

# A very deep *Provanna* (Gastropoda: Abysochrysoidea) discovered from the Shinkai Seep Field, Southern Mariana Forearc

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*The ‘Shinkai Seep Field’ is a serpentinite-hosted chemosynthetic ecosystem in the Southern Mariana Forearc. In June 2015 the site was revisited and a number of rissoiform gastropods were collected. Taxonomic investigations revealed that these specimens represent a hitherto undescribed species of Provanna (Gastropoda: Abysochrysoidea), described herein as Provanna cingulata n. sp. This new species is characterized by numerous spiral keels, lack of significant axial sculpture, rounded and inflated whorls, and large size for the genus. With the shell height exceeding 16.5 mm (may reach 20 mm), it is the largest Provanna species known thus far. Phylogenetic analysis using 411 bp of the cytochrome oxidase c subunit I (COI) gene confirmed its systematic placement within the genus Provanna. This is the only gastropod from a family endemic to chemosynthetic ecosystems thus far known from the ‘Shinkai Seep Field’. Furthermore, with a collection depth of 5687 m, it represents the deepest known bathymetric range for the superfamily Abysochrysoidea as a whole.*

**Keywords:** Chemosynthetic ecosystems, Mollusca, new species, Provannidae, serpentinization, serpentinite-hosted

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## INTRODUCTION

Since the first encounter with hydrothermal vents on the Galápagos Rift was published in 1977 (Lonsdale, 1977; Corliss & Ballard, 1977), scientific interest and effort in exploring deep-sea chemosynthetic ecosystems have remained very high. Chemosynthetic ecosystems are now known to manifest in a variety of ways and settings other than vents such as cold seeps, and organic falls and are considered to be widespread (Baker *et al.*, 2010). The discovery of the Lost City hydrothermal field illuminated the presence of serpentinite-hosted chemosynthetic systems (Kelley *et al.*, 2001, 2005, 2007). Serpentinization produces methane, CH<sub>4</sub>, the anaerobic oxidation of which results in the production of hydrogen sulphide, H<sub>2</sub>S, both of which are key to powering chemosynthetic ecosystems through chemosynthetic microbes (Kelley *et al.*, 2005; Ohara *et al.*, 2012). Unlike hydrothermal vents, the fluids of which are usually highly acidic (Van Dover, 2000), the fluids from such serpentinite-hosted systems are often highly alkaline (Kelley *et al.*, 2005; Takai *et al.*, 2005). Megafauna dependent on chemosynthesis have been reported

to be common in such environments, although the details are little-known (Kelley *et al.*, 2007).

Gastropod molluscs comprise a major constituent of mega-fauna communities in various chemosynthetic ecosystems, and have received considerable taxonomic effort (reviewed in Warén & Bouchet, 2001; Sasaki *et al.*, 2010). Gastropods in the superfamily Abysochrysoidea currently assigned to the family Provannidae are restricted to chemosynthetic ecosystems (Johnson *et al.*, 2010). The genus *Provanna* within it includes medium-sized snails (generally < 15 mm in shell height) and is a common endemic of such communities around the world (Sasaki *et al.*, 2010). They are deposit feeders grazing on bacteria and detritus, and produce lecithotrophic larvae (Warén & Bouchet, 1986, 1993; Levesque *et al.*, 2006; Sasaki *et al.*, 2010). It is the most species-rich genus of Abysochrysoidea, currently with 22 described extant species (Johnson *et al.*, 2010) and eight fossil species (Amano & Jenkins, 2013; Amano & Little, 2014).

Serendipitously discovered in September 2010 in a submersible dive originally aimed to investigate the geology of the Southern Mariana Forearc, the ‘Shinkai Seep Field’ (SSF; Figure 1) is a serpentinite-hosted system located on a steep inner slope of the Mariana Trench, Southern Mariana Forearc (Ohara *et al.*, 2012). The dominant fauna is a large vesicomyid clam, *Calyptogena (Abyssogena) mariana* Okutani *et al.*, 2013. In June 2015, further detailed investigations and sampling of the SSF was undertaken on the YK15-11 cruise

