

DNA Barcoding of *Scoelepis (Parascoelepis) papillosa* (Annelida, Spionidae) in Korea, with Additional Taxonomic Notes

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ABSTRACT

Scoelepis (Parascoelepis) papillosa (Okuda, 1937), originally described from a single incomplete individual from Jeju Island in Korea, was collected from the intertidal sandflats of Soan Island (Jeollanam-do province) in Korea. The examined specimens of *S. (P.) papillosa* agree well with the original description in having the papillae on the basal sheath of the palps, presence of occipital antenna, absence of notochaetae in chaetiger 1, branchiae completely fused with notopodial postchaetal lamellae at the anterior chaetigers, and neuropodial hooded hooks appearing from chaetiger 16. In this study, the sequences of partial mitochondrial cytochrome *c* oxidase subunit I (*COI*), 16S ribosomal DNA (16S rDNA), and the nuclear 18S ribosomal DNA (18S rDNA) of the species were determined. We also provide the detailed description and illustrations on this species based on the complete specimens newly collected in this study.

Keywords: 16S rDNA, 18S rDNA, *COI*, Korean waters, taxonomy

INTRODUCTION

The genus *Scoelepis* Blainville, 1828 is one of the most speciose taxa of spionid polychaetes comprised of 70 species in the subgenus *Scoelepis* Blainville, 1828 and 14 species in the subgenus *Parascoelepis* Maciolek, 1987 (Blake et al., 2019). The *Parascoelepis* species have multidentate hooks with a curved shaft that can be distinguished from those of *Scoelepis* (Maciolek, 1987; Delgado-Blas, 2006). Among them, five *Parascoelepis* species, *S. (P.) geniculata* Imajima, 1992, *S. (P.) globosa* Wu and Chen, 1964, *S. (P.) papillosa* (Okuda, 1937), *S. (P.) texana* Foster, 1971, and *S. (P.) yamaguchii* (Imajima, 1959), have been recorded in Northeast Asia (Okuda, 1937; Imajima, 1959, 1992; Zhou et al., 2009; Abe and Sato-Okoshi, 2021). One *Scoelepis* species, *S. (P.) papillosa*, is considered native to Korea (Okuda, 1937).

Okuda (1937) originally described *S. (P.) papillosa* as *Nerinides papillosus* based on a single incomplete individual (42 chaetigers) from Jeju Island (Quelpart Island) in Korea. Pettibone (1963) erected the subgenus *Nerinides* Mesnil, 1896 based on the absence of notches on neuropodial postchaetal lamellae. Later, Maciolek (1987) suggested that subgenus

Nerinides is invalid, and the species should be assigned to the subgenus *Parascoelepis* Maciolek, 1987 based mainly on the morphology of the hooded hooks. During the survey of Korean spionid polychaetes, the specimens of *S. (P.) papillosa* agreed well with the original description of the diagnostic features collected from Soan Island (Jeollanam-do province) in Korea. The sequences of partial mitochondrial cytochrome *c* oxidase subunit I (*COI*), 16S ribosomal DNA (16S rDNA), and the nuclear 18S ribosomal DNA (18S rDNA) are provided.

The samples were collected from intertidal sandflats on the southern coast of Korea (Fig. 1) using 500 µm-mesh sieves. The observation was carried out under both live and fixed materials. The live materials were relaxed in a 10% magnesium chloride (MgCl₂) solution, and morphological observations were then carried out under a stereomicroscope (MZ125; Leica, Germany). The photographs were taken using a digital camera (Dhyana 400DC; Tucsen, China) with a capture program (Mosaic version 15; Tucsen). A methyl green staining was performed according to the method of Meißner (2005). After the observations, the specimens were fixed in 4% formaldehyde for the morphological study and in 95% ethanol for the molecular study. Voucher specimens were deposi-

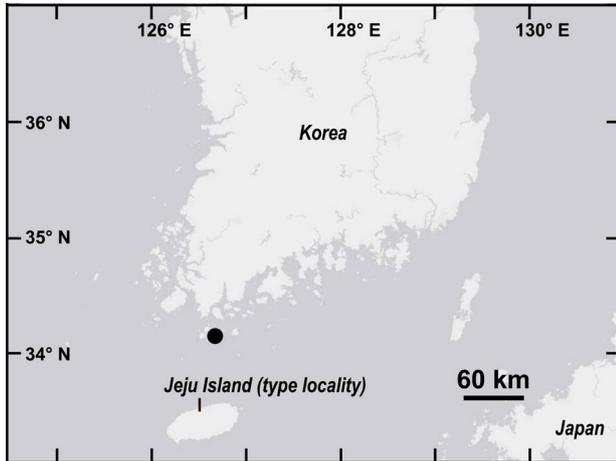


Fig. 1. Map showing the location of the sampling station (●) where *Scoelepis (Parascoelepis) papillosa* (Okuda, 1937) was collected in this study.

