



# A new deep-sea hot vent stalked barnacle from the Mariana Trough with notes on the feeding ecology of *Vulcanolepas*

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## Abstract

A new species of deep-sea neolepadid stalked barnacle, *Vulcanolepas verenae* **sp. nov.**, is described from Hafa Adai hydrothermal vent field in the Mariana back-arc basin, northwest Pacific. *Vulcanolepas verenae* **sp. nov.** is differentiated from other described species in the family morphologically by a mandible with a very small first tooth and small, non-protruding peduncular scales. Its gross external morphology exhibits a range of plasticity, similar to those previously reported from other neolepadid stalked barnacles. This is the first report of the genus *Vulcanolepas* in the northern hemisphere, and we discuss the phylogeography of Neolepadidae with a phylogenetic reconstruction based on the cytochrome *c* oxidase subunit I gene. We also discuss the relationship between key morphological characteristics and the feeding habits in the family, showing that, a number of *Vulcanolepas* species including *Vulcanolepas verenae* **sp. nov.** are specialized in culturing filamentous bacteria on their cirri.

**Keywords** Neolepadidae · Epibiont · Chemoautotrophic bacteria · Phylogeography

## Introduction

Stalked barnacles in the family Neolepadidae (Yamaguchi et al. 2004) are commonly found in diffuse flow and marginal areas of deep-sea hydrothermal vent fields in Pacific, Indian, and Southern oceans (Marsh et al. 2012; Chan et al. 2019). The family currently consists of four extant genera including *Neolepas* Newman, 1979, *Leucolepas* Southward & Jones, 2003, *Vulcanolepas* Southward & Jones, 2003, and *Ashinkailepas* Yamaguchi, Newman & Hashimoto, 2004, supported by molecular taxonomic analyses, and the extinct genus *Stipilepas* Gale, 2020 (Yamaguchi et al. 2004; Herrera

et al. 2015; Buckeridge et al. 2013; Watanabe et al. 2018; Gale et al. 2020). *Ashinkailepas* is the only neolepadid genus occurring in both hydrothermal vents and hydrocarbon seeps, currently containing two recent and one fossil species (Gale et al. 2020). The genus shows highly specific morphologies with prominent apico-basal ridges of capitular plates and strongly projected scale plates; therefore, the other three neolepadid genera are currently placed in the tribe Neolepadini, and an arrangement supported by molecular phylogeny (Herrera et al. 2015). *Neolepas* was the first described vent barnacle genus reported from the East Pacific Rise (Newman 1979), and the second and third genera, *Leucolepas* and *Vulcanolepas*, were established by Southward and Jones (2003) based on morphological characteristics. Molecular phylogenetics have indicated that *Vulcanolepas* is likely not monophyletic and their gross external morphologies have been shown to be highly plastic within a single genetically homogeneous species, under different environmental conditions (Herrera et al. 2015; Buckeridge et al. 2013; Watanabe et al. 2018). *Leucolepas* is monotypic and only includes the type species *Leucolepas longa* Southward & Jones, 2003.

Eight species have been described in the *Neolepas/Vulcanolepas* group, all restricted to hydrothermal vents. These include *Neolepas zeviniae* Newman, 1979 from the

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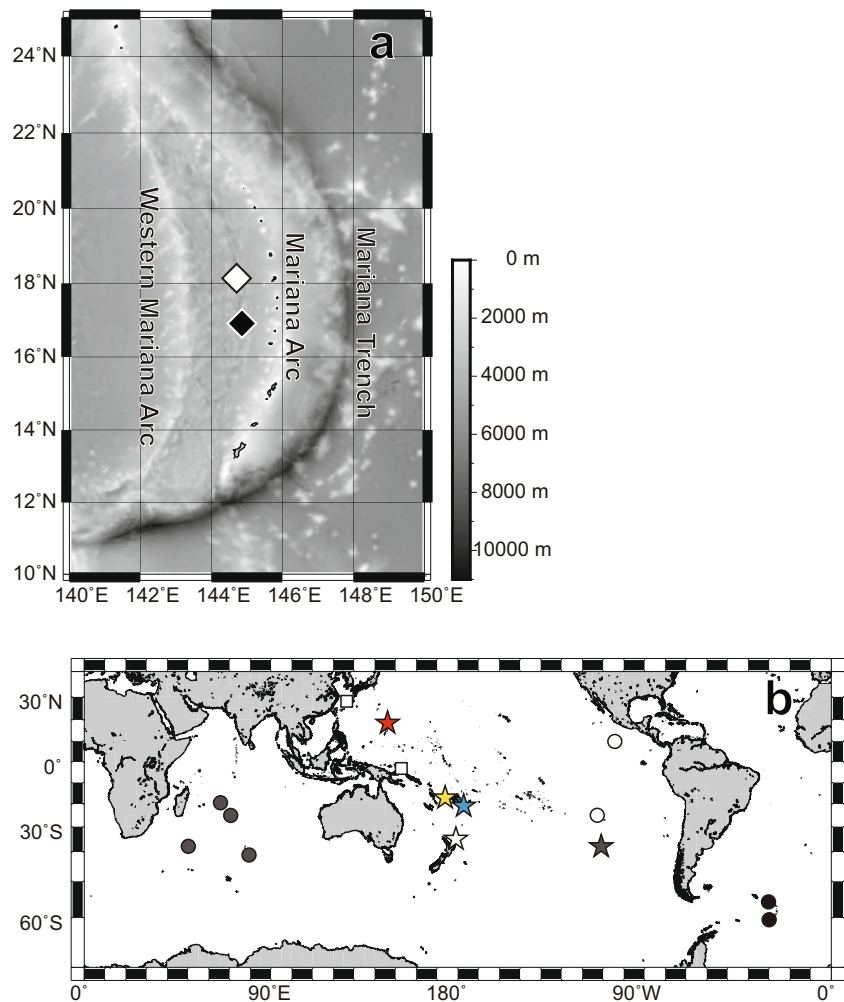
northeast Pacific, *Neolepas rapanui* Jones, 1993 from the southeast Pacific, *Neolepas scotiaensis* (Buckeridge and Linse, 2013 in Buckeridge et al. 2013) from the Southern Ocean, *Neolepas marisindica* Watanabe, Chen & Chan in Watanabe et al. 2018 from the Indian Ocean, *Vulcanolepas osheai* (Buckeridge, 2000) from Brothers Seamount on the Kermadec Arc, *Vulcanolepas parensis* Southward, 2005 from the Pacific-Antarctic Ridge, *Vulcanolepas buckeridgei* Chan & Chang, 2018 from the Lau Basin, and *Vulcanolepas fijiensis* Chan, Ju & Kim, 2019. Among them, a specialized feeding habit of cultivating epibiotic symbionts on their feeding appendages (i.e., cirri) has been reported in *V. osheai* and *V. buckeridgei* (Suzuki et al. 2009). Due to the high level of plasticity in the gross external morphology (i.e., the stalk and plates) on which the identification of barnacles often relies, the identification and taxonomy of these vent barnacles require a total evidence approach that puts weight also on other characteristics such as arthropodal morphology, ecological, and physiological characteristics and molecular data. Previous studies have shown that arthropodal (mouth part) morphology is able to reliably distinguish different species of *Vulcanolepas* (Watanabe et al. 2018; Chan et al. 2019).

