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Source: Zoological Science, 34(3):252-260.

Published By: Zoological Society of Japan

<https://doi.org/10.2108/zs160154>

URL: <http://www.bioone.org/doi/full/10.2108/zs160154>

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# Description of a New Species for Asian Populations of the “Cosmopolitan” *Perinereis cultrifera* (Annelida: Nereididae)

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An Asian species of *Perinereis*, which has been regarded as a local population of a cosmopolitan species *P. cultrifera* (Grube, 1840) (type locality: Italy), is described as a new species, *P. euiini*, based on Korean specimens. This new species is distinguishable from *P. cultrifera* and other similar species such as *P. floridana* (Ehlers, 1868) (type locality: USA), *P. anderssoni* Kinberg, 1866 (type locality: Brazil), and *P. helleri* (Grube, 1878) (type locality: Philippines) by the character combination of the absence of lateral group of paragnaths in area III on the proboscis, the presence of small notopodial prechaetal lobe in anterior parapodia, and the greatly expanded dorsal ligule in posterior parapodia. All previous records of “*P. cultrifera*” from eastern Asia (Taiwan, China, Japan, and Korea) are judged to belong to *P. euiini* sp. nov., leading to the conclusion that *P. cultrifera* is not a cosmopolitan species. Specimens previously identified as *P. cultrifera* var. *floridana* (or *P. cultrifera floridana*) in eastern Asia are also judged to belong to *P. euiini* sp. nov., although they have different paragnath number in area V (one instead of three). Their aberrant paragnath number was regarded as an intraspecific variation of *P. euiini* sp. nov. The comparison of DNA sequences of the mitochondrial cytochrome c oxidase subunit I (COI) between *P. euiini* sp. nov. and “*P. cultrifera*” from China and Portugal also supports the erection of the new species.

**Key words:** cosmopolitan species, Eastern Asia, Nereididae, new species, *Perinereis cultrifera*

## INTRODUCTION

*Perinereis* Kinberg, 1865 is one of the most diverse genera in the family Nereididae, currently including 19 species and subspecies in eastern Asia (Wu, 1967; Imajima, 1996; Paik, 1989; Sun and Yang, 2004; Glasby and Hsieh, 2006; Salazar-Vallejo et al., 2014; Tanaka, 2016). Among them, *Perinereis cultrifera* (Grube, 1840) (type locality: Naples, Italy) has been regarded as a cosmopolitan species, recorded from the Atlantic Ocean, Mediterranean Sea, Red Sea, Arabian Sea, Indian Ocean, Yellow Sea, and Pacific Ocean (Grube, 1840; Fauvel, 1932, 1936, 1953; Uschakov and Wu, 1965; Imajima, 1972, 1996, 2003; Paik, 1977, 1979, 1989; Wu et al., 1985; Hutchings et al., 1991; Wehe and Fiege, 2002). *Perinereis floridana* (Ehlers, 1868) (type locality: Florida, USA), which was relegated to one of varieties of *P. cultrifera* by Fauvel (1932), has also been known as a widely distributed species, recorded from the Caribbean Sea, Atlantic Ocean, Arabian Sea, Indian Ocean, Yellow Sea, and Pacific Ocean (Ehlers, 1864–1868; Fauvel, 1932, 1953; Uschakov and Wu, 1965; Imajima, 1972, 1996; Rho and Lee, 1982; Wu et al., 1985; Paik, 1989; de Leon-Gonzalez and Solis-Weiss, 1998; Wehe and Fiege, 2002).

In the present study, to clarify whether *P. cultrifera* and *P. floridana* are true cosmopolitan species, we reexamined the Asian specimens that have been previously identified as *P. cultrifera* and *P. cultrifera* var. *floridana* (or *P. cultrifera floridana*) by comparison with type and non-type materials of these species and their recent redescrptions. Our results demonstrate that the Asian specimens are morphologically different from both *P. cultrifera* and *P. floridana*, and judged to belong to an undescribed species, which is described here as a new species. Additionally, DNA sequences of the mitochondrial cytochrome c oxidase subunit I (COI) are compared between the new species from Korea and “*P. cultrifera*” from China and Portugal.

## MATERIALS AND METHODS

### Sample collection and morphological observation

Two syntypes of *Nereis cultrifera* Grube, 1840, and newly collected Korean specimens keying out to “*Perinereis cultrifera*”, following the identification keys provided by Imajima (1972) and Wu et al. (1985), were examined. Worms were collected from sessile organisms attached to both intertidal and subtidal rocky areas. We picked sessile organisms attached to intertidal rocky area with digging knife during low tide. In the case of subtidal rocky area, sessile organisms were collected by SCUBA diving. Collected samples were rinsed by seawater in a bucket. The worms were separated from sessile organisms by sieving. To make newly collected worms’ proboscis protrude, 7% MgCl<sub>2</sub> was added for anesthetization and

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doi:10.2108/zs160154

slight pressure was applied to their pharyngeal region. Specimens were then fixed in 7% neutralized formalin or 80% ethanol and subsequently preserved in 80% ethanol. Their paragnaths in each area on the proboscis were counted. Photographs were taken with a built-in digital camera attached to a stereoscopic microscope (Leica EZ4 HD). Drawings were prepared with a camera lucida attached to a stereoscopic microscope (Nikon SMZ1500). Non-type materials deposited in museum collections as *Perinereis anderssoni* Kinberg, 1866, *P. helleri* (Grube, 1878), or *P. cultrifera* var. *floridana* (Ehlers, 1868), were also examined for comparison.

Terminology of morphological characteristics followed that of Bakken and Wilson (2005). Abbreviations of specimen repositories are as follows: NIBR, National Institute of Biological Resources, Incheon, Korea; ZMB, Museum für Naturkunde, Berlin, Germany; USNM, National Museum of Natural History, Washington DC, USA; NSMT, National Museum of Nature and Science, Tsukuba, Japan.