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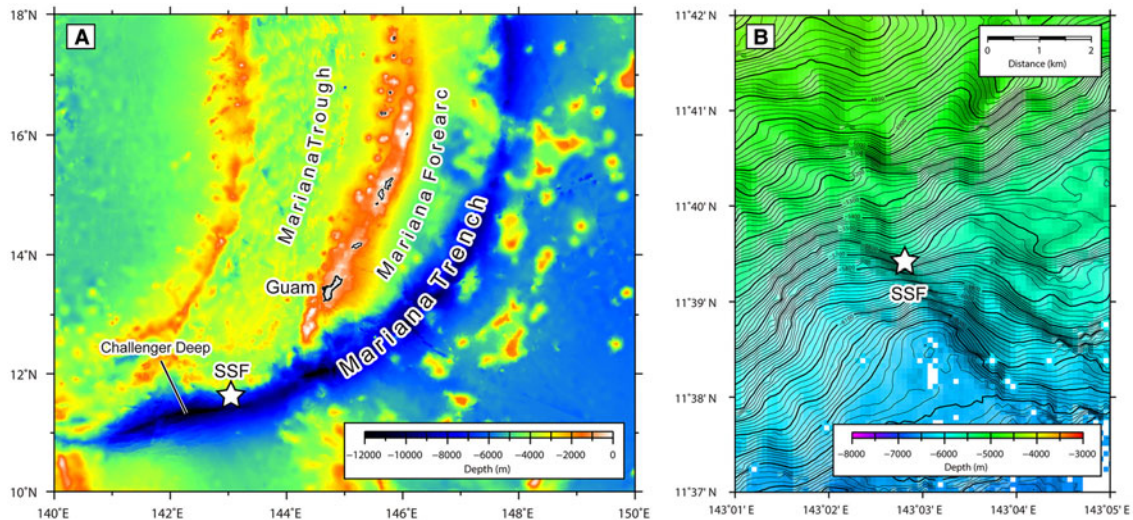


Fig. 1. Location of the Shinkai Seep Field (SSF): (A) index map indicating the SSF by an asterisk; (B) detailed bathymetry of the SSF area (after Ohara *et al.*, 2012). Contours in 20 m intervals.

of R/V *Yokosuka*. Biological samples were taken during the DSV *Shinkai 6500* dive 1433, and numerous specimens of a rissiform gastropod were collected. Taxonomic investigations which followed revealed that these specimens represented a highly characteristic *Provanna* species previously unknown to science. It is among the first Abyssochrysoid gastropod discovered from serpentinite-hosted ecosystems along with a *Desbruyeresia* species from South Chamorro Seamount (Chen *et al.*, 2016). Here this new species is formally described and named, as *Provanna cingulata* n. sp., and the bathymetric distribution range of the genus *Provanna* is discussed.

## MATERIALS AND METHODS

### Sample collection

All specimens of the new *Provanna* were collected from the SSF by the DSV *Shinkai 6500* dive 1433 during cruise YK15-11. Upon recovery onto R/V *Yokosuka*, the specimens were immediately placed into 99% ethanol to dehydrate for preservation and storage.

### Morphology

Morphological investigation and dissection were carried out under an Olympus SZX9 dissecting microscope. The radula was dissected and protoconch was illustrated from specimens preserved in 99% ethanol. Scanning electron microscopy (SEM) and shell morphometric measurements were carried out as in Chen *et al.* (2016).

Type specimens are deposited in the University Museum, the University of Tokyo (UMUT), the American Museum of Natural History, New York City (AMNH), the National Museum of Nature & Science, Tsukuba (NSMT) and Japan Agency for Marine-Earth Science and Technology (JAMSTEC).

### Genetics

Two specimens of the provannid from the SSF were sequenced for the barcoding gene cytochrome oxidase *c* subunit I (COI).

In addition, COI of one specimen of *Provanna shinkaiiae* Okutani & Fujikura, 2002 from the JAMSTEC collection (No. 051346-051358, 70% ethanol, from methane seep, Japan Trench, 39°6.432'N 143°53.478'E, 5352 m, ROV *KAIKO* Dive #258, R/V *Kairei* cruise KR02-09) was also sequenced for comparison. DNA extraction and quality checks were performed as in Chen *et al.* (2016). The COI region was amplified with the primer pairs LCO1490 and HCO2198 (Folmer *et al.*, 1994) as well as Pg501L (5'-TATACGATGACGGGGAATGC-3') and Pg1253R (5'-TGTTGAGGAAAGAAAGTAATATTA-3') (Ogura *et al.*, unpublished). The polymerase chain reaction was carried out in 20 µl reactions, including 1 µl DNA template (15–30 ng µl<sup>-1</sup>), 1 µl each of forward and reverse primers (10 µM), 1.6 µl dNTP mixture (TaKaRa Bio, Japan), 2 µl 10 × buffer, TaKaRa Ex Taq DNA polymerase solution (TaKaRa Bio, Japan), and 13.25 µl double-distilled water. A Veriti 200 Thermal Cycler (Applied Biosystems) was used for thermo cycling. The protocol used was: 95°C for 1 min followed by 35 cycles of [95°C for 15 s, 40°C for 15 s, 72°C for 30 s], ending with 72°C for 7 min. Amplification was confirmed with 1.4% agarose gel electrophoresis using ethidium bromide. ExoSAP-IT (Affymetrix) was used following standard protocols to purify successful PCR products. Details of cycle sequencing reaction and purification are as listed in Chen *et al.* (2016). Sequences were resolved from precipitated products using Applied Biosystems 3130xl DNA sequencer.