Here, we report the fifth species of *Vulcanolepas*, collected from the Hafa Adai hydrothermal vent field in the Mariana Trough, northwestern Pacific (Fig. 1a; Baker et al. 2017). Mariana Trough is a back-arc basin located between Mariana and Western Mariana arcs (Fig. 1a). This is the second report of stalked barnacle from Mariana Trough vents after a single sighting in the Alice Springs field in the central part of Mariana Trough (Fig. 1a), but unfortunately no specimen was collected from Alice Springs (Hessler and Lonsdale 1991). We characterize and describe the *Vulcanolepas* species from Hafa Adai using both morphology and genetics and show that its arthropodal morphology is distinct from other described species.

## Materials and methods

**Sampling** A single stalked barnacle was collected at the Hula Hoop site (16°57.701'N, 144°52.152'E, Depth = 3278 m) in the Hafa Adai hydrothermal vent field, Mariana Trough by ROV *SuBastian* on-board the Schmidt Ocean Institute R/V

**Fig. 1** Map showing locations of Hafa Adai (black diamond) and Alice Springs (white diamond) hydrothermal vent fields (a) and distribution of currently known neolepadid stalked barnacles (b). Red star: *Vulcanolepas verena* sp. nov., white star: *V. osheai*, gray star: *V. fijiensis*, yellow star: *V. buckeridgei*, white circle: *Neolepas zevinae/rapanui* complex, black circle: *N. scotiaensis*, gray circle: *N. marisindica*, white square: *Leucolepas longa*



*Falkor* (Fig. 1b). The specimen is deposited in the National Museum of Nature and Science, Tsukuba (NSMT), Japan.

**Morphological observation:** The gross morphology of the stalked barnacle was observed in distilled and deionized water. The soft body was carefully extracted with detaching muscles and membranes from the inner side of the plates by sharpened tweezers. The cirrus on the left side of the body and oral cones were dissected, examined, and photographed using a digital camera (Panasonic DMC-GH3) under a light microscope (Olympus BX51). The terminology of descriptions of the capitular plates and arthropodal characters coined by Darwin (1854) are followed here.

**Molecular phylogenetic reconstruction:** Genomic DNA was extracted using DNeasy Blood and Tissue Kit (QIAGEN) from the adductor muscle. Partial sequence of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was amplified by PCR using the universal primer pair (LCO1490 and HCO2198) designed by Folmer et al. (1994) and the Hot Start version of Premix *ExTaq* (TaKaRa) in the following steps: initial denaturation at 94 °C for 3 min followed by 35 cycles of denaturation (94 °C for 30 s), annealing (50 °C for 30 s), and extension (72 °C for 90 s). The PCR products were purified using Exo-SAP-it (usb). BigDye Terminator reaction was carried out using BigDye Terminator ver 3.1, and the products were then sequenced using an ABI3130 automated sequencer (Applied Biosystems, Thermo Fisher). The electropherograms obtained were checked by eye and assembled with Geneous ver. 9 (Biomatters Limited). The resulting sequence is deposited in DDBJ, with the accession number of: MT662001.

The obtained sequence was aligned with currently available neolepadid sequences in the International Nucleotide Sequence Database Collaboration database, using Clustal X mounted on MEGA ver 7.0.26 (Tamura et al. 2013). The model selection program in MEGA ver. 7.0.26 was applied to select the best model for maximum likelihood algorithm, which selected the Tamura 3-parameter + Gamma distribution model. Phylogenetic reconstruction was carried out using MEGA with the genus *Ashinkailepas* set as the outgroup, with 2000 bootstrap replicates for the 588 bp-length alignment.

## Results

### Systematics

**Superorder Thoracica** Darwin, 1854

**Order Scalpelliformes** Buckeridge & Newman, 2006

**Family Neolepididae** Yamaguchi, Newman & Hashimoto, 2004

**Tribe Neolepadini** Yamaguchi, Newman & Hashimoto, 2004

**Genus *Vulcanolepas*** Southward & Jones, 2003

*Vulcanolepas* Southward and Jones 2003:81.-Southward 2005: 148.-Buckeridge et al. 2013: 556.

**Type species.** *Neolepas osheai* Buckeridge, 2000

**Diagnosis** (emended from Southward and Jones 2003). Neolepadid stalked barnacles whose adults have a ratio of peduncle length to capitulum length of between 1:1 and 7:1. Peduncle armed with numerous small scales, 20 or more scales per whorl in upper half of peduncle; scales less than 1 mm wide, almost flat, projecting up to 0.5 mm beyond cuticle. Capitulum broad, tergal apex blunter than in other neolepadid genera. Basal angle of scutum close to capitulo-peduncle margin. Basal angle of tergum slightly above capitulo-peduncular margin.

**Distribution.** Kermadec Arc, Southwest Pacific; North Fiji Basin, Southwest Pacific; Lau Basin, Southwest Pacific; Mariana Trough, Northwest Pacific; Pacific-Antarctic Ridge (depth range: 1200–3278 m).

**Remarks.** The latest diagnosis for this genus was provided by Buckeridge et al. (2013). The major diagnostic character of *Vulcanolepas* emended by Buckeridge et al. (2013) included the ratio of peduncle length to capitulum length reaching 20:1 and they also removed the degree of projection of peduncular scale in order to accommodate *Vulcanolepas scotiaensis* (Buckeridge et al. 2013), but that species is now considered to be a *Neolepas* and properly known as *Neolepas scotiaensis* from molecular evidence (Watanabe et al. 2018). Morphologically, *N. scotiaensis* has very large degree of peduncular projection (up to 1.5 mm beyond the cuticle), whilst in described *Vulcanolepas* species, this is only <0.5 mm beyond the cuticle. Large peduncular scale projection is one of the key morphological characters of *Neolepas*. We therefore reverted back to the diagnosis of Southward and Jones (2003) and Southward (2005) and updated it to include the range of characters observed from the recently described *V. buckeridgei* and *V. fijiensis* as well as the new species *Vulcanolepas verenae* **sp. nov.** described herein, including the ratio of peduncle length to capitulum length of between 1:1 and 7:1 and peduncular scales less than 1 mm wide, almost flat, projecting up to 0.5 mm beyond cuticle. Among Neolepadini, the key character that distinguish *Neolepas* from *Vulcanolepas* is the larger degree of peduncular projection (up to 1.5 mm beyond the cuticle, vs 0.5 mm in *Vulcanolepas*) as mentioned above. *Leucolepas* consists of a single species, *L. longa*, which can be distinguished from *Vulcanolepas* by the separated capitular plates, narrower tergum, and the longer peduncle (more than five times longer than the capitulum).

