**On the systematics and ecology of two new species of *Provanna* (Abyssochrysoidea: Provannidae) from deep-sea hydrothermal vents in the Caribbean Sea and Southern Ocean**

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SHORT RUNNING HEAD
ECOLOGY OF TWO NEW *PROVANNA* SPECIES

# ABSTRACT

The recent discoveries of deep-sea hydrothermal vent fields on the Mid-Cayman Spreading Centre, Caribbean (Beebe Vent Field, 4956–4972 m depth) and the East Scotia Ridge (ESR), Southern Ocean (E2 and E9 vent fields, 2394–2641 m depth) provided comprehensive collections of two new provannid species, *Provanna beebei* sp. nov. and *P. cooki* sp. nov. Morphological and molecular taxonomy (530 bp of the mitochondrial COI gene) confirmed *Provanna beebei* sp. nov. and *P. cooki* sp. nov. as distinct species which are formally described with notes on their distribution, habitats, and species associations. Bayesian and Maximum Likelihood analyses support the placement of *P. beebei* sp. nov. and *P. cooki* sp. nov. within the genus *Provanna* and indicate a well-supported clade containing the two deep-sea new species and the abyssal West Pacific *P. cingulata*. These are the first records of *Provanna* at hydrothermal vents in the Caribbean Sea and Southern Ocean, extending the known geographic range of the genus. For the first time, intraspecific phenotypic variation and size fragmentation have been reported for *Provanna*. At the ESR the size-frequency distributions and medians of shell size in *P. cooki* sp. nov. varied significantly between E2 and E9 as well as between diffuse flow and high temperature venting habitats within each field. Variation in shell sculpture with habitat was also observed in *P. cooki* sp. nov.

# INTRODUCTION

Hydrothermal vent environments have become important models for understanding the origins and limits of life (e.g., Martin *et al*., 2008; Takai, 2011; Clarke, 2014), and the evolution of island-like communities in the deep sea (Rogers *et al*., 2012), in addition to being a potential source of natural products (Thornburg *et al*., 2010) and mineral resources (Boschen *et al*., 2013). Assemblages of vent-endemic species and their habitats are patchy and transient in nature (Collins *et al*., 2013), linearly spread along oceanic ridges or haphazardly distributed throughout back-arc basins at low to high latitudes (Beaulieu *et al*., 2013).

Since the discovery of hydrothermal vents along the Galápagos Rift in 1977, gastropods have become recognised as a major component of the chemosynthesis-based faunal assemblages at vent fields in western Pacific back-arc basins, on the Central- and Southwest-Indian Ridges, on the Mid-Atlantic Ridge, and the Mid-Cayman Spreading Centre (Nakamura *et al*., 2012; Nye *et al*., 2013a and references therein; Wheeler *et al*., 2013). Gastropods are one of the most species-rich macrofaunal taxa from hydrothermal vents and hydrocarbon seeps (Warèn, Bouchet & von Cosel, 2006) and with large species including *Alvinoconcha* spp. (Okutani & Ohta, 1988; Johnson *et al*., 2015), *Ifremeria nautilei* Bouchet & Warèn, 1991, or morphologically unusual ones, such as the scaly-foot gastropod (Warèn *et al*., 2003), *Chrysomallon squamiferum* Chen *et al*., 2015, gaining considerable attention. Various types of symbiotic relationships with bacteria have also widened the interest in gastropods in deep-sea chemosynthetic environments (e.g., Borowski *et al*., 2002; Suzuki *et al*., 2006; Bates, 2007), and other gastropod species associated with vents, seeps, and organic-falls (animal carcasses, wood) graze on bacterial biofilms (Johnson *et al*., 2010).

The superfamily Abyssochrysoidea Tomlin, 1927, as currently understood (Sasaki *et al*., 2010), includes the extant families Abyssochrysidae Tomlin, 1927 and Provannidae Warén & Ponder, 1991. Based on reconstruction of phylogenetic relationships within the Abyssochrysoidea using a multi-gene data set, Johnson *et al*. (2010) suggest the paraphyly of the Provannidae. This family includes the extant genera *Provanna* Dall, 1918, *Alviniconcha* Okutani & Ohta, 1988, *Cordesia* Warèn & Bouchet, 2009, *Desbruyeresia* Warèn & Bouchet, 1993, and *Ifremeria* Bouchet & Warèn, 1991 (Reynolds *et al*. 2010; Sasaki *et al*. 2010), and is known from vents,

seeps, and organic falls in the deep ocean (Amano & Little, 2014). *Provanna* is the most species-rich of these genera, so far represented by 19 described species with an overall bathymetric range of 450 to 5687 m depth (Table 1), and seven fossil species of *Provanna* have been formally described (Amano & Little, 2014). Most *Provanna* species graze on filamentous bacteria, although some are deposit feeders (Sasaki *et al*., 2010). Bergquist *et al*. (2007) suggest that *P. variablis* Warèn & Bouchet, 1986 may host symbiotic bacteria, but doubts have been raised about that interpretation based on the anatomy of the species (Sasaki *et al*., 2010).

During recent deep-sea expeditions to newly discovered hydrothermal vent fields on the Mid-Cayman Spreading Centre (RV 'Atlantis' voyage 18-16 and RSS James Cook voyage JC82; Connelly *et al*., 2012) and the East Scotia Ridge (RSS James Cook voyage JC42; Rogers *et al*., 2012), provannid gastropods of different morphotypes and sizes were observed (Fig. 1) and sufficiently sampled for subsequent taxonomic and ecological analyses. Species from chemosynthetic habitats like vent and seep are often endemic and newly discovered sites often led to descriptions of new taxa. Vrijenhoek (2009) recognised the challenges in the identification of chemosynthetic fauna based on insufficient morphological information and genealogical information, and the uncertainties in accounting species diversity by the presence of cryptic species as well as species with high phenotypic plasticity. Insufficient sampling within chemosynthetic sites that exhibit fine-scale heterogeneity in physical and chemical parameters (Luther *et al*., 2001) may only collect a subset of the intraspecific morphological variability present or miss size-or sex-based habitat partitioning in species microdistributions (Bates, 2008). Ecological knowledge on deep-sea species is still scarce compared with that on intertidal or terrestrial species (e.g. Wilding, Butlin & Grahame, 2001; Irie, 2006; Nakano & Spencer, 2007; Charrier *et al*., 2013), and far less is known about the relative importance of local adaptation vs ecophenotypic variation as the causes for morphometric variability in deep sea habitats.

The aims of this paper are therefore: (1) to describe the new species of *Provanna* and compare their morphologies with those of other described *Provanna* species; (2) to use COI mtDNA sequence data to determine if these species are genetically distinct from each other and all other provannid species in the GenBank database; (3) to determine if there is significant variation in the size-frequency distributions of the sampled populations of the new species, within and between vent

fields where samples allow, and (4) to assess spatial variation in morphology among populations of vent-endemic species.