The complementary sequences from the forward and reverse primers were aligned to check the sequencing accuracy using a software Alignment Explorer mounted on the software package MEGA 6 (Tamura *et al.*, 2013). Phylogenetic analyses were carried out using the same package using the resulting sequences plus the abyssochrysoid COI sequences available on GenBank. Sequences of the whelk *Neptunea amianta* (Dall, 1890), *N. antiqua* (Linnaeus, 1758), and the periwinkle *Littorina littorea* (Linnaeus, 1758) from distantly related gastropod groups were included as outgroup taxa (after Johnson *et al.*, 2010).

The Model Selection (ML) program in MEGA 6 was applied to the dataset to select the most suitable nucleotide substitution model, which was HKY + G + I. Then the maximum-likelihood (ML) tree was generated using this model also in

MEGA 6. The ML tree was bootstrapped 2000 times. Restricted by the length of some shorter sequences on GenBank, the sequence length used in the final phylogenetic analyses was 411 bp. New sequences generated from this study are deposited in DNA Data Bank of Japan (DDBJ) under the accession numbers LC094443, LC094444 and LC095875.

#### SYSTEMATICS

Clade CAENOGASTROPODA Cox, 1960  
 Superfamily ABYSSOCHRYSOIDEA Tomlin, 1927  
 Family PROVANNIDAE Warén & Ponder, 1991  
 Genus *Provanna* Dall, 1918  
*Provanna cingulata* n. sp.  
 (Figures 2–4)

#### ZOOBANK REGISTRATION

urn:lsid:zoobank.org:act:F7E7D074-0924-4F4B-9980-3CE3A1E144EE

#### TYPE LOCALITY

Shinkai Seep Field, 11°39.3652'N, 143°02.8786'E, 5687 m in depth. Collected during the R/V *Yokosuka* cruise YK15-11, DSV *Shinkai 6500* Dive 1433, 2015/7/15, by Tomoyo Okumura.

#### TYPE MATERIAL

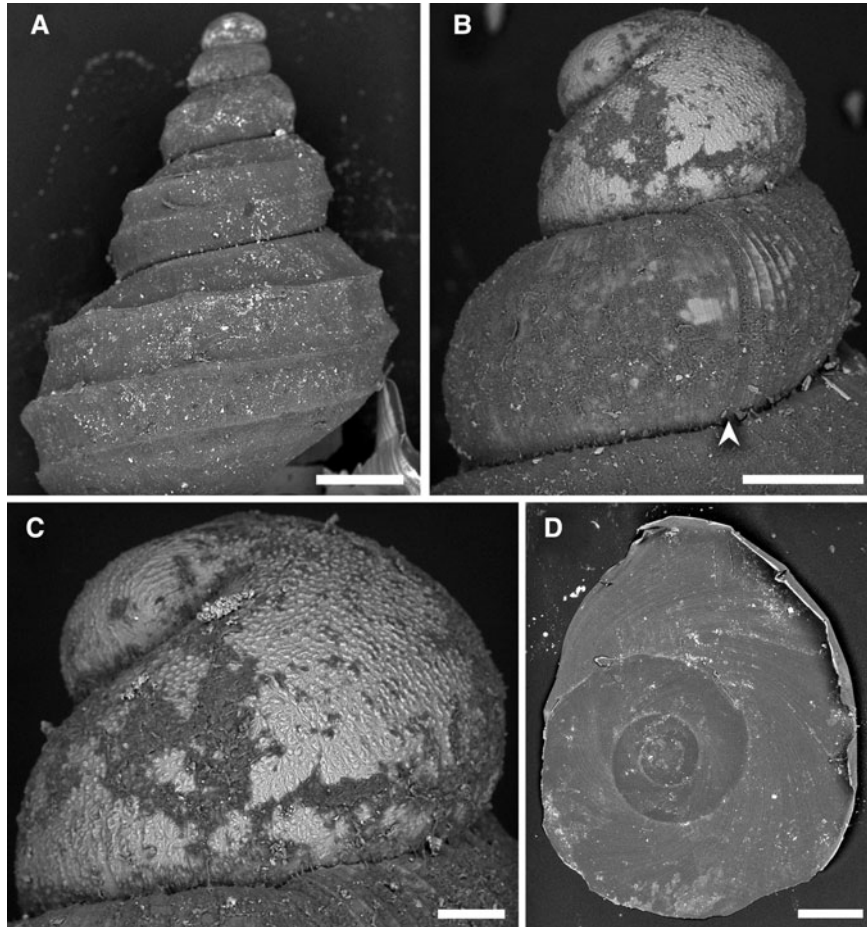
Holotype: Shell height (SH) 11.0 mm, shell width (SW) 6.8 mm, live collected, 99% ethanol, Figure 2A–D (UMUT RM32589).