The distribution of the genus *Vulcanolepas* was extended considerably with the collection of *Vulcanolepas verenae* **sp. nov.**, as this is the first distribution record of *Vulcanolepas* in the northern hemisphere and extends the bathymetric range of the genus by 649 m (previously the deepest record was 2629 m held by *V. buckeridgei*; Chan and Chang 2018). This depth rivals that of the deepest known vent barnacle



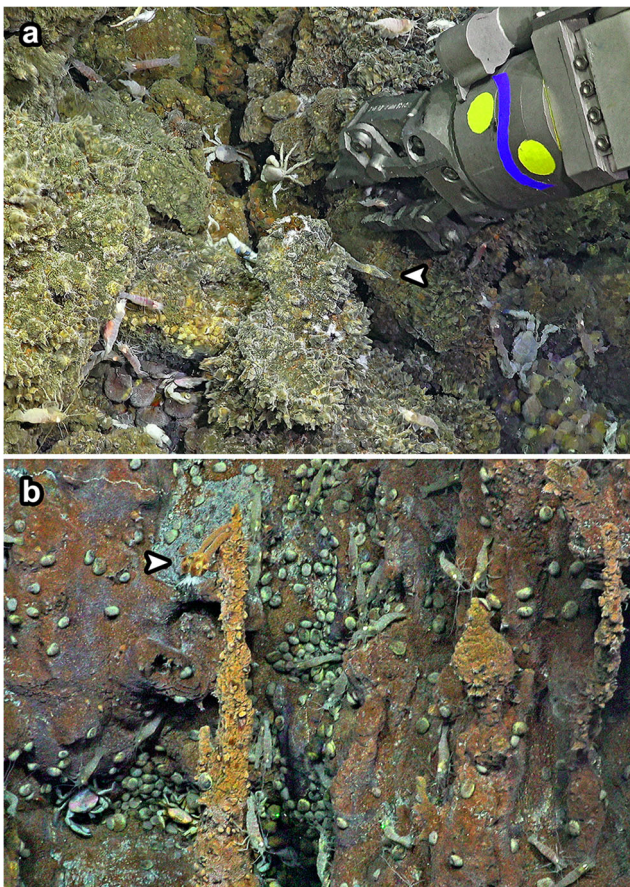
genus, the balanomorph barnacle *Eochionelasmus* Yamaguchi in Yamaguchi and Newman 1990, which has a depth range of 1764–2500 m for *E. ohtai* Yamaguchi in Yamaguchi and Newman 1990 in North Fiji, Lau and Manus Back-Arc Basins, Pacific Ocean (Yamaguchi and Newman 1997a). *Eochionelasmus paquensis* Yamaguchi and Newman, 1997b is known from sites up to 2578 m deep in the East Pacific Rise (Yamaguchi and Newman 1997b), Eastern Pacific, and *E. coreana* Chan et al. 2020 from 2625 m deep at Solitaire Vent field, Indian Ocean (Chan et al. 2020).

***Vulcanolepas verenae* sp. nov.**

<http://zoobank.org/B01E0DE9-537F-4C67-A2BC-172AF1671D8D>

**Type locality.** Hula Hoop site (16°57.701'N, 144°52.152'E, Depth = 3278 m), Hafa Adai hydrothermal vent field, Mariana Trough (Fig. 1).

**Type material.** Holotype (NSMT-Cr 28234, Figs. 2, 3, 4, 5, 6, 7). Collected live from the type locality during dive #42 of



**Fig. 2** In situ observations of *Vulcanolepas verenae* sp. nov. in Hafa Adai vent field, Mariana Trough. **a** The holotype (NSMT-Cr 28234) just before collection during ROV *SuBastian* dive #42; **b** Sighting of an uncollected cluster during ROV *SuBastian* dive #43

ROV *SuBastian* on-board the Schmidt Ocean Institute R/V *Falkor*, cruise FK161129. Preserved in 99.5% ethanol.

**Diagnosis.** *Vulcanolepas* with small peduncular scales, about 0.8 mm in length and protruding 0.3 mm from peduncle. First tooth of mandible small. Proximal segment of posterior and anterior rami of cirrus I protuberant. Segments of anterior and posterior segment of cirrus II protuberant. Setae of cirri III to VI extreme long, length of setae about 10 times the length of an individual segment. Surface of segment in cirri III to VI bear a line of spines.

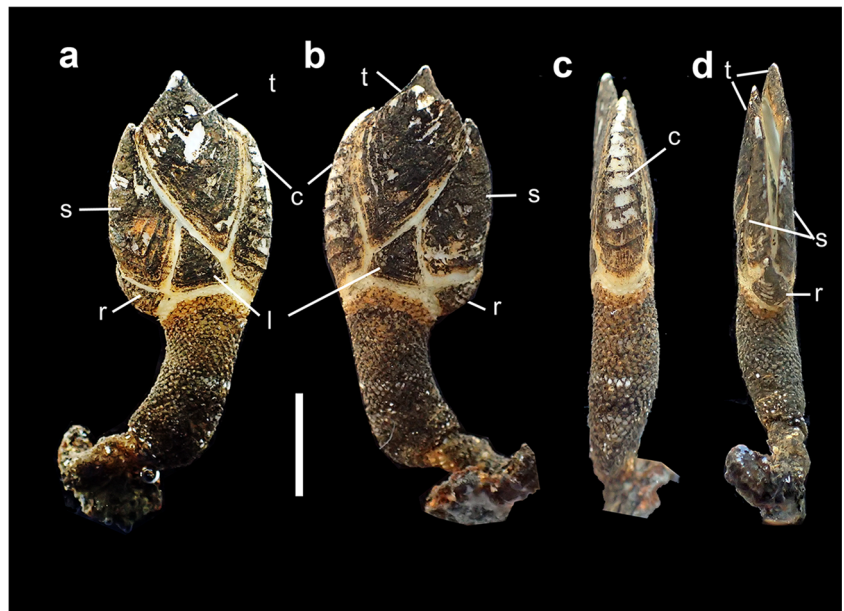
**Description.** Hermaphrodite. Capitulum higher than wide; capitulum height to width ratio 1.4:1 (Fig. 3). Capitulum with eight approximate plates; plates with black mineral coating, inter-plate spaces occupied by thin cuticular membranes (Fig. 3). Carina umbo apical, slightly bowed, surface with 10 horizontal growth lines; carina height about 4/5 capitulum height (Fig. 3). Tergum quadrangular with apex angle 58°, surface with 10 V-shaped growth lines; basal angle of tergum (68°) slightly elevated from capitulum-peduncle edge, about 1/5 total height of capitulum (Fig. 3). Scutum quadrangular, umbo apical, apical angle 31°, located at capitulum-peduncle edge (Fig. 3), surface with 7 V-shaped growth ridges parallel to basal margin, basal angle 101°. Medial latus equilateral triangular, umbo apical, apical angle 53°, surface with eight straight horizontal growth lines. Rostrum triangular, with six horizontal growth lines (Fig. 4e).

