

# A new species and new record of Pacificincolidae (Bryozoa: Cheilostomata) from Korea

Bum Sik Min<sup>1</sup>, Hyun Sook Chae<sup>2</sup>, Ho Jin Yang<sup>2</sup>, Geon Woo Noh<sup>3</sup>, Dong Hee Lee<sup>4</sup> and Ji Eun Seo<sup>5,\*</sup>

<sup>1</sup>Marine Research Center, National Park Research Institute, Korea National Park Service, Yeosu 59723, Republic of Korea

<sup>2</sup>Marine Bryozoans Resources Bank of Korea, Chungbuk 27841, Republic of Korea

<sup>3</sup>Department of Food Science & Biotechnology, Graduate School, Woosuk University, Jeonbuk 55338, Republic of Korea

<sup>4</sup>Department of Life Science, Graduate School, Woosuk University, Chungbuk 27841, Republic of Korea

<sup>5</sup>Department of Life Science, Woosuk University, Chungbuk 27841, Republic of Korea

\*Correspondent: jeseo@woosuk.ac.kr

We performed a taxonomic study on Korean Pacificincolidae (Bryozoa: Cheilostomata) with the materials collected from 39 localities (ports, intertidal and subtidal zones) in the coastal seas of South Korea from 1981 to 2020. Korean *Mucronella perforata* reported by Rho and Seo (1985) turned out to be not *Pacificincola perforata*, but *Primavelans glabricollaris* n. sp. based on morphological data. *Pacificincola perforata*, which was transferred from *Mucronella perforata* (Okada and Mawatari, 1937), is also added to the Korean fauna. This study is the first to use mitochondrial 16S rRNA gene sequences to study the family Pacificincolidae. The NJ analysis also reconfirmed the validity of the genus *Primavelans*, established on the basis of the early astogeny by De Blauwe in 2006, with the mitochondrial 16S rRNA sequences. The inter-genetic distance among two species belong to the Pacificincolidae was in the range of  $13.39 \pm 1.83$  nm. *Primavelans glabricollaris* n. sp. is thus distinguished from *Pacificincola perforata* with both morphological and molecular data. A new species, *Primavelans glabricollaris*, and a new record, *Pacificincola perforata* from Korea are herein described in detail and illustrated by scanning electron microscopy.

Keywords: 16S rRNA, Bryozoa, Korea, morphology, new record, new species, Pacificincolidae

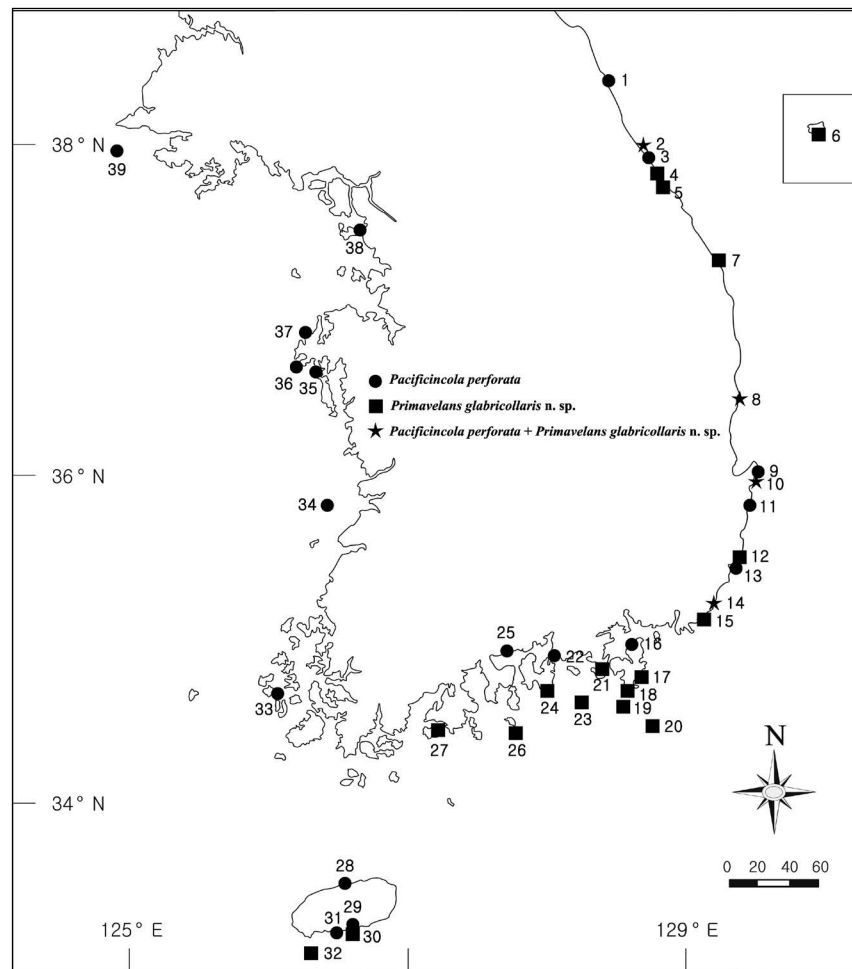
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## INTRODUCTION

The family Pacificincolidae Liu and Liu, 1999 comprises five species belonging to three genera (*Burdwoodipora* López Gappa, Liuzzi and Zelaya, 2017; *Pacificincola* Liu and Liu, 1999; and *Primavelans* De Blauwe, 2006): *Burdwoodipora paguricola* López Gappa, Liuzzi and Zelaya, 2017; *Pacificincola aviculifera* (Osburn, 1914); *Pacificincola perforata* (Okada and Mawatari, 1937); *Primavelans insculpta* (Hincks, 1882); and *Primavelans mexicana* (Soule, Soule and Chaney, 1995). Liu and Liu (1999) established the family Pacificincolidae and the genus *Pacificincola* for *Mucronella perforata* Okada and Mawatari, 1937, and then *Schizoporella insculpta* Hincks, 1883 were synonymized into this genus (Dick *et al.*, 2005). Afterwards, the new genus *Primavelans* belonging to the family Pacificincolidae was established by De Blauwe (2006) on the basis of the early astogeny. *Pacificincola insculpta* (Hincks, 1883) and *Hippoporina mexicana*

*Soule, Soule and Chaney, 1995* were synonymized into the genus *Primavelans* (De Blauwe, 2006). In addition, *Phylactellipora aviculifera* (Osburn, 1914) from Florida was also transferred to the genus *Pacificincola* (De Blauwe, 2006). However, one of two species belonging to *Phylactellipora*, *Phylactellipora punctigera* (Waters, 1899), was provisionally synonymized into *Neolagenipora punctigera* (<http://bryozoa.net>, 2017), and the other, *Phylactellipora lyrulata* (Calvet, 1909), was synonymized into *Lageneschara lyrulata*. *Phylactaellipora* was recently synonymized into *Neolagenipora* (WoRMS, Apr. 26, 2021). Korean *Mucronella perforata* reported by Rho and Seo (1985) thus needed to be reviewed.