#### DNA extraction, PCR amplification, and molecular analysis

Three non-type specimens of *P. euiini* sp. nov. (NIBRIV0000502105: Pcgcoje1–3) were selected for genomic DNA extraction. Total genomic DNA was extracted from ventral soft tissue using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. PCR amplification of COI was conducted using a set of universal primers LCO1490 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO1490 5'-TAAACTTCAGGGTGACCAAAAATCA-3' (Folmer et al., 1994). The PCR thermal cycling was 94°C for 30 sec; 35 cycles of 98°C for 10 sec, 47°C for 30 sec, and 72°C for 1 min; and a final extension of 72°C for 10 min. Amplified PCR products were purified using the QIAquick PCR purification Kit (Qiagen, Valencia, CA, USA). The sequencing reaction was performed with BigDye Terminator ver. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) using the same primers. The products were then electrophoresed in an ABI 3730 sequencer (Applied Biosystems, Foster City, CA, USA); both directions were sequenced. The obtained sequences were aligned using Clustal W implemented in MEGA ver. 7 (Kumar et al., 2016). Pairwise distances were calculated using the Kimura-2-parameter model (Kimura, 1980).

## RESULTS

### Taxonomic description

Family **Nereididae** Blainville, 1818

Genus ***Perinereis*** Kinberg, 1865

***Perinereis euiini*** sp. nov.

(Korean name: Han-tomak-nunssup-cham-gaetjireongi)

(Figs. 1, 2, 3A, 4A, B, 5A, B)

*Nereis cultrifera*: Izuka, 1912: 151–153, pl. 16, figs. 7–14.

*Perinereis cultrifera*: Fauvel, 1936: 62–63; Okuda, 1938: 92; Okuda, 1950: 52, figs. i–j; Okuda and Yamada, 1954: 185, fig. 3F; Imajima and Hartman, 1964: 152; Uschakov and Wu, 1965: 200–201 (trans. 1979: 64); Wu, 1967: 66–67; Imajima, 1972: 88–91, figs. 24a–l, 27; Imajima, 1996: 128–129, figs. 102, 102'; Paik, 1977: 174–176, fig. 17a–g; Paik, 1979: 41, 53, 58, fig. 7e, f; Paik, 1989: 314–315, pl. 27, figs. 64 (1–4), 74a–c; Wu et al., 1985: 213; Khlebovich, 1996: 145–146, fig. XLV1–7; Imajima, 2003: 176; Sun and Yang, 2004: 186–190, figs. 106a–j, 107a–e.

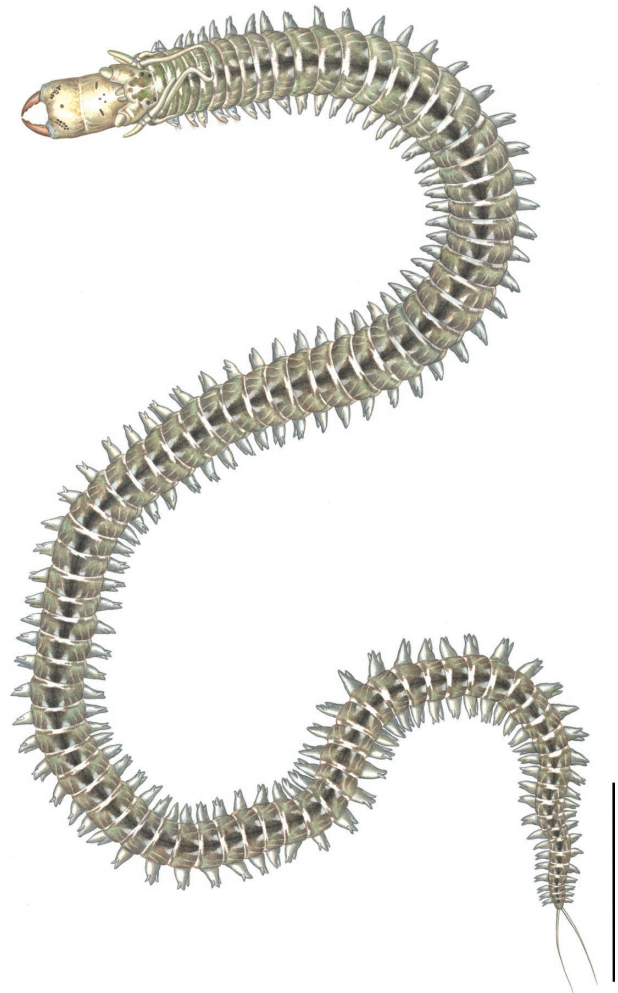
*Perinereis cultrifera* var. *typica*: Khlebovich and Wu, 1962: 39; Khlebovich, 1963: 57; Uschakov and Wu, 1965: 200–201 (trans. 1979: 64).

*Perinereis cultrifera typica*: Wu et al., 1985: 214, fig. 118a–j (not fig. 121a–j).

*Perinereis cultrifera* var. *floridana*: Khlebovich and Wu, 1962: 39, 51; Khlebovich, 1963: 57; Uschakov and Wu, 1965: 200–201 (trans. 1979: 64); Imajima, 1972: 91, fig. 25a–b.