Peduncle to capitulum ratio about 1:1 (Fig. 3). Peduncle with 52 rounded scales per whorl just below the capitulum region, 26 scales per whorl at mid region of capitulum, scales width 0.83 mm (averaged from 3 scales), height 0.66 mm (averaged from 3 scales) projected from peduncle.

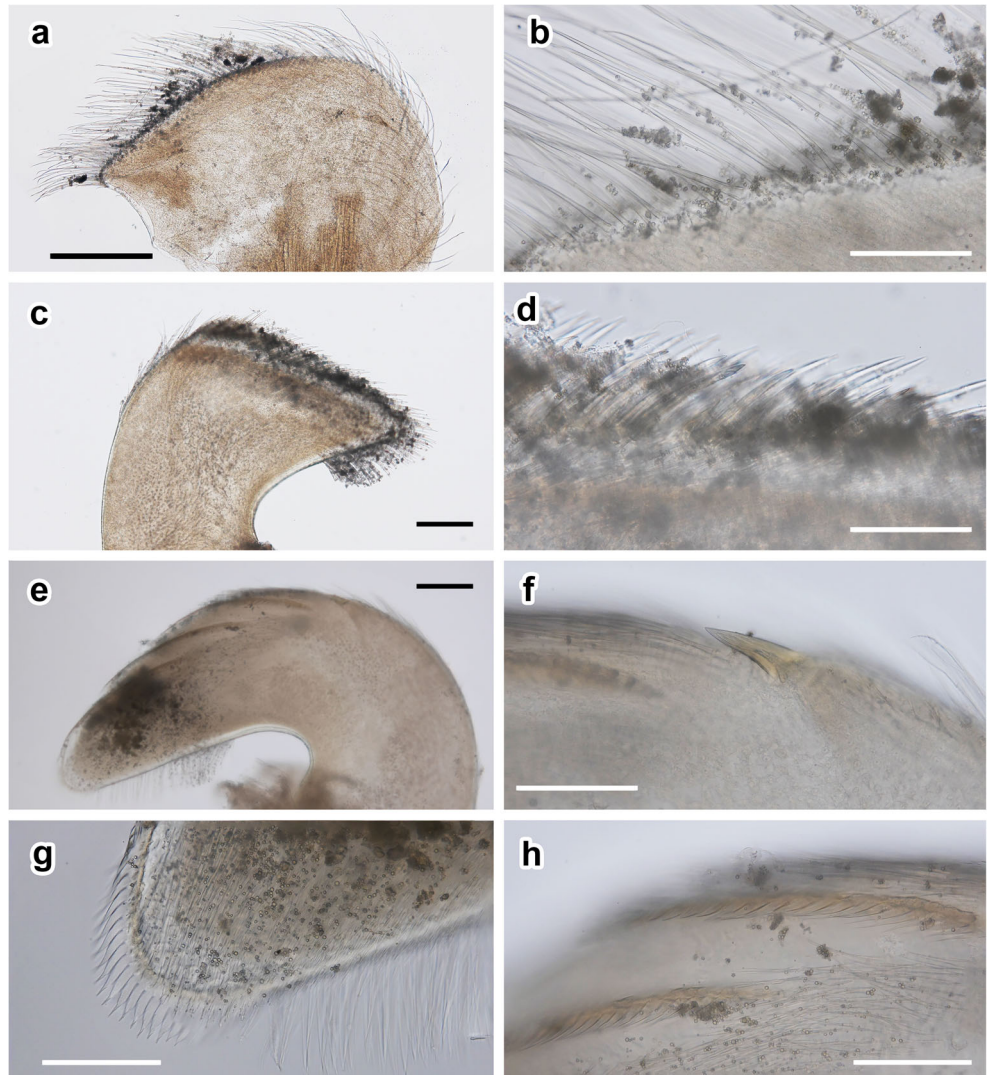
Trophi. Maxilla subtriangular, with a convex exterior margin, simple type setae on exterior margin (Fig. 4a, b). Maxillule cutting edge straight, with dense simple type setae on cutting margin; interior margin straight (Fig. 4c, d). Mandibles tridentoid, first teeth separated from the remainder, small and sharp, can only be observed from inner side of mandible (Fig. 4e, f). Second and third teeth comb-shaped, with more than 30 sharp spines on cutting edge (Fig. 4g, h). Cutting edge of second and third teeth long, each occupy about half of mandible's total length; inferior angle circular, composed of a series of sharp and large spines (Fig. 4e). Mandibular palp circular, with relatively sharp tip, with simple setae on tip and outer margin (Fig. 5a, b). Labrum not bullate, with single array of small sharp teeth on cutting edge (Fig. 5c, d).

Cirri. All six pairs of cirri are located close to each other. Cirrus I, both anterior and posterior rami are similar in length, proximal segments with width to length ratio about 2, rami become antenniform when approaching to the distal ends, with segment width to length ratio about 0.5 (Figs. 5e, g, 7a). The last 7 proximal segments of the posterior and last 8 proximal of anterior ramus protuberant,

**Fig. 3** External morphology of *Vulcanolepas verenae* sp. nov., holotype (NSMT-Cr 28234). **a** left view; **b** right view; **c** carinal view; **d** rostral view. Abbreviations: paired tergum (t), scutum (s), unpaired carina (c), lateral (l), rostrum (r). Scale bars: 1 cm for all parts