Since the mitochondrial, large-subunit, 16S rRNA gene has been used in the molecular phylogenetic analysis to examine sister-group relationships among gymnoleamate bryozoans (Dick *et al.*, 2000), subsequent molecular phylogenetic studies by using 16S rRNA had been followed to confirm the species status, morphological



**Fig. 1.** The map showing the localities where the examined materials were collected from 1981 to 2020. 1, Geojin Port (Goseong-gun); 2, Jodo Island (Yangyang-gun); 3, Namae Port (Yangyang-gun); 4, Jumunjin Port (Gangneung-si); 5, Gyeongpodae (Gangneung-si); 6, Neunggeol (Ulleungdo Island); 7, Deoksan Port (Samcheok-si); 8, Ganggu Port (Yeongdeok-gun); 9, Guryongpo Port (Pohang-si); 10, Yangpo Port (Pohang-si); 11, Gampo Port (Gyeongju-si); 12, Bangeojin Port (Ulsan); 13, Jangsaengpo Port (Ulsan); 14, Daebyeon Port (Busan); 15, Mipo Port (Busan); 16, Suyabangdo Island (Geoje-si); 17, Gujora Port (Geoje-si); 18, Daetseom Island (Geoje-si); 19, Daeguelbido Island (Tongyeong-si); 20, Hongdo Island (Tongyeong-si); 21, Tongyeong Consignment Market (Tongyeong-si); 22, Sindo Island (Tongyeong-si); 23, Yokjido Island (Tongyeong-si); 24, Sangju-ri (Namhae-gun); 25, Gwangyang Port (Gwangyang-si); 26, Almado Island (Yeosu-si); 27, Gyedo Island (Goheung-gun); 28, Jeju Port (Jeju-do); 29, Seogwipo Port (Jeju-do); 30, Munseom Island (Jeju-do); 31, Gangjeong (Jeju-do); 32, Gapado Island (Jeju-do); 33, Hauido Island (Sinan-gun); 34, Hoenggyeongdo Island (Gunsan-si); 35, Cheongpodae Beach (Taeon-gun); 36, Sinjindo (Taeon-gun); 37, Hagampo Beach (Taeon-gun); 38, Incheon Port (Incheon); 39, Baengnyeongdo Island (Incheon). ●, *Pacificincola perforata*; ■, *Primavelans glabricollaris* n. sp.; ★, *Pacificincola perforata* + *Primavelans glabricollaris* n. sp.

and molecular characterization of species, and perform phylogeographical and phylochronological studies (Dick *et al.*, 2003; Dick and Mawatari, 2005; Hao *et al.*, 2005; Nikulina *et al.*, 2010; Fehlaue-Ale *et al.*, 2011; 2015). The objective of this work was to clarify the fauna of Pacificincolidae in the coastal seas and islands of South Korea and review Korean *Mucronella perforata* using both morphological description and phylogenetic analyses of nucleotide sequence data. Their nucleotide sequence data are expected to build the phylogenetic tree of a total of six species belonging to the family Pacificincolidae in the near future.

## MATERIALS AND METHODS

### Sampling

Taxonomic study on Korean Pacificincolidae (Bryozoa: Cheilostomata) was done with the materials collected from 39 localities (Fig. 1) in ports, coastal seas and islands of South Korea from 1981 to 2020. Most of these specimens were obtained from rocks, oyster shells, worm tubes, anthozoans, hydrozoans and plastics, in the intertidal zone and subtidal zone by SCUBA diving, and they were preserved in 95% ethyl alcohol. Images were

**Table 1.** List of species, localities of sampling and GenBank accession numbers of specimens included in the present study. Number of locality in Fig. 1 is indicated in the parentheses.

Classification	Locality	Accession No.
Suborder Ascophora		
Superfamily Schizoporelloidea		
Family Pacificincolidae		
1. <i>Primavelans glabricollaris</i> n. sp.	Almado Island, Jul 20, 2017 (25)	SUB9573201
2. <i>Primavelans glabricollaris</i> n. sp.	Hongdo Island, Apr 26, 2018 (19)	SUB9573221
3. <i>Primavelans glabricollaris</i> n. sp.	Hongdo, Island, Apr 26, 2018 (19)	SUB9573262
4. <i>Pacificincola perforata</i>	Sindo Island, May 7, 2020 (21)	SUB9573286
5. <i>Pacificincola perforata</i>	Sindo Island, May 7, 2020 (21)	SUB 9573997
6. <i>Pacificincola perforata</i>	Sindo Island, May 7, 2020 (21)	SUB 9574008
Outgroups	Locality	Accession No.
Family Schizoporellidae		
7. <i>Schizoporella erratoidea</i>	Dongshan Island, Fujian Orov., China	AY789102
Family Microporellidae		
8. <i>Microporella ciliata</i>	Pacific Bio-Marine, California, USA	AF156286

taken by a digital camera (G9, Canon, Japan). Colonies were examined with a stereomicroscope (Stemi SV6, Carl Zeiss, Germany). Holotype and paratype colonies were coated with gold in an ion-sputter coater (Cressington 108 auto sputter coater, Cressington, England and MCM-100, SEC, Korea), and examined with the scanning electron microscope (JSM-6701F, JEOL, Japan and SNE-3200M Mini-SEM, SEC, Korea) at 5–10 kV accelerating voltage. The holotype and paratype specimens described here are deposited in the National Marine Biodiversity Institute of Korea (MABIK), Seocheon-gun, Korea). The remainder of the materials are stored at the Marine Bryozoans Resources Bank of Korea (MBRBK), Woosuk University, Korea.

For the phylogenetic analyses, we used eight sequences of four species of the superfamily Schizoporelloidea including 2 Pacificincolidae species (Table 1).

#### Isolation and purification of total genomic DNA

About 0.5 g clean bryozoan material was ground into powder and put into one 10 mL centrifuge tube containing 2.0 mL DNA isolation buffer (0.5% SDS, 15 mmol/L EDTA, 5 mmol/L NaCl, 10 mmol/L Tris-HCl, pH 7.6) and 20 µL Proteinase K (20 µg/mL), bathed at 55°C for 2–3 h, centrifuged at 4000 rpm for a few seconds; then about 400 µL of the supernatant was removed into one 1.5 mL Eppendorf tube containing 500 µL 8 mol/L GuSCN and 100 µL 50% clean glass milk liquor, bathed at 37°C, for 2–3 h; centrifuged at 4000 rpm for 2 min, the supernatant was removed, and the sediments were cleaned with 70% alcohol twice, acetone once, dried in a

vacuum concentrator at 45°C for about 15 min; then 100 µL of TE (10 mmol/L Tris-Cl, 1 mmol/L EDTA, pH 8.0) was bathed at 55°C for 30 min, centrifuged at 8000 rpm for 2 min. The supernatant was removed and preserved at –20°C until use.

#### The designing and synthesizing of primers

One pair of universal oligonucleotide primers for Pacificincolidae: 16Sa (5'-CGCCTGTTTATCAAAAA CAT-3') and 16Sb (5'-CCGGTCTGAACTCAGAT-3') were synthesized by Macrogen Inc.

#### PCR amplification, DNA sequencing and Phylogenetic analyses

PCR was performed in 50 µL volumes containing 5–10 ng DNA, 10× Buffer, 2.5 mmol/L MgCl<sub>2</sub>, 0.1 µmol/L each dNTP, 0.1 µmol/L each primer, 5% BSA and 1.0 units Taq DNA polymerase using the standard technique. The cycling parameters were as follows; pre-denaturation at 94°C for 5 min; then for a total of 40 cycles: denaturation at 94°C for 30 sec; annealing at 54°C for 30 sec and extension at 72°C for 1.5 min; final extension at 75°C for 10 min. PCR products were stored at –20°C until use. PCR products were purified QIAquick PCR purification kit. The purified DNA amplification fragments were directly cloned into the bacteriophage M13 vector, then 1–2 µL of the ligation reaction were transformed into the commercially prepared HB101 *E. coli* bacterial cells. Positive colonies identified by antibiotic resistance and blue/white selection

were cultured in LB medium. DNA preparations from 24 positive clones were extracted and sequenced with the universal M13 primers (reverse 5'-TTCACACAG GAAAA-3') on an ABI-377 automated DNA sequencer.

The sequences were verified through BLAST analysis of GenBank databases and sequence alignment was performed using Geneious Prime v11.0.9 (Biomatters, Auckland, New Zealand). Since the family Pacificincolidae belongs to the superfamily Schizoporelloidea, Schizoporellidae and Microporellidae of the same superfamily were selected as the outgroups, and the molecular trees were reconstructed in MEGA (version 2.0) and PHYLIP (version 3.5c) software packages using the neighbor-joining (NJ) method. NJ analysis is based on the distance model of Kimura's-two parameters. Bootstrap analyses (1000 replicates) with internal branch test were performed to estimate the support levels for the nodes in the resultant topology from the NJ analysis.

## SYSTEMATIC ACCOUNTS

Phylum Bryozoa Ehrenberg, 1831  
 Class Gymnolaemata Allman, 1856  
 Order Cheilostomata Busk, 1852  
 Suborder Ascophora Levinsen, 1909  
 Superfamily Schizoporelloidea Jullien, 1883

### Family Pacificincolidae Liu and Liu, 1999

**Diagnosis.** Colony encrusting or encrusting-erect and sometimes rising into forming frilled massive colony when older. Zooids irregularly rectangular to subhexagonal. Frontal wall uniformly perforated and granulated. Orifice semicircular with a pair of condyles forming sinus, and without oral spines. Peristome distinct or confined to a raised rim, with proximal raised umbo and a small opening of a heterozooid between the umbo and the orifice. Spines lacking. Ovicell hyperstomial, prominent, endooecium imperforate or perforated on the surface, with membranous ectooecium thickened by secondary calcification. The frontal wall of the ancestrula is covered by lateral daughter zooids or remains free.

#### A Key to the genera of Pacificincolidae

Frontal shield of ancestrula not covered by daughter zooids..... *Pacificincola*  
 Frontal shield of ancestrula covered by daughter zooids..... *Primavelans*

### Genus *Pacificincola* Liu and Liu, 1999

**Diagnosis.** Characters as for family Pacificincolidae, Spines and avicularia lacking. Ancestrula similar to later

zooids, ancestrula not covered by lateral zooids. Orifice, kenozooidal opening, except for a narrow proximo-lateral gymnocyst.

Type species: *Mucronella perforata* Okada and Mawatari, 1937

#### A key to the species of *Pacificincola*

Ovicell with tuberculate radiating ribs.....  
 ..... *Pacificincola perforata*  
 Ovicell without tuberculate radiating ribs.....  
 ..... *Pacificincola aviculifera*

### *Pacificincola perforata* (Okada and Mawatari, 1937) (Fig. 2)

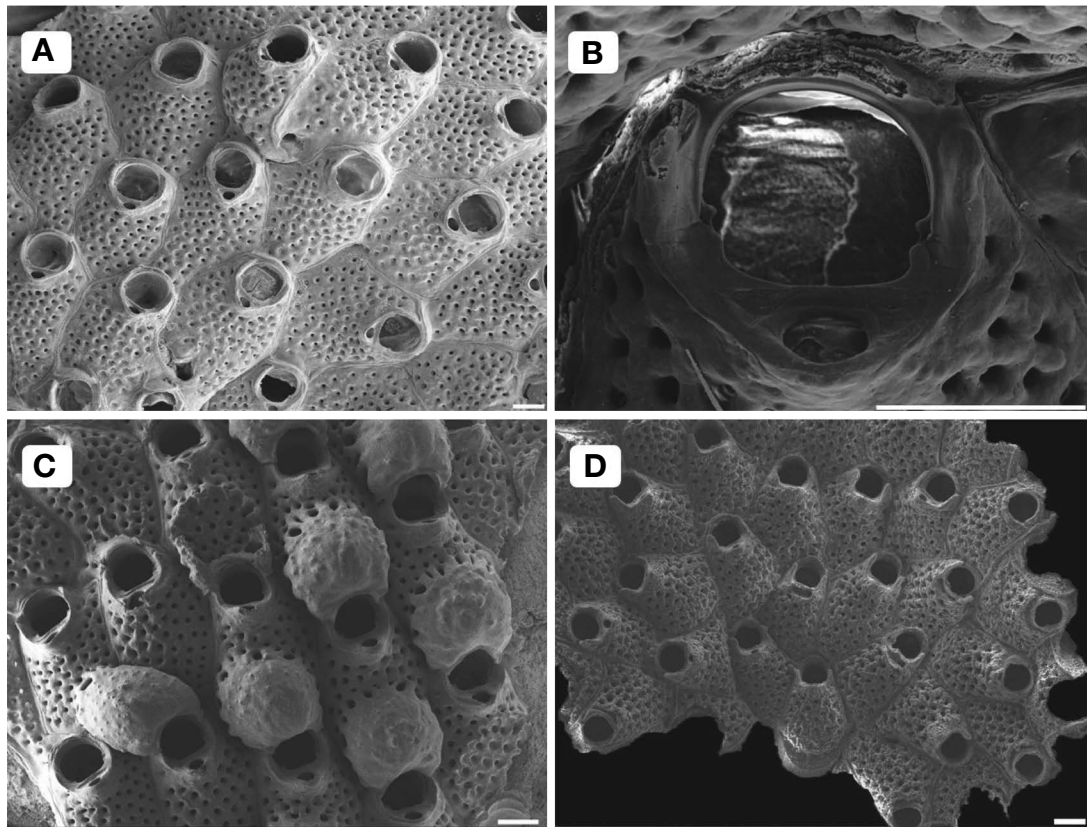
*Mucronella perforata* Okada and Mawatari, 1937, p. 442, pl. 11, fig. 8, text fig. 4a-c.

*Hippoporina perforata*: Huang *et al.* 1990, p. 744, fig. 8, c.  
*Pacificincola perforata*: Liu and Liu, 1999, p. 340, text-fig. 1a-c, pl. 1, figs. 1-5; Liu *et al.*, 2001, p. 572, pl. 44, figs. 1, 2; De Blauwe, 2006, p. 140, figs. 1-3; Grischenko *et al.*, 2007, p. 1122, fig. 30.

**Material examined.** Baengnyeongdo Island, 27 Nov. 2007; Incheon Port, 4 Sep. 2008; 1 Jul. 2010; 27 Jun. 2011; 25 Aug. 2012; Hagampo Beach, 13 Feb. 2009; 16 May 2010; Daeyeon Port, 30 Jun. 2009; Sinjindo, 26 Jul. 2009; Gwangyang Port, 16 Aug. 2009; Jodo Island, 4 May 2010; Hauido Island, 21 Jun. 2010; Jangsaengpo Port, 25 Jun. 2010; 24 Jun. 2011; 25 Feb. 2014; Jeju Port 28 Jun. 2010; Seogwipo Port, 28 Jun. 2010; Cheongpoda Beach, 12 Jul. 2010; 17 Feb. 2011; Ganggu Port, 24 Jun. 2011; 17 Jan. 2012; Gampo Port, 24 Jun. 2011; 3 Mar. 2015; Gangjeong, 10 Jul. 2011; Guryongpo Port, 22 Aug. 2012; Yangpo Port, 22 Aug. 2012; Hoenggyeongdo Island, 17 Apr. 2013; Suyabangdo Island, 14 Oct. 2013; Namae Port, 26 Feb. 2014; MABIK IV00170720, 27 Feb. 2014; Sindo Island, 7 May 2020.

**Substratum.** Rocks, stones, shells (oysters, bivalves, gastropods), calcareous tubeworm, seaweeds, plastics.

**Description.** Colony encrusting, coherent, greyish white, forming unilaminar patch, up to about 2 cm in diameter, light yellow color when alive. Zooids subhexagonal, elongate ovoid, or irregularly rectangular, 0.43-0.68 mm long (average  $0.51 \pm 0.07$  mm), 0.23-0.39 mm wide (average  $0.33 \pm 0.04$  mm), separated by a groove and suture line. All zooids of one type. Frontal wall slightly convex, granulated, evenly perforated with circular pores. Orifice semicircular, longer than wide, 0.09-0.15 mm long (average  $0.12 \pm 0.01$  mm), 0.08-0.14 mm wide (average  $0.12 \pm 0.01$  mm); wide shallow sinus laterally confined by a pair of pointed small triangular condyles. Peristome distinct lateral lappets, not developed distally, with proximal raised umbo and a small opening of kenozooid between umbo and orifice. Ovicell globose, prominent, hyper-



**Fig. 2.** *Pacificincola perforata*. (A) Baengnyeongdo Island, 27 Nov. 2008; (B, C) Sinjindo Island, 26 Jul. 2009; (D) Cheongpodae Beach, 12 Jul. 2010. A, zooids; B, orifice; C, ovicells; D, ancestrula and daughter zooids. Scale bars: A–D=0.10 mm.

stomial, 0.21–0.24 mm long (average  $0.23 \pm 0.01$  mm), 0.23–0.25 mm wide (average  $0.25 \pm 0.01$  mm), closed by operculum, recumbent on frontal wall of distal zooid and partially overhanging orifice, frontally imperforate, irregular nodular, sometimes with finely tuberculate radiating ribs, with series of marginal pores. No spines and avicularia. Interzooidal communication via multiporous septula. Ancestrula elongate oval, 0.05–0.06 mm long (average  $0.06 \pm 0.01$  mm), 0.08–0.09 mm wide (average  $0.08 \pm 0.00$  mm), similar to autozooids, frontal wall finely granulated and evenly perforated with numerous pores. Ancestrular orifice roughly circular. Ancestrula buds smaller zooids, two distolaterally and two laterally, surrounded by zooids.

**Distribution.** Japan (Yatarojima near Onagawa Bay, northern Honshu, 34 m in depth; Akkeshi Bay on the Pacific side of Hokkaido). China (Qingdao in Shandong Province; Shipu in Zhejiang Province; Pingtan Island, Xiamen, and Dongshan Island in Fujian Province; Daya Bay and Dapeng Bay in Guangdong Province, and Hong Kong and its neighboring waters, 0–15 m in depth), France (Bay of Arcachon), Netherlands (Goesse Sas in the Oosterschelde; Yerseke).

**Remarks.** According to Liu and Liu (1999), the width of orifice in Chinese specimen ranges from 0.07 to 0.11

mm (average  $0.09 \pm 0.02$  mm), which is smaller than the one of European materials (De Blauwe, 2006). On the other hand, the zooidal length and width from France are smaller than the others from the Pacific (Table 2).

**Ecology.** This species is a fouling bryozoan which attaches to seaweeds, hydroids, rocks, stones, shells, buoys, fishing nets and synthetic materials. This is known to be one of the commonest bryozoan foulers in the coastal waters off the southern China seas (Liu and Liu, 1999). Also in Korea, this species attaches to stones, oyster shells (*Crassostrea gigas*), bivalve shells (*Mytilus galloprovincialis*), worm tubes, seaweeds, plastics from intertidal zone. Japanese specimens were found on seaweed, rock and bivalve shell only, not on the man-made substratum, such as fishing nets and buoys (Grischenlo *et al.*, 2007). The East Pacific bryozoan *Pacificincola perforata* has been introduced to the Northeast Atlantic Ocean. The most likely route of introduction is via importations of pacific oysters (*Crassostrea gigas*). Its currently known distribution in the Atlantic is restricted to estuaries in France and the Netherlands (De Blauwe, 2006). It can be expected that *Pacificincola perforata* will rapidly spread on the Atlantic coast of Europe.

**Table 2.** Measurements of zooid length, zooid width and orifice width in mm of *Pacificincola perforata* from China (Liu and Liu, 1999), Japan (Grischenko *et al.*, 2007), France (De Blauwe, 2006), and Korea (this study).

	Korea	China	Japan	France
Zooid length (mm)	0.43–0.68	0.49–0.75	0.40–0.60	0.37–0.56
Zooid width (mm)	0.23–0.39	0.22–0.33	0.25–0.43	0.18–0.28
Orifice width (mm)	0.08–0.14	0.07–0.11	0.10–0.15	0.12–0.15

### Genus *Primavelans* De Blauwe, 2006

**Diagnosis.** Characters as for family Pacificincolidae, but with frontal wall evenly perforated. Orifice with a pair of condyles forming sinus. No spines. Ovicell hyperstomial. Ancestrula budding a triplet of daughter zooids; frontal wall of ancestrula covered by two lateral daughter zooids, having a median longitudinal ridge, only the ancestrular orifice remaining free.

Type species: *Schizoporella insculpta* Hincks, 1883

#### A Key to the species of *Primavelans*

- Ovicell perforated ... *Primavelans glabricollaris* n. sp.  
Ovicell not perforated ..... 2
- Peristome well raised ..... *Primavelans mexicana*  
Peristome scarcely raised ..... *Primavelans insculpta*

#### *Primavelans glabricollaris* n. sp. (Fig. 3)

*Mucronella perforata*: Rho and Seo, 1985, p. 4, pls. 9, 10.  
*Pacificincola perforata* Hirose, 2010, pp. 124, 125, pl. 214  
A–E. Not *Pacificincola perforata* (Okada and Mawatari, 1937).

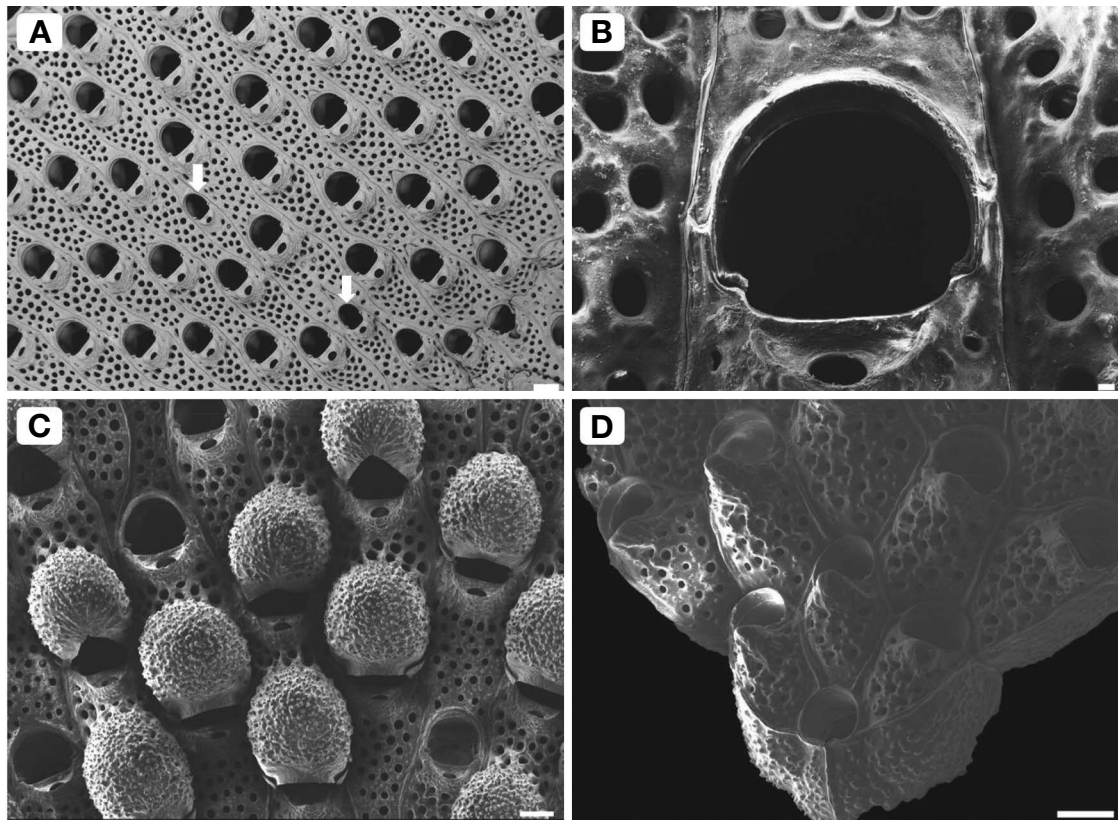
**Type.** Holotype: MABIK IV00170721, One intact colony, collected by 21 Nov. 2009 at rocky shore of Munseom Island, Jeju-do, (B.S. Min and H.J. Yang) by SCUBA diving from 25–30 m in depth. Paratype: MABIK IV00170722, Bangeojin Port, 13 Aug. 2009, by B.S. Min, and H.J. Yang.

**Additional material examined.** Yeon-daedo Island, 10 Jul. 1964; Yeonhwado Island, 19 Jul. 1978; Seogwipo, 13 Jul. 1979; Sangju-ri, 20 May 1981; Mipo, 25 May 1981; 8 Dec. 1981; Dodong, 11 Jul. 1984; Neunggeol, 26 Aug. 2003; Deoksan Port, 7 Jul. 2002; Gyedo Island, 24 Jun. 2008; Yangpo Port, 12 Aug. 2009; 24 Jun. 2010; 24 Jun. 2011; 22 Aug. 2012; 22 Dec. 2012; 25 Feb. 2014; 3 Mar. 2015; Bangeojin Port, 13 Aug. 2009; Gujora Port, 15 Aug. 2009; Jumunjin Port, 30 Aug. 2009; Daebyeon Port, 19 Sep. 2009; Munseom Island, 6 Oct. 2009 (J.E. Seo, B.S. Min and H.J. Yang) by SCUBA diving from 30 m in depth; 21 Nov. 2009; 19 Jan. 2010 (B.S. Min and H.J. Yang) by SCUBA diving from 25–30 m in depth; Jodo Island, 5 May 2010 (B.S. Min and H.J. Yang) by SCUBA diving from 10 m in depth; Yokjido Island, 1 Nov. 2011

(B.S. Min and S.G. Ra) by SCUBA diving from 10–15 m in depth; 2 Nov. 2011 (B.S. Min and S.G. Ra) by SCUBA diving from 10–15 m in depth; Ganggu Port, 17 Jan. 2012; Daeguelbido Island, 25 Jun. 2013 (B.S. Min) by SCUBA diving from 10–15 m in depth; Daetseom Island, 26 Jun. 2013; Tongyeong Port, 2 Mar. 2015; Gyeongpoda (Gangneung-si) 5 May 2015 (B.S. Min and J.K. Jung) by SCUBA diving from 15 m in depth; Gapado Island (Jeju-do) 5 Jul. 2015 (B.S. Min, H.J. Yang and J.K. Jung) by SCUBA diving from 25 m in depth; Almado, 20 Jul 2017 (B.S. Min) by SCUBA diving from 25 m in depth; Hongdo, 26 Apr. 2018 (B.S. Min) by SCUBA diving from 25 m in depth.

**Substrata.** Anthozoans (sea fans), hydrozoans, oyster shells, rocks, buoys (plastic or styrofoam).

**Description.** Colony unilaminar, encrusting when young; erect and massive, growing up into bilaminar to about 20 cm in diameter when older; light yellow, or orange when alive. Zooids elongate, rounded-hexagonal, or barrel-shaped, 0.45–0.84 mm long (average  $0.62 \pm 0.10$  mm), 0.20–0.29 mm wide (average  $0.26 \pm 0.04$  mm). Frontal wall slightly convex, evenly perforated, pustulose between perforations. Orifice subcircular, slightly wider than long, 0.14–0.21 mm long (average  $0.18 \pm 0.02$  mm), 0.16–0.21 mm wide (average  $0.19 \pm 0.02$  mm), with a wide, shallow sinus flanked by blunt condyles. Zooids of two types, male with a smaller orifice and female with a larger orifice. Male zooid, 0.49–0.5 mm long (average  $0.48 \pm 0.03$  mm), 0.12–0.19 wide (average  $0.16 \pm 0.04$  mm). Orifice of male zooid, 0.10–0.13 mm long (average  $0.18 \pm 0.01$  mm), 0.09–0.11 wide (average  $0.10 \pm 0.01$  mm). Peristome not well developed. Small heterozooid proximal to orifice forms lunate umbo small, elliptical foramen. Ovicell conspicuous, globose, reaching to condyles and leaving only 1/3 of orifice when mature, surface pustulose, 0.29–0.35 mm long (average  $0.31 \pm 0.02$  mm), 0.25–0.29 mm wide (average  $0.27 \pm 0.01$  mm); generally with a few tiny pores on surface. Developing ovicells with thin ribs radiating from proximal margin; mature ovicells lacking ribs but having a smooth zone along proximal margin and paired, thin, raised basal flanges at proximal corners, and appear to be closed by zooidal operculum (Fig. 3C). Spines and avicularia lacking. Ancestrula oval in outline, 0.11 mm long (average  $0.11 \pm 0.00$  mm), 0.10–



**Fig. 3.** *Primavelans glabricollaris* n. sp. (A) Hongdo, 26 Apr. 2018; (B–D) Jumunjin Port, 30 Aug. 2009. A, autozooids, arrow indicates male zooid; B, D-shaped orifice; C, ovicells with minute pores, paired proximolateral flanges, and smooth zone along proximal margin, which appears to conform shape of operculum; D, ancestrula with triplet of primary daughter zooids, with secondary calcification covering ancestrula proximal to orifice. Scale bars: A, C, D=0.10 mm, B=0.01 mm.

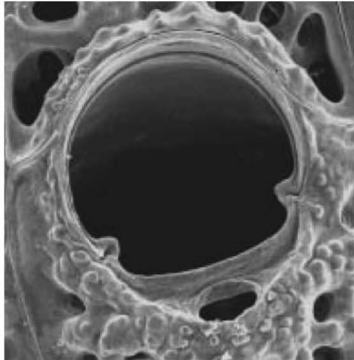
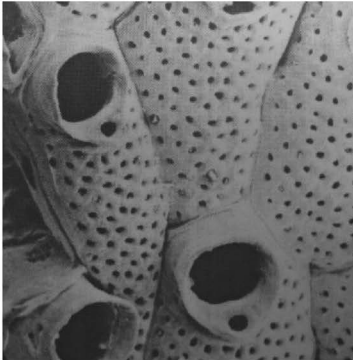
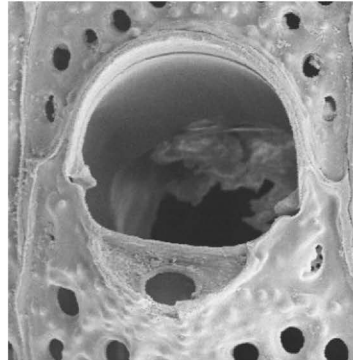
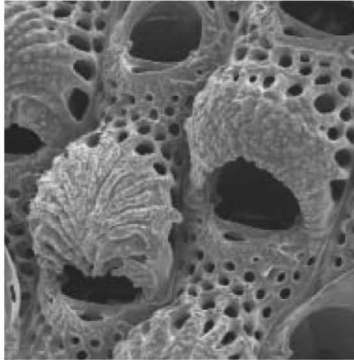
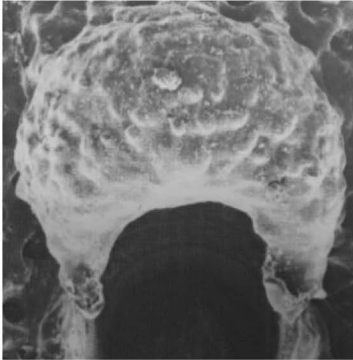
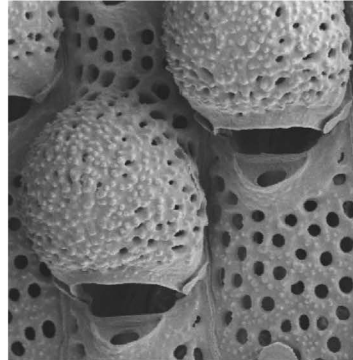
0.13 mm wide, (average  $0.12 \pm 0.2$  mm) budding a distal and two distolateral primary daughter zooids; frontal wall of ancestrula covered by the two lateral daughter zooids, having a median longitudinal ridge, only the ancestrular orifice remaining free.

**Etymology.** The species name is from the Latin *glaber* (smooth), *collarium* (collar), reflecting to the mature ovicell having with smooth collars.

**Remarks.** There are three species belonging to the genus *Primavelans*, *Primavelans insculpta* and *Primavelans mexicana* from the east Pacific, and *Primavelans glabricollaris* n. sp. The proximal lip by blunt and downcurved condyles of the orifice in our new species is more straight than slightly convex by sharp condyles in *Primavelans insculpta*. The condyles of *Primavelans mexicana* are much smaller than the rest two species of *Primavelans*, which it form the proximal lip arched slightly in the middle (Soule, Soule and Chaney, 1995). Both *Primavelans glabricollaris* n. sp. and *Primavelans insculpta* has two types of zooids, male with a smaller orifice and female with a larger orifice. However, the former species has more rare male zooids than the latter (Nielsen, 1981). The

ovicell in *Primavelans glabricollaris* n. sp. is minutely perforated and has thin flanges at the proximolateral corners and a smooth zone along the proximal margin, which seems to conform to the shape of the zooidal operculum. In addition, *Primavelans glabricollaris* n. sp. in lacking ridges on the mature ovicell differs from *Primavelans insculpta*, which has few small pores closed by advanced calcification (Soule, Soule and Chaney, 1995). *Primavelans mexicana* has the imperforate ovicell with lateral oral flanges showing difference from the one of *Primavelans glabricollaris* n. sp. (Soule, Soule and Chaney, 1995) (Fig. 3). Japanese specimen from Sagami Bay (Hirose, 2010) does not show the ancestrula of the genus *Primavelans*; however it is clearly different from *Pacificincola perforata* in having perforate ovicell with thin flanges at its proximolateral corners and deeply covering zooidal orifice, when mature (Hirose, 2010). Thus, of Japanese specimens, the one from Sagami Bay seems to be different from the one from Akkeshi Bay, Hokkaido.

**Ecology.** *Primavelans glabricollaris* n. sp. is initially unilaminar but then grows and rises into irregular, bilaminar and frilled massive colony in case of materials collected

	<i>Primavelans insculpta</i>	<i>Primavelans mexicana</i>	<i>Primavelans glabricollaris</i> n. sp.
Peristome			
	scarcely raised	well raised	scarcely raised
Ovicell			
	hyperstornial, imperforate, finely tuberculated surface	hyperstornial, imperforate, nodular surface	hyperstornial, perforate, thin flanges at corners
Distribution	East Pacific (Alaska to California)	East Pacific (California to Costa Rica)	Pacific (Korea, Japan)

**Fig. 4.** Comparison of characters among *Primavelans glabricollaris* n. sp. and its two congeners.

**Table 3.** P-distance values among three families belonging to Schizoporelloidea

Family	1	2	3
Pacificincolidae			
Microporellidae	0.2702		
Schizoporellidae	0.2766	0.2527	

**Table 4.** P-distance values among four species of four different genera belonging to the superfamily Schizoporelloidea.

Genus	1	2	3	4
<i>Primavelans glabricollaris</i> n. sp.				
<i>Pacificinicola perforata</i>	0.1339			
<i>Microporella cilaiata</i>	0.2651	0.2753		
<i>Schizoporella erratoidea</i>	0.2679	0.2853	0.2527	

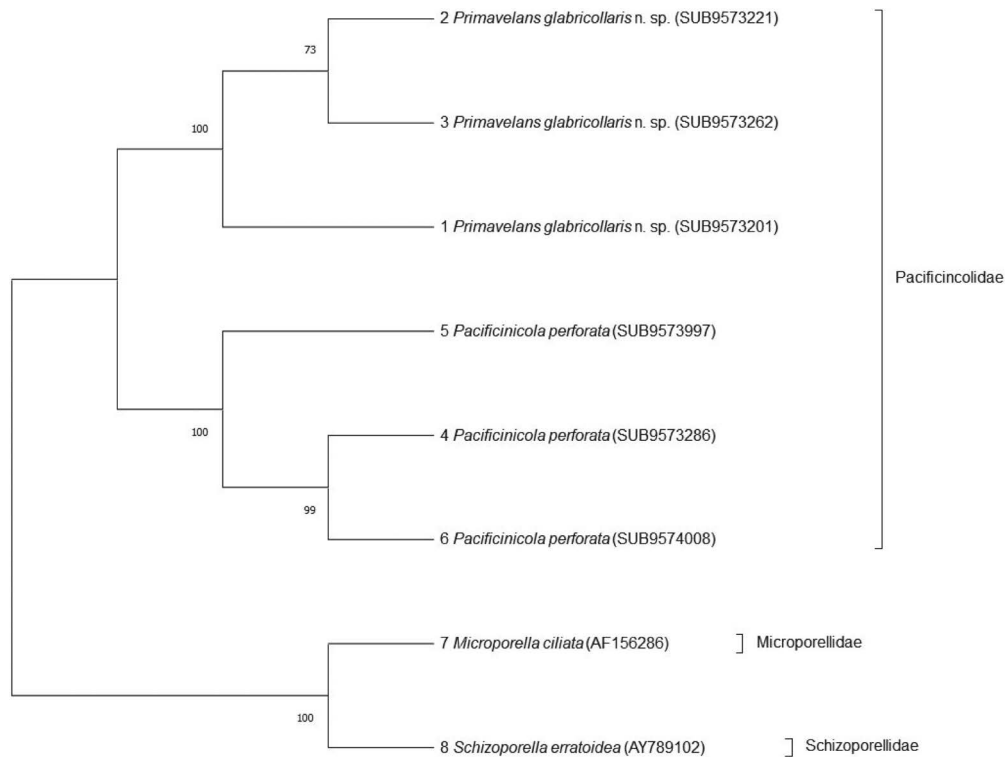
from a depth of about 25 m. This new species is a fouling bryozoan attached to the ropes hanging on wharfs or aquaculture cages. Most colonies collected from Jeju-do and eastern and southern shoreline of Korea are commonly attached to buoys such as plastics or styrofoam in ports. In scallop farms, colonies of the new species encrust the aquaculture cages, and interrupt the growth of young scallops, causing a disturbance to the flow of water. This new species on hydroids seems to be found in Europe (personal

communication). Occasionally in Jeju-do, other invertebrates, such as decapods and starfish, are found within the colony of this species.

## RESULTS AND DISCUSSION

Three genera belonging to the family Pacificincolidae





**Fig. 5.** Neighbor-Joining (NJ) phylogenetic tree based on approximately 480 bp of 16S rRNA gene from four Schizoporelloids species, based on the kimura-2 parameter distance model. Bootstrap values indicated on each node. Species is provided with both serial number of material and GenBank accession number.

**Table 5.** Pairwise distances of two Pacificincolidae species and two species belonging to Schizoporelloidea as the outgroups, performed by the kimura 2-parameter distance model with 1000 bootstrap replicates.

Species	GenBank Accession No.	1	2	3	4	5	6	7	8
1 <i>Primavelans glabricollaris</i> n. sp.	SUB9573201								
2 <i>Primavelans glabricollaris</i> n. sp.	SUB9573221	0.0045							
3 <i>Primavelans glabricollaris</i> n. sp.	SUB9573262	0.0045	0.0000						
4 <i>Pacificincola perforata</i>	SUB9573286	0.1468	0.1406	0.1406					
5 <i>Pacificincola perforata</i>	SUB9573997	0.1334	0.1296	0.1296	0.0204				
6 <i>Pacificincola perforata</i>	SUB9574008	0.1306	0.1269	0.1269	0.0109	0.0022			
7 <i>Microporella ciliata</i>	AF156286	0.2665	0.2644	0.2644	0.2867	0.2695	0.2695		
8 <i>Schizoporella erratoidea</i>	AY789102	0.2672	0.2682	0.2682	0.2942	0.2747	0.2869	0.2527	

Liu and Liu, 1999 were recorded worldwide so far: *Pacificincola* Liu and Liu 1999, *Primavelans* De Blauwe, 2006 and *Burdwoodipora* López Gappa, Liuzzi and Zelaya, 2017. *Mucronella perforata* was reported by Rho and Seo (1985) from the Korean waters. As a result of taxonomic study on Korean Pacificincolidae (Bryozoa: Cheilostomata), Korean *Mucronella perforata* turns out to be not *Pacificincola perforata*, but *Primavelans glabricollaris* n. sp. by the following morphological data. The genus *Primavelans* was established on the basis of

the early astogeny by De Blauwe in 2006. Korean *Mucronella perforata*, which the frontal shield of ancestrula is covered by triplet lateral daughter zooids with the exception of the ancestrular orifice, was herein transferred into this new genus. Furthermore, Korean *Primavelans* n. sp. which has thin flanges at the proximolateral corners and smooth zone along the proximal margin of ovicell, definitely differs from *Primavelans insculpta* and *Primavelans mexicana* from East Pacific. Our new species also has a few pores and lacks the ridge on mature ov-

**Table 6.** Mean of p-distance values within two genera, *Primavelans* and *Pacificincola* belonging to the Pacificincolidae.

Species	No. of localities	Mean
<i>Primavelans glabricollaris</i> n. sp.	3	0.0030
<i>Pacificincola perforata</i>	3	0.0111

icell, whereas *Primavelans insculpta* has a few pores closed by advanced calcification and the ridge on mature ovicell. On the other hand, *Pacificincola perforata* which was transferred from *Mucronella perforata* (Okada and Mawatari, 1937) by Liu and Liu (1999), who established both a new family and genus, Pacificincolidae and *Pacificincola*, is also added to the Korean fauna.

For the purpose of reconfirmation on the family Pacificincolidae and the genus *Pacificincola*, the mitochondrial 16S rRNA gene was sequenced from these two species and the other two species belonging to other families as outgroups to identify them and their phylogenetic relationships for the first time. Three families belonging to the superfamily Schizoporelloidea, which are Pacificincolidae, Schizoporellidae and Microporellidae, are clearly separated by the pairwise distance (p-distance) and NJ analysis. The pairwise distance value between Pacificincolidae and Schizoporellidae show slightly higher ( $27.66 \pm 2.84\%$ ) than the one of between Pacificincolidae and Microporellidae ( $27.02 \pm 2.72\%$ ) (Table 3). This is not congruent with Hao *et al.* (2005), who inferred that *Microporella ciliata* was the basal branch in the ascophorans group.

The NJ analysis inferred that the Pacificincolidae was a sister group of Schizoporellidae and Microporellidae, and monophyly supported by high bootstrap values (Fig. 5). *Primavelans glabricollaris* and *Pacificincola perforata* within the group of Pacificincolidae were separated by a mean genetic distance of  $13.39 \pm 1.83\%$  (Table 4).

*Primavelans glabricollaris* n. sp. is thus distinguished from *Pacificincola perforata* by sequences data as well as aforementioned morphological data. In terms of intra-specific genetic variation of the two species, p-distance values of sequences of specimens between Almado Island (SUB9573201) and Hongdo Island (SUB9573221 and SUB9573262) of the new species showed  $0.45\% \pm 0.31\%$  with a mean of 0.30%, and the value among three different colonies (SUB9573286, SUB9573997 and SUB9574008) from same locality of *Pacificincola perforata* ranges 0.00–2.66% with the mean of 1.11%, which are negligible in both species (Tables 5, 6).

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