Paratypes: #1 (Figure 2E–F): SH 11.4 mm, SW 7.3 mm, live collected, 99% ethanol (AMNH\_IZC 250202). #2: SH 8.8 mm, SW 5.3 mm, live collected, 99% ethanol (NSMT-Mo 78974). #3 (Figure 2G, H): SH 14.3 mm, SW 10.1 mm, dead collected shell only, 99% ethanol (NSMT-Mo 78975). #4 (Figure 2I–L): SH 16.5 mm, SW 11.0 mm, dead collected shell only, 99% ethanol (UMUT RM32590). #5: Juvenile specimen, dead collected with broken aperture, SH 3.6 mm, SW 2.3 mm, dried and mounted for protoconch SEM (UMUT RM32591). #6: Two live collected specimens, dissected for radula and a



Fig. 2. *Provanna cingulata* n. sp.: (A–D) holotype, shell height 11.0 mm (UMUT RM32589); (E–F) paratype #1, shell height 11.4 mm (AMNH\_IZC 250202); (G–H) paratype #3, shell height 14.3 mm (NSMT-Mo 78975); (I–L) paratype #4, shell height 16.5 mm (UMUT RM32590). Scale bars: A–L = 2 mm.

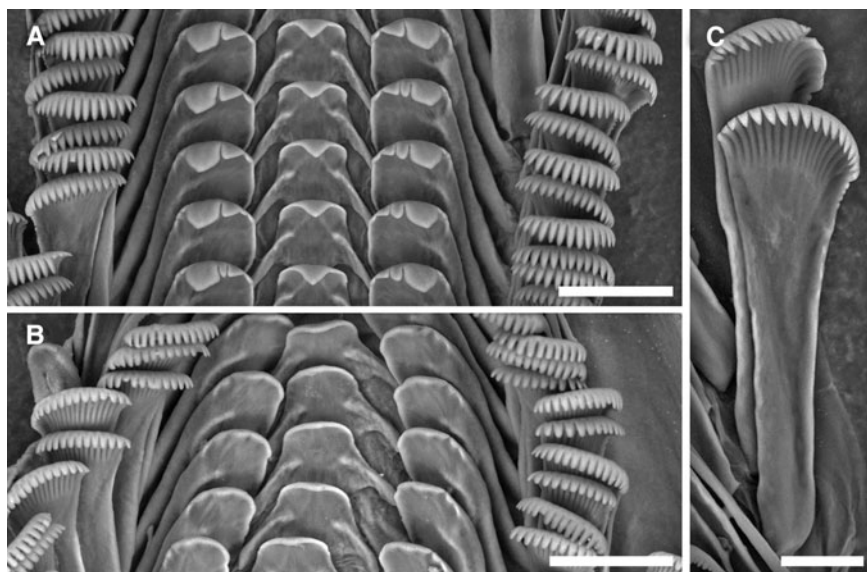




**Fig. 3.** *Provanna cingulata* n. sp.: (A) early whorls of a juvenile specimen (paratype #4, UMUT RM32591); (B–C) protoconch, white arrow in (B) indicates boundary between the protoconch and the teleoconch; (D) operculum. Scale bars: A, D = 500  $\mu$ m; B = 200  $\mu$ m; C = 50  $\mu$ m.

section of foot taken for DNA, 99% ethanol (UMUT RM32592).  
 #7: Five intact specimens in 99% ethanol (JAMSTEC 1150051773).

All type materials originate from the type locality with identical collection data (original accession number for the lot is 12541-15002-6 K#1433-Bo6).



**Fig. 4.** *Provanna cingulata* n. sp., radula: (A) middle section of an adult radula ribbon; (B) Most anterior section of the same adult radula ribbon, showing obvious dental wear; (C) a single marginal teeth. Scale bars: A–B = 50  $\mu$ m; C = 20  $\mu$ m.