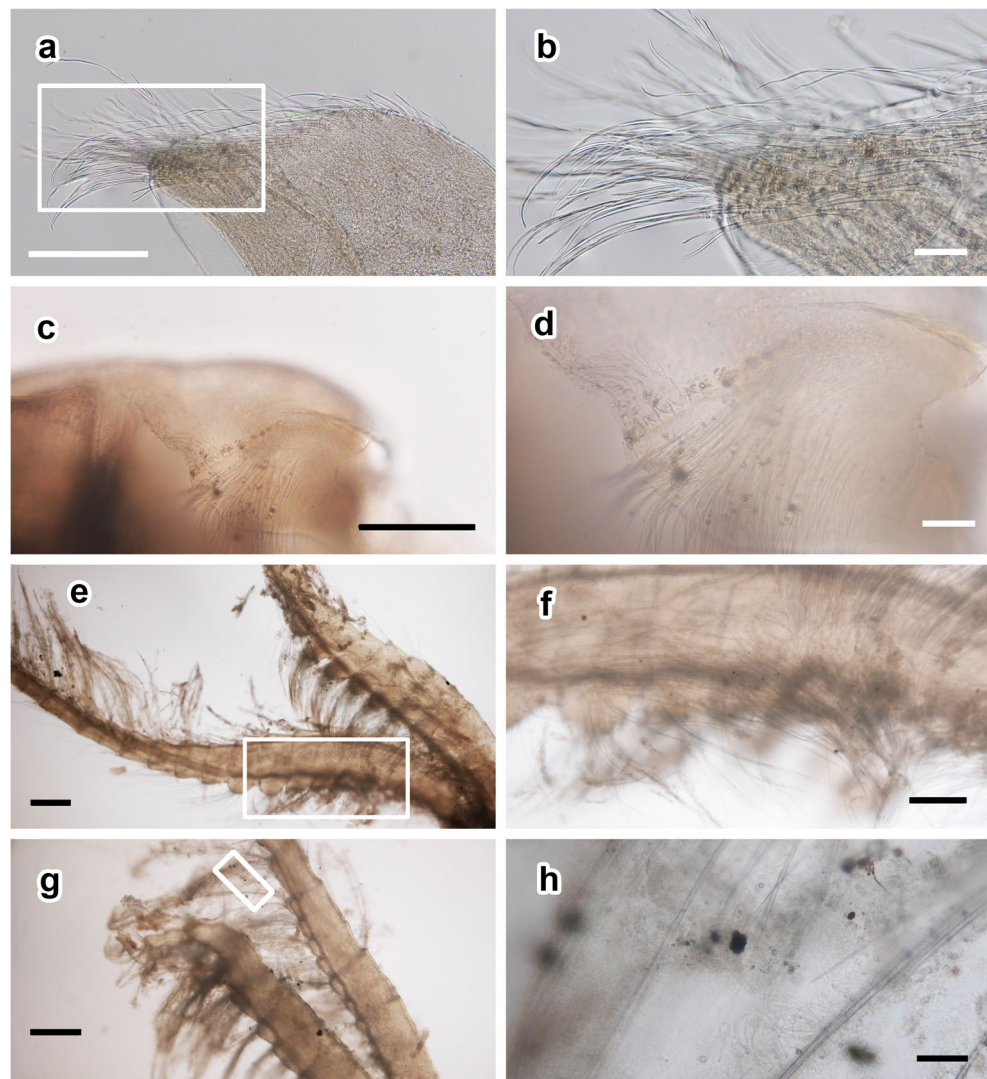


**Fig. 4** *Vulcanolepas verenae* sp. nov., holotype (NSMT-Cr 28234). **a** maxilla; **b** setae on margin of maxilla; **c** maxillule; **d** setae on cutting margin of the maxillule; **e** mandible; **f** first tooth of the mandible; **g** inferior angle; **h** second and third teeth of the mandible. Scale bars: a, c, e: 500 µm; b, d, f, g, h: 100 µm





**Fig. 5** *Vulcanolepas verenae* sp. nov., holotype (NSMT-Cr 28234). **a** mandibular palps; **b** setae on palps in the box in **a**; **c** labrum with fine, sharp teeth on edge; **d** magnified view of teeth on labrum; **e** cirrus I; **f** simple type setae on cirrus I, which was magnified in the box in **e**; **g** cirrus II; **h** simple type setae with filamentous bacteria on cirrus II, which was magnified in the box in **g**. Scale bars: a, b, c, d, f, h: 100  $\mu$ m; e, g: 1 mm



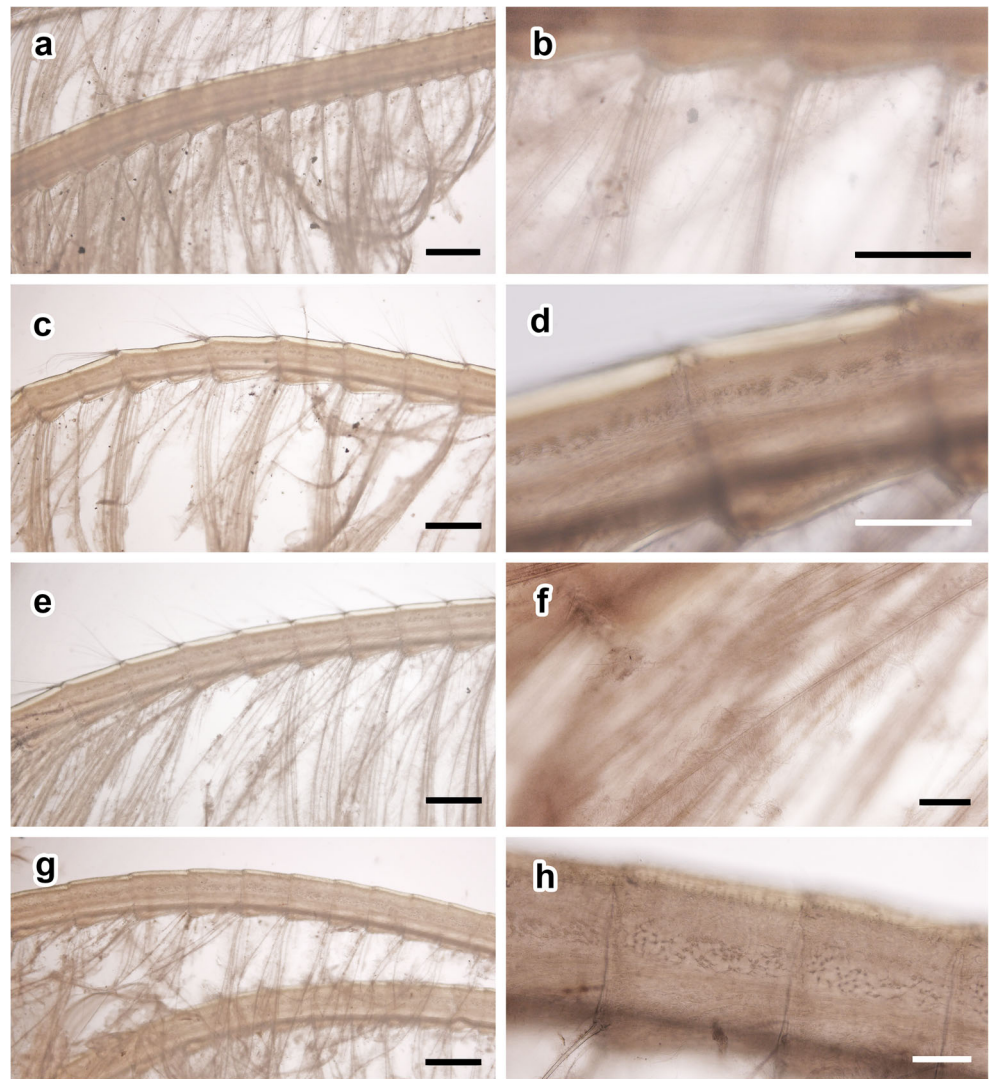
bearing dense bundles of simple setae (Fig. 7a). Cirrus II broken in holotype, segments protuberant at proximal region, with denser simple-type setae (Figs. 5g, h, 7b). Cirrus III incomplete in holotype, both anterior and posterior ramus bear very long setae (Fig. 6a, b). Length of setae about 10 times the length of an individual segment (Fig. 7c). Setae simple and surface coated by filamentous bacteria (Fig. 6f). Cirri IV–VI similar in morphology, anterior and posterior rami similar in length. Segments at distal 1/3 end of both rami become width twice greater than height (Figs. 6c–h, 7d–f). Setae are long, about 10 times the length of an individual segment, coated with filamentous bacteria (Fig. 6f). Intermediate segments of cirri IV–VI bear two pairs of short setae at distal end and bundles of long setae (Figs. 6c–h). Intermediate segment with scattered spines on lateral surfaces (Figs. 6c–h). Caudal appendage unsegmented, short, blunt, length about 1/8 length of pedicel on cirrus VI (Fig. 7h). Penis without basi-dorsal point, being half length of cirrus VI (Fig. 7g).