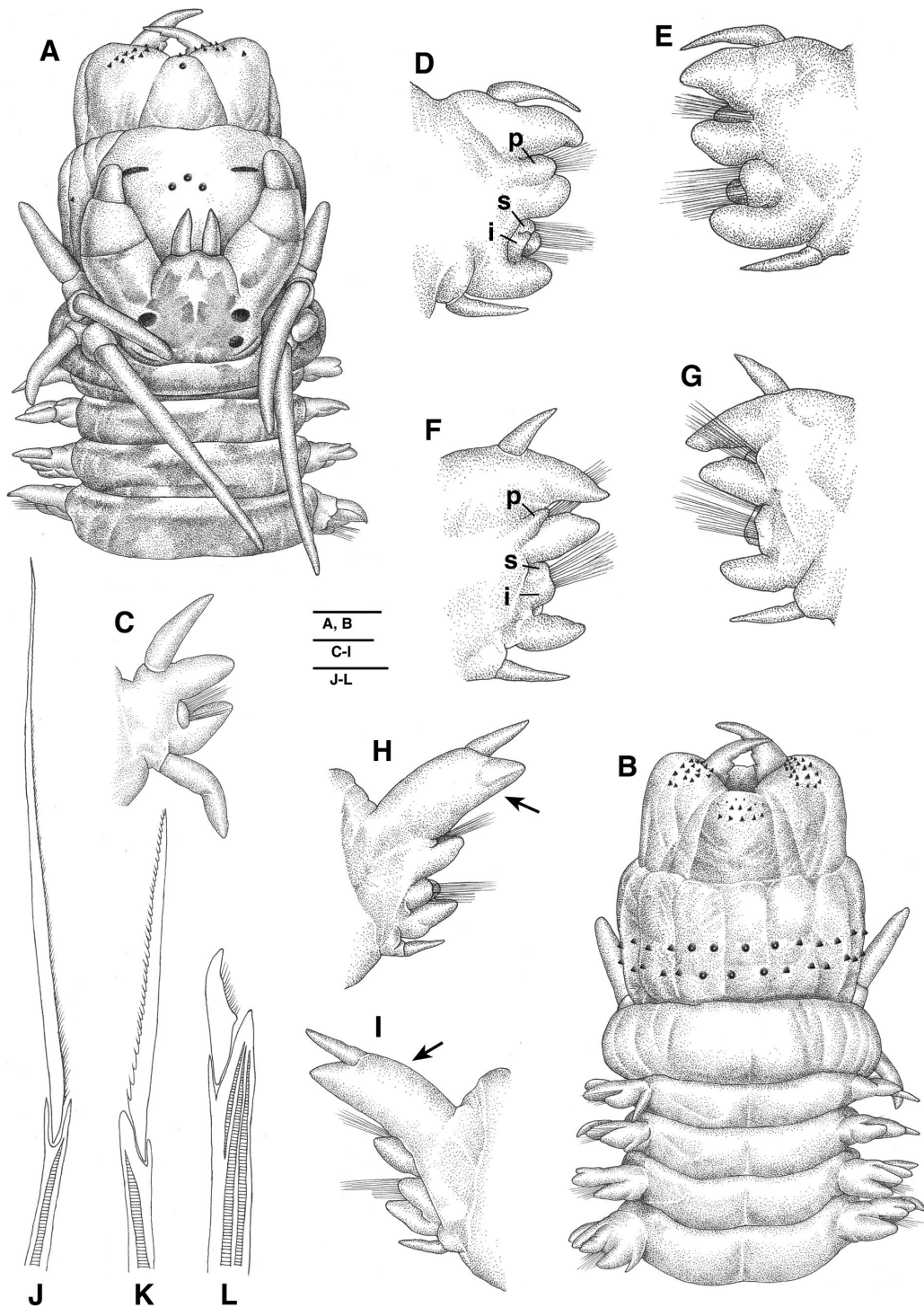
*Perinereis cultrifera floridana*: Rho and Lee, 1982: 39, pl. 3, figs. 2–3; Wu et al., 1985: 218, fig. 123a–b; Paik, 1989: 316, pl. 27, figs. 65 (1–2), 75; Imajima, 1996: 130, fig. 103; Khlebovich, 1996: 146; Sun and Yang, 2004: 186–190, fig. 108a–b.

**Type materials.** Holotype, NIBRIV0000502083, associated with algal roots in intertidal rocky shore, Gusan-myeon, Masan-si, Gyeongsangnam-do, Korea (35°6'41"N, 128°36'4"E), 28 June 2006, collected by T. Park, fixed in 7% formalin. Paratypes: (1) NIBRIV0000502100, 1 ind., associated with mussels in intertidal boulder shore, Gagybeach, Jindo-gun, Jeollanam-do, Korea, 8 October 2008, collected by T. Park, fixed in 80% ethanol. (2) NIBRIV0000502101, 2 inds., associated with oysters in intertidal rocky shore, Dolsan-eup, Yeosu-si, Jeollanam-do, Korea (34°35'56"N, 127°47'45"E), 10 October 2008, collected by T. Park, fixed in 80% ethanol. (3) NIBRIV0000502102, 1 ind., associated with algal roots in intertidal rocky shore, Gunnae-ri, Wando-



**Fig. 1.** Drawing of *Perinereis euiini* sp. nov. based on a paratype (NIBRIV0000502103). Scale bar, 10 mm.

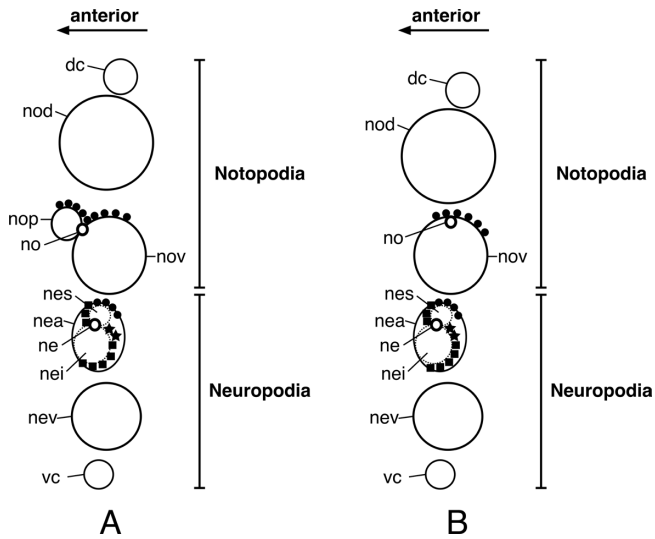




**Fig. 2.** *Perinereis euiini* sp. nov., holotype, NIBRIV0000502083. (A, B) Dorsal and ventral views of anterior end with the everted proboscis. (C) Anterior view of parapodium 1. (D, E) Anterior and posterior views of parapodium 9. (F, G) Anterior and posterior views of parapodium 46. (H, I) Anterior and posterior views of parapodium 79. (J) Homogomph spiniger from notopodial fascicle in parapodium 46. (K) Heterogomph spiniger from neuropodial infra-acicular fascicle in parapodium 9. (L) Heterogomph falciger from neuropodial infra-acicular fascicle in parapodium 9. i, inferior lobe; p, prechaetal lobe; s, superior lobe. Arrows indicate greatly expanded notopodial dorsal ligule. Scale bar: 1 mm in (A, B); 0.5 mm in (C–I); 0.03 mm in (J–L).