**Table 1.** Collection conditions and shell measurements of all available specimens (both type series and non-types).

	Collecting condition	Shell height (mm)	Shell width (mm)	Aperture height (mm)	Aperture width (mm)
<i>Holotype</i>	Live	11.0	6.8	4.9	3.8
<i>Paratype 1</i>	Live	11.4	7.3	5.4	3.7
<i>Paratype 2</i>	Live	8.8	5.3	3.8	2.9
<i>Paratype 3</i>	Dead	14.3	10.1	6.6	4.9
<i>Paratype 4</i>	Dead	16.5	11.0	7.5	5.6
<i>Paratype 5</i>	Dead	3.6	2.3	<i>Aperture broken</i>	
<i>Paratype 6-1</i>	Live	9.1	6.1	4.3	3.1
<i>Paratype 6-2</i>	Live	10.4	6.5	4.7	3.2
<i>Paratype 6-3</i>	Live	8.1	5.2	3.8	2.9
<i>Paratype 7-1</i>	Live	10.4	6.5	4.8	3.0
<i>Paratype 7-2</i>	Live	9.3	6.4	4.8	3.5
<i>Paratype 7-3</i>	Live	8.8	5.5	4.0	2.8
<i>Paratype 7-4</i>	Live	6.9	4.5	3.7	2.8
<i>Paratype 7-5</i>	Live	5.1	3.3	2.5	1.7
<i>Non-type 01</i>	Dead	12.9	7.5	5.3	4.0
<i>Non-type 02</i>	Live	7.6	5.4	3.6	2.8
<i>Non-type 03</i>	Live	6.5	4.4	3.3	2.6
<i>Non-type 04</i>	Live	6.8	4.8	3.4	2.6
<i>Non-type 05</i>	Dead	6.8	4.6	<i>Aperture broken</i>	
<i>Non-type 06</i>	Live	6.6	4.2	3.2	2.3
<i>Non-type 07</i>	Live	6.8	4.4	3.3	2.5
<i>Non-type 08</i>	Live	6.3	4.1	3.2	2.3
<i>Non-type 09</i>	Live	5.6	3.6	2.7	1.9
<i>Non-type 10</i>	Live	8.0	5.6	4.0	2.9
<i>Non-type 11</i>	Dead	<i>Fragmented shell only</i>			
<i>Non-type 12</i>	Dead	<i>Fragmented shell only</i>			
<i>Non-type 13</i>	Dead	<i>Fragmented shell only</i>			
<i>Non-type 14</i>	Dead	<i>Fragmented shell only</i>			
<i>Non-type 15</i>	Dead	<i>Fragmented shell only</i>			
<i>Non-type 16</i>	Dead	<i>Fragmented shell only</i>			
<i>Non-type 17</i>	Dead	<i>Fragmented shell only</i>			
<i>Non-type 18</i>	Dead	<i>Fragmented shell only</i>			

**FURTHER MATERIAL EXAMINED**

There were 18 further specimens collected together from the type locality, which were also examined. Of these, eight were live collected, two were dead collected, and eight specimens were only shell fragments (Table 1).

**DIAGNOSIS**

A large *Provanna* up to 16.5 mm in shell height with rounded whorls and surface sculpture consisting of numerous spiral keels, lacking axial sculpture except for part of protoconch.

**DESCRIPTION**

Shell (Figure 2). Rissoiform, about 5.5 whorls. Large sized for the genus, up to 16.5 mm in height. Protoconch (Figure 3B, C) preserved in juveniles, slightly corroded in adults. Only protoconch I present, protoconch II lacking. White, about 1.5 whorls and 0.6 mm in height. Earlier part generally smooth with evenly dispersed fine granulation. Granules gradually becoming to disappear, continually replaced by axial ribbing. Teleoconch whorls rather inflated, rounded, convex. Suture moderately deep, impressed. Surface sculpture consists of spiral cords, increasing in number with growth, cross section always triangular. Very earliest teleoconch smooth but two prominent spiral cords emerging after 0.5 whorls and a third emerging from interspace after another whorl (Figure 3A). Lower of two initial cords especially sharp and strong throughout all growth stages. Penultimate whorl with eight to nine spiral cords above suture,

weak and strong cords alternating. Strength of spiral cords increasing with growth. On body whorl of adults most cords of equal strength, only most peripheral one significantly stronger. Spiral cords present in close proximity on base, decreasing in strength anteriorly. Cord numbers increasing with growth (8–12 in specimens investigated). No axial ribbing or clearly visible growth lines present on teleoconch. Aperture rounded, slightly taller than wide. Outer-lip sharp, not thickened. Anterior siphonal notch shallow but distinct. Inner lip simple and smooth with no plicae or extension of parietal callous. Columella straight. No umbilical opening present. Ostracum thin, white, partly or completely corroded in body whorl leaving only periostracum layer. Periostracum golden brown in colouration, decreasing in intensity towards apex.