# MATERIAL AND METHODS


*Sample collection*

Specimens were collected from the Beebe Vent Field (4956–4972 m depth) at the Mid-Cayman Spreading Centre, Caribbean (Connelly *et al*., 2012), and the E2 and E9 vent fields (2394–2641 m depth, 400 km distance apart), East Scotia Ridge, Southern Ocean (Rogers *et al*., 2012), using the suction samplers of the 'Jason-II' and 'Isis' remotely operated vehicles (ROVs) (Fig. 1). Detailed site descriptions and maps of the Beebe Vent Field were given in Connelly *et al*. (2012) and for E2 and E9 in Rogers *et al*. (2012). During both expeditions, provannid specimens were preserved in 96% pre-cooled ethanol. Note that 96% ethanol often makes tissue brittle.


*Morphology*

Shell morphometrics (length, width) were taken to the nearest 0.1 mm using digital Vernier callipers. Scanning electron microscopy of shells, opercula (both uncoated), and radulae (gold coated) was performed using a Hitachi TM3000 scanning electron microscope at the British Antarctic Survey. Shell and operculum were placed in an ultrasonic cleaning bath for three minutes. Radulae were prepared by dissecting the radula sacs and dissolving them in 25% potassium hydroxide to expose the radulae, then cleaned using fine brushes and placed onto carbonate sticker SEM stubs.


*Molecular Genetics*

Genomic DNA was successfully extracted from *Provanna* from the Beebe field with the DNeasy Blood & Tissue Kit (Qiagen, Crawley, West Sussex, United Kingdom) as directed by the manufacturer. PCR amplifications of the COI mtDNA region were performed using high-fidelity velocity polymerase (Bioline, London, UK) in 15-mL

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reactions with 0.2-mM LCO and HCO variant primers (Goddall-Copestake, 2014), c.3 ng of template and a thermocycling profile of 50 s at 98°C, 35 cycles of [10 s at 98°C, 10 s at 56°C], ending with 90 s at 72°C. Sequencing was performed at Source Biosciences, Cambridge, UK.

For *Provanna* from E2 and E9 fields in the ESR, genomic DNA was extracted using the DNeasy Blood & Tissue Kit and the COI region was amplified with the primer pair LCO1490 and HCO2198 (Folmer *et al.,* 1994). The polymerase chain reaction was carried out in 20 μl reactions, including 1 μl each of primer mix at 10 μM and 15–30 ng of template (15–30 ng/μl). PCR protocol was 1 min at 95°C followed by 35 cycles of [15 s at 95°C, 15 s at 40°C, 30 s at 72°C], ending with 7 min at 72°C. Successful PCR products were purified using ExoSAP-IT (Affymetrix), following standard protocols. Cycle sequencing reactions were carried out in the following protocol: 1 min at 96°C, 25 cycles of [10 s at 96°C, 5 s at 50°C, 75 s for 60°C], ending with 4 min at 60°C. Sequences were resolved using an Applied Biosystems 3130xl DNA sequencer (JAMSTEC).

The complimentary sequences from the forward and reverse primers were aligned to check the sequencing accuracy using the software package GENEIOUS 5.6 (Drummond *et al.,* 2011). The resulting sequences were used for the phylogenetic analyses also using MEGA 6, with abyssochrysoid COI sequences available on GenBank. Sequences of the whelks *Neptunea amianta* (Dall, 1890) and *N. antiqua* (Linnaeus, 1758), and the periwinkle *Littorina littorea* (Linnaeus, 1758) from distantly related gastropod groups were included as outgroup taxa following Johnson *et al*. (2010) and Chen *et al.* (2016).

Bayesian analysis was conducted using MrBayes 3.2 (Ronquist *et al.,* 2012). Prior to the Bayesian analyses, the most suitable nucleotide substitution model was selected, using the Bayesian information criterion in PartitionFinder v. 1.0.1 (Lanfear *et al.*, 2012), which was GTR+I+G for the first and the second codon and HKY+G for the third codon. Metropolis-coupled Monte Carlo Markovchains were run for 5 million generations. Topologies were sampled every 100 generations, with the first 25% discarded as 'burn-in' to ensure that chains had converged. Convergence was checked using Tracer v. 1.6 (Rambaut, Suchard & Drummond, 2013) to determine the adequate 'burn-in' values. The maximum-likelihood (ML) tree was generated in the software MEGA 6 (Tamura *et al*., 2013) with 2000 bootstraps using the GTR+I+G

substitution model, determined as the most suitable by the Model Selection program incorporated in the same programme.

The GenBank accession numbers for the new generated partial sequences of COI MtDNA are MK790057–MK790067.

*Type Repositories*

The holotypes are deposited in the invertebrate collection at the Natural History Museum, London (NHMUK), paratypes as well as the specimens used in the morphometric analysis are deposited in the Natural History Museum, London, UK (NHMUK), the Cambridge Zoology Museum, Cambridge, UK (UMZC), the Museum National d'Histoire Naturelle (MNHN), Paris, France, and the University Museum, the University of Tokyo (UMUT).

# SYSTEMATIC DESCRIPTIONS

**Superfamily ABYSSOCHRYSOIDEA Tomlin, 1927**
**Family PROVANNIDAE Warén and Ponder, 1991**
**Genus *Provanna* Dall, 1918**

***Provanna beebei* sp. nov.**

(Figs 1–3)

*Provanna* sp.- Plouviez *et al*., 2015: Table 1, fig. 2E

*ZooBank registration*: urn:lsid:zoobank.org:act:92F95A7B-3BD0-4DF2-964D-CCB87CB80D20

*Type material*: Holotype (NHMUK 20190543; Fig. 2G, H): Beebe Chimlets, Beebe Vent Field, Mid-Cayman Spreading Centre, 4972 m depth, 18°32.799′N, 81°43.139′W, sampled 11 January 2014, ROV 'Jason-2', RV 'Atlantis' voyage 18-16, 96% ethanol.

Paratypes: Paratype 1 (NHMUK 20190544; Fig. 2A, B), Paratype 2 (NHMUK 20190545; Fig. 2C, D), Paratype 3 (NHMUK 20190546; Fig. 2E, F) have the same

collecting data and preservation as the holotype. Paratype 4 (NHMUK 20190547; Fig. 2I, J), Paratype 5 (NHMUK 20190548; Fig. 3A, B), Paratype 6 (NHMUK 20190549; Fig. 3C): Anemone Field, Beebe Vent Field, Mid-Cayman Spreading Centre, 4956 m depth, 18°32.815′N, 81°43.101′W, sampled 24 February 2014, ROV 'Isis', RRS 'James Cook' voyage JC82. Paratypes 5 and 6 are dissected and mounted for SEM.