eup, Wando-gun, Jeollanam-do, Korea (34°18'6"N, 126°46'12"E), 4 September 2013, collected by T. Park, fixed in 80% ethanol. (4) NIBRIV0000502103, 3 inds., 13 m in depth, associated with sessile organisms in subtidal rocky

area near Baesim port, Dongdo-ri, Samsan-myeon, Yeosu-si, Jeollanam-do, Korea (34°3'32"N, 127°19'34"E), 23 April 2013, collected by T. Park, fixed in 80% ethanol. (5) NIBRIV0000771297, 1 ind., 15 m in depth, associated with



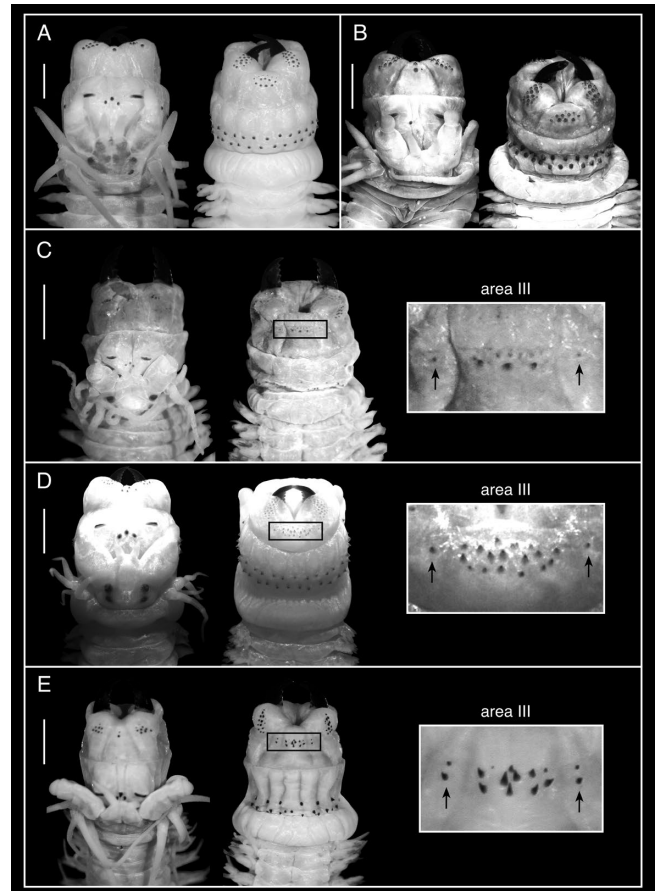
**Fig. 3.** Schematic diagrams of atokous chaetal arrangement in distal view of anterior left parapodium. (A) *Perinereis euiini* sp. nov. and *P. cultrifera*. (B) *P. anderssoni*, *P. floridana*, and *P. helleri*. (●): homogomph spiniger. (★): heterogomph spiniger. (■): heterogomph falciger. dc, dorsal cirrus; nod, notopodial dorsal ligule; nop, notopodial prechaetal lobe; no, notoacacula; nov, notopodial ventral ligule; nes, neuropodial superior lobe; nea, neuropodial acicular lobe; ne, neuroacacula; nei, neuropodial inferior lobe; nev, neuropodial ventral ligule; vc, ventral cirrus.

sessile organisms subtidal artificial reef near Yulim beach, Samsan-myeon, Yeosu-si, Jeollanam-do, Korea, 30 April 2013, collected by T. Park, fixed in 80% ethanol. (6) NSMT-Pol P-621, 1 ind., 3 m in depth, associated with mussels in subtidal rocky area, Iyeo port, Seodo-ri, Samsan-myeon, Yeosu-si, Jeollanam-do, Korea (34°3'48"N, 127°16'91"E), 24 April 2013, collected by T. Park, fixed in 80% ethanol.

**Non-type materials examined.** NIBRIV0000502104, 5 inds., associated with algal roots in intertidal rocky shore, Happo village, Wonpo-dong, Jinhae-gu, Changwon-si, Gyeongsangnam-do, Korea (35°5'48"N, 128°41'56"E), 29 June 2006, collected by T. Park, fixed in 7% formalin. NIBRIV0000502105, 5 inds., associated with oysters, in intertidal rocky shore, Oklim-ri, Ilun-myeon, Geoje-si, Gyeongsangnam-do, Korea (34°51'5"N, 128°43'11"E), 20 March 2012, collected by T. Park and J. Y. Kim, fixed in 80% ethanol.

**Comparative materials examined.** Syntypes of *Nereis cultrifera* Grube, 1840 (ZMB 5653), Mediterranean Sea, Naples, Italy, 2 of 19 inds. Non-types of *Perinereis anderssoni* Kinberg, 1866 (USNM 24255), among masses of oysters, Sao Francisco, South Atlantic Ocean, Brazil, 7 October 1925, 6 of many inds., collected by W. L. Schmitt. Non-types of *Perinereis helleri* (Grube, 1878) (NIBRIV0000502065), St. Johns Island, Southern shore of Singapore, 26 November 2003, 5 inds., collector unknown. Non-types of *Perinereis cultrifera* var. *floridana* (Ehlers, 1868) (NSMT-Pol. 18672–18675), Mukaishima, Hiroshima Pref., Japan (34°21.7'N, 133°13.2'E), May 1964, 1 of 4 inds., collected by M. Imajima.

**Diagnosis.** Greenish brown pigmentation present on dorsum in live specimens. Single shield-shaped paragnath on each of area VI; three conical paragnaths arranged usually in triangle in area V; lateral group of paragnaths absent



**Fig. 4.** Dorsal (left) and ventral (right) views of everted proboscis of four *Perinereis* species. (A, B) *P. euiini* sp. nov.: (A) holotype; (B) a Japanese specimen previously identified as *P. cultrifera* var. *floridana* (NSMT-Pol. 18672–18675). (C) *P. cultrifera* (one of syntypes, ZMB 5653). (D) *P. anderssoni* (USNM 24255). (E) *P. helleri* (NIBRIV0000502065). Images of the enlargement of area III on the proboscis are added in (C–E). Arrows indicate lateral groups of paragnaths on area III. Scale bars, 1 mm.