Operculum (Figure 3D). Paucispiral, nucleus eccentric, a little more than 3.5 volutions. Thin, semi-transparent, corneous. Yellowish-brown in colouration. Oval shaped, bluntly pointed at top.

Radula (Figure 4A). Taenioglossate, formula 2 + 1 + 1 + 1 + 2. Teeth solid. Central tooth triangular with one single strong cusp having a triangular cutting edge, laterally supported on both sides and weakly in the centre. Lateral teeth laterally thickened, with two to three moderately strong inner cusps increasing in size outwards, one strong central cusp, and two to three very weak outer cusps. A weakly raised ridge present under outer lateral cusps. Variation in cusp numbers seen between rows, even within single radular membrane. Marginal teeth (Figure 4C) moderate in length,

marginals truncated apically. Distal end evenly serrated into well-separated denticles, numbering ~15 in inner marginals and ~18 in outer. Shafts carry very fine, shallow serrations on outer side near distal end.

Soft parts. Head-foot simple with two cephalic tentacles of equal length. No penis or neck furrow observed. Position of eyes indicated by an unpigmented bulge near base of cephalic tentacles. Gill monopectinate, not hypertrophied. Epipodial tentacles lacking, both anterior and posterior pedal glands present. All available material were fixed and preserved in 99% ethanol, tissues are thus brittle and bleached, preventing further detailed anatomical investigations.

#### DIMENSIONS

Largest known specimen 16.5 mm in shell height (Paratype #4) but with much corroded apex; maximum size is estimated to be up to 20 mm. Shell measurements for all available specimens are shown on Table 1.

#### COMPARATIVE REMARKS

*Provanna cingulata* n. sp. is most similar to *Provanna macleani* Warén & Bouchet, 1989 from wood falls of the East Pacific (Johnson *et al.*, 2010), which has very strong, numerous spiral cords like *P. cingulata* n. sp. and although axial sculpture is present it is very weak and indistinct (Warén & Bouchet, 1989). The periostracum colouration of both species is yellowish brown. There are, however, more numerous spiral cords which are more closely spaced in *P. cingulata* n. sp. compared with *P. macleani* (7–8 above suture vs 6 above suture, 8–12 basal ribs vs 5 basal ribs). Furthermore, the radula of *P. macleani* is very different from that of *P. cingulata* n. sp. in having a reduced and membranaceous central tooth (Warén & Bouchet, 1989), as the central tooth of *P. cingulata* n. sp. is prominent and solid. *Provanna macleani* is also much smaller in size with specimens only reaching 7.1 mm (Warén & Bouchet, 1989).

*Provanna cingulata* n. sp. may also confused with young specimens of *Provanna reticulata* Warén & Bouchet, 2009 from seeps off West Africa. Although the adult shells of *P. reticulata* have strong axial ribbing lacking in *P. cingulata* n. sp., in addition to spiral ribbing, and therefore easily separable, the young shells only have spiral ribbing (Warén & Bouchet, 2009, figure 10I) and are very similar to those of *P. cingulata* n. sp. The radula differs slightly between the two species, however, with regards to the inner cusps of laterals. In *P. reticulata* there are always two inner cusps, whereas in *P. cingulata* n. sp. there are two or three (variable among rows within same specimen). Otherwise the juveniles of the two species are difficult to separate.

Except these two, *Provanna cingulata* n. sp. differs from the other described species of *Provanna* by having the sculpture consisting entirely of spiral cords and completely lacking in axial ribbing. Furthermore, its size when fully grown is unusually large for the genus.

The shell of *Provanna cingulata* n. sp. bears a certain resemblance to *Cordesia provannooides* Warén & Bouchet, 2009 but it lacks the characteristic penis. *Provanna cingulata* n. sp. also has radula and protoconch characteristic of genus *Provanna*, which significantly differs from those of *Cordesia* (Warén & Bouchet, 2009). Therefore we can safely exclude the possibility of the new species being a member of *Cordesia* and place it in the genus *Provanna* with good confidence.

Only specimens up to 11.4 mm shell height (Paratype #2) were collected alive, all large specimens were dead collected.

In such dead collected specimens the apex was always corroded with only about four whorls remaining, although they were also clearly corroded from the aperture inwards indicating they may have been dead for quite a while. If the protoconch is intact when alive, the largest specimens could perhaps reach 20 mm in shell length. The vast majority of the live collected specimens had a well-preserved apex.

