# First Record of Two *Pseudopolydora* (Annelida: Spionidae) Species in Korea

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#### ABSTRACT

Two *Pseudopolydora* polychaetes, *P. bassarginensis* and *P. reticulata*, originally described from Peter the Great Bay in Russia and Taiwan, respectively, were recorded firstly in Korea with DNA information. Two species are known to have distinct morphological characteristics that are separated from other *Pseudopolydora* species. They are characterized by reticulate pigmentations on the dorsal sides of the anterior chaetigers, a longitudinal black band-like pigmentation on the caruncle, and black paired spots on the ventral sides of the anterior chaetigers. These two species can be distinguished morphologically from each other by the length of the caruncle. Methyl green staining pattern of the species is a good method for delimiting *Pseudopolydora* species. The partial sequences of the mitochondrial cytochrome *c* oxidase subunit I (*COI*), 16S ribosomal DNA (16S rDNA), and the nuclear 18S ribosomal DNA (18S rDNA) from Korean specimens of the two species were determined. The morphological descriptions and images of the two *Pseudopolydora* species are provided.

Keywords: Korea Strait, molecular analysis, Polychaeta, taxonomy, Yellow Sea

#### INTRODUCTION

Member of genus Pseudopolydora Czerniavsky, 1881, mostly known from the western Pacific, occurs mainly in silty sand or muddy intertidal sand along the coasts (Radashevsky and Migotto, 2009). They are characterized by the absence of the notochaetae in chaetiger 1, branchiae present from posterior to chaetiger 5, and having two types (falcate and pennoned) of modified spines in the notopodium of chaetiger 5 (Blake, 1996). Thus far, 23 Pseudopolydora species have been reported worldwide (Read and Fauchald, 2021). Among Pseudopolydora species, P. bassarginensis (Zachs, 1933) and P. reticulata Radashevsky & Hsieh, 2000 had been confused because Japanese populations have intermediate morphological characteristics of two species (Abe et al., 2016). Abe et al. (2016) identified that the Japanese populations have reticulated pigmentation on dorsum, black band-like pigmentation along the caruncle, and black paired spots on the ventral side of anterior chaetigers. But the Japanese populations have caruncle usually extending to the middle of chaetiger 4 which is a diagnostic characteristic to distinguish P. bassarginensis from P. reticulata (see Radashevsky and Hsieh, 2000). Except for the length of the caruncle, the Japanese specimens showed most likely to *P. reticulata* from Taiwan (type locality), and they tentatively identified the Japanese specimens as P. cf. reticulata (see Abe et al., 2016). Due to the brief original description of P. bassarginensis without illustration, the status of the species is still unclear, and the taxonomic work based on the morphological and molecular analyses is needed to clarify this problem (Radashevsky and Hsieh, 2000; Abe and Sato-Okoshi, 2021). The sequences of 16S ribosomal DNA(16S rDNA) and the nuclear 18S ribosomal DNA (18S rDNA) are known to be useful molecular markers for identification of Pseudopolydora species (Simon et al., 2017). The partial mitochondrial cytochrome c oxidase subunit I (COI) also has been used frequently as a tool for species identification among polychaetes (Carr et al., 2011; Park and Kim, 2017; Choi et al., 2020).

In this study, two species of *P. bassarginensis* and *P. reticulata* are reported for the first time in Korean fauna with describing of methyl green staining patterns.

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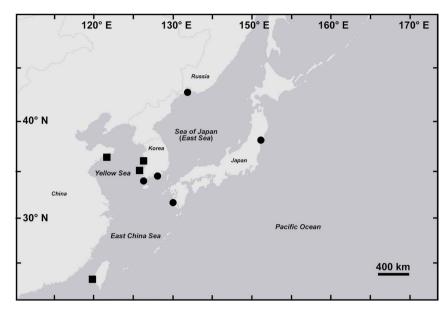


Fig. 1. Distributions of Pseudopolydora bassarginensis (Zachs, 1933) (•) and Pseudopolydora reticulata Radashevsky & Hsieh, 2000 (■).