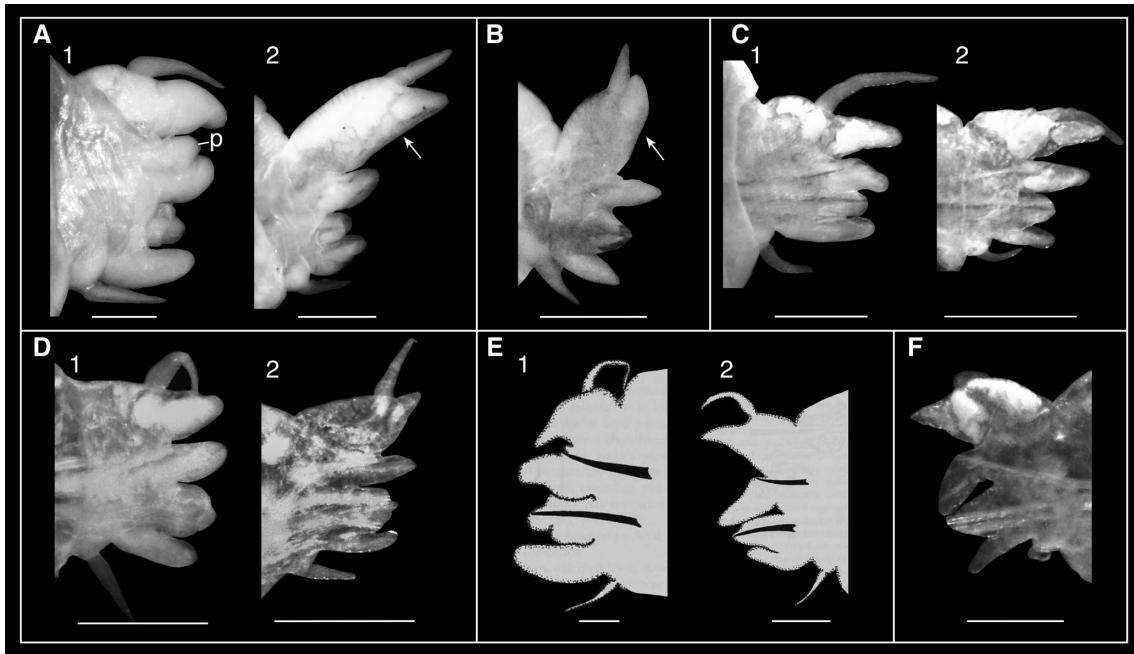
in area III. Notopodial dorsal ligules gradually and greatly expanded posteriorly. Small notopodial prechaetal lobe present in anterior and middle parapodia.

**Description of atokes.** Holotype, complete with 90 chaetigers, 102 mm long, 3.5 mm and 5.3 mm wide excluding and including parapodia at chaetiger 10, respectively.

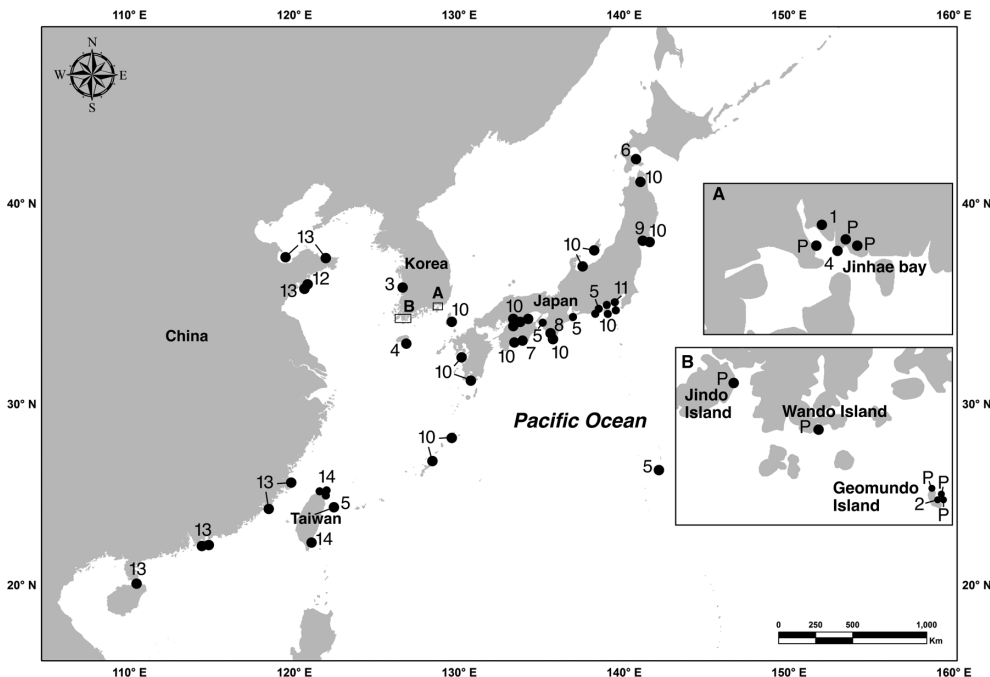
Body gradually tapered posteriorly toward pygidium. Dorsum convex, venter relatively flat with longitudinal mid-ventral groove. Dorsum with stout pigmentation of greenish brown color in live individuals (Fig. 1), with pale pigmentation of brownish cream color in preserved ones.

Prostomium pyriform, slightly wider than long, with pair of smooth, tapered antennae inserted at anterior end. Pair of palps with palpophores and shorter round palpostyles. Two pairs of eyes arranged trapezoidally; anterior pair slightly larger than posterior pair; gap of anterior pair slightly wider than posterior pair (Fig. 2A).