#### DISTRIBUTION

So far only known from the type locality, the Shinkai Seep Field.

#### ETYMOLOGY

'*Cingulatus*' (Latin) meaning girdled or belted, referring to the numerous spiral cords prominently girdling the whorls. Used as an adjective.

#### MOLECULAR PHYLOGENY

Figure 5 shows the phylogenetic tree produced by ML (HKY + G + I) method, clearly showing that *P. cingulata* n. sp. is nested within a monophyletic clade with other species currently classified in the genus *Provanna*. All other currently recognized abyssochrysoide genera are also grouped together as monophyletic clades. This result supports the morphological characteristics in placing *P. cingulata* n. sp. in the genus *Provanna*. According to the resulting tree, of those species whose COI sequences are available on GenBank, *Provanna cingulata* n. sp. is closest related to an undescribed *Provanna* species from Beebe vent field on the Mid-Cayman Spreading Centre represented by a single COI sequence on GenBank ('*Provanna* sp. SP-2014' from Plouviez *et al.*, 2015). The two specimens of *P. cingulata* n. sp. sequenced differed only by 0.16% pairwise distance in their COI sequence (1197 bp), while their pairwise distance from the undescribed Mid-Cayman *Provanna* was 5.4–5.8%. *Provanna cingulata* n. sp. was genetically distinct from *P. macleani*, the morphologically most similar described species, with the two separated by the undescribed *Provanna* from Mid-Cayman.

#### DISCUSSION

*Provanna cingulata* n. sp. co-occurs with several other gastropod species in the SSF, including *Bayerius arnoldi* (Lus, 1981), a trochoid and a xylo-disculid. However, this is the only gastropod collected from the SSF that belongs to an obligatory chemosynthetically associated group (Sasaki *et al.*, 2010). *Provanna cingulata* n. sp. is thus likely to require chemosynthetically influenced habitats for survival; like *Calyptogena (Abyssogena) mariana* from the same seep field. It is also among the first definitive records of the superfamily Abyssochrysoidea from serpentinite-hosted chemosynthetic ecosystems, in addition to a recently described species of *Desbruyeresia* (Chen *et al.*, 2016). As the gill of *P. cingulata* n. sp. is not hypertrophied, it is unlikely to house endosymbiotic chemosymbionts. The radula ribbon shows significant and obvious wear in the anterior section (Figure 4B) compared with a more posterior, pristine, section (Figure 4A), strongly suggesting a deposit feeder which uses the radula to graze bacteria and perhaps also other particles. The same has been suggested for other *Provanna* species, for example *P. variabilis* Warén & Bouchet, 1986 (Warén & Bouchet, 1993); and all *Provanna* species are presumed to have similar feeding habits (Sasaki *et al.*, 2010). The well-