### MATERIALS AND METHODS

Adult samples were collected from western and southern coasts of intertidal sand in Korea using 500 µm-mesh sieves (Figs. 1, 2A). The live specimens were relaxed in a 10% magnesium chloride solution (MgCl<sub>2</sub>), and morphological observation was carried out under a stereomicroscope (MZ125; Leica Microsystems, Wetzlar, Germany). Their appendages were dissected in a petri-dish using dissection forceps and surgical knives under a stereomicroscope and observed by optical microscopy (DM2500; Leica Microsystems). After the observations, the specimens were fixed in 4% formaldehyde for the morphological observations and 95% ethanol for molecular analyses. All voucher specimens were deposited at the National Institute of Biological Resources in Korea (NIBR).

The genomic DNA was extracted from a palp of collected specimens using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Polymerase chain reaction amplification was performed with primers for three gene regions: Dorid\_COI.3F/1R or Dorid\_COI. 1F/1R for *COI* (Williams et al., 2017), 16Sar and 16Sbr for 16S rDNA (Kessing et al., 1989), and 18E and 18B for 18S rDNA (Mincks et al., 2009). The newly determined sequences were registered in the GenBank. Pairwise genetic distances (p-distances) were calculated using MEGA X (Kumar et al., 2018) between two *Pseudopolydora* species and their congeners, *P. cf. reticulata*, *P. kempi japonica* Imajima & Hartman, 1964, *P. paucibranchiata* (Okuda, 1937), *P. pulchra* (Carazzi,

Korean name: 1\*무늬선녀얼굴갯지렁이(신칭)

1893), and *P. uphondo* Simon, Sato-Okoshi & Abe, 2017, currently available in GenBank (Simon et al., 2017; Radashevsky et al., 2020; Abe and Sato-Okoshi, 2021).

#### RESULTS

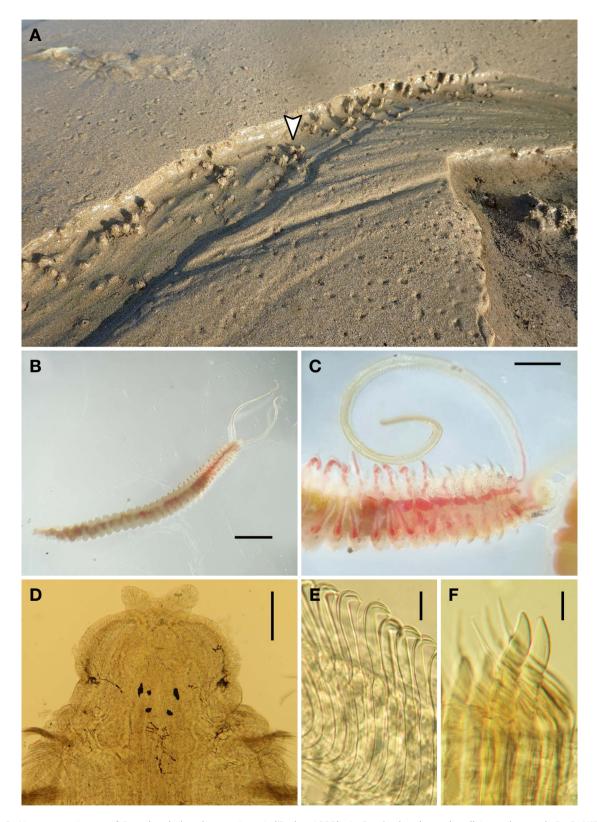
Order Spionida *sensu* Rouse & Fauchald, 1997 Family Spionidae Grube, 1850 Genus *Pseudopolydora* Czerniavsky, 1881