Peristomium longer than other chaetigers, with four pairs of tentacular cirri; posterior dorsal tentacular cirri longest, reaching back to chaetiger 5 in holotype (4–8 in other materials examined) (Fig. 2A).



**Fig. 5.** Anterior view of parapodia of *Perinereis euiini* sp. nov. (**A, B**) and four other closely related species (**C–F**). Images of chaetae are removed. (**A, B**) *Perinereis euiini* sp. nov.: (**A**) holotype, anterior (1) and posterior (2) parapodia; (**B**) a Japanese specimen previously identified as *P. cultrifera* var. *floridana* (NSMT-Pol. 18672–18675), posterior parapodium. p, prechaetal lobe. Arrows indicate greatly expanded notopodial dorsal ligule. (**C**) *P. helleri* (NIBRIV0000502065), anterior (1) and posterior (2) parapodia. (**D**) *P. anderssoni* (USNM 24255), anterior (1) and posterior (2) parapodia. (**E**) *P. floridana* (modified from de Leon-Gonzalez and Solis-Weiss, 1998), anterior (1) and posterior (2) parapodia. (**F**) *P. cultrifera* (one of syntype, ZMB 5653), posterior parapodium. Scale bar, 0.5 mm in (**A–D**) and (**F**), 0.1 mm in (**E**).



**Fig. 6.** Distribution of *Perinereis euiini* sp. nov. in eastern Asia based on the present study (P) and the literature (1–14). 1: Paik (1977), 2: Paik (1979), 3: Rho and Lee (1982), 4: Paik (1989), 5: Izuka (1912), 6: Fauvel (1936), 7: Okuda (1938), 8: Okuda (1950), 9: Okuda and Yamada (1954), 10: Imajima (1972), 11: Imajima (2003), 12: Uschakov and Wu (1965), 13: Wu et al. (1985), 14: Wu (1967).

Proboscis with pair of dark brown amber jaws, each with 5–6 teeth of serrated inner margin (Fig. 2A, B). Conical paragnaths present on both maxillary and oral rings except

area VI; single, shield-shaped paragnath on each side of area VI; paragnaths on oral ring slightly larger than those on maxillary ring. Paragnath numbers and arrangements in holotype as follows (range in other material given in parentheses): area I, 2 (1–3) in longitudinal arrangement; area II, 11 on right and 12 on left (8–16), arranged in oblique triangular patch; area III, 14 (10–15) in oval patch without lateral groups; area IV, 22 on right and 21 on left (16–26), without bar-shaped paragnath, arranged in about three oblique rows; area V, 3 (3–3) in triangular arrangement; area VI, single shield-shaped paragnath on each side; areas VII–VIII, 33 (33–40) in two irregular rows in central area (area VII) and single row in lateral sides (area VIII) (Figs. 2A, B; 4A, B).

Parapodia of first two chaetigers sub-biramous, all following posterior parapodia biramous. Sub-biramous parapodia without notoacacula and with reduced notopodia consisting of dorsal cirrus and dor-



**Table 1.** Variation in paragnath number in areas I to VII–VIII on proboscis of *Perinereis euiini* sp. nov. Ranges (mean  $\pm$  standard deviation) are shown.

Locality (Reference)	I	II*	III total	III lateral	IV*	V	VI*	VII–VIII
Korea (Present study, n=20)	1–3 (2 $\pm$ 0.4)	8–16 (12.1 $\pm$ 2)	10–15 (13 $\pm$ 1.6)	absent	16–26 (21.4 $\pm$ 2.4)	3	1	33–40 (36.6 $\pm$ 2)
Korea (Paik, 1977, 1989 as <i>P. floridana</i> )	2	13	15	absent	20–22	3	1	two rows
Korea (Rho and Lee, 1982; Paik, 1989 as <i>P. c. floridana</i> )	?	?	?	?	?	1	1	two rows
Japan (Imajima, 1972, 1996 as <i>P. cultrifera</i> )	2	12–13	12–14	absent	20–22	3	1	two rows
Japan (Present study, as <i>P. c. var. floridana</i> , n=1)	2	15	10	absent	20–23	1	1	34
China (Wu et al., 1985, as <i>P. c. typica</i> )	1–2	10–26	10–15	absent	20–30	3	1	two rows
China (Wu et al., 1985, as <i>P. c. floridana</i> )	1–2	?	?	?	?	1	1	?
Taiwan (Wu, 1967, as <i>P. cultrifera</i> )	2–3	15–16	15–16	absent	22	3	1	38

\*Paragnath numbers on each side.

sal ligule, and with neuroacacula and neuropodia consisting of acicular lobe, ventral ligule, and ventral cirrus (Fig. 2C).

Notopodia consisting of dorsal cirrus, dorsal ligule, small prechaetal lobe, and ventral ligule in biramous parapodia (Figs. 2D–G; 5A, B). Dorsal cirri slender, slightly shorter than (or same in length as) notopodial dorsal ligule in anterior and middle parapodia, same in length as notopodial ventral ligule in posterior parapodia (Fig. 2H, I).

Notopodial dorsal ligule oval with bluntly tapering tip in anterior parapodia (Fig. 2D, E), gradually expanding in middle parapodia and greatly expanding up to three times length of notopodial ventral ligule in posterior parapodia (Fig. 2H, I). Notopodial prechaetal lobe shorter than notopodial dorsal and ventral ligules, gradually reducing posteriorly (Fig. 2D, F, H). Notopodial ventral ligule oval with bluntly tapering tip throughout.

Neuropodia consisting of acicular lobe, ventral ligule, and ventral cirrus throughout. Superior and inferior lobes present in neuropodial acicular lobe in anterior and middle parapodia (Fig. 2D–G), gradually reduced posteriorly (Fig. 2H, I). Neuropodial ventral ligule oval with bluntly tapering tip throughout. Ventral cirri slender, shorter than ventral ligules throughout.

Notochaetae all homogomph spinigers; blades long with finely serrated edge (Fig. 2J). Upper neurochaetae consisting of heterogomph falcigers with serrated blades at anterior

**Table 2.** Data used in the comparison of DNA sequences of *P. euiini* sp. nov. from Korea, "*P. cultrifera*" from China and Portugal.