ted at the National Institute of Biological Resources in Korea (NIBR). The genomic DNA was extracted from a palp from three specimens using a LaboPass Tissue Mini (Cosmo GENE-TECH, Seoul, Korea) according to the manufacturer's instructions. Polymerase chain reaction amplification of the sequences of the three gene regions was carried out using the following primer sets: LCO1490 and HCO709 for *COI* (Blank et al., 2008), 16Sar and 16Sbr for 16S rDNA (Kessing et al., 1989), and 18E and 18B for 18S rDNA (Mincks et al., 2009). Molecular analyses were performed using the partial sequences aligned by Geneious 8.1.9 (Biomatters Auckland, New Zealand).

RESULTS AND DISCUSSION

The partial mitochondrial *COI*, 16S rDNA, and nuclear 18S rDNA sequences from three specimens (NIBRIV0000890432–4) of *S. (P.) papillosa* were determined. The newly determined sequences were registered at the GenBank (accession Nos. OK514649–51 for *COI* [up to 677 bp], OK523336–8 for 16S rDNA [up to 519 bp], and OK523332–4 for 18S rDNA [up to 1,791 bp]). The intra-specific genetic distances were 0.1–0.3% in the *COI* (669 bp), 0.0–0.2% in 16S rDNA (519 bp) and no variations were detected in 18S rDNA (1,791 bp). Based on the available molecular data from the GenBank, *S. (P.) papillosa* showed a 17.5% (107/611 bp) difference with *S. (S.) daphoinos* Zhou, Ji & Li, 2009 (GU362687, Zhou et al., 2010) in *COI*, 12.9% (61/473 bp) with *Scoelepis* sp. (MZ338352) (Li et al., unpublished) in 16S rDNA, and 0.2%



Fig. 2. *Scoelepis (Parascoelepis) papillosa* (Okuda, 1937), NIBRIV0000890436, with palps, fixed in formalin. Scale bar=2.0 mm.

(4/1,625 bp) with *Scoelepis* sp. (MW590231, Wang and Ke, unpublished) in 18S rDNA.

The DNA sequences determined in this study along with additional morphological observations (see below) will be useful information for further taxonomic or phylogenetic studies of the genus *Scoelepis*.

Order Spionida *sensu* Rouse and Fauchald, 1997
 Family Spionidae Grube, 1850
 Genus *Scoelepis* Blainville, 1828
 Subgenus *Parascoelepis* Maciolek, 1987

1Scoelepis (Parascoelepis) papillosa* (Okuda, 1937)**

Nerinides papillosus Okuda, 1937: 219, figs. 1, 2.

Scoelepis (Nerinides) papillosa Pettibone, 1963: 93.

Scoelepis (Parascoelepis) papillosa Maciolek, 1987: 20.

Material examined. Korea: 5 individuals, Jeollanam-do: Wando-gun, Soan-myeon, Gahak-ri, Soan Island, 34°9'56.1" N, 126°39'29.8"E, intertidal sandflats, 25 May 2021, Lee GH, Lee HE (NIBRIV0000890432–5 fixed in 95% ethanol, NIBRIV0000890436 fixed in formalin).

Description. Body complete (NIBRIV0000890436) (Fig. 2) with 70 chaetigers, 1.8 mm in width (measured at anterior body region) and 45.9 mm in length; Body dorsoventrally flattened anteriorly, cylindrical posteriorly. Yellowish-white color in both live and formalin-fixed specimens (Figs. 2, 4A). Prostomium elongated, anteriorly conical, distally pointed, posteriorly extended into caruncle attached to dorsum; caruncle extending to end of chaetiger 2 without elevation; two pairs of