*Additional materials examined:* 103 specimens from the same lot as the holotype and Paratypes 1–3 (NHMUK 20190550); 45 specimens from the same lot as Paratypes 4–6 (NHMUK 20190551) and DNA was extracted from 2 specimens of these. All specimens are preserved in 96% ethanol.

*Etymology*: The new species is named after the type locality, the Beebe Vent Field.

*Description*: Shell (Fig. 2) sturdy, length greater than width, maximum dimensions 12.7 mm long, 8.8 mm wide. Surface and apical region corroded in all specimens with protoconch and early teleoconch whorls lost. Surface covered by metallic, golden periostracum beneath a black entrustment, varying in thickness and coverage between specimens.

Shell surface cancellated in appearance as a result of spiral and axial sculptures. Penultimate and body whorls have 3 or 4 spiral ribs with occasional secondary ones that are crossed by weakly defined axial ribs with granules at intersections. The base of the body whorl is weakly inflated, bearing 4−9 strong spiral ribs that diminish in prominence and become smooth towards the abapical end. Umbilicus absent.

Aperture pyriform in outline. No nacre or lustre on internal wall or shell exterior. Outer lip thin and crenulated; crenulations correspond to extremities of spiral ribs and grooves between ribs, less pronounced in more corroded specimens to give appearance of smoother outer lip. Inner lip mildly curved, continuing to weakly twisted columellar lip which forms shallow sinus.

Operculum (Fig. 3A) smaller than aperture, transparent, paucispiral and oval in outline, length 3.3 mm, width 2.5 mm (from a specimen 9.1 mm in shell length, 7.3 mm in shell width; 2 whorls remaining but apex corroded; same lot as Paratype 5 and 6). Opercular retraction is deep.

Radula (Fig. 3B, C) taenioglossate, bilaterally symmetrical with the formula 2-1-1-1-2. Length 1.4–3.5 mm, width 0.2–2.1 mm, with 78–120 transverse rows. Central tooth differentiated in form from lateral tooth; low trapezoid shape, wider proximally than distally, with elongated and pointed basal edges, and single incurved triangular blunt cusp. Lateral tooth of similar size to central tooth, elongate oval shape with incurved inner edge featuring large, round central cusp with 2 or 3 smaller blunt cusps on either side which decrease in size away from central cusp. Inner and outer marginal teeth (Fig. 3C) similar in size, mushroom shaped with elongated stalk and broadened recurved top rake-like, bearing 13–17 comb-like cusps with several flanking serrations.

Soft parts opaque white after preservation, with no visible eye.

*Distribution and habitat*: Known only from the type locality, the Beebe Vent Field, Mid-Cayman Spreading Centre, Caribbean in 4956–4972 m water depth (18°32.799′–18°32.815′N, 81°43.101′–81°43.139′W), and in densities of 263 $\pm$ 67 *s.d.* individuals m$^{-2}$ (*n* = 8 images analysed, area estimated using lasers with known distances apart). See Connelly *et al*. (2012) for an overview of the geological, geochemical and biological setting of the Beebe vent field.

The new species has been observed at and sampled from the Anemone Field area (Fig. 1C, D) on the upper slopes of the west mound of the Beebe Vent Field, at temperatures of 4.07–4.75°C, in close proximity to diffuse hydrothermal flow from fissures. Here it is found on black rock with mats of filamentous microbes, actinostolid anemones, and the alvinocaridid shrimp *Rimicaris hybisae* Nye, Copley & Plouviez, 2012 (Fig. 1C, D).

The new species has also been sampled and recorded on black rock near to small micro-chimneys and diffuse flow sources with *Rimicaris hybisae* and the ophiuroid *Ophioctinella acies* Tyler *et al*., 1995 at the Beebe Chimlets (Fig. 1A, B) on the northeast slope of the west mound of the Beebe vent field.

*Remarks*: *Provanna beebei* sp. nov. superficially resembles *Provanna chevalieri* Warén & Bouchet, 2009 and *P. macleani* Warén & Bouchet, 1989. The new species is readily distinguished from *P. chevalieri* by the absence of a siphonal canal and matt (vs shiny) periostracum. *Provanna beebei* sp. nov. exhibits spiral and axial shell

sculpture, whereas *P. macleani* has primarily spiral sculpture, and a radula characterised by a reduced, thin central tooth, without basal supports.

*Provanna beebei* sp. nov. differs from the recently described *P. cingulata* Chen, Watanabe & Ohara, 2018 and from *P. cooki* sp. nov. in having a spiral and axial sculpture while the latter two species have only spiral ribs.

The new species is geographically closest to *P. ademetoides* (Florida Escarpment) and *P. sculpta* (Louisiana Slope) from cold seeps, but differs markedly in sculpture from these species. This record extends the known geographic range of *Provanna* into the Caribbean (see Table 1).

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***Provanna cooki* sp. nov.**

(Figs 1, 4–6)

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Provannid gastropod - Rogers *et al*., 2012: 7, 9

Provannid sp. 1 - Rogers *et al*., 2012: Table 2

Provannid sp. 2 - Rogers *et al*., 2012: Table 2

Provannidae sp.1 and sp. 2 – Reid *et al*., 2013: 3, 8, Table 3

Provannidae sp.1 and sp. 2 – Rogers & Linse, 2014: 243, Table 1

*ZooBank registration*: urn:lsid:zoobank.org:act:9C808A95-4F19-4AC9-8B05-E45E8DDCF237

*Type material*: Holotype (NHMUK 20190552; Fig. 4K, L): SW Field, East Scotia Ridge segment E9, 2396 m depth, 60°02.823′S, 29°58.696′W, sampled 2 Feb 2010, ROV 'Isis', RRS 'James Cook' voyage JC42, 96% ethanol. Shell length 7.2 mm, ultimate whorl width 4 mm, 7 whorls, 3 distinct spirals.
Paratypes: Paratype 1 (MNHN-IM-2000-34740; Fig. 4A, B), Paratype 2 (UMZC 2019.5; Fig. 4C, D): Anemone Field, East Scotia Ridge segment E2, 2603 m depth, 56°05.277′S, 30°19.087′W, sampled 24 Jan 2010, ROV 'Isis', RRS 'James Cook' voyage JC42. Paratype 3 (UMZC 2019.6; Fig. 4E, F): Diffuse flow under the Dog's Head chimney, East Scotia Ridge segment E2, 2627 m depth, 56°05.335′S, 30°19.100′W, sampled 23 Jan 2010, ROV 'Isis', RRS 'James Cook' voyage JC42. Paratype 4 (UMUT RM33144; Fig. 4G, H): Anemone Field, East Scotia Ridge segment E2, 2603 m depth, 56°05.277′S, 30°19.087′W, sampled 24 Jan 2010, ROV