Species	Location	GenBank accession No.	Voucher No.	References
<i>P. euiini</i> sp. nov.	Korea (present study)	KY249122	NIBRIV0000502105-Pcgeoje1	present study
		KY249123	NIBRIV0000502105-Pcgeoje2	
		KY249124	NIBRIV0000502105-Pcgeoje3	
" <i>P. cultrifera</i> "	China	KC800624	–	Y. Deng*
		KC800625	–	
		KC800627	–	
" <i>P. cultrifera</i> "	Portugal	KR916910	SFP16-005	Lobo et al. (2016)
		KR916911	SFP18-002	
		KR916912	LMBP18-003	

\*Unpublished data.

position, and homogomph spinigers with long serrated blades at posterior position (Fig. 3A). Lower neurochaetae consisting of heterogomph falcigers with serrated blades (Fig. 2L) at both anterior and posterior positions, and heterogomph spinigers with long serrated blades (Fig. 2K) at superior posterior position (Fig. 3A).

Pygidium with anus on dorsal side, with pair of tapering cylindrical anal cirri (Fig. 1).

**Variation in paragnath number.** Paragnath numbers in *Perinereis euiini* sp. nov. from four countries are summarized in Table 1.

**Habitats.** Intertidal and subtidal zones (up to 10 m deep). Under stones in mudflats or rocky shores or associated with sessile organisms such as algae, mussels, and oysters.

**Distribution.** Eastern Asia (China, Taiwan, Korea, Japan) (Fig. 6).

**Etymology.** The species is named in honor of the late

**Table 3.** Pairwise genetic distances (K2P distances) based on 658 positions of COI sequences among *P. euiini* sp. nov. from Korea, "*P. cultrifera*" from China and Portugal.

Species	GenBank accession no.	<i>P. euiini</i> sp. nov.			" <i>P. cultrifera</i> " from China			" <i>P. cultrifera</i> " from Portugal		
		KY249122	KY249123	KY249124	KC800624	KC800625	KC800627	KR916910	KR916911	KR916912
<i>P. euiini</i> sp. nov.	KY249122	–	0.002	0.002	0.002	0.005	0.003	0.249	0.256	0.249
	KY249123	0.002	–	0.000	0.000	0.003	0.002	0.247	0.254	0.247
	KY249124	0.002	0.000	–	0.000	0.003	0.002	0.247	0.254	0.247
" <i>P. cultrifera</i> " from China	KC800624	0.002	0.000	0.000	–	0.003	0.002	0.247	0.254	0.247
	KC800625	0.005	0.003	0.003	0.003	–	0.002	0.244	0.251	0.244
	KC800627	0.003	0.002	0.002	0.002	0.002	–	0.247	0.254	0.247
" <i>P. cultrifera</i> " from Portugal	KR916910	0.249	0.247	0.247	0.247	0.244	0.247	–	0.005	0.000
	KR916911	0.256	0.254	0.254	0.254	0.251	0.254	0.005	–	0.005
	KR916912	0.249	0.247	0.247	0.247	0.244	0.247	0.000	0.005	–

**Table 4.** Comparison of key characteristics of *Perinereis euiini* sp. nov. with four closely similar species.

Species (Locality and reference)	Range of paragnath number								Notopodial prechaetal lobe	Posterior notopodial dorsal ligule
	I	II*	III total	III* lateral	IV*	V	VI*	VII–VIII		
<i>P. euiini</i> sp. nov. (Korea, present study, 20 type and non-types)	1–3	8–16	10–15	absent	16–26	3	1	33–40	present	expanded up to 3 times longer than notopodial ventral ligule
<i>P. cultrifera</i> (Naples, Hutchings et al., 1991, 19 syntypes)	1–2	3–15	5–11	present	6–20	2–5	1	20–50	present	expanded up to 2 times longer than notopodial ventral ligule
	(Naples, present study, 2 syntypes)	1	4–7	10–12	1–2	10–16	3	1	32–36	present
<i>P. anderssoni</i> (Brazil, present study, 6 non-types)	4–6	11–17	17–23	0–1	28–37	3	1	39–43	absent	expanded up to 2 times longer than notopodial ventral ligule
	(Brazil, de León-González & Solís-Weiss, 1998)	4	13	19	1**	27	3	1	45	absent
<i>P. floridana</i> (Mexico, de León-González & Solís-Weiss, 1998)	2	9	16	?	18	1	1	24	absent	not enlarged
<i>P. helleri</i> (Philippines, Australia, Hutchings et al., 1991, Type and non-types)	2	4–17	11–20	2–3	10–19	3	1	21–40	absent	slightly enlarged
	(Singapore, present study, 5 non-types)	2	6–10	12–17	2–3	12–17	3–4	1	25	absent

\*Paragnath numbers on each side.

\*\*Personal information from Jesús Angel de León-González.

Prof. Eui-In Paik who pioneered taxonomic studies on Korean polychaetes.

### Comparison of DNA sequences

A total 658 bp of partial COI sequences was obtained from three individuals of *P. euiini* sp. nov. No insertions or deletions were detected in the three sequences. The COI sequences of six specimens identified as *P. cultrifera* from China and Portugal were mined from GenBank for comparison (Table 2). The pairwise genetic distances calculated by the Kimura-2-parameter model are listed in Table 3. Mean intraspecific distance was 0.2% (range 0–0.5%). Mean pairwise distance between *P. euiini* sp. nov. and "*P. cultrifera*" from Portugal was 25% (range 24.7–25.6%) while *P. euiini* sp. nov. and "*P. cultrifera*" from China was only 0.2% (range 0–0.5%).

### DISCUSSION

*Perinereis euiini* sp. nov. is similar to *P. cultrifera*. However, it clearly differs from *P. cultrifera* in the following two diagnostic characteristics: (1) the absence of lateral groups of paragnaths on area III (Fig. 4A, B), in contrast to the presence of those in *P. cultrifera* (Fig. 4C, Table 4), and (2) notopodial dorsal ligules greatly expanded (up to three times longer than notopodial ventral ligule) in posterior parapodia (Fig. 5A–2, B), in contrast to those not so greatly expanded (up to two times longer than notopodial ventral ligule) in *P. cultrifera* (Fig. 5F, Table 4).