### <sup>1\*</sup>Pseudopolydora bassarginensis (Zachs, 1933) (Figs. 2, 3C, 4A, C, E)

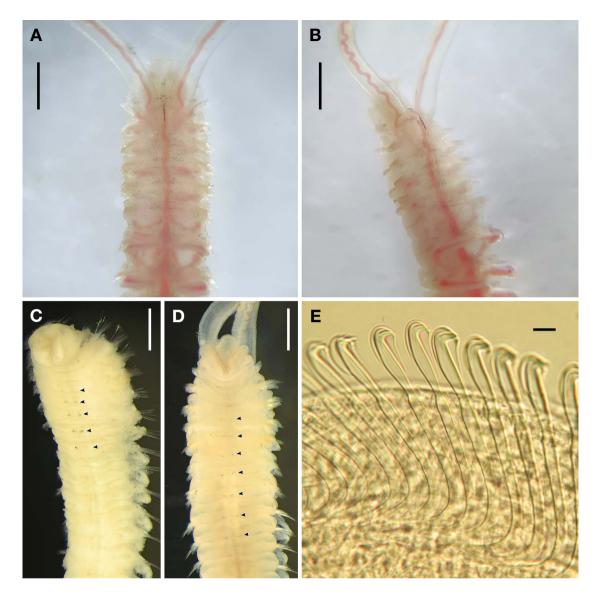
Polydora (Carazzia) bassarginensis Zachs, 1933: 129. Pseudopolydora cf. kempi: Sato-Okoshi, 2000: 448 in part. Pseudopolydora cf. reticulata: Abe et al., 2016: 652, fig. 2a-c; Abe and Sato-Okoshi, 2021: fig. 9E, F.

**Material examined.** Korea Strait, Korea: 15 inds. (NIBRIV 0000862798), Jeollanam-do: Jindo-gun, Jodo-myeon, Sinyukri, 34°17'4"N, 126°4'45"E, 25 Oct 2018, collected by Korea National Park Service; 20 inds. (NIBRIV0000886075–79), Gyeongsangnam-do: Namhae-gun, Sangju-myeon, Yanga-ri, 34°43'45.1"N, 127°57'09.7"E, 19 Aug 2020, 64 inds., same locality, 20 Aug 2020, 33 inds., same locality, 34°43'02.5"N, 127°57'29.0"E, 20 Aug 2020, collected by Lee GH.

**Description.** Body yellowish-white with black reticulated pigmentation present on dorsal side of anterior chaetigers (Fig.



**Fig. 2.** Korean specimens of *Pseudopolydora bassarginensis* (Zachs, 1933). A, Sand tubes (arrowhead) in a silty sand; B, C, NIBRIV 0000886079, dorsal view of live specimen (B), Dorsal view of anterior end of live specimen (C); D, Dorsal view of the prostomium with two chaetigers; E, Hooded hooks from posterior chaetiger; F, Pennoned and falcate spines from chaetiger 5. Scale bars: B=2 mm, C=0.5 mm, D=0.2 mm, E, F=20 µm.



**Fig. 3.** Korean specimens of *Pseudopolydora reticulata* Radashevsky & Hsieh (A, B, D, E, NIBRIV0000886074) and *Pseudopolydora bassarginensis* (Zachs, 1933) (C, NIBRIV0000886078). A, Dorsal view of live specimen; B, Dorsolateral view of live specimen; C, D, Ventral views of anterior end of formalin-fixed specimen, ventral black paired spots (arrowheads); E, Hooded hooks from the posterior chaetiger. Scale bars: A-D=0.5 mm, E=20 µm.

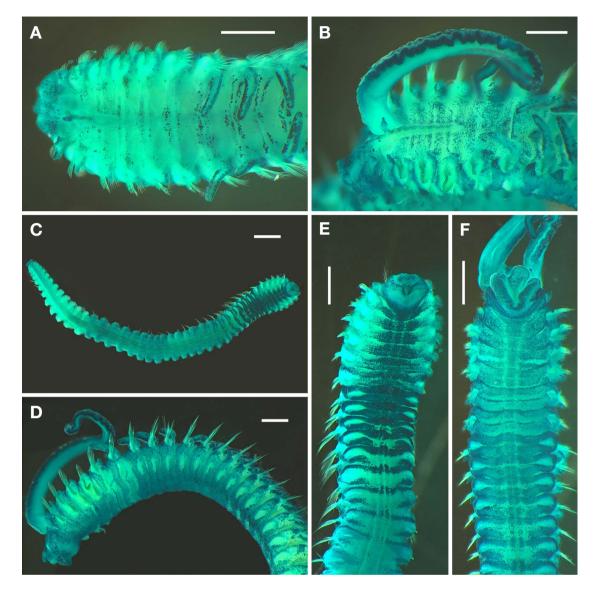
2B, C). Prostomium anteriorly bifid, extending posteriorly to usually front to middle of chaetiger 4 (rarely end of chaetiger 4) as low caruncle; longitudinal black band-like pigmentation present along midline of caruncle (Fig. 2D). Ventral black spots rarely (24 specimens in 114 examined) present along posterior end of anterior chaetigers in fixed specimens (Fig. 3C). Occipital antenna present on caruncle. Four black eyes arranged in trapezoid; anterior pair often divided into spots, appearing as 2–3 eyes (Fig. 2D). Palps reaching 10–15 chaetigers with longitudinal groove lined with fine cilia. Chaetiger 1 without notochaetae. Anterior row of notochaetae in chaetigers 3, 4, 6, and 7 slightly modified, arranged in weak

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J-shape. Chaetiger 5 similar in size as neighbor segments with superior capillaries, 2 kinds of modified spines arranged in J-shaped series, ventral capillaries; pennoned spines in anterior row with curved pointed tips about 20 in series and simple falcate spines in posterior row about 15 in series (Fig. 2F). Bidentate hooded hooks in neuropodia from chaetiger 8 to posterior end, not accompanied by capillaries (Fig. 2E). Branchiae present on chaetiger 7 to 18–24, free from notopodial postchaetal lamellae. Pygidium large flaring disc with dorsal gap and erect process on each dorsolateral side.

Habitat. Fine sand of intertidal zone.

Distribution. Japan (Abe et al., 2016), southern coast of Korea



**Fig. 4.** Methyl green staining patterns of *Pseudopolydora bassarginensis* (Zachs, 1933) (A, C, E) and *Pseudopolydora reticulata* Radashevsky & Hsieh (B, D, F), all specimens fixed in formalin. A, Dorsal view of anterior end, palps removed; B, Dorsal view of anterior end, left palp removed; C, Ventral view of whole body; D, Lateral view of anterior end; E, Ventral view of anterior end; F, Ventral view of anterior end (NIBRIV0000886074). Scale bars: A, B, D-F=0.5 mm, C=1.0 mm.

(present study), Peter the Great Bay in Russia (type locality, Zachs, 1933) (Fig. 1).

**Methyl green staining pattern.** Anterior part of prostomium and peristomium, distal part of occipital antennae, ciliated groove of palps, margins of branchiae, notopodial postchaetal lamellae, ventral side of anterior body region, and pygidium intensively stained (Fig. 4A, C, E). In 42 segments specimen, stained dots scattered in the middle region of chaetigers 1–6 and bands of scattered dots of chaetigers 7–14 most intensively stained ventrally (Fig. 4C, E). **Molecular data.** The sequences of partial *COI* (829 bp), 16S rDNA (486 bp), and 18S rDNA (1,779 bp) from three Korean specimens were determined (GenBank accession number: MZ317544–6 for *COI*, MZ323187–9 for 16S rDNA, and MZ317516–8 for 18S rDNA).

### <sup>1\*</sup>*Pseudopolydora reticulata* Radashevsky & Hsieh, 2000 (Figs. 3A, B, D, E, 4B, D, F)

*Pseudopolydora reticulata* Radashevsky and Hsieh, 2000: 229, fig. 8; Zhou et al., 2010: 10.

Korean name: 1\*그물선녀얼굴갯지렁이(신칭)

**Material examined.** Yellow Sea, Korea: 2 inds. (NIBRIV000 0886072), Jeollabuk-do: Buan-gun, Byeonsan-myeon, Mapori, 35°39'16.4"N, 126°29'26.0"E, 19 Sep 2020, 1 ind. (NIBR IV0000886071), same locality, 21 Sep 2020, collected by Lee GH; 1 ind., Buan-gun, Byeonsan-myeon, Daehang-ri, 35°40'44.2"N, 126°31'29.7"E, 21 Sep 2020, collected by Lee GH; 1 ind. (NIBRIV0000886073), Jeollanam-do: Sinan-gun, Jaeun-myeon, Hanun-ri, Dunjiang Beach, 34°55'12.0"N, 126° 03'26.2"E, 2 Oct 2020, collected by Lee GH; 1 ind. (NIBRIV 0000886074), Chungcheongnam-do: Seocheon-gun, Seomyeon, Chunjangdae Beach, 36°09'41.2"N, 126°31'11.1"E, 20 Oct 2020, collected by Lee GH.

Description. Body yellowish-white with black reticulated pigmentation present on dorsal side of anterior part of body (Fig. 3A). Prostomium anteriorly bifid, extending posteriorly to usually middle to end of chaetiger 5 as low caruncle; longitudinal black band-like pigmentation present along midline of caruncle (Fig. 3B). Ventral black spots present along posterior end of anterior chaetigers (in 6 specimens examined) (Fig. 3D). Occipital antenna present on caruncle (Fig. 3A, B). Four black eyes arranged in trapezoid. Palps reaching about 10 chaetigers with longitudinal groove lined with fine cilia. Chaetiger 1 without notochaetae. Anterior row of notochaetae in chaetigers 3, 4, 6, and 7 slightly modified, arranged in weak J-shape. Chaetiger 5 similar in size as neighbor segments with superior capillaries, two kinds of modified spines arranged in J-shaped series, ventral capillaries; pennoned spines in anterior row with curved pointed tips about 20 in series, and simple falcate spines in posterior row, about 15 in series. Bidentate hooded hooks in neuropodia from chaetiger 8 to posterior end, not accompanied by capillaries (Fig. 3E). Branchiae present on chaetiger 7 to about 18-20, free from notopodial postchaetal lamellae. Pygidium large flaring disc with dorsal gap and erect process on each dorsolateral side.

Habitat. Fine sand of the intertidal zone.

**Distribution.** Yellow Sea of China (Zhou et al., 2010) and Korea (present study), western coast of Taiwan (type locality, Radashevsky and Hsieh, 2000) (Fig. 1).

**Methyl green staining pattern.** Anterior part of prostomium and peristomium, distal part of occipital antennae, ciliated groove of palps, margins of branchiae, notopodial postchaetal lamellae, ventral side of anterior body region, and pygidium intensively stained (Fig. 4B, D, F). In 56 segments specimen, bands of scattered dots on chaetigers 1–34 intensively stained ventrally (Fig. 4D, F).

**Molecular data.** The sequences of partial *COI* (822 bp), 16S rDNA (496 bp), and 18S rDNA (1,776 bp) from three Korean specimens were determined (GenBank accession number: MZ317547–9 for *COI*, MZ323190–2 for 16S rDNA, and MZ317519–21 for 18S rDNA).

#### DISCUSSION

Pseudopolydora bassarginensis is originally described as having reticulate pigmentations on the dorsum and caruncle with longitudinal black pigmentation usually extending to the middle of chaetiger 4 (Zachs, 1933). He did not describe ventral spots. Its congener, P. reticulata, is defined as having the reticulate pigmentation on the dorsum and caruncle with longitudinal black pigmentation usually extending to the end of chaetiger 5, and black paired spots on the ventral side in anterior chaetigers (Radashevsky and Hsieh, 2000). Abe et al. (2016) identified that the Japanese populations showed intermediate morphological characteristics between P. reticulata and P. bassarginensis in having the caruncle with longitudinal pigmentation usually extending to the middle of chaetiger 4 and black paired spots on the ventral side in anterior chaetigers. For these reasons, the status of this species had been unclear and Japanese populations were tentatively referred to as P. cf. reticulata (Abe et al., 2016; Abe and Sato-Okoshi, 2021).

Against this background, two Pseudopolydora species collected from the southern and western coasts of Korea were identified as P. bassarginensis and P. reticulata, respectively, based on the morphological and molecular analyses. Two species are quite similar in having the netlike reticulate pigmentation on the dorsum, caruncle with longitudinal black pigmentation, and black paired spots on the ventral side in anterior chaetigers. The conspicuous difference showed in the length of the caruncle (usually extending to the middle of chaetiger 4 in P. bassarginensis vs. end of chaetiger 5 in P. reti*culata*). The specimens from the southern coast well agreed with the original description of P. bassarginensis from Russia except for the presence of ventral paired spots. We examined that this feature exhibits very rarely (appearing on 24 among 114 examined specimens). This can easily lead to erroneous observations in the original description. We suggested that the specimens from the south coast of Korea be identified as P. bassarginensis. The Japanese specimens of P. cf. reticulata (Abe et al., 2016) clearly showed this characteristic and we synonymized it to P. bassarginensis. The specimens from the western coast of Korea well agreed with the original description of P. reticulata in the presence of reticulated pigmentation on the dorsum, the caruncle with longitudinal black band-like pigmentation usually extending to the middle of chaetiger 5, and the presence of ventral black spots (Radashevsky and Hsieh, 2000). This species differs P. bassarginensis from the length of the caruncle (see above).

The methyl green staining patterns of the two species were provided based on the Korean specimens (see above). The methyl green staining patterns of *P. bassarginensis* clearly differ from *P. reticulata* as follows: stained dots scattered in

No.	Species	Location	Voucher No.	GenBank accession No.	1	2	3	4	5	9	7	Data source
Ч	P. bassarginensis	Korea Strait, Korea	NIBRIV0000886075	MZ323187								Present study
2	P. cf. reticulata	Japan	I	LC595760	0.003							Abe and Sato-Okoshi (2021)
m	P. bassarginensis	Sea of Japan, Russia	VIR 11473	MG460894	0.007	0.003						Radashevsky et al. (2020)
4	P. reticulata	Yellow Sea, Korea	NIBRIV0000886071	MZ323191	0.059	0.056	0.053					Present study
ß	P. kempi japonica	Sea of Japan, Russia	VIR 11474	MG460897	0.082	0.076	0.082	0.072				Radashevsky et al. (2020)
9	P. pulchra	France	VIR 18571	MG460932	0.119	0.116	0.119	0.129	0.145			Radashevsky et al. (2020)
7	P. uphondo	Japan	I	LC107865	0.158	0.151	0.155	0.164	0.174	0.162		Simon et al. (2017)
ω	P. paucibranchiata	Sea of Japan, Russia	VIR 11785	MG460899	0.158	0.154	0.151	0.168	0.161	0.195	0.186	Radashevsky et al. (2020)

the middle region of chaetigers 1-6 that do not extend laterally to the parapodia and bands of scattered dots on chaetigers 7-14(in 42 segments) in *P. bassarginensis* while bands of scattered dots on chaetigers 1-34 (in 56 segments) in *P. reticulata*. The result seems methyl green staining pattern is a very useful character for species in the genus *Pseudopolydora* (Simon et al., 2017).

The sequences of three gene regions (COI, 16S rDNA, and 18S rDNA) of P. bassarginensis and P. reticulata were determined from the Korean specimens. The intra-specific genetic distances were 0.3-0.9% in COI, 0.2-0.6% in 16S rDNA, and no variation was detected in 18S rDNA in P. bassarginensis, and 0.2-0.9% in COI, and no variation was detected in 16S rDNA and 18S rDNA in P. reticulata. The species identification was supported by a comparison of the partial DNA sequences of 16S rDNA from the specimens of the type locality, Russia (Table 1). The genetic distance among the P. bassarginensis specimens from Korea and Russia was 0.0-0.7% in 16S rDNA (0-2/312 bp). The Japanese specimens also showed high molecular similarity with Korean and Russian species indicating these three specimens are conspecifics. The inter-specific genetic distances between P. bassarginensis and its congeners currently available were 5.3-18.3% in 16S rDNA (Table 1). Genetically P. bassarginensis is closest to P. reticulata among the available data from the Gen-Bank in three gene regions.

In conclusion, the status of *P*. cf. *reticulata* from East Asia is now clarified by the taxonomic works combined with morphological and molecular analyses based on the Korean specimens. This study showed that the methyl green staining pattern is a good diagnostic character in this genus *Pseudopolydora*. The molecular makers of three gene regions are also powerful tools for species identification. The determined DNA information along with morphological observations will be valuable resources for further taxonomic and phylogenetic studies.

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## **CONFLICTS OF INTEREST**

Seong Myeong Yoon and Gi-Sik Min, contributing editors of the Animal Systematics, Evolution and Diversity, were not involved in the editorial evaluation or decision to publish this article. The remaining author has declared no conflicts of interest.

**Table 1.** The pairwise genetic distances of 16S rDNA sequences of *Pseudopolydora* polychaetes available in GenBank (312 bp)

#### ACKNOWLEDGMENTS

This study was supported by the research funds from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR 201902204, NIBR202102203). This study was also supported by the Korea National Park Service and Inha University.

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Received June 22, 2021 Revised October 13, 2021

Accepted January 10, 2022