meng W, Zhu Y, Zhou J, Liu L, 2013. Assessing benthic ecological status in stressed Liaodong Bay (China) with AMBI and M-AMBI. *Chinese Journal of Oceanology and Limnology*, 31:482-492. <https://doi.org/10.1007/s00343-013-2177-0>
- 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, 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>
- Hardy SM, Carr CM, Hardman M, Steinke D, Corstorphine E, Mah C, 2011. Biodiversity and phylogeography of Arctic marine fauna: insights from molecular tools. *Marine Biodiversity*, 41:195-210. <https://doi.org/10.1007/s12526-010-0056-x>
- Imajima M, 1997. 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, Tokyo. National Science Museum Monographs, 13:1-131.
- Imajima M, Hartman O, 1964. The polychaetous annelids of Japan. Allan Hancock Foundation Publications, Occasional Paper, 26:1-452.
- Izuka A, 1912. The errantiate polychaeta of Japan. Journal of the College of Science, Imperial University of Tokyo, 30:1-262.
- Jimi N, Yasuoka N, Kajihara H, 2018. Polychaetes collected from floats of oyster-farming rafts in Kure, the Seto Inland Sea, Japan, with notes on the pest species *Polydora hoplura* (Annelida: Spionidae). Bulletin of the Kitakyushu Museum of Natural History and Human History Series A, 16:1-4.
- Liu R, 2008. Checklist of marine biota of China Seas. China Science Press, Beijing, pp. 1-1267.
- Marenzeller E, 1879. Südjapanische Anneliden I. Amphinomea, Aphroditea, Lycoridae, Phyllodocea, Hesionea, Syllidea, Eunicea, Glycera, Sternaspidea, Chaetoptera, Cirratulea, Amphitenea. Denkschriften der Mathematisch-naturwissenschaftlichen Classe der Kaiserliche Akademie der Wissenschaften, 41:109-154 (in German).
- Monro CCA, 1928. Notes on some unnamed polynoids in the British museum. Annals and Magazine of Natural History, 10:311-316. <https://doi.org/10.1080/00222932808672785>
- Park J, Kajihara H, Jung J, 2019. First record of the interstitial annelid *Pharyngocirrus uchidai* (Annelida: Saccocirridae) from Korea, confirmed by topotypic DNA barcoding data from Japan. Animal Systematics, Evolution and Diversity, 35:33-36. <https://doi.org/10.5635/ASED.2019.35.1.002>
- 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>
- Park TS, Kim W, 2007. A taxonomic study on *Perinereis nuntia* species group (Polychaeta: Nereididae) of Korea. Korean Journal of Systematic Zoology, 23:75-85. <https://doi.org/10.5635/KJSZ.2007.23.1.075>
- Salazar-Vallejo SI, Carrera-Parra LF, Muir AI, de León-González JA, Piotrowski C, Sato M, 2014. Polychaete species (Annelida) described from the Philippine and China Seas. Zootaxa, 3842:1-68. <https://doi.org/10.11646/zootaxa.3842.1.1>
- Seidler HJ, 1924. Beiträge zur Kenntnis der polynoiden I. Archiv für Naturgeschichte, Berlin, 89:1-217 (in German).
- Stecher G, Tamura K, Kumar S, 2020. Molecular Evolutionary Genetics Analysis (MEGA) for macOS. Molecular Biology and Evolution, 37:1237-1239. <https://doi.org/10.1093/molbev/msz312>
- Uschakov PV, 1982. Polychaetes of the Suborder Aphroditiformia of the Arctic Ocean and the northwestern Part of the Pacific Ocean, Families Aphroditidae and Polynoidae. Fauna of the U.S.S.R., Mnogoshchetinkovyye chervil (Fauna of the USSR, Polychaeta), 2:1-272 (in Russian).
- Uschakov PV, Wu BL, 1959. Polychaete worms of families Phyllococeidae and Aphroditidae (Polychaeta Errantia) from the Yellow Sea. Archives of the Institute of Chinese Oceanology, 1:1-40.
- Uschakov PV, Wu BL, 1965. The Polychaeta Errantia of the Yellow Sea (translated from Russian in 1979, Amerind Publishing, New Delhi, pp. 1-137). Explorations of the Fauna of the Seas, 3:145-258.
- Yan T, Yan W, Dong Y, Wang H, Yan Y, Liang G, 2006. Marine fouling of offshore installations in the northern Beibu Gulf of China. International Biodeterioration and Biodegradation, 58:99-105. <https://doi.org/10.1016/j.ibiod.2006.07.007>

Received March 30, 2020
 Revised July 13, 2020
 Accepted July 13, 2020