**Etymology.** Named after Verena Tunnicliffe, University of Victoria, Canada, for her contribution to hydrothermal vent research including the discovery of the present new species.

**Distribution.** At present, only collected from the Hafa Adai vent field. A stalked barnacle has previously been reported from the Alice Spring field, Mariana Trough (Hessler and Lonsdale 1991), but as no specimen was collected we cannot be sure if this record represents *V. verenae* sp. nov.

**Remarks.** This is the fifth species included in the genus *Vulcanolepas*. All *Vulcanolepas* have distinct morphological diagnostic characteristics (Watanabe et al. 2018; Chan et al. 2019). *Vulcanolepas verenae* sp. nov. is similar to *V. osheai* and *V. buckeridgei* in having extreme long setae, extending up to more than 3 mm on cirri III–VI in *V. verenae* sp. nov., and those setae are coated with filamentous bacteria. Mandibles of *V. buckeridgei*, *V. osheai*, and *V. verenae* sp. nov. all have very small first tooth, which differ from other members of *Vulcanolepas*. *Vulcanolepas verenae* sp. nov. differs from

**Fig. 6** *Vulcanolepas veranae* sp. nov., holotype (NSMT-Cr 28234). **a** anterior ramus on cirrus III; **b** intermediate segment on cirrus III; **c** posterior ramus on cirrus IV; **d** spines on the surface of segments of cirrus IV; **e** posterior ramus on cirrus V; **f** simple setae on cirrus V coated by dense filamentous bacteria; **g** cirrus VI; **h** spines on the surface of segment of cirrus VI. Scale bars: a, c, e, g: 1 mm; b, f: 100  $\mu$ m, d: 500  $\mu$ m



*V. buckeridgei* in having smaller peduncular scales. The number of scales just below the capitulum reached 52 in *V. veranae*. The number of scales just below capitulum in *V. buckeridgei* is about 23. In addition, peduncular scales in *V. buckeridgei* protrude more outwards from the peduncle in the basal region.

The first mandible tooth of *V. parensis* and *V. fijiensis* is much larger than the first tooth in *V. buckeridgei* and *V. veranae* sp. nov. In addition, the inferior angle of the mandible of *V. scotiaensis* contains dense setae, whilst the inferior angle of *V. buckeridgei* and *V. veranae* sp. nov. has several sharp spines, without any setae. Mandibles of *V. oshaei* have four teeth, which differs from the other four *Vulcanolepas* species. These characters together distinguish *V. veranae* sp. nov. from other described *Vulcanolepas* species.

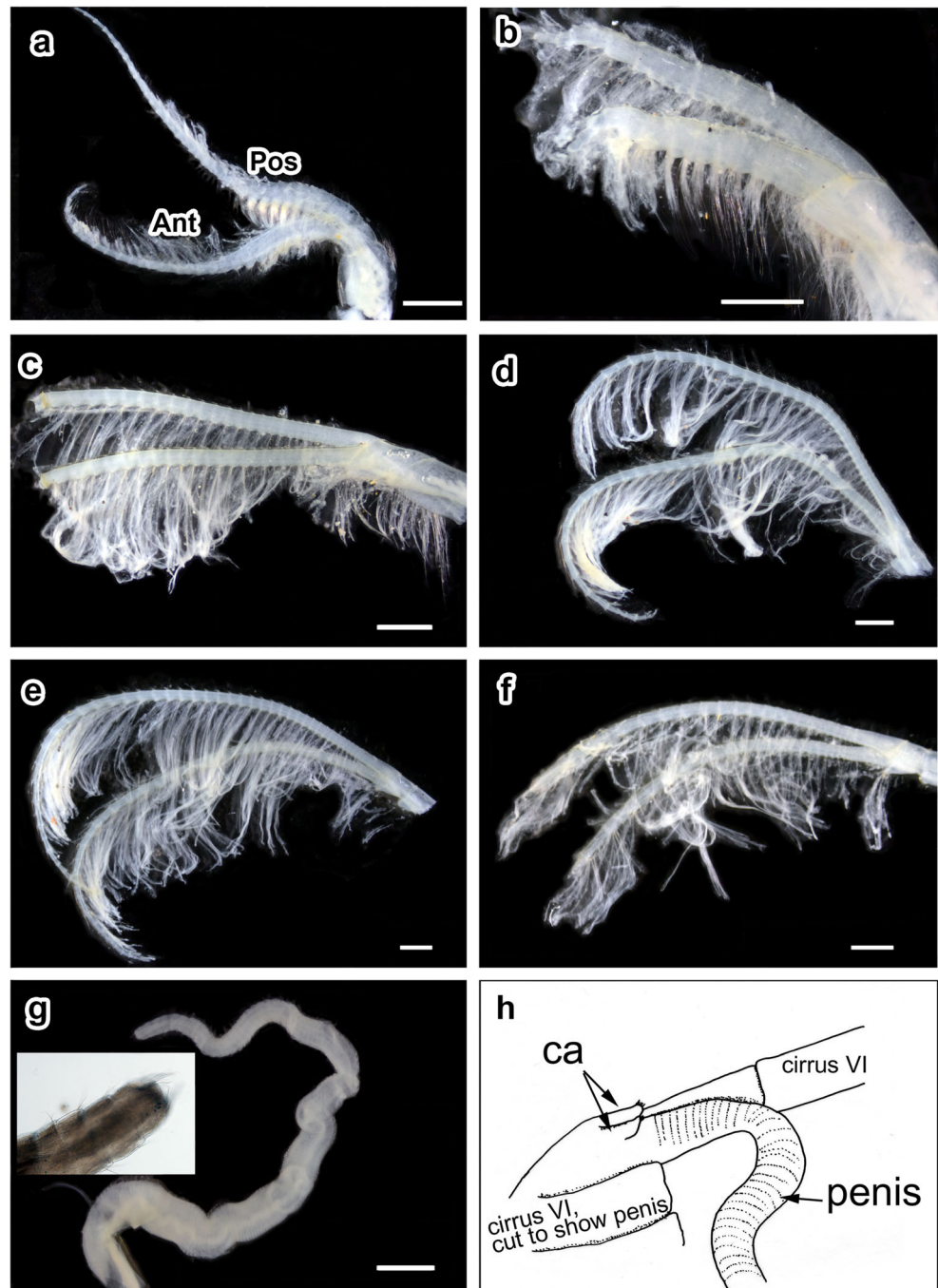
**Molecular phylogeny.** Although only a single specimen was available for *V. veranae* sp. nov., the number of nucleotide substitution to the other species showed clear

difference and barcoding gap (more than 15 bp in a 588 bp alignment) to the intraspecific nucleotide divergence in the genus (less than 5 bp; Table 1). The ratio of transition and transversion of the nucleotide substitution were larger than 1, and the region used in the present analyses does not appear to be saturated for the analyses.

The phylogenetic reconstruction showed that *V. veranae* sp. nov. is mostly closely related to *V. oshaei* and *V. buckeridgei*. *Vulcanolepas* was recovered as paraphyletic, with *Leucolepas longa* being recovered sister to *V. fijiensis*, and a problematic position of *Neolepas* embedded within *Vulcanolepas* (Fig. 8). All species included, except for the *N. zeviniae/rapanui* complex, were strongly supported (92% bootstrapping replicates in Maximum Likelihood and Bayesian posterior probability of 1.00). However, phylogenetic relationships among the genera were not resolved with strong support.



**Fig. 7** *Vulcanolepas verenae* sp. nov., holotype (NSMT-Cr 28234). Overview of **a** cirrus I; **b** cirrus II; **c** cirrus III; **d** cirrus IV; **e** cirrus V; **f** cirrus VI; **g** penis, insert showing the tip under light microscopy; **h** drawing of caudal appendages (ca) and the penis. Pos: posterior, Ant: anterior. Scale bars: 1 mm for all parts



## Discussion

The present results showed that the stalked barnacle from a hydrothermal vent field in the Mariana Trough constitutes a lineage independent from the previously described vent stalked barnacles in Neolepadidae. The mandibular and cirral morphologies, as well as molecular data (Figs. 4, 5, 6, 7, 8), support the Mariana Trough vent barnacle as an undescribed species, described as *V. verenae* sp. nov. herein. There appears to be two different feeding ecology among *Vulcanolepas* species, either harboring or feeding on epibiotic bacteria on their cirri, or simply

suspension feeding. The former is applicable to *V. osheai*, *V. buckeridgei*, and *V. verenae* sp. nov. Dense bacteria on the cirri of *Vulcanolepas* was first observed in *V. buckeridgei* (referred to as “neolepadine Lau A” in Southward and Newman 1998) in Hine Hina site, Lau Basin. The relationship between bacteria on the cirri and the host barnacle was examined in *V. osheai* from the Brothers Caldera, Kermadec Arc (Suzuki et al. 2009). The combination of bacterial metabarcoding, in situ hybridization, as well as fatty-acid and carbon isotopic composition showed that the microbes on cirri of *V. osheai* mainly consisted of sulfur-oxidizing bacteria in Epsilonproteobacteria



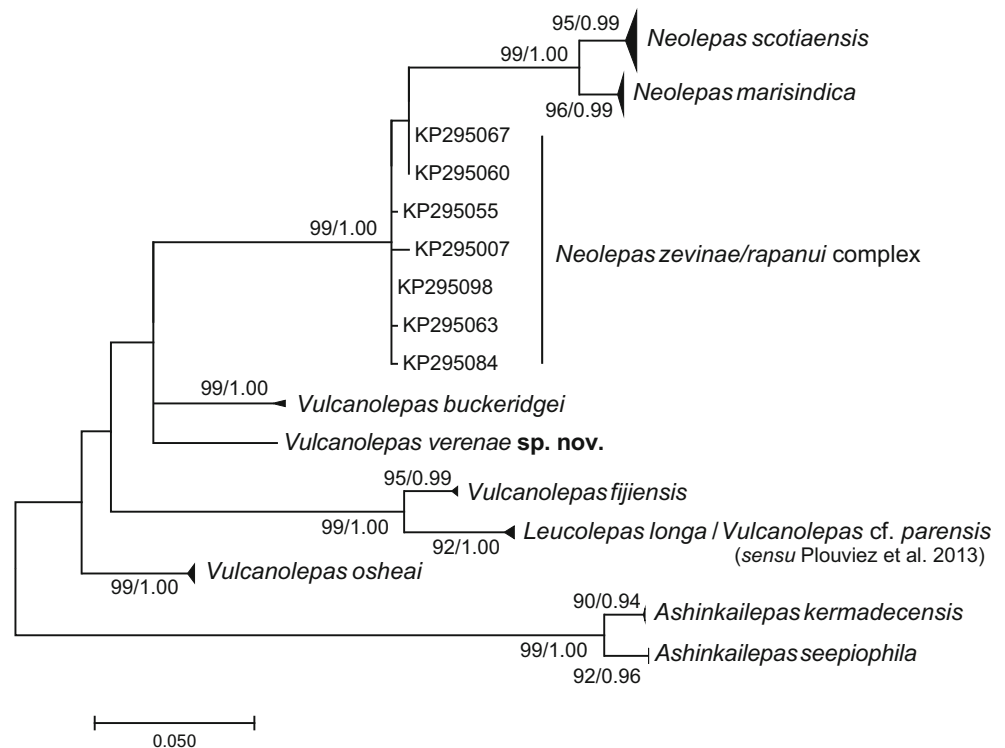
**Table 1** Mean number of nucleotide base substitution within species and among species, of 588 bp in COI sequences

		1	2	3	4	5	6	7	8	9	10
1	<i>V. verenae</i> <b>sp. nov.</b>	n.c.									
2	<i>V. buckeridgei</i>	35.000	4.000								
3	<i>V. osheai</i>	41.667	45.000	2.267							
4	<i>V. fijiensis</i>	56.000	58.000	59.833	1.333						
5	<i>N. zeviniae/rapanui</i> complex	49.857	44.429	49.619	63.143	3.143					
6	<i>N. marisindica</i>	58.000	51.000	59.256	63.231	32.945	1.513				
7	<i>N. scotiaensis</i>	56.895	51.579	59.746	68.263	34.218	16.227	2.480			
8	<i>L. longa</i>	58.000	59.250	61.042	25.333	68.464	67.615	72.263	2.667		
9	<i>A. seepiophila</i>	71.000	81.500	73.500	81.000	88.429	87.846	86.474	79.500	0.000	
10	<i>A. kermadecensis</i>	68.250	77.250	71.250	80.750	84.179	86.673	85.961	80.250	13.250	0.500

and Gammaproteobacteria and were likely consumed by the host barnacle (Suzuki et al. 2009). Similar dense bacteria were observed in *V. verenae* **sp. nov.**, which is inferred to have a similar feeding ecology. These three species of *Vulcanolepas* possess elongated setae (2.59 mm in *V. osheai*, 4.6 mm in *V. buckeridgei* on average, over 3 mm in *V. verenae* **sp. nov.**) compared with the other *Vulcanolepas* species without bacteria on their cirri; 1.62 mm on average in *V. paretensis*; and just shy of 2 mm in *V. fijiensis* (Southward and Newman 1998, Southward 2005, Chan and Chang 2018). Neither *Neolepas scotiaensis* (originally described in *Vulcanolepas* and later transferred to *Neolepas*) nor *Leucolepas longa* harbor bacteria on their cirri and

setae (Tunnicliffe and Southward 2004; Marsh et al. 2012; Buckeridge et al. 2013). Basic natural history observations and information can be valuable in separating closely related taxa (Sigwart and Chen 2018). Indeed, *V. fijiensis* apparently co-occurred with another stalked barnacle lacking ectosymbionts (“neolepadine Lau B” in Southward and Newman 1998) in the Lau Basin, and the two had different microhabitat preferences (Southward and Newman 1998). The feeding ecology of vent stalked barnacles and the behavior of cultivating epibionts may also be key characters to consider in the taxonomy of neolepadid stalked barnacles.

**Fig. 8** Phylogenetic reconstruction of neolepadid stalked barnacles based on 588 bp of the partial mitochondrial COI gene. Numbers on the branch show the Maximum Likelihood bootstrap value/Bayesian posterior probability supports. Only those higher than 70% / 0.70 are shown



A key for presently known *Vulcanolepas* species is provided below:

1. First tooth of mandible large.....2  
    First tooth of mandible small.....3
2. Length ratio of antenniform to robust segments in both rami of cirrus I almost equal (~ 1).....*V. fijiensis*  
    Length ratio of antenniform to robust segments in both rami of cirrus I larger than 1.....*V. parensis*
3. Setae on cirri IV–VI very long, length ratio of annulus to length of setae > 10.....4  
    Length ratio of annulus to length of setae in cirri IV–VI < 10 .....*V. osheai*
4. Peduncular scales small and non-protruded.....*V. verenae* **sp. nov.**  
    Peduncular scales large and protruded.....*V. buckeridgei*

The distribution of each *Vulcanolepas* species is limited to a specific area of the Pacific Ocean: *V. osheai* on the Kermadec Arc, *V. buckeridgei* in the Lau Basin, *V. parensis* on the Pacific-Antarctic Ridge, *V. fijiensis* in the North Fiji Basin, and *V. verenae* **sp. nov.** in the Mariana Trough. This differs from most species in the other Neolepadini genera (i.e., *Leucolepas* and *Neolepas*) which are more widely distributed (Fig. 1). Although the larval dispersal mechanisms of *Vulcanolepas* are not yet known, the limited existing knowledge on their reproductive traits suggest limited capability of larval dispersal. *Vulcanolepas osheai* brood the largest eggs among currently known neolepadids (1.05 mm in length; Buckeridge 2000), and brooding is not frequently observed, unlike in *L. longa* (Tunnicliffe and Southward 2004). The fragmented distribution of *Vulcanolepas* associated with specific geographic regions may be due to the larval dispersal and specialized in certain types of hydrothermal vents.

Our single-locus phylogenetic analysis could not fully resolve the phylogeny of the family Neolepadidae; however, the nodes with high statistical support were consistent with a multi-locus phylogenetic analyses of deep-sea vent barnacles by Herrera et al. (2015). The examination of *V. verenae* **sp. nov.** and comparisons with other *Vulcanolepas* species suggest that members of the genus *Vulcanolepas* may share a number of key characteristics, summarized in the genus diagnosis above. Nevertheless, results from the molecular phylogeny casts doubts on if all these characters are taxonomically informative, since *Vulcanolepas* was not recovered as monophyletic, a condition shared with previous studies (Herrera et al. 2015; Watanabe et al. 2018; Chan et al. 2019). *Vulcanolepas fijiensis*, which does not possess dense bacterial aggregation on the cirri, was recovered as the sister species of *L. longa*, both in the present analysis and in Chan et al. (2019). *Vulcanolepas* sp. from Hine Hina, Lau Basin, was also recovered in a clade with *L. longa* in a three-gene phylogeny (18S, 28S, and H3) (Pérez-Losada et al. 2008; Herrera et al. 2015). These infer that these two species may

be more closely related to *L. longa* than other *Vulcanolepas* species. For the problematic *V. cf. parensis* sensu Plouviez et al. (2013) from the Manus Basin, the partial COI sequences (from Plouviez et al. (2013) were actually genetically indistinguishable from *L. longa* and should be regarded as misidentification of *L. longa*, as previously pointed out by Watanabe et al. (2018). Genetic data from the true *V. parensis*, from specimens confirmed to agree morphologically with the holotype, is therefore still unavailable. The placement of *V. osheai*, which has ectosymbionts, was not well-resolved in the present single-locus analysis (Fig. 8), but previous multi-locus analyses have resolved it in a basal position among a monophyletic clade with other ectosymbiont-hosting *Vulcanolepas* species and *Neolepas* species, instead of being closely related to *L. longa* (Pérez-Losada et al. 2008, Herrera et al. 2015). Together, these suggest that species currently included in *Vulcanolepas* may consist of two groups, one housing ectosymbionts that is more closely related to *Neolepas* (including *V. osheai*, *V. buckeridgei*, and *V. verenae* **sp. nov.**) and one without ectosymbionts that is more closely related to *Leucolepas* (including *V. fijiensis* and *V. parensis*). Given the topology of recent molecular phylogenies with *Neolepas* and *Leucolepas* species embedded within a paraphyletic *Vulcanolepas*, it is possible that hosting ectosymbionts is an ancestral character present in Neolepadini, subsequently lost in some species. Further phylogenetic analyses, as well as detailed examinations of morphological and ecological characters, will help resolve the issues with genera assignments in Neolepadidae and provide insights on the speciation and adaptation of these deep-sea barnacles.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** The study species is an invertebrate crustacean and no experimental manipulation was undertaken on live animals in this study. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

**Sampling and field studies** All necessary permits for sampling and observations have been obtained by the authors or contributors mentioned in the acknowledgments, where applicable.

**Data availability** The datasets generated in the current study are available in the NCBI GenBank repository, with accession number MT662001. Materials used in the present study are deposited in the National Museum of Nature and Science, Tsukuba (NSMT), Japan.

**Author contributions** HKW and CC conceived and designed the research. HKW and BKKC conducted morphological observation and description. HKW conducted molecular phylogenetic analysis. All authors wrote, read and approved the manuscript.

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