'Isis', RRS 'James Cook' voyage JC42. Paratype 5 (MNHN-IM-2000-34741; Fig. 4I, J), Marsh Tower, East Scotia Ridge segment E9, 2605 m depth, 60°02.807′S, 29°58.708′W, sampled 2 Feb 2010, ROV 'Isis', RRS 'James Cook' voyage JC42. Paratype 6 (NHMUK 20190553; Fig. 5A–C), Dog's Head, East Scotia Ridge segment E2, 2605 m depth, 56°05.313′S, 30°19.087′W, sampled 20 Jan 2010, ROV 'Isis', RRS 'James Cook' voyage JC42. Paratype 7 (NHMUK 20190554; Fig. 5D, E), Deep Castle, East Scotia Ridge segment E2, 2639 m depth, 56°05.325′S, 30°19.057′W, sampled 24 Jan 2010, ROV 'Isis', RRS 'James Cook' voyage JC42. Paratypes 1–5 are preserved in 96% ethanol, Paratypes 6 and 7 are dissected for SEM.

*Additional materials examined*: 107 specimens from same lot as the holotype (NHMUK 20190555) and of these 3 specimens extracted for DNA; 146 specimens from the same lot as Paratypes 1, 2, and 4 (MNHN-IM-2000-34742) and of these 3 specimens extracted for DNA; 20 specimens from the same lot as Paratype 3 (UMZC 2019.7) and of these 3 specimens extracted for DNA; 22 specimens from the same lot as Paratype 5 (MZC 2019.8); 1 egg case from the same lot as Paratype 5 (NHMUK 20190556); 1 specimen from the same lot as Paratype 6 (NHMUK 20190557); 13 specimens from the same lot as Paratype 7 (UMUT Mo-RM33145). All specimens are preserved in 96% ethanol.

*Etymology*: The new species is named after Captain James Cook, who discovered the South Sandwich Islands during his second voyage in 1775. The RRS 'James Cook' was the research ship used for the sampling of the new species, during its first voyage to the Southern Ocean.

*Description*: Shell (Figs 4, 5A) rather high, slender, turreted, length greater than width, maximum dimensions 15.0 mm long, 8.2 mm wide. Surface covered by a metallic, golden periostracum, beneath a rust-coloured or black encrustment. Surface and apical region corroded or covered in crust in specimens to varying degrees, with protoconch and early teleoconch whorls lost in some specimens. Protoconch about 1.5 whorls and no clear boundary between PI and teleoconch whorl visible (Fig. 5C). The absence of a PII indicates direct development in this species. The initial protoconch sculpture is finely granulated followed by an increasing presence of fine spiral lines.

The first teleoconch whorl diameter is about 240 μm and the about 300 μm at 1.5 whorls.

Shell sculpture exhibits marked variation between individuals, resulting in two morphotypes, one rather smooth (Figs 4A–H, 5A) and the other with spiral sculptures (Fig. 4I–L). In spiral ribbed specimens the penultimate and body whorls have 2–4 spiral ribs that are crossed by weekly defined axial ribs. Base of the body whorl weakly inflated, smooth towards the abapical end. Umbilicus absent.

Aperture oval in outline. Nacre and lustre absent on internal wall and shell exterior. Thin outer lip crenulated; crenulations correspond to extremities of spiral ribs and grooves between ribs, less pronounced in more corroded specimens to give appearance of smoother outer lip. Inner lip curved mildly, continuous with columellar lip.

Operculum (Fig. 5B) smaller than aperture, transparent, paucispiral with oval outline, length 1.3–3.1 mm, width 1.0–2.3 mm in specimens ranging from 4 to 12.4 mm in shell length. Opercular retraction is deep.

Radula (Fig. 5D, E) taenioglossate, bilaterally symmetrical with the formula 2-1-1-1-2. Length 0.8–3.6 mm, width 0.1–0.2 mm, with 23–128 transverse rows in specimens ranging from 4 to 12.4 mm in shell length. Central tooth differentiated in form from lateral teeth; low trapezoid shape, broader proximally than distally, with basal edges elongated and pointed, and an incurved triangular pointed cusp. Lateral tooth similar size to central tooth, elongate oval shape with incurved inner edge bearing 5 or 6 pointed cusps, sub-central cusp most prominent. Inner and outer marginal teeth (Fig. 5E) similar in size, mushroom shaped with elongated stalk and broadened incurved top bearing 13–17 comb-like cusps with several flanking serrations.

Preserved soft parts opaque white, with no visible eye.

*Distribution and habitat*: Known only from the type locality, hydrothermal vent and diffuse flow fields in 2394–2641 m water depth on the E2 and E9 segments of the East Scotia Ridge (ESR), Southern Ocean (56°05'S–60°02'S, 29°58'–30°19'W). The hydrothermal settings at the E2 and E9 segments of the ESR are characterised by black-smoker chimneys and diffuse flow areas of different low temperatures. Rogers *et al*. (2012) provide an overview of the geological, geochemical and biological setting of the E2 and E9 vent fields, and Marsh *et al*. (2012) describe the specific

chimney structures and diffuse flow areas within the E9 vent field. These authors also reveal the microdistributions of the fauna and defined specific assemblage types characterised by abiotic (e.g., distance from the vent fluid exit and temperature), and biotic parameters (e.g., presence and dominance of chemosynthetic taxa).

At the E2 vent field the 'smooth' morphotype of *Provanna cooki* sp. nov. dominated specimens collected from the sides and bottom of the Dogs' Head chimneys, where it was found with the crab *Kiwa tyleri* Thatje 2015 in Thatje *et al.*, 2015 and actinostolid anemones (Fig. 1E). The 'smooth' morphotype also dominated collections from the low-venting and peripheral diffuse flow fields at Diffuse, which also hosted the 'robust' morphotype of the stalked barnacle *Neolepas scotiaensis* (Buckeridge & Linse in Buckeridge, Linse & Jackson, 2013), and from the diffuse flow at Deep Castle. Most of the specimens were also coated with black, orange or both colours of mineral precipitates.

At the E9 vent field, only the 'ribbed' morphotype of *P. cooki* was present in collections, and the shells were not covered in mineral precipitates. The gastropods were collected from the 'barnacle assemblage' (as defined by Marsh *et al.*, 2012) at Marsh Tower (Fig. 1F) and SW Field. The 'barnacle assemblage' is characterised by the 'gracile' morphotype of *Neolepas scotiaensis* (Buckeridge *et al.*, 2013). Other biota observed in close proximity to the new species at the E9 vent field also included amphipods and microbial mats (Fig. 1F).