Morphological characteristics of all specimens previously reported as *P. cultrifera* from eastern Asian waters, i.e., China, Taiwan, Korea, and Japan (Izuka, 1912; Fauvel, 1936; Okuda, 1938, 1950; Okuda and Yamada, 1954; Khlebovich and Wu, 1962; Khlebovich, 1963, 1996; Imajima



and Hartman, 1964; Uschakov and Wu, 1965; Wu, 1967; Imajima, 1972, 1996, 2003; Paik, 1977, 1979, 1989; Rho and Lee, 1982; Wu et al., 1985; Sun and Yang, 2004) agree well with those of *P. euiini* sp. nov. (Table 1). Therefore, it is concluded that they all belong to *P. euiini* sp. nov., and that *P. cultrifera* is not a cosmopolitan species.

This conclusion is supported by the comparison of DNA sequences of COI between *P. euiini* sp. nov., and "*P. cultrifera*" from China and Portugal. The DNA sequences of specimens from Portugal markedly differ from *P. euiini* sp. nov., but, those from China is identical with *P. euiini* sp. nov. (Table 3), indicating that the Portugal specimens belong to *P. cultrifera sensu stricto*, whereas the Chinese specimens belong to not *P. cultrifera* but *P. euiini* sp. nov. According to Hebert et al. (2003), the mean pairwise distance at COI among congeneric annelid species pairs is 15.7%. Also, Park and Kim (2007) found that the mean pairwise distances between species pairs among four *Perinereis* species (*P. mictodonta*, *P. nuntia*, *P. vallata*, and *P. wilsoni*) ranged 18.95 to 25.05%. These values are comparable with the mean pairwise distance between *P. euiini* sp. nov. and *P. cultrifera* (25%) in the present study.

*Perinereis euiini* sp. nov. is also similar to *P. floridana*. However, it differs from *P. floridana* in the following two diagnostic characteristics: (1) the presence of notopodial prechaetal lobes (Fig. 5A-1), in contrast to the absence of notopodial prechaetal lobes in *P. floridana* (Fig. 5E-1, Table 4), and (2) notopodial dorsal ligules greatly expanded in posterior parapodia, in contrast to those not expanded in *P. floridana* (Fig. 5E-2, Table 4) (de Leon-Gonzalez and Solis-Weiss, 1998; de Leon-Gonzalez and Goethel, 2013).

Fauvel (1932) has relegated *P. floridana* to one of varieties of *P. cultrifera* based on materials collected from India and adjacent areas. In eastern Asia (i.e., China, Korea, and Japan), Khlebovich and Wu (1962), Khlebovich (1963, 1996), Uschakov and Wu (1965), Imajima (1972, 1996), Rho and Lee (1982), Wu et al. (1985), Paik (1989), and Sun and Yang (2004) have recorded *P. cultrifera* var. *floridana* (or *P. cultrifera floridana*) which is different from the Asian "*P. cultrifera*" in paragnath number on area V (one instead of three). In the present study, we confirmed that the general morphology of the Asian specimens reported as *P. cultrifera* var. *floridana* (or *P. cultrifera floridana*) agreed well with that of *P. euiini* sp. nov. (Figs. 4B, 5B, Table 1). Therefore, we judged that they did not belong to *P. floridana sensu stricto*, but to *P. euiini* sp. nov., regarding their aberrant paragnath number in area V as an intraspecific variation of *P. euiini* sp. nov.

Wu et al. (1985) have reported six ecological subspecies of *P. cultrifera*. Among them, *P. cultrifera typica* and *P. cultrifera floridana* are synonymized as *P. euiini* sp. nov. in the present study. On the other hand, all of four varieties of *P. cultrifera*, which were erected by Fauvel (1932) based on Indian specimens, do not belong to *P. euiini* sp. nov. because Fauvel's (1932: p. 105) following description does not agree with the diagnostic characteristic of *P. euiini* sp. nov.: "In all these forms, the posterior feet are not materially modified. The dorsal ligule, in fact, protrudes over the ventral division, but is not enlarged into a large flag-like process bearing the dorsal cirrus near its tip..." In addition, a not greatly expanded notopodial dorsal ligule in posterior parapodia is shown in Fauvel's (1953: p. 207) figure (fig. 106c).

*Perinereis euiini* sp. nov. is also similar to *P. anderssoni* Kinberg, 1866 (type locality: Rio de Janeiro, Brazil) and *P. helleri* (Grube, 1878) (type locality: Bohol Island, Philippines). However, it is different from them in the following two diagnostic characteristics: (1) the absence of lateral groups of paragnaths on area III, in contrast to the presence of those in *P. anderssoni* (Fig. 4D) and *P. helleri* (Fig. 4E), and (2) the presence of notopodial prechaetal lobes, in contrast to the absence of those in *P. anderssoni* (Fig. 5D, Table 4) and *P. helleri* (Fig. 5C, Table 4).

#### ACKNOWLEDGMENTS

The authors are indebted to Drs. Birger Neuhaus (ZMB), the late Kristian Fauchald (USNM), and Hironomi Komatsu (NSMT) for their help in the examination of museum specimens. The authors are also grateful to Professor Masanori Sato at Kagoshima University and Professor Hiroshi Kajihara at Hokkaido University for their helpful comments to improve the manuscript. TP would like to also thank Ms. Yun Kyoung Kim for her worthwhile assistance with the drawing. The present study was supported by a grant (NIBR201501111) from the National Institute of Biological Resources (NIBR) funded by the Ministry of Environment (MOE), Republic of Korea.

#### COMPETING INTERESTS

The authors have no competing interests to declare.

#### AUTHOR CONTRIBUTIONS

TP performed research, analyzed data, and wrote the paper. WK designed research and analyzed data.

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(Received September 18, 2016 / Accepted February 19, 2017)