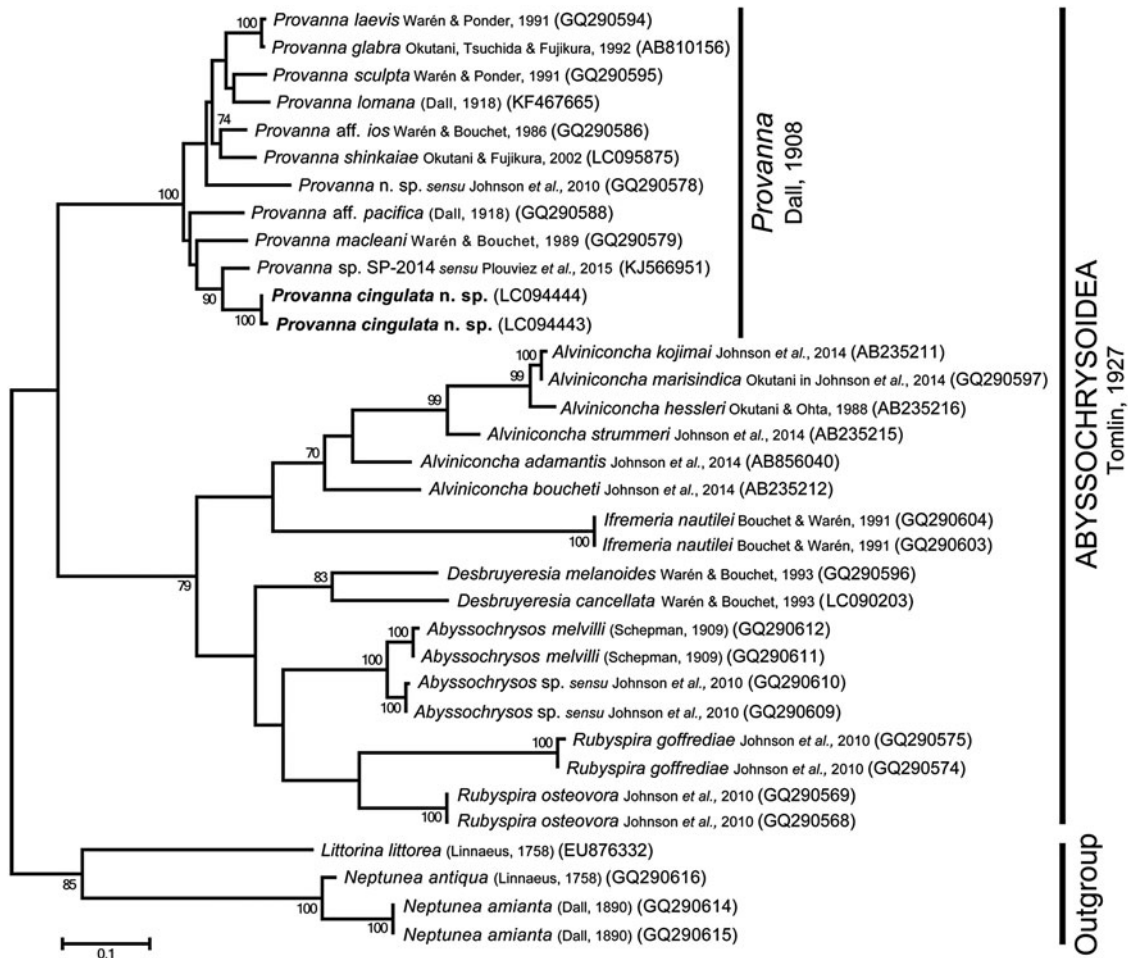


Fig. 5. Phylogenetic tree of Abysochrysoidea based on 411 bp of the COI gene, showing the systematic position of *Provanna cingulata* n. sp. within the genus *Provanna*. Node values are ML (HKY + G + I) bootstrap values. GenBank accession numbers of the COI sequences used are shown in parentheses.

preserved protoconch in *P. cingulata* n. sp. is virtually identical to that of the *Provanna* spp. protoconch illustrated in Warén & Ponder, 1991 (Figure 1), and is indicative of lecithotrophic development without a planktotrophic stage (Sasaki *et al.*, 2010).

With a maximum shell height of 16.5 mm and estimated to reach 20 mm if the apex is intact, *P. cingulata* n. sp. is the largest *Provanna* species so far known. Of the 25 described recent and fossil *Provanna* species, the largest is *P. reticulata* Warén & Bouchet, 2009 from West Africa seeps which reaches a shell height of 14.0 mm (see Table 1 in Amano & Little, 2014). Another large *Provanna* species from the Antarctic hydrothermal vents of East Scotia Ridge (Rogers *et al.*, 2012) is currently under description, but that species only reaches 15.0 mm in shell height (Katrin Linse, pers. comm.).

Furthermore, *Provanna cingulata* n. sp. was collected from 5687 m deep, which is so far the deepest occurrence record of not only *Provanna* but also the whole superfamily Abysochrysoidea. Prior to the discovery of *P. cingulata* n. sp., the deepest abysochrysoidea known were *P. abyssalis* Okutani & Fujikura, 2002 and *P. shinkaiiae* from methane seeps on the landward slope of Japan Trench (Okutani & Fujikura, 2002; Fujikura *et al.*, 2012). *Provanna abyssalis* was collected from a depth of 5379 m and *P. shinkaiiae* from 5343 m. These are followed by a yet-undescribed species of *Provanna* from the Beebe hydrothermal vent field of the Mid-Cayman Spreading Centre, the deepest hydrothermal

vent field associated with a spreading centre on earth (German *et al.*, 2010), reaching the depth of 4966 m (Connelly *et al.*, 2012; Plouviez *et al.*, 2015). All other abysochrysoidea have bathymetric ranges reaching less than 4000 m. The discovery of *P. cingulata* n. sp. thus extends the bathymetric range of Abysochrysoidea by more than 300 m.

Interestingly, although *P. cingulata* n. sp. is closely related to the Mid-Cayman species, these two are not closely related to *P. shinkaiiae* which was also included in the phylogenetic analyses. This rejects the possibility of a single deep clade within *Provanna*, and the adaptation to living in very deep habitats probably evolved more than once in the genus. To further elucidate and consolidate the evolutionary history of *Provanna* species, multi-gene phylogenetic analyses with divergence time constraints including these species should be carried out in the future to be added to the current knowledge as presented by Johnson *et al.* (2010).

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