*Remarks*: *Provanna cooki* lay oval-shaped egg capsules of ~1.5 x 1 x 1 mm size that contain 5 or 6 eggs which hatch as juveniles through intracapsular metamorphosis (Fig. 6). The egg capsules can be connected to each other. This observation supports the suggestion by Gustafson & Lutz (1994) that in species of the genus *Provanna* a planktotrophic larval stage does not occur. The observed protoconch sculpture and whorl numbers in *P. cooki* sp. nov. are in agreement with the illustrations for *Provanna* sp. of Warén & Ponder (1991) and *P. variabilis* Gustafson & Lutz (1994) while the protoconch size is considerably smaller with ~300 μm for *P. cooki* sp. nov. to 411 μm in *Provanna* sp. and ~390 μm in *P. variabilis*.

The two new species described herein are readily distinguished from each other by differences in shell and radula characters, especially the shapes of the central and lateral teeth. *Provanna cooki* sp. nov. is morphologically similar to *P. cingulata* by displaying only a spiral shell sculpture but differs from this species by having a

lower number of spiral ribs and wider interspaces. The two also differ significantly in radular characteristics with the laterals of *Provanna cooki* sp. nov. bearing only a single inner cusp before the strong central cusp while the laterals of *P. cingulata* bear 2 or 3 inner cusps. The outer cusps of the lateral teeth of *P. cooki* sp. nov. is also much more prominent compared to *P. cingulata.*

This record furthers our existing knowledge of deep-sea biodiversity, as it is by far the southernmost record of *Provanna*, extending the known geographic range into the Southern Ocean (see Table 1). The Antarctic Ocean is defined as limit 60°S, the Southern Ocean is the waters within the Polar Front, which reach far further north than 60°S.

*Size-frequency analyses*

Shell measurements were taken from 148 specimens of *Provanna beebei* sp. nov. and 465 specimens of *Provanna cooki* sp. nov., of which 199 specimens belonged to the 'smooth' morphotype and came from E2 and 266 specimens to the 'ribbed' morphotype and from E9. Shell length correlated positively with shell width in both new species (Spearman correlation: *Provanna beebei* sp. nov., $R = 0.80$, $P < 0.0001$; *P. cooki* sp. nov., $R = 0.96$, $P < 0.0001$; Fig. 7). Specimens of *Provanna beebei* sp. nov. ranged in shell size from 1.5 to 12.7 mm long and 1.1 to 8.8 mm wide (Table 2; Fig. 8). The median shell lengths of this species were significantly larger at the Anemone Field than the Beebe Chimlets (Mann-Whitney $U$ test, $U = 1771.0$, $N_1 = 45$, $N_2 = 103$, $P < 0.01$). Specimens of *Provanna cooki* sp. nov. ranged in shell size from 1.3 to 15.0 mm long and 0.9 to 8.2 mm wide (Table 5; Fig. 8). Overall, specimens were significantly larger at the E9 Vent Field (median length 5.7 mm, IQR 3.5−8.5) than the E2 Vent Field (median length 4.0 mm, IQR 3.0−5.0) (Mann-Whitney $U$ test, $U = 16578.5$, $N_1 = 266$, $N_2 = 199$, $P < 0.001$). There was also significant variation in the size-frequency distributions of *Provanna cooki* sp. nov. collected from different locations within the E2 and E9 vent fields (Kruskal-Wallis multisample test, $H = 78.9$, 4 df, $P < 0.001$; Fig. 8). At the E2 vent field, median shell length was significantly larger at Diffuse than Deep Castle (Mann-Whitney $U$ test, $U = 1091.0$, $N_1 = 20$, $N_2 = 167$, $P < 0.05$), and at Dog's Head than Deep Castle (Mann-Whitney $U$ test, $U = 471.5$, $N_1 = 12$, $N_2 = 167$, $P < 0.01$). Within the E9 vent field, median shell length of *P.*

*cooki* sp. nov. was significantly larger at Marsh Tower than SW Field (Mann-Whitney $U$ test, $U = 3907.5$, $N_1 = 66$, $N_2 = 200$, $P < 0.001$).

*Species delimitation analysis*

Partial sequences of the COI mtDNA barcoding regions were amplified from two specimens of *Provanna beebei* sp. nov. and nine specimens of *P. cooki* sp. nov. The two new partial COI sequences of *P. beebei* sp. nov. were identical to each other but differed by 1.4% uncorrected p-distance (over 623 bp; only 0.2% over 530 bp used for phylogenetic reconstruction, see below) from the previously published sequence for specimen *Provanna* sp. SP-2014 (KJ566951) collected during the R/V *Atlantis* expedition AT18-16 from the Mid-Cayman Spreading Centre vents (Plouviez *et al.*, 2015). Partial COI sequences of the nine specimens of *P. cooki* sp. nov. provided two haplotypes, which varied only by 0.2% uncorrected p-distance from each other (over 638 bp). Within the three specimens sequenced from the same lot as Paratype 3, both haplotypes were present when the morphology of all three specimens were ribbed. The dominant haplotype (with seven individuals out of nine) occurred across all three lots sequenced for COI. These results confirmed the 'smooth' and 'ribbed' morphotypes as one MOTU (molecular operational taxonomic unit).

The Bayesian and ML analyses using a 530 bp alignment of the COI gene provided the same topology within the genus *Provanna* but differed in the relative position of other abyssochrysoid genera (although all currently accepted genera were well-supported in both) and the positions of species within the genus *Alviniconcha*. Here the Bayesian topology is shown with Bayesian posterior probabilities and ML bootstrap support values on concurrent nodes, where ML values are lacking the ML topologies differed (Fig. 8). The species of *Provanna* clustered in a single supported clade within the Abyssochrysoidea (Bayesian/ML support of 99%/80%), although the ML support is not very strong. *Provanna beebei* sp. nov. and *P. cooki* sp. nov. came out as well supported MOTUs (Bayesian/ML support of 99%/100% and 99%/99%, respectively) with a p-distance of 4.5% between each other over 530 bp used for the analyses (3.9% over 638 bp), both distinct from all other species of *Provanna* (Fig. 9). *Provanna beebei* sp. nov. and *P. cooki* sp. nov. were recovered as sister taxa within *Provanna* and together with *P. cingulata* they formed a well-supported clade (Bayesian/ML support of 99%/95%; Fig. 9).

# DISCUSSION

Taxonomic analysis of provannid gastropods from the Beebe Vent Field and the E2 and E9 vent fields reveals two new species of *Provanna* that are distinguished from each other, and from previously described species, by a combination of molecular and morphological features. The specimen from the Cayman Trough sequenced in Plouviez *et al.* (2015) is suggested to be the same species as *P. beebei* sp. nov. as described herein. The two new species appear to be genetically closely related based on their COI sequences, and forms a clade with *P. cingulata* from the Western Pacific.

*Provanna cooki* sp. nov. exhibits considerable variation in shell morphology. Appreciable variation is described in *P. variabilis* Warén & Bouchet, 1986 (within and between specimens), but this variation in *P. variabilis* does not appear to follow any clear pattern, such as size dependency. The two morphotypes of *P. cooki* sp. nov. differing in shell sculpture (initially identified as two morphotypes, see Rogers *et al.*, 2012) is similar to that found in the stalked barnacle *Neolepas scotiaensis* at the E2 and E9 vent fields (Buckeridge *et al.*, 2013). The 'smooth' morphotype of *P. cooki* sp. nov. and the 'robust' morphotype of *N. scotiaensis* occupy sites of low hydrothermal activity at the E2 vent field, whereas the 'ribbed' morphotype of *P. cooki* sp. nov. and the 'gracile' morphotype of *N. scotiaensis* inhabit areas near the venting source at the E9 vent field. The lepetodrilid limpet *Leptodrilus* sp., which is known from the same vent sites at E2 and E9 but also in the nearby hydrothermally active Kemp Caldera, showed an extraordinary range in shell morphometrics between E2/E9 and the Kemp Caldera, but no variability in shell pattern linked to low and high venting habitats (K. Linse *et al.*, unpubl. data).

*Provanna* belongs to the superfamily Abyssochrysoidea, a group that seems to display high morphological plasticity. Warén & Bouchet (1993: 74) remarked that the genus displays simple and highly variable shells, often with two morphotype at the same site making indisputable species identification difficult. Since then, several species of *Provanna* have been described (Table 1) but no intraspecific morphometric data comparable to those on *P. beebei* sp. nov. and *P. cooki* sp. nov. have been

available. The initial molecular analysis presented here based on COI diversity on *P. cooki* sp. nov. leads to the speculation that two distinct ecophenotypes may be present in this species and makes it a suitable species for further studies on the environmental and molecular drivers of plasticity in vent species. Since E2 was dominated by the smooth form and only the ribbed form was present in E9, we speculate that variations in abiotic factors (e.g., temperature, transition metals, and sulfide) between E2 and E9 vent fields (Rogers *et al.*, 2012; Hawkes *et al.*, 2013; James *et al.*, 2014) may have driven the morphological differences. James *et al.* (2014) reported the composition and concentrations of hydrothermal fluids from diffuse flow sites at E2 and E9 in their close vicinity. Although temperatures of fluids at the habitat of *P. cooki* sp. nov. at E2 (diffuse flow below Dog's Head chimney, 20°C; Anemone Field, 3.5°C) and E9 (Marsh Tower, 5°C; SW Field, 20°C) were within a similar range, some chemical concentrations differed significantly between the E2 and E9 sites, including Cs (E2: 3.5–26.5 nmol/kg; E9: 0.37–6.33 nmol/kg;), Mn (E2: 9.76–94.2 µmol/kg; E9: 0.82–18.9 µmol/kg), and Fe (E2: 2.17–4.13 µmol/kg; E9: 0.0–5.4µmol/kg).

Spatial variation in size within the E2 and E9 vent fields is another feature of *Provanna cooki* sp. nov., and has also been reported for *Leptodrilus* sp. and *Neolepas scotiaensis* from the same assemblages (Buckeridge *et al.*, 2013; K. Linse *et al.*, unpubl. data). Similar spatial size variation has been found in other vent taxa and explained by either size partitioning based on age or sex as in the lepetodrilid *L. fucensis* (Bates, 2008; Kelly & Metaxas, 2008), the shrimp *Rimicaris hybisae* (Nye, Copley & Tyler, 2013b), and the alvinellids *Paralvinella palmiformis* and *P. hessleri* (Copley *et al.*, 2003; V. Nye & J. Copley, unpubl. data) or by size difference in ecophenotypes like in the tubeworm *Ridgeia piscesae* (Tunnicliffe, St Germain & Hílario, 2014) and the stalked barnacle *Neolepas scotiaensis* (Buckeridge *et al.*, 2013).

The two new species reported herein extend the known distribution of *Provanna* and provide the first records of the genus in the Caribbean and Southern Ocean. Although the vent fauna at the Mid-Cayman Spreading Centre (MCSC) appears to be largely distinct at the species level (Nye, 2014), the presence of shrimp-dominated faunal assemblages at the Beebe and Von Damm vent fields supports higher-level taxonomic affinities with the Mid-Atlantic Ridge (MAR) vent fauna (Connelly *et al.*, 2012; Nye, Copley & Plouviez, 2012; Plouviez *et al.*, 2015). However, the MCSC vent fauna also exhibits faunal affinities with the West Pacific

and other provinces (Nye, 2014; Plouviez *et al*., 2015). *Provanna beebei* sp. nov. appears to be morphologically closest to *P. macleani* from cold seeps and organic fall in the East Pacific while genetically (COI gene only) forms a clade with the Southern Ocean *P. cooki* sp. nov. and West Pacific *P. cingulata*, thus further complicating the interpretation of the biogeographic alliance of the MCSC vent fauna with that of other provinces.

Biogeographic analyses suggest that the East Scotia Ridge (ESR) vent fauna forms a distinct biogeographic province with a unique faunal assemblage and structure, but with some fauna shared with vents in the west and southwest Pacific, and the MAR (Rogers *et al*., 2012; Nye, 2014). The discovery of a new species of *Provanna* at the ESR vents suggests that the environmental conditions of the Southern Ocean do not act as a barrier for this genus, and highlights the unique species composition of the ESR vent fauna assemblage.

The recent exploration and investigation of hydrothermal vent fields in the Caribbean and Southern Ocean has provided an opportunity to enhance existing knowledge of biodiversity in the deep sea and the biogeography and evolutionary history of vent fauna. The discovery of these two new species underscores the existence of undiscovered biodiversity in off-axis vents, Antarctic and deep-sea settings. The molecular Bayesian and ML analyses based on the COI gene only resulted in a clade of varying support containing the Caribbean *P. beebei* sp. nov., the West Pacific *P. cingulata* and Southern Ocean *P. cooki* sp. nov. but was unable to resolve the phylogenetic relationships within *Provanna*. A multi-gene analysis is required to elucidate the evolutionary history, phylogenetic and biogeographic relationships of the recent *Provanna* species.


# ACKNOWLEDGEMENTS

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# Figure Legends

**Figure 1.** *Provanna beebei* sp. nov. (**A−D**) and *P. cooki* sp. nov. (**E, F**) *in situ*. **A.** Beebe Chimlets with shrimp *Rimicaris hybisae*. **B.** Beebe Chimlets with ophiuroid *Ophioctinella acies* and actinostolid anemones. **C.** Anemone Field with *R. hybisae* and actinostolid anemones. **D.** Beebe Chimlets with microbial matt and actinostolid anemones. **E.** E2 Dog's Head with yeti crab *Kiwa* sp. and actinostolid anemones. **F.** E9 Marsh Tower with stalked barnacles *Neolepas scotiaensis*, amphipods and microbial matt. Images taken using the remotely operated vehicle (ROV) 'Isis'.
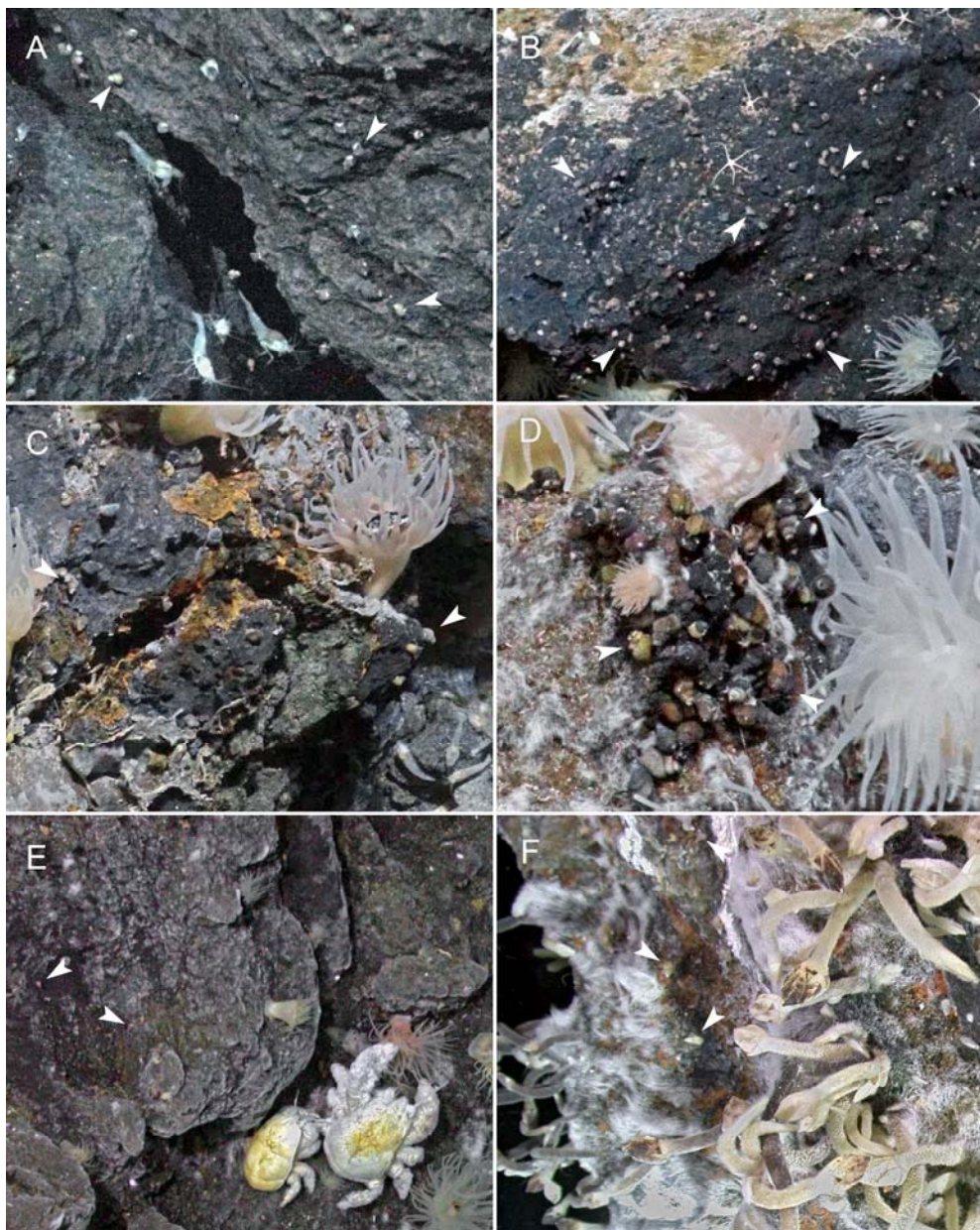
**Figure 2.** *Provanna beebei* sp. nov., Beebe Vent Field. **A, B.** Paratype 1 (NHMUK 20190544). **C, D.** Paratype 2 (NHMUK 20190545). **E, F.** Paratype 3 (NHMUK 20190546). **G, H.** Holotype (NHMUK 20190543). **I, J.** Paratype 4 (NHMUK 20190547). Scale bars = 1 mm.
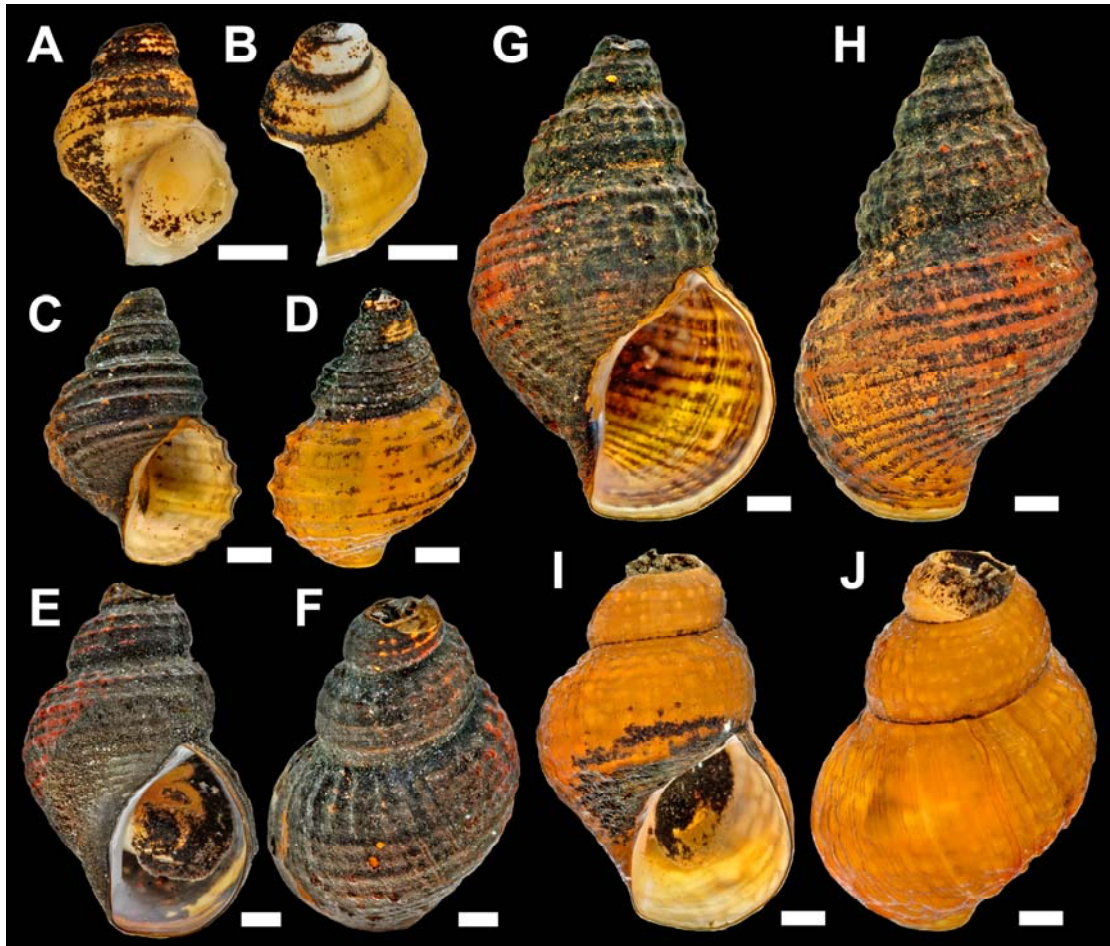
**Figure 3.** *Provanna beebei* sp. nov., SEM images. **A.** Operculum (Paratype 5, NHMUK 20190548). **B.** Radula (Paratype 5, NHMUK 20190548). **C.** Lateral teeth of radula (Paratype 6, NHMUK 20190549). Scale bars: **A** = 1 mm; **B, C** = 50 μm.
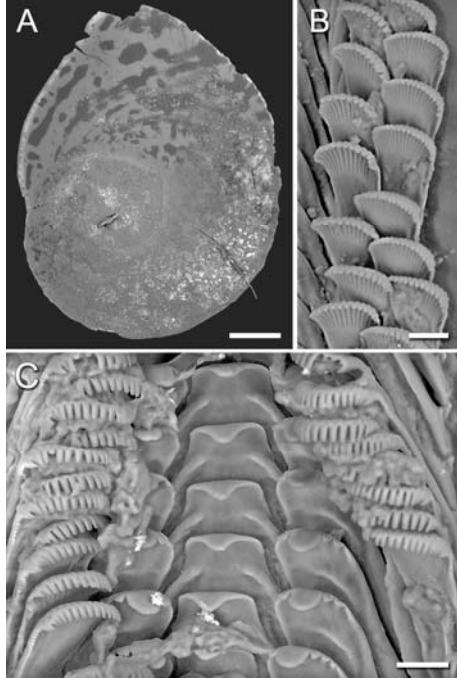
**Figure 4.** *Provanna cooki* sp. nov. **A, B.** Paratype 1 (MNHN-IM-2000-34740). **C, D,** Paratype 2 (UMZC 2019.5). **E, F.** Paratype 3 (UMZC 2019.6). **G, H.** Paratype 4 (UMUT RM33144). **I, J.** Paratype 5 (MNHN-IM-2000-34741). **K, L.** Holotype (NHMUK 20190552), SW Field. Scale bars = 1 mm.
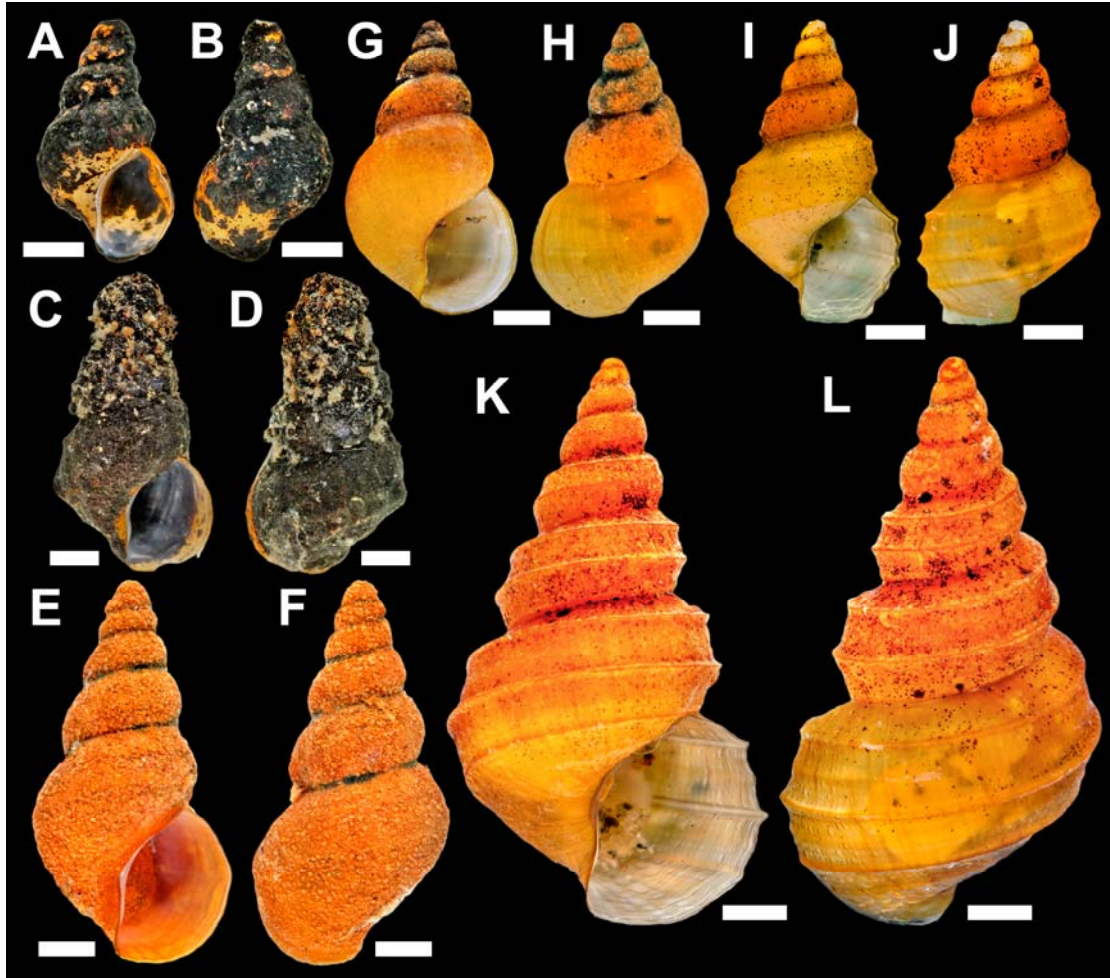
**Figure 5.** *Provanna cooki* sp. nov., SEM images. **A.** Shell (Paratype 6, NHMUK 20190553), 3.2 mm long, 1.6 mm wide. **B.** Operculum (Paratype 6, NHMUK 20190553). **C.** Apical region of the shell (Paratype 6, NHMUK 20190553). **D.** Radula (Paratype 7, shell 8.9 mm long, 4.6 mm wide; NHMUK 20190554). