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First discovery of the sessile barnacle *Eochionelasmus* (Cirripedia: Balanomorpha) from a hydrothermal vent field in the Indian Ocean

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Abstract

A new species of chionelasmatid sessile vent barnacle, *Eochionelasmus coreana* sp. nov., is described and illustrated on the basis of specimens collected from the Solitaire hydrothermal vent field in the Central Indian Ridge of the Indian Ocean. This new species is morphologically very similar to *E. ohtai*, the type species of the genus *Eochionelasmus*. However, it differs from *E. ohtai* in its distribution, the status of the notch on the maxillule, and the positions of rl1 and cl1 on whorls of the imbricating plates. In addition, a molecular phylogenetic tree indicated that the chionelasmatid *Eochionelasmus* was closely related to the waikalasmatid *Waikalasma* with high supporting values rather than the other chionelasmatid *Chionelasmus*. The new species is not only the first record of a sessile vent barnacle from outside of the Pacific Ocean, but is also the first sessile barnacle from the Indian Ocean.

Introduction

Some taxa of thoracican barnacles are known to be endemic to chemosynthetic hydrothermal vents and seeps on the deep-sea floor (Chan & Chang, 2018). Most of these endemic species occur in the Pacific Ocean; only two stalked barnacles, *Vulcanolepas scotiaensis* Buckeridge & Linse, 2013 (East Scotia Ridge) and *Neolepas marisindica* Watanabe *et al.*, 2018 (Indian Ocean), are known outside of the Pacific. Seventeen extant species belonging to eight genera have been described, and are separated into two molecular phylogenetic lineages descended from different common ancestors settled in hydrothermal vent ecosystems by two independent events: the major clade including brachylepadomorphs, neoverrucids and neolepadids, and the balanomorphan *Eochionelasmus* clade (Herrera *et al.*, 2015; Chan, 2018).

Among the extant descendants of hydrothermal vent barnacles, the *Eochionelasmus* clade includes only the genus *Eochionelasmus* Yamaguchi, 1990, which is characterized by six principal wall plates and multiple whorls of basal imbricating plates (Yamaguchi & Newman, 1990, 1997*a*, 1997*b*). Two *Eochionelasmus* species, *E. ohtai* Yamaguchi, 1990 and *E. paquensis* Yamaguchi, 1997, have been recorded in different vent fields, the South-west Pacific Ocean and East Pacific Rise, respectively (Yamaguchi & Newman, 1997*a*, 1997*b*; Figure 1). *Eochionelasmus ohtai*, which is distributed widely in hydrothermal vent fields of the South-west Pacific Ocean, comprises two subspecies, *E. ohtai ohtai* for the North Fiji-Lau populations and *E. ohtai manusensis* for the Manus population, based on distinct differences in the ontogenetic development of the imbricating plates (Yamaguchi & Newman, 1997*a*, 1997*b*). Phylogenetic and population genetic analyses have been conducted to elucidate their taxonomic position and dispersal capacity (Plouviez *et al.*, 2013; Herrera *et al.*, 2015; Kim *et al.*, 2018); however, DNA sequences of *E. paquensis* are unavailable in public databases.

The Indian Ocean ridges have an intermediate spreading rate (50–60 mm year^{-1}) compared with those of the East Pacific Rise and Mid-Atlantic Rise, and the organisms living there remain poorly studied (Beedessee *et al.*, 2013). The stalked neolepadid *Neolepas marisindica* Watanabe *et al.*, 2018 is currently the only known vent-endemic barnacle in the Indian Ocean and is distributed widely among five hydrothermal vent fields of the Indian Oceanic ridges: Karei, Solitaire, Onnuri, Longqi and Site 21 (Watanabe *et al.*, 2018; Ryu *et al.*, 2019).

In this study, we describe a new species of the chionelasmatid *Eochionelasmus* found in the Solitaire hydrothermal vent field of the Central Indian Ridge; its *CO1* barcodes for DNA taxonomy have been deposited in GenBank. We also discuss the phylogenetic position of the new species with known congener sequences.

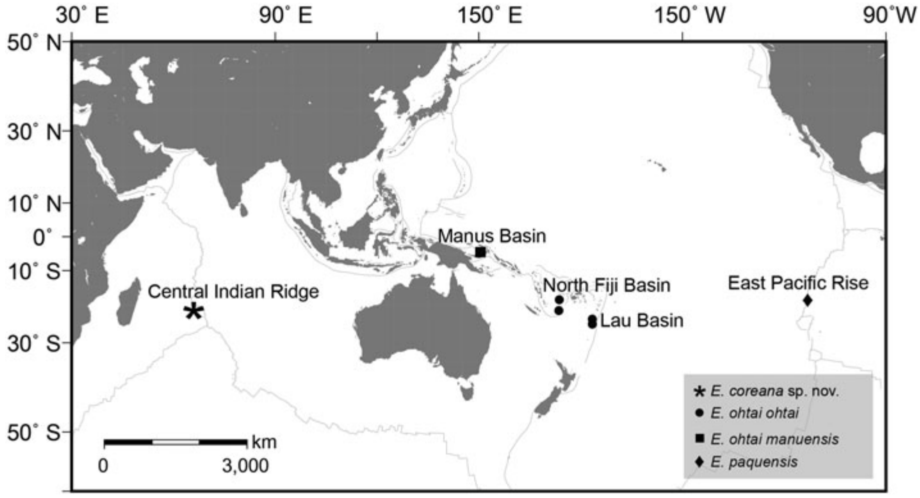

Fig. 1. Distribution map of *Eochionelasmus* species based on Yamaguchi & Newman (1990, 1997*a*, 1997*b*) and this study.

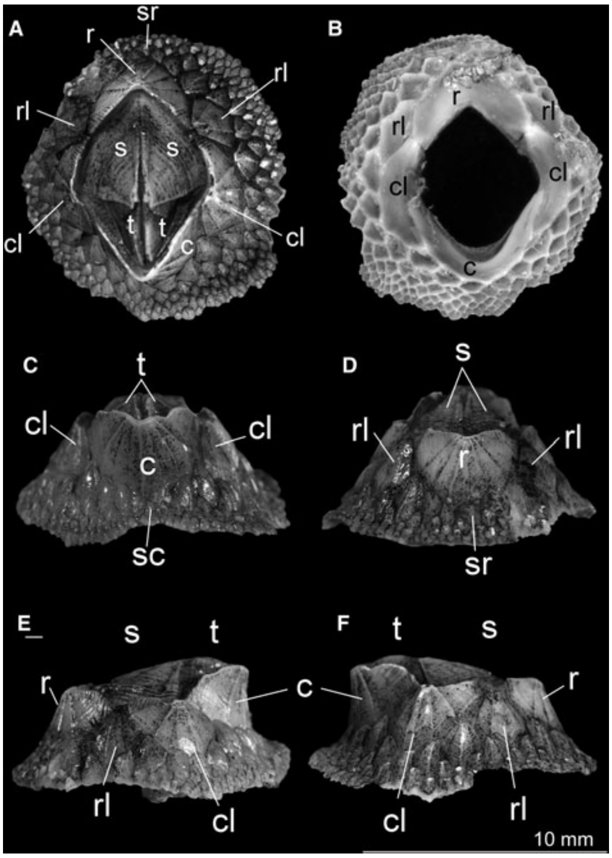

Fig. 2. External shell of *Eochionelasmus coreana* sp. nov. (holotype). (A) Top view. (B) Basal view showing the sheath is formed by the combination of rl, paired cl and c. rl is located outside the sheath. (C) Carinal view. (D) Rostral view. (E) Left view. (F) Right view. R, rostrum; c, carina; cl, carinal latus; rl, rostral latus; s, scutum; t, tergum; sc, subcarina; sr, subrostrum.

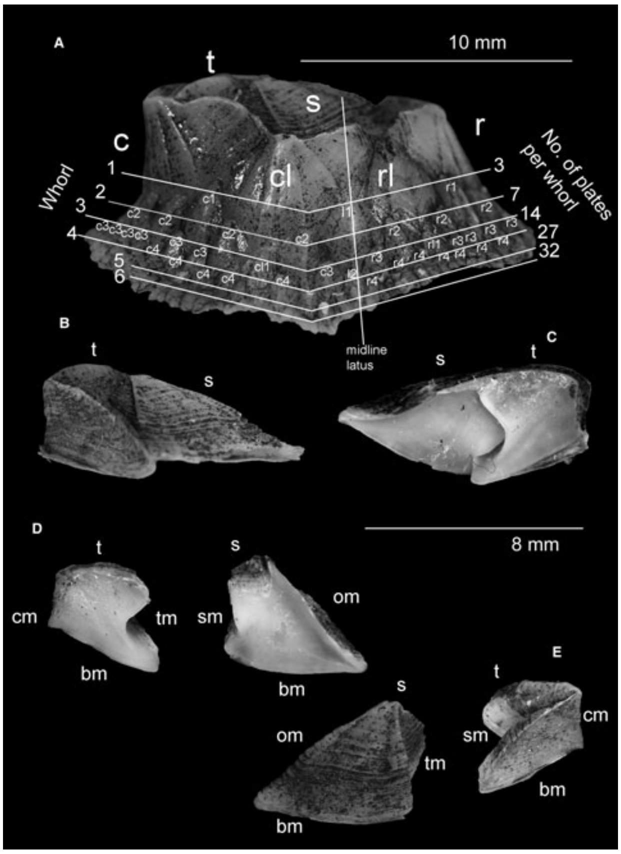

Fig. 3. (A) Side view of *Eochionelasmus coreana* sp. nov. (holotype), showing the number of whorls of imbricating plates and number of imbricating plates per row. Naming of imbricating plates follows the system in Yamaguchi & Newman (1997*a*, 1997*b*). Imbricating plates on the carinal side from the latera are abbreviated as c. Imbricating plates on the rostral side from the latera are abbreviated as r. The numeral of the imbricating plates indicates the number of whorls from their first appearance. cl1 indicates the first cl that appears directly under cl. rl1 indicates the first rl that appears directly under cl. l is the latera. Note that not all the imbricating plates are labelled for clarity reasons. (B) Outer side of inter-locked scutum and tergum. (C) Inner side of inter-locked scutum and tergum. (D) Inner side of tergum and scutum. (E) Outside of scutum and tergum. cm, carinal margin; om, occludent margin; bm, basal margin; tm, tergal margin; sm, scutal margin; t, tergum; s, scutum.

Materials and methods

Vent barnacle sampling

Eochionelasmus specimens were collected at depths of >2600 m from the Solitaire hydrothermal vent field on the Central Indian Ridge (Figure 1) using a TV-guided grab sampler equipped by the research vessel 'ISABU' during a vent ecosystem study in the Indian mid-ocean ridge by the Korean Institute of Oceanographic Science and Technology. The specimens were immediately frozen and kept at − 80 °C for morphological and molecular studies.

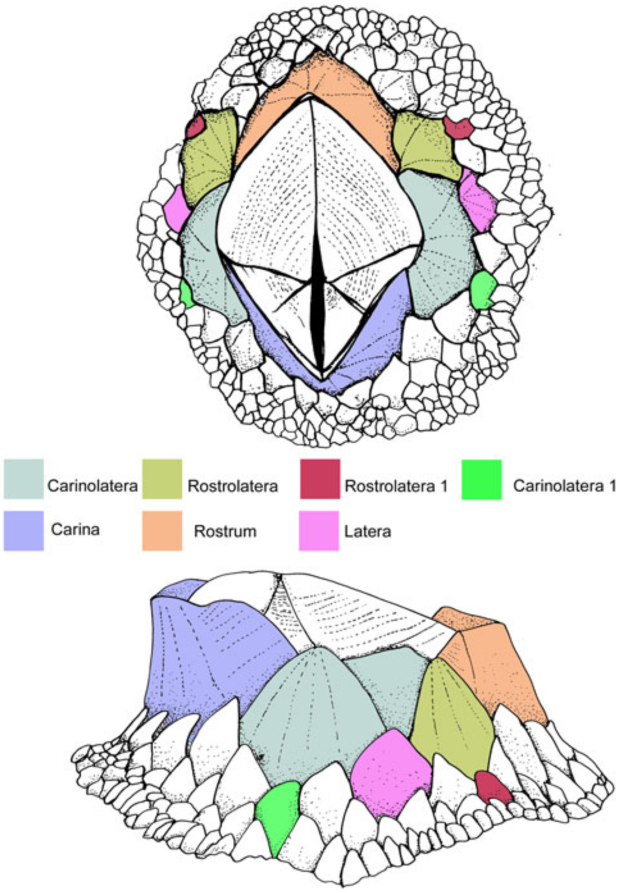

Fig. 4. Drawing of *Eochionelasmus coreana* sp. nov. (A) Plan view and (B) Lateral view, showing the arrangement of shell plates and imbricating plates. Note latera appears on the third whorl of imbricating plates (also see Fig. 3).

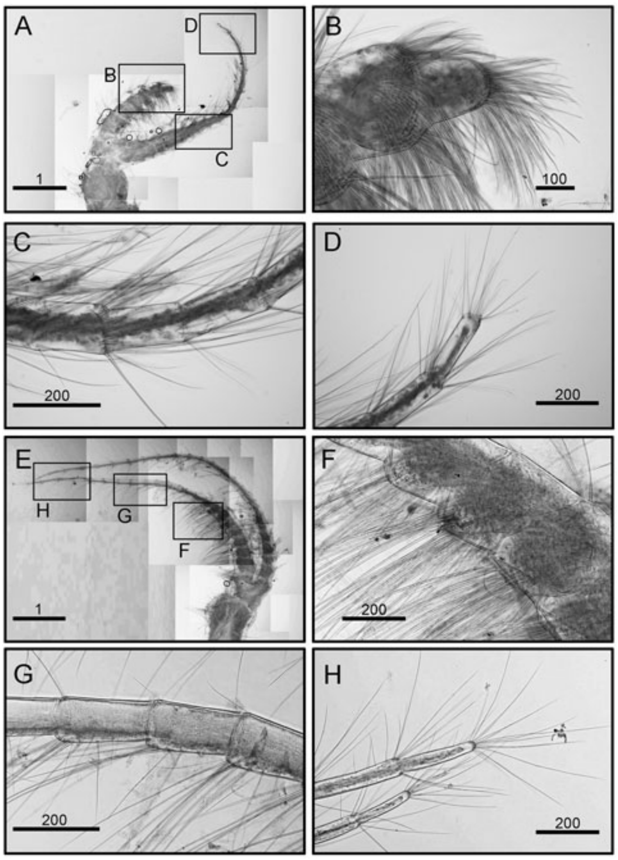

Fig. 5. *Eochionelasmus coreana* sp. nov. (holotype). (A) Cirrus I, showing anterior and posterior ramus. (B) Simple setae on posterior ramus. (C) Simple setae on anterior ramus. (D) Setae on distal end of anterior ramus. (E) Cirrus II. (F) Dense simple setae on the robust segment of anterior ramus. (G) Intermediate segment on posterior ramus. (H) Distal segment of posterior ramus. Scale bars in A, E in mm; others in µm.

Morphological examination

Each specimen was examined and dissected under stereomicroscopes. Soft parts including the mouth parts, cirri and penis were photographed using a compound microscope (Zeiss Axioplan compound microscope installed with objective APO40 × and APO20 × lenses; Germany). Morphological terminology generally follows Yamaguchi & Newman (1997*a*, 1997*b*) for descriptions of the imbricating plates and arthropodal characters, and Chan *et al.* (2008) for setal classification. The dissected holotype and the dissected paratype have been deposited in the Korea National Institute for Biological Resources (Incheon, Korea, NIBR) and the Biodiversity Research Museum, Academia Sinica, Taiwan (ASIZCR), respectively.

Phylogenetic analysis

A small amount of muscle tissue was dissected from the *Eochionelasmus* specimens for DNA extraction. Total genomic DNA was extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The partial sequences of three mitochondrial (12S, 16S rDNA and *CO1*) and two nuclear (18S rDNA and histone 3) genes were determined using the universal primers (Folmer *et al.*, 1994; Chan *et al.*, 2017). PCR amplification was performed in a total volume of 50 µl containing 1 µl of genomic DNA, 4 µl of dNTP mixture (2.5 mM each), 1 µl of each primer (10 pmol), 5 µl of 10 × ExTaq Buffer (Mg^{2+} plus), and 1.25 U of Takara Ex Taq DNA Polymerase (Takara Biotechnology Co., Tokyo, Japan) under the following conditions: initial denaturation at 94 °C for 2 min, followed by 40 cycles of denaturation (15 s at 95 °C), primer annealing (30 s at 50 °C in the first 10 cycles and 30 s at 55 °C in the last 30 cycles), and extension (2 min at 72 °C), with a final extension step at 72 °C for 5 min. Then, 5 µl of each PCR product was run on a 1.0% agarose gel and visualized under UV light. Finally, Sanger sequencing was performed at Macrogen Service (Macrogen, Seoul, Korea) using the ABI PRISM 3730XL Analyzer (Applied Biosystems, Foster City, CA, USA) with a BigDye(R) Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems).

The newly determined sequences from this study were trimmed and annotated, and then aligned with the other balanomorph genera retrieved from GenBank using T-Coffee (Notredame *et al.*, 2000). To construct a phylogenetic tree of balanomorph barnacles, individual 12S rRNA, 16S rDNA, 18S rDNA and histone 3 gene alignments were concatenated to form a single multiple-sequence alignment with DAMBE5 (Xia, 2013). The best-fitting model of nucleotide substitution was then determined using the Akaike information criterion in jModelTest 2.1.7 (Darriba *et al.*, 2012); the model GTR + I + G was selected as the best evolution model. Maximum likelihood (ML) and Bayesian inference (BI) tests were performed using RAxML version 8.2.11 (Stamatakis, 2014) and MrBayes 3.2.6 (Ronquist *et al.*, 2012), respectively, implemented in Geneious Prime with the gene partition option. Confidence in the resulting balanomorph relationships was assessed based on the bootstrap proportion (BP) with 200 replications for the ML model. For BI analysis, four Markov chain Monte Carlo chains were run for 1000,000 generations and sampled every 200 generations. Bayesian posterior probability (BPP)

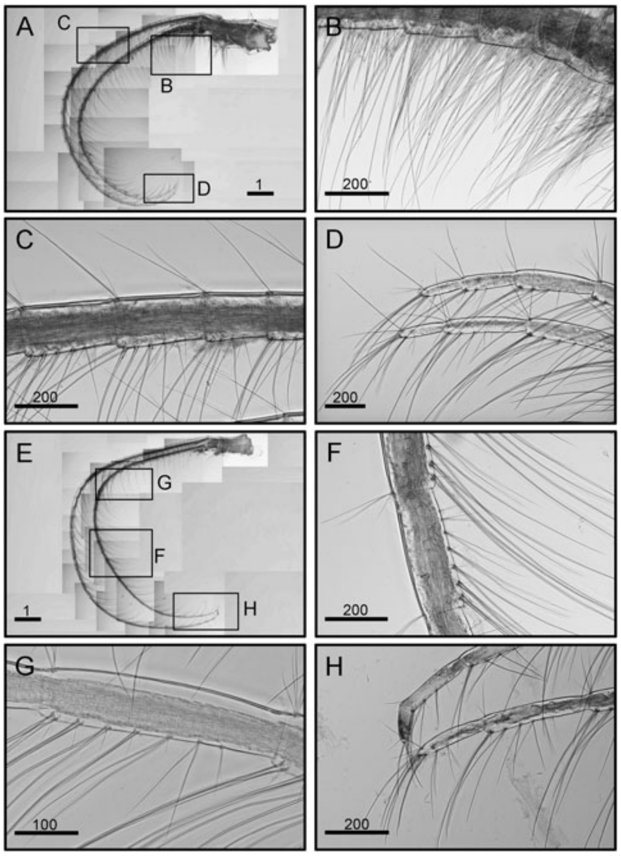

Fig. 6. *Eochionelasmus coreana* sp. nov. (holotype). (A) Cirrus III. (B) Dense setae on proximal region of rami. (C) Intermediate segments. (D) Distal segments. (E) Cirrus IV. (F) Intermediate segment. (G) Proximal segments. (H) Distal segments. Scale bars in A, E in mm; others in μm.

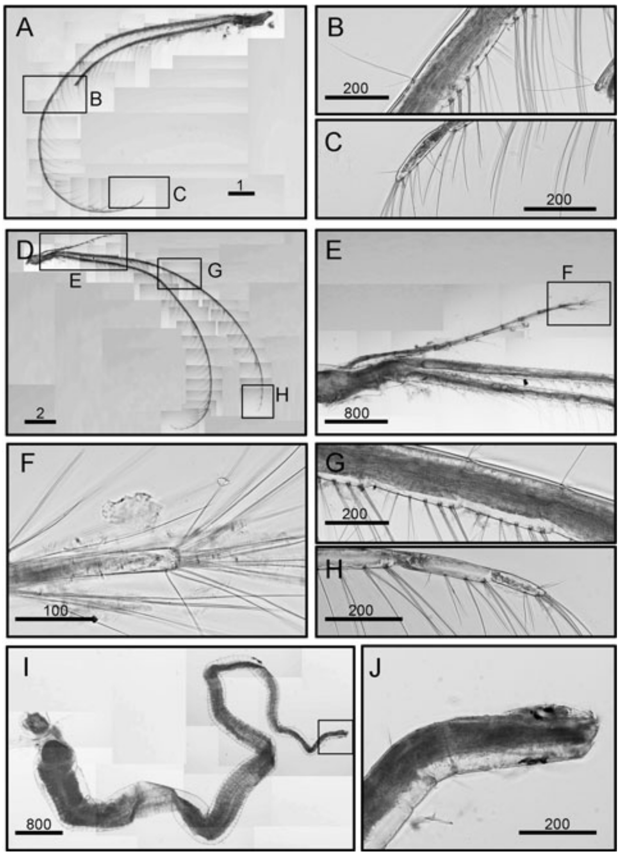

Fig. 7. *Eochionelasmus coreana* sp. nov. (holotype). (A) Cirrus V. (B) Intermediate segments. (C) Distal segments. (D) Cirrus VI and caudal appendages. (E) Caudal appendages. (F) Distal segments. (G) Intermediate segments. (I) Penis. (J) Tip of penis. Scale bars in A, D in mm; others in μm.

values were estimated after the initial 500 (10%) trees were discarded as burn-in.

Results

Systematics

Superorder THORACICA Darwin, 1854
Order SESSILIA Lamarck, 1818
Suborder BALANOMORPHA Pilsbry, 1916
Superfamily CHIONELASMATOIDEA Buckeridge, 1983
Family CHIONELASMATIDAE Buckeridge, 1983
Genus *Eochionelasmus* Yamaguchi, 1990
Eochionelasmus coreana sp. nov.
(Figures 2–10)

Type material

Holotype: NIBR-IV0000865932. Solitaire vent fields, Indian Ocean Ridge (19° 33′ 39″ S 65° 50′ 89″ E; water depth: 2625 m); collected using a TV-guided grab sampler, coll. D.-S. Kim, 19 June 2018.

Paratype: ASIZCR-000433, same data as holotype.

Diagnosis

Eochionelasmus with 6–7 whorls of imbricating plates, carinolatera with wide alae, maxillule without notch on upper portion, third tooth of mandibles with numerous denticles on cutting edge. Location of carinolatera 1 and rostrolatera 1 on the third whorl of imbricating plates.

Description

Primary shell wall six-plated; composed of carina, paired carinolatera, paired rostrolatera, and rostrum (c-cl-rl-r-rl-cl); conical and depressed (height ∼1/2 of rostro-carino diameter) (Figure 2). Orifice large and rhomboidal (Figure 2). Sheath formed by carina, rostrum, paired carinolatera, but rostrolatera not entered (Figure 2B).

Shells were surrounded by 6–7 whorls of triangular imbricating plates (Figures 2C–F & 3A). Size of imbricating plates increased from the shell rim to the upper whorls (Figure 2C–F). The first whorl of imbricating plates started on the lower portion of primary shell wall (Figures 3A, 4A, B). First whorl with three imbricating plates, carina 1 (c1), latus 1 (l1) and rostrum 1 (r1); second whorl with seven imbricating plates; third whorl with 14 imbricating plates, carinolatera 1 (cl1) and rostrolatera 1 (rl1) occurred; fourth whorl with 27 imbricating plates; fifth whorl with 40 imbricating plates (Figures 3A, 4A, B).

Scutum and tergum strongly articulated (Figure 3B, C). Scutum triangular, dorsal surface with horizontal growth lines. Occludent and basal margins straight. Articular ridge high and articular furrow deep. Tergal margin with *V*-shaped articular ridge. Adductor muscle and depressor muscle scars in scutum absent (Figure 3D, E). Tergum quadrangular, dorsal surface with horizontal growth lines, scutal margin strongly concaved. Carinal margin straight, basal margin long and straight, spur sharp and triangular. Pits for depressor muscle in tergum absent (Figure 3D, E).

Cirrus I, anterior ramus short, seven segmented, posterior rami long, with antenniform segments starting from half length of rami, 20 segmented (Figure 5A). All segments in both rami

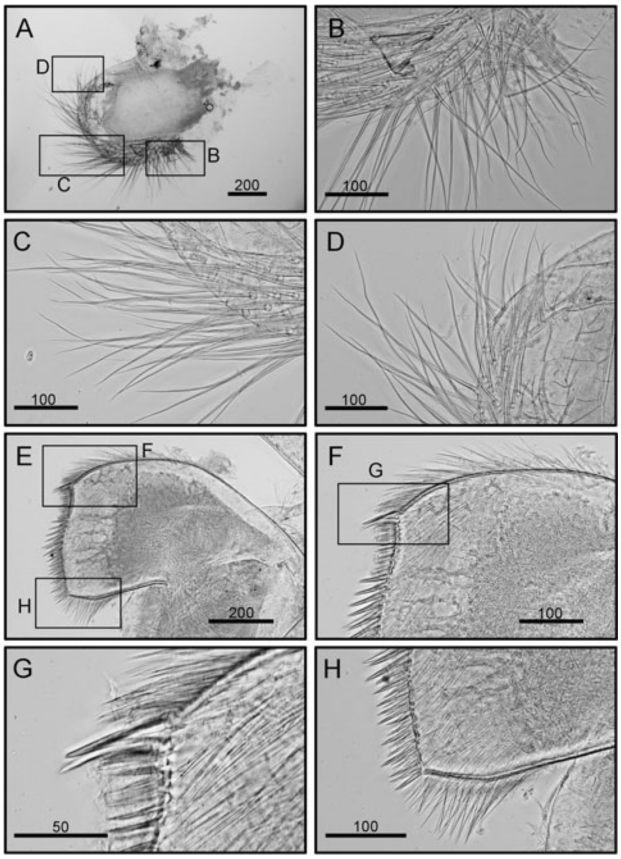

Fig. 8. *Eochionelasmus coreana* sp. nov. (holotype). (A) Maxilla. (B) Setae on exterior margin. (C) Setae on outer margin. (D) Setae on inner margin. (E) Maxillule. (F) Cutting edge of maxillule. (G) Upper portion of cutting edge. (H) Lower portion of cutting edge. Scale bars in μm.

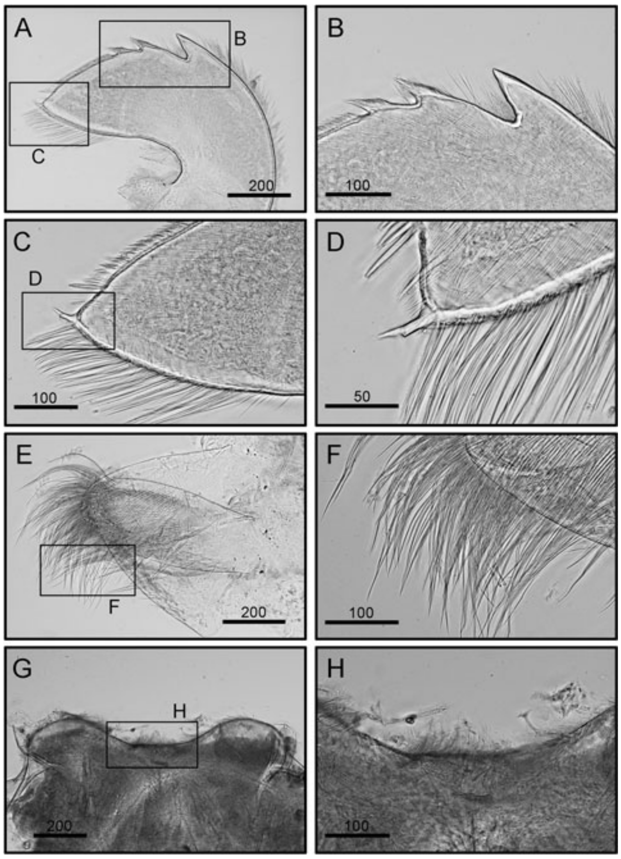

Fig. 9. *Eochionelasmus coreana* sp. nov. (holotype). (A) Mandible. (B) The three teeth of the mandibles. (C) Inferior angle. (D) Outer margin. (E) Mandibulatory palp. (F) Setae on mandibulatory palp. (G) Labrum. (H) Cutting edge of labrum. Scale bars in μm.

bear simple setae (Figure 5B–D). Cirrus II, anterior and posterior rami subsequal, anterior rami 22 segmented, distal half of ramus bear antenniform, proximal half with robust segments (Figure 5E, F). Antenniform segment bears three pairs of long simple setae and one pair of short simple setae (Figure 5G, H). Proximal region of segments bears dense long simple setae (Figure 5F). Posterior ramus 25 segmented, with antenniform segments starting from 1/4 of the proximal region. All segments bear simple setae. Cirri III, IV, V and VI are similar in morphology, all with long and slender rami (segment counts, see Table 1) (Figures 6 & 7). Proximal segments bear dense simple setae (Figure 6B). Intermediate segments of cirrus III bear four pairs of long setae and two pairs of short setae (Figure 6C, D). Intermediate segments of cirrus V and VI bear five pairs of long setae and two pairs of short setae (Figures 6E–H & 7A–G). Caudal appendage long, 16 segmented (Figure 7D, E). Penis long, without basal point (Figure 7I, J).

Maxilla oval, single-lobed, simple setae distally (Figure 8A) and along inferior margin (Figure 8B–D). Maxillule cutting edge slightly convex, without notch, the lower 2/3 portion of cutting edge slightly protruded. Two distinct large setae on top of cutting edge, followed by a row of nine smaller setae on the 1/3 upper portion of cutting edge, protruded 2/3 lower portion of cutting edge with 28–30 large setae (Figure 8E–H) (maxilla morphology consistent between holotype and paratype; Figure 10A, B). Mandible with three teeth (Figure 9A–D), first tooth slightly departed from second and third teeth, lower margin long. First teeth largest and sharp (Figure 9B), second and third with dense row of setae on cutting edge. Lower margin long and straight, with dense row of setae. Inferior angle with two large setae (Figures 9C, D & 10C–F). Mandibular palp triangular, bearing dense serrulate setae distally (Figure 9E, F) and along interior margin. Labrum concaved, notch absent, cutting edge with extreme small teeth (Figure 9G, H; consistent in two specimens, Figure 10G, H).

Distribution

At present, only known from the Solitaire hydrothermal vent field on the Central Indian Ridge.

Etymology

The name coreana represents the Latin name of Korea, 'corea'. This name acknowledges the Korean deep-sea research team (Korean Institute of Oceanography, Science and Technology, KIOST) that collected the new species in the Solitaire hydrothermal vent field on the Central Indian Ridge.

Remarks

The third *Eochionelasmus* species, *E. coreana*, is not only the first record of a sessile vent barnacle from outside the Pacific Ocean, but is also the first discovery of *Eochionelasmus* from the Indian Ocean; the distributions of *E. ohtai* and *E. paquensis* are restricted to the South-west Pacific Ocean and the East Pacific Rise, respectively.

Eochionelasmus coreana and *E. ohtai* are morphologically similar in their external shell structure, and the shapes of the scutum and tergum. However, *Eochionelasmus coreana* differs from *E. ohtai* in the cutting edge on the maxillule (notch absent in *E. coreana vs* shallow notch in *E. ohtai*), and the location of rl1 and cl1 on whorls of the imbricating plates (third in *E. coreana*

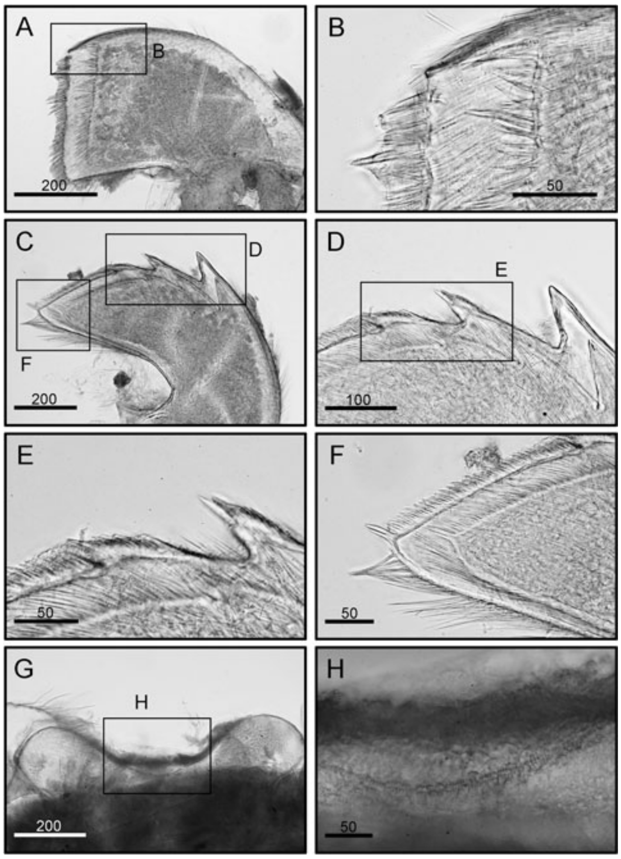

Fig. 10. *Eochionelasmus coreana* sp. nov. (paratype). (A) Maxillule. (B) Upper portion of cutting edge. (C) Mandible. (D) The three teeth of the mandibles. (E) Third tooth of mandible. (F) Inferior angle. (G) Labrum. (H) Cutting edge of labrum. Scale bars in µm.

vs fourth in *E. ohtai*). Meanwhile, *E. coreana* is different from *E. paquensis* in the characteristics of the mandible and the length ratio between the rami parts on cirrus I (Table 1).

GenBank accession numbers of CO1 DNA barcode

MT008257 for the holotype; MT008258 for the paratype.

Phylogenetic position of Eochionelasmus *within the balanomorph lineage*

Four newly obtained sequences (12S, 16S, 18S and histone 3) of *E. coreana* were registered in GenBank (accession nos. MT008251–MT008253, MT008255). Additionally, in this study, the nuclear gene sequences (accession nos. MT008254 for 18S and MT008256 for *H3*) of *E. ohtai* were obtained from the same specimen using the complete mitochondrial genome (NC_036957) determined by Kim *et al.* (2018). We could not include sequences of *E. paquensis* in all analyses because they were not available from open-access sequence databases. The phylogenetic trees were constructed using the concatenated sequence alignment of four genes from 10 balanomorph genera and one verrucomorph species, *Verruca stroemia*, as an outgroup (Figure 11; Table 2). The tree strongly supported two *Eochionelasmus* species, *E. coreana* and *E. ohtai*, as monophyletic taxa, with high support values of 97% BP and 1.00 BPP. The chionelasmatid *Eochionelasmus* species and the waikalasmatid *Waikalasma dianajonesae* also had strong support for monophyly (97% BP and 1.00 BPP). However, three chionelasmatid species, *E. coreana*, *E. ohtai* and *Chionelasmus darwini*, were not monophyletic.

In terms of nucleotide sequence divergence, the four genes showed different variations, in decreasing order: 16S, 12S, *H3* and 18S (Table 3). The sequence divergence between *E. ohtai* and *E. coreana* was 16.7% for 16S, 12.9% for 12S, 5.3% for *H3* and 2.0% for 18S; all values were consistent with the

Table 1. Comparison of morphological characters of *Eochionelasmus coreana* sp. nov., the generalized *E. ohtai* from North Fiji Basin, and the holotype of *E. paquensis.* The contents of the table have been revised from Yamaguchi & Newman (1997*a*, 1997*b*)

Characters	*E. coreana* sp. nov.	*E. ohtai*	*E. paquensis*
Location of rl1	Third whorl	Fourth whorl	Third whorl
Location of cl1	Third whorl	Fourth whorl	Third whorl
Location of r3 and c3	Third whorl	Third whorl[a]	Third whorl
Boundary between ala and paries	Distinct	Distinct	Indistinct
Basal length/tergal length of scutum	Basal > tergal	Equal	Basal > tergal
Tergal margin of scutum	Concaved	Straight	Not straight
Articular ridge of scutum	Very high	Very high	Absent
Adductor muscle pit	Very faint	Faint	Absent
Pit for lateral depressor muscle	Absent	Absent	Present
Tergal depressor crest along basal margin	Absent	Faint	Absent
First tooth of mandible	Large erect	Large erect	Low small
Third tooth of mandible	With dense setae	With dense setae	–
Lower margin of mandible	Straight to inferior angle	Straight to inferior angle	Rounding to inferior angle
Notch of maxillule	Absent	Shallow	Absent
Ratio of anterior *vs* posterior rami of cirrus I	2.4:1	2.7:1	1.7:1
Ratio of segment no. of anterior *vs* posterior rami of cirrus I	2.8:1	2.6:1	1.8.1
Length of anterior ramus of cirri I to III	Long	Long	Short
Distribution	Central Indian Ridge	Southwest Pacific Ocean	East Pacific Rise

[a] In Table 1 of Yamaguchi & Newman (1997*a*, 1997*b*), the location of r3 and c3 of *E. ohtai* was listed as the fourth whorl. In Figure 4 of Yamaguchi & Newman (1997*a*, 1997*b*), which showed the generalized pattern of *E. ohtai*, the r3 and c3 were located on the third whorl. We adopted the arrangement of the generalized pattern of *E. ohtai* from Figure 4 of Yamaguchi & Newman (1997*a*, 1997*b*) and concluded the location of r3 and c3 should be the third whorl.

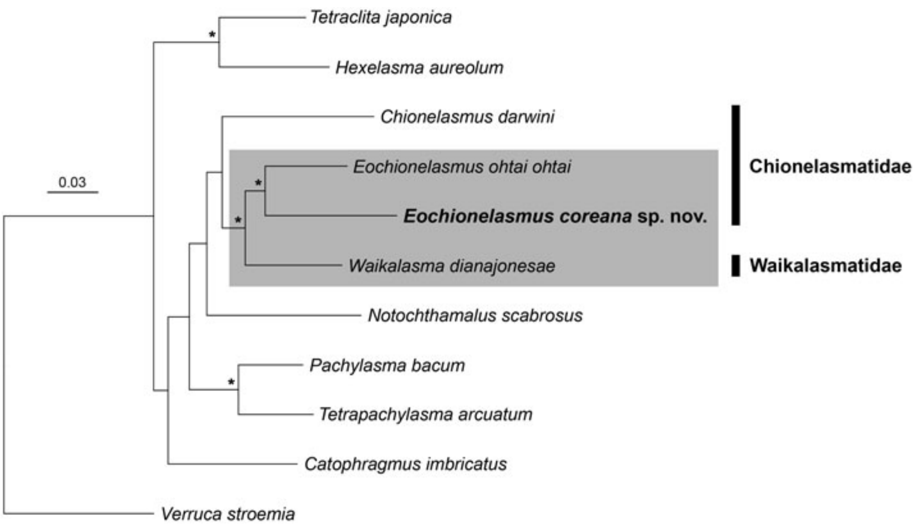

Fig. 11. Phylogenetic tree of *Eochionelasmus coreana* sp. nov. and the other balanomorph barnacles based on two mitochondrial (12S and 16S rDNA) and two nuclear (18S rDNA and histone 3) genes. Asterisks indicate the maximum likelihood bootstrap proportions >97% and Bayesian posterior probabilities >0.99.

Table 2. Information of the sequences used for constructing the phylogenetic tree in this study

			GenBank accession no.			
			Mitochondrial gene		Nuclear gene	
Superfamily	Family	Species	12S	16S	18S	Histone 3
Chionelasmatoidea	Chionelasmatidae	*Eochionelasmus coreana* sp. nov.	MT008251	MT008252	MT008253	MT008255
Chionelasmatoidea	Chionelasmatidae	*Eochionelasmus ohtai*	NC_036957	NC_036957	MT008254	MT008256
Chionelasmatoidea	Chionelasmatidae	*Chionelasmus darwini*	KX230850	KX230926	KX230999	KX231144
Chionelasmatoidea	Waikalasmatidae	*Waikalasma dianajonesae*	KX230876	KX230952	KX231023	KX231164
Pachylasmatoidea	Pachylasmatidae	*Pachylasma bacum*	KX230870	KX230946	KX231018	KX231160
Pachylasmatoidea	Pachylasmatidae	*Tetrapachylasma arcuatum*	KX230873	KX230949	KX231020	KX231153
Tetraclitoidea	Bathylasmatidae	*Hexelasma aureolum*	KX230892	KX230966	KX231036	KX231152
Chthamaloidea	Catophragmidae	*Catophragmus imbricatus*	KX230902	KX230975	JX083887	KX231177
Chthamaloidea	Chthamalidae	*Notochthamalus scabrosus*	KX230909	KX230983	KX231060	KX231192
Tetraclitoidea	Tetraclitidae	*Tetraclita japonica*	KX230916	KX230989	KX231067	KX231199
Suborder						
Verrucomorpha	Verrucidae	*Verruca stroemia*	KX230925	KX230998	KX231058	KX231206

inter-generic variations among balanomorph genera. Based on the concatenated sequences of four genes, the minimum and maximum variations were 6.1% between *Pachylasma bacum* and *Tetrapachylasma arcuatum*, and 11.8% between *Hexelasma aureolum* and *Notochthamalus scabrosus*, respectively. The variation (8.1%) between two *Eochionelasmus* species from the concatenated sequences had an intermediate value compared with the range of inter-generic variations among balanomorphs.

Discussion

Geotectonic events, ecological/hydrographic barriers, hydrothermal fluid characteristics, and length of larval development are considered important factors for distribution, migration, settlement and speciation of hydrothermal vent organisms (Watling *et al*., 2013). However, the origin, migration and distribution of hydrothermal vent fauna among disconnected vent habitats with repeating cycles of creation and extinction are still not fully understood. In this study, we discovered the first sessile *Eochionelasmus* barnacle, *E. coreana*, in the Indian Ocean. This raises an interesting question of how three *Eochionelasmus* species in three different oceans originated (Figure 1). Thus, if we can understand their distributions and environmental adaptation strategies, we may gain new insights into the association between adaptive radiation of hydrothermal vents organisms and geotectonic events on Earth.

Despite morphological and phylogenetic affinities of the genus *Eochionelasmus* with Balanomorpha, the phylogenetic position of *Eochionelasmus* within the balanomorphs is uncertain because of lineage-specific evolutionary rates and/or taxonomic undersampling (Pérez-Losada *et al*., 2014; Herrera *et al*., 2015). In this study, we identified a new species in *Eochionelasmus* from the Indian Ocean, and then re-constructed the phylogenetic tree with related genera, *Waikalasma* Buckeridge, 1983 and *Chionelasmus* Pilsbry, 1907 (Figure 10). Based on the tree, the chionelasmatid *Eochionelasmus* was closely related to the waikalasmatid *Waikalasma* with high support rather than the other chionelasmatid *Chionelasmus*. Although chionelasmatids and waikalasmatids were clustered in a single clade in the present study, the bootstrap value was not high enough to support the

Table 3. Sequence divergence among the partial sequences of two mitochondrial (12S and 16S rDNA) and two nuclear (18S rDNA and histone 3) genes from balanomorph genera

		1	2	3	4	5	6	7	8	9	10
1	*Eochionelasmus coreana* sp. nov.		12.9/16.7/2.0/5.3	17.4/24.9/1.3/6.4	14.5/21.8/1.2/4.3	15.4/24.0/1.2/5.7	15.8/23.4/1.3/6.7	21.5/24.0/1.8/9.3	16.7/24.5/1.1/8.5	14.9/25.9/1.1/10.6	18.8/25.3/1.5/8.2
2	*Eochionelasmus. ohtai*	8.1		15.1/24.5/1.3/6.8	12.6/16.3/1.2/5.7	12.3/22.3/1.2/6.7	13.9/21.0/1.3/7.1	20.1/24.2/2.0/9.6	12.9/21.6/1.1/10.6	11.7/23.3/1.1/11.0	15.6/24.1/1.5/10.6
3	*Chionelasmus darwini*	10.4	9.9		16.5/23.4/0.6/5.7	16.8/24.0/0.6/6.4	18.8/24.1/0.7/6.8	21.2/25.2/1.1/7.1	15.5/24.9/0.5/9.3	16.2/24.2/0.4/8.9	20.5/24.8/0.9/6.8
4	*Waikalasma dianajonesae*	9.2	7.7	9.6		13.2/20.4/0.5/5.3	18.1/21.6/0.6/5.7	19.9/26.2/1.2/8.2	14.5/24.9/0.4/8.9	12.6/24.5/0.3/9.6	19.5/24.7/0.8/8.9
5	*Pachylasma bacum*	10.0	9.1	10.0	8.4		10.3/15.9/0.1/2.5	18.6/23.1/0.8/7.8	13.8/23.5/0.1/9.6	14.6/24.0/0.2/7.4	16.6/24.4/0.5/7.8
6	*Tetrapachylasma arcuatum*	9.9	9.1	10.6	9.6	6.2		20.6/21.1/0.9/6.4	15.5/20.3/0.2/10.3	17.8/23.7/0.2/6.0	17.6/23.1/0.6/7.1
7	*Hexelasma aureolum*	11.5	11.5	11.2	11.3	10.1	9.9		19.2/20.7/0.8/11.7	22.5/26.9/0.9/7.8	12.0/18.5/0.7/3.6
8	*Catophragmus imbricatus*	10.2	9.6	10.4	10.0	9.4	8.9	10.4		14.9/26.3/0.1/11.7	16.9/20.9/0.5/11.7
9	*Nesochthamalus scabrosus*	11.1	9.7	10.0	9.7	9.4	9.6	11.8	10.5		17.9/24.6/0.6/7.8
10	*Tetraclita japonica*	11.3	10.8	10.8	10.8	10.0	9.8	7.5	9.8	10.3	

Nucleotide sequence variations (%) were calculated using the *p*-distance method in Mega 10.0.5. Upper right is for the 12S/16S/18S/H3 genes and lower left the concatenated sequences of the four genes.

common ancestry of the *Eochionelasmus–Waikalasma* clade and the genus *Chionelasmus*. Thus, the phylogenetic relationship between chionelasmatid and waikalasmatid members remains uncertain and will require additional molecular markers for analysis.

Conversely, based on traditional taxonomy, these three genera have been considered the most basal taxa of balanomorph barnacles, with *Eochionelasmus* considered more closely related to *Chionelasmus* than *Waikalasma* (Jones, 2000; Chan *et al.*, 2017). However, in terms of habitat, *Eochionelasmus*, which lives in hydrothermal vents, differs from *Chionelasmus* and *Waikalasma*, which live in general deep-sea environments. Considering that principal food sources change under different habitat conditions, it has been assumed that *Eochionelasmus* developed fine setae and weaker spines on its mouth parts to obtain chemosynthetic bacteria as a food source through filter-feeding. Such morphological specialization in mouth parts were also observed in neolepadid barnacles which live in hydrothermal vents (Newman, 1979; Buckeridge, 2000). Conversely, *Chionelasmus* and *Waikalasma* may have developed stronger spines on their mouth parts for capturing and feeding on zooplankton (Jones, 2000; Chan *et al.*, 2017). To improve our understanding of the position and the relationship of these basal groups in the balanomorph phylogeny, further research is needed, including discovery of additional taxa, and mitogenome-based phylogenetic and biogeographic studies.

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References

Beedessee G, Watanabe H, Ogura T, Nemoto S, Yahagi T, Nakagawa S, Nakamura K, Takai K, Koonjul M and Marie DEP (2013) High Connectivity of Animal Populations in Deep-Sea Hydrothermal Vent Fields in the Central Indian Ridge Relevant to Its Geological Setting. *PLoS ONE* **8**, e81570.

Buckeridge JS (1983) The fossil barnacles (Cirripedia: Thoracica) of New Zealand and Australia. *New Zealand Geological Survey Paleontological Bulletin* **50**, 1–152.

Buckeridge JS (2000) *Neolepas osheai* sp. nov., a new deep-sea vent barnacle (Cirripedia: Pedunculata) from the Brothers Caldera, south-west Pacific Ocean, New Zealand. *Journal of Marine and Freshwater Research* **34**, 409–418.

Chan BKK (2018) Modern molecular phylogenetic groups of thoracican barnacles: systematics, biology and ecology. In Kado R, Mimura H and Endo N (eds), *Barnacles: Recent Progress in Biology and Antifouling*. New York, NY: Nova Science Publishers, pp. 3–32.

Chan BKK and Chang YW (2018) A new deep-sea scalpelliform barnacle, *Vulcanolepas buckeridgei* sp. nov. (Eolepadidae: Neolepadinae) from hydrothermal vents in the Lau Basin. *Zootaxa* **4407**, 117–129.

Chan BKK, Hoeg JT and Garm A (2008) Setal morphology and setation patterns of barnacle cirri: adaptations and implications for thoracican evolution. *Journal of Zoology* **275**, 294–306.

Chan BKK, Corbari L, Rodriguez PA and Tsang LM (2017) Molecular phylogeny of the lower acorn barnacle families (Bathylasmatidae, Chionelasmatidae, Pachylasmatidae and Waikalasmatidae) (Cirripedia: Balanomorpha) with evidence for revisions in family classification. *Zoological Journal of the Linnean Society* **180**, 542–555.

Darriba D, Taboada GL, Doallo R and Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**, 772.

Folmer O, Black M, Hoeh W, Lutz R and Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**, 294–297.

Herrera S, Watanabe H and Shank TM (2015) Evolutionary and biogeographical patterns of barnacles from deep-sea hydrothermal vents. *Molecular Ecology* **24**, 673–689.

Jones DS (2000) Crustacea Cirripedia Thoracica: Chionelasmatoidea and Pachylasmatoidea (Balanomorpha) of New Caledonia, Vanuatu and Wallis and Futuna Islands, with a review of all currently assigned taxa. In Crosnier A (ed.), *Résultats des Campagnes MUSORSTOM, Volume 21. Mémoires du Muséum national d'Histoire naturelle* **184**, 141–283.

Kim SJ, Lee WK, Kim RO and Ju SJ (2018) Complete mitochondrial genome of the hydrothermal vent barnacle *Eochionelasmus ohtai* (Cirripedia, Thoracica). *Mitochondrial DNA Part B* **3**, 46–47.

Newman WA (1979) A new scalpellid (Cirripedia); a Mesozoic relic living near an abyssal hydrothermal spring. *Transactions of the San Diego Society of Natural History* **19**, 153–167.

Notredame C, Higgins DG and Heringa J (2000) T-Coffee: a novel method for fast and accurate multiple sequence alignment. *Journal of Molecular Biology* **302**, 205–217.

Pérez-Losada M, Høeg JT, Simon-Blecher N, Achituv Y, Jones D and Crandall KA (2014) Molecular phylogeny, systematics and morphological evolution of the acorn barnacles (Thoracica: Sessilia: Balanomorpha). *Molecular Phylogenetics and Evolution* **81**, 147–158.

Plouviez S, Schultz TF, McGinnis G, Minshall H, Rudder M and van Dover CL (2013) a new species of *Vulcanolepas* in the North Fiji basin hydrothermal-vent barnacles in Manus Basin. *Deep Sea Research Part I: Oceanographic Research Papers* **82**, 73–79.

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Suchard MA and Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**, 539–542.

Ryu T, Woo S and Lee N (2019) The first reference transcriptome assembly of the stalked barnacle, *Neolepas marisindica*, from the Onnuri Vent Field on the Central Indian Ridge. *Marine Genomics* **48**, 100679.

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312–1313.

Watanabe HK, Chen C, Marie DP, Takai K, Fujikura K and Chan BKK (2018) Phylogeography of hydrothermal vent stalked barnacles: a new species fills a gap in the Indian Ocean 'dispersal corridor' hypothesis. *Royal Society Open Science* **5**, 172408. http://dx.doi.org/10.1098/rsos.172408.

Watling L, Guinotte J, Clark MR and Smith CR (2013) A proposed biogeography of the deep ocean floor. *Progress in Oceanography* **111**, 91–112.

Xia X (2013) DAMBE5: a comprehensive software package for data analysis in molecular biology and evolution. *Molecular Biology and Evolution* **30**, 1720–1728.

Yamaguchi T and Newman WA (1990) A new and primitive barnacle (Cirripedia: Balanomorpha) from the North Fiji Basin Abyssal Hydrothermal Field, and its evolutionary implications. *Pacific Science* **44**, 135–155.

Yamaguchi T and Newman WA (1997*a*) *Eochionelasmus paquensis*, new species (Cirripedia, Balanomorpha) from 17°25′S, north of Easter Island: first record of a hydrothermal sessile barnacle from the East Pacific Rise. *Journal of Crustacean Biology* **17**, 488–496.

Yamaguchi T and Newman WA (1997*b*) The hydrothermal vent barnacle *Eochionelasmus* (Cirripedia, Balanomorpha) from the North Fiji, Lau and Manus Basins, South-West Pacific. *Zoosystema* **19**, 623–649.