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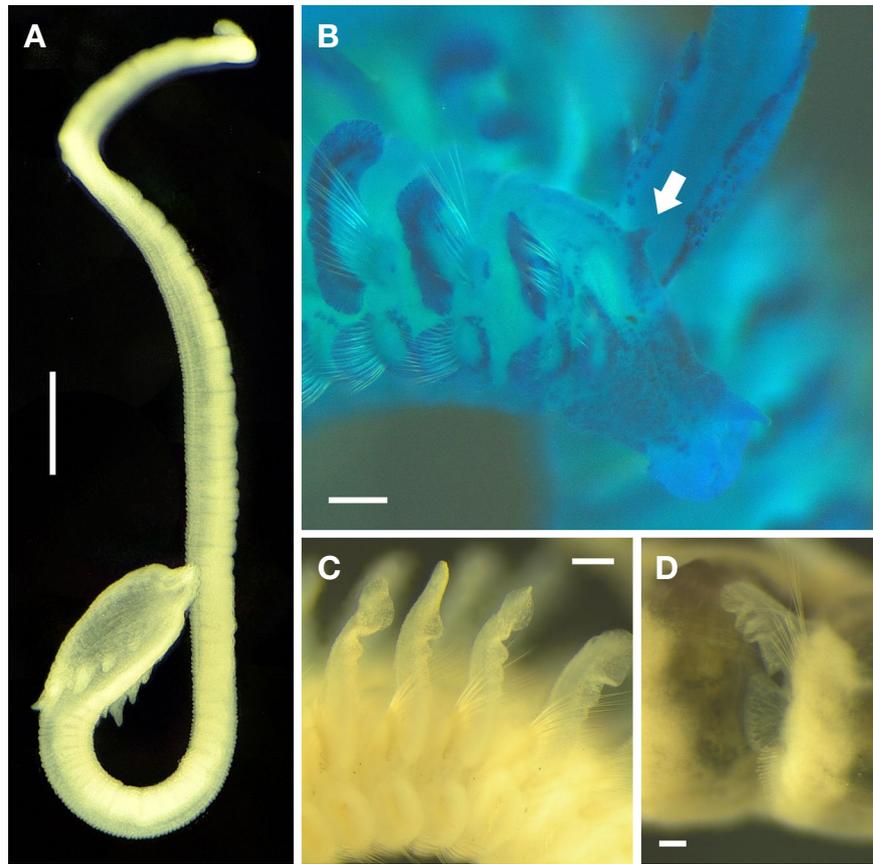


Fig. 3. *Scolelepis (Parascolelepis) papillosa* (Okuda, 1937), NIBRIV0000890436, right palp removed. A, Right palp; B, Anterior end stained with a methyl green solution, right palp removed, lateral view, occipital antenna (arrow); C, Chaetigers 11–14, lateral view; D, Chaetiger 44, lateral view. Scale bars: A=0.5 mm, B, C=0.2 mm, D=0.1 mm.

reddish eyes arranged in trapezoid (some specimens without eyes), anterolateral pair larger, crescent-shaped, widely apart, and posterior pair smaller, rounded; occipital antenna present on middle part of caruncle; prostomium separated from peristomium by furrow (Fig. 3B). Peristomium well-developed, separated from prostomium, forming well-developed lateral wings. Palps reaching to about chaetiger 17, with papillated basal sheath (Fig. 3A); papillae present on upper side of basal sheath; 8–10 irregular white spots exhibited on palps in living specimens (Fig. 4A), but gradually fade out after fixation and then completely disappear. Low but distinct transversal ciliated bands present throughout body.

Chaetiger 1 well developed, with rounded postchaetal lamellae in both rami; notochaetae absent. Branchiae from chaetiger 2, present throughout body; longest branchiae on chaetigers 8–18 (Fig. 3C); accessory branchiae absent. Notopodial postchaetal lamellae foliate and folded, completely fused to branchiae (Fig. 3C, D). Neuropodial postchaetal lamellae broadly rounded. Prechaetal notopodial lamellae small and rounded. Anterior chaetae all capillaries granulated

with narrow sheaths, arranged in two rows in both rami; notochaetae slightly granulated and neurochaetae stout, heavily granulated; notochaetae more posteriorly arranged in bundle. Neuropodial hooded hooks appearing from chaetiger 16 (chaetiger 17 in two specimens) (Fig. 4B), numbering up to 21 per fascicle; hooks with small 1 pair of teeth and uppermost tooth surmounting well-developed main fang (Fig. 4C); hooks accompanied by 1–2 capillaries.

Pygidium with ventral, more or less bilobed cushion and dorsal anus.

Methyl green staining pattern. Prostomium, peristomium, basal sheath of palp (especially papillae), occipital antenna, notopodial postchaetal lamellae, neuropodial postchaetal lamellae, pygidium most intensely stained (Fig. 3B).

Habitat. Sandflats of intertidal zone.

Distribution. Southern coast and Jeju Island, Korea.

Remarks. The specimens of *S. (P.) papillosa* morphologically agree well with the original description in the following characteristics: the presence of papillae on the basal sheath of palps, occipital antenna, the absence of notochaetae in

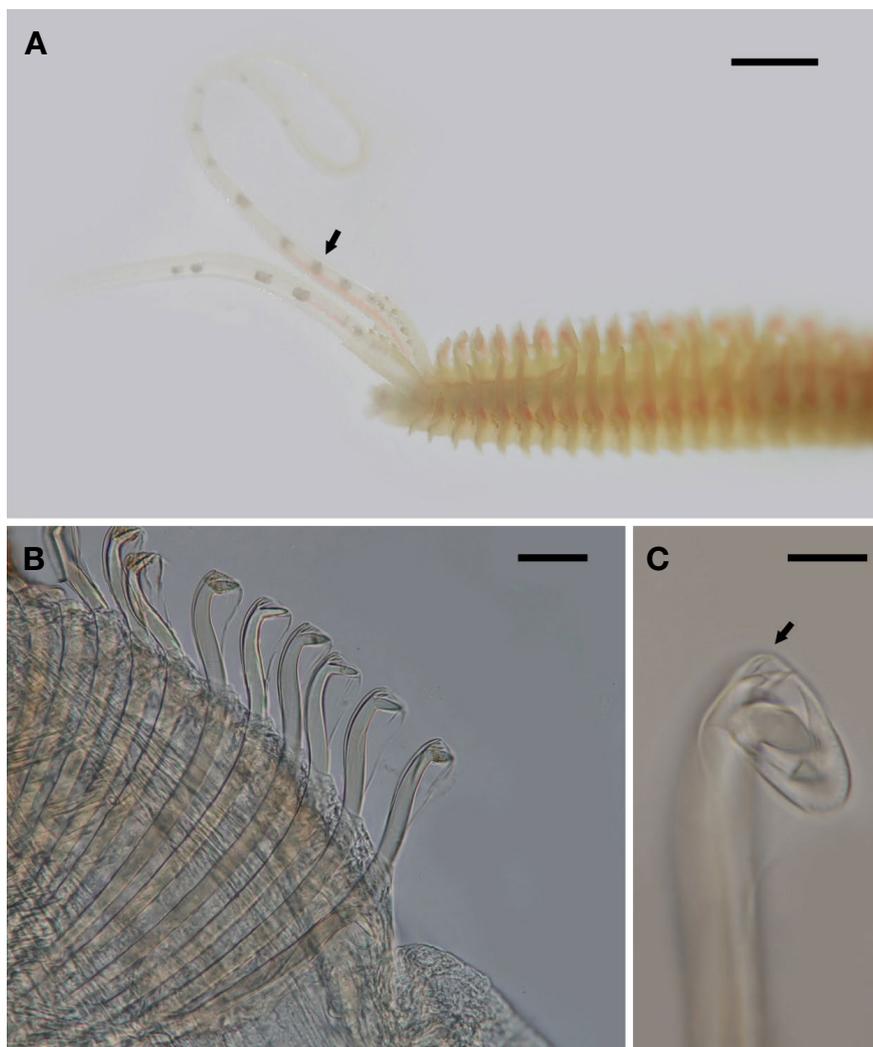


Fig. 4. *Scolelepis (Parascolelepis) papillosa* (Okuda, 1937), NIBRIV0000890436 (A), NIBRIV0000890435 (B, C). A, Live specimen in seawater, dorsolateral view, irregular white spot (arrow); B, Neuropodial hooded hooks in chaetiger 71, lateral view; C, Neuropodial hooded hook in chaetiger 42, a small uppermost tooth (arrow), frontolateral view. Scale bars: A=2.0 mm, B, C=50.0 μ m, D=20.0 μ m.

chaetiger 1, the branchiae completely fused with notopodial postchaetal lamellae in anterior chaetigers, and neuropodial hooded hooks appearing from chaetiger 16 (Okuda, 1937). We newly observed the taxonomic characteristics based on the complete specimens as follows: irregular white spots on the palps in live specimens, two pairs of reddish eyes arranged in trapezoid (absent in some specimens), notopodial postchaetal lamellae fully fused to branchiae throughout the body, neuropodial hooded hooks with four teeth, and pygidium with ventral bilobed cushion (see above).

Scolelepis (Parascolelepis) papillosa is most similar to *S. (P.) yamaguchii* from Japan in having the papillae on the basal sheath of palps, occipital antenna, branchiae completely fused to notopodial postchaetal lamellae in the anterior chaetigers, and bilobed pygidium (Imajima, 1959). However, it

differs from the latter by having the caruncle extending to the end of chaetiger 2 (vs. chaetiger 1), absence of notochaetae in chaetiger 1 (vs. present), the branchiae present on throughout the body (vs. absent at posterior region), and neuropodial hooded hooks with three small teeth above the main fang appearing from chaetigers 16–17 (vs. hooks with four small teeth above the main fang appearing from chaetigers 20–21 (Imajima, 1959, 1992).

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CONFLICTS OF INTEREST

Gi-Sik Min, a contributing editor of the *Animal Systematics, Evolution and Diversity*, was not involved in the editorial evaluation or decision to publish this article. All remaining authors have declared no conflicts of interest.

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REFERENCES

- Abe H, Sato-Okoshi W, 2021. Molecular identification and larval morphology of spionid polychaetes (Annelida, Spionidae) from northeastern Japan. *ZooKeys*, 1015:1-86. <https://doi.org/10.3897/zookeys.1015.54387>
- Blake JA, Maciolek NJ, Meissner K, 2019. Spionidae Grube, 1850. In: *Handbook of Zoology: Annelida, Vol. 2. Pleistoannelida, Sedentaria II*. De Gruyter, Berlin, pp. 1-102.
- Blank, M, Laine AO, Jürss K, Bastrop R, 2008. Molecular identification key based on PCR/RFLP for three polychaete sibling species of the genus *Marenzelleria*, and the species' current distribution in the Baltic Sea. *Helgoland Marine Research*, 62:129-141. <https://doi.org/10.1007/s10152-007-0081-8>
- Delgado-Blas VH, 2006. Partial revision of *Scoelepis* (Polychaeta: Spionidae) from the Grand Caribbean Region, with the description of two new species and a key to species recorded in the area. *Contributions to Zoology*, 75:75-97. <https://doi.org/10.1163/18759866-0750102003>
- Imajima M, 1959. A description of a new species of the Spionidae (Polychaeta), *Nerinides yamaguchii* n. sp., with notes on its development. *Journal of Hokkaido Gakugei University*, 10:155-165.
- Imajima M, 1992. Spionidae (Annelida, Polychaeta) from Japan VIII. The genus *Scoelepis*. *Bulletin of the National Science Museum, Tokyo, Series A (Zoology)*, 18:1-34.
- Kessing B, Croom H, Martin A, McIntosh C, Owen McMillian W, Palumbi S, 1989. The simple fool's guide to PCR. Department of Zoology, University of Hawaii, Honolulu, HI, pp. 1-47.
- Maciolek NJ, 1987. New species and records of *Scoelepis* (Polychaeta: Spionidae) from the East coast of North America, with a review of the subgenera. *Bulletin of the Biological Society of Washington*, 7:16-40.
- Meißner K, 2005. Revision of the genus *Spiophanes* (Polychaeta: Spionidae); with new synonymies, new records and descriptions of new species. *Zoosystematics and Evolution*, 81:3-65. <https://doi.org/10.1002/mmnz.200310001>
- Mincks SL, Dyal PL, Paterson GLJ, Smith CR, Glover AG, 2009. A new species of *Aurospio* (Polychaeta, Spionidae) from the Antarctic shelf, with analysis of its ecology, reproductive biology and evolutionary history. *Marine Ecology*, 30:181-197. <https://doi.org/10.1111/j.1439-0485.2008.00265.x>
- Okuda S, 1937. Spioniform polychaetes from Japan. *Journal of the Faculty of Science, Hokkaido Imperial University, Series 6, Zoology*, 5:217-254.
- Pettibone MH, 1963. Revision of some genera of polychaete worms of the family Spionidae, including the description of a new species of *Scoelepis*. *Proceedings of the Biological Society of Washington*, 76:89-104.
- Zhou H, Zhang Z, Chen H, Sun R, Wang H, Guo L, Pan H, 2010. Integrating a DNA barcoding project with an ecological survey: a case study on temperate intertidal polychaete communities in Qingdao, China. *Chinese Journal of Oceanology and Limnology*, 28:899-910. <https://doi.org/10.1007/s00343-010-9131-1>
- Zhou J, Ji W, Li X, 2009. A new species of *Scoelepis* (Polychaeta: Spionidae) from sandy beaches in China, with a review of Chinese *Scoelepis* species. *Zootaxa*, 2236:37-49. <https://doi.org/10.11646/zootaxa.2236.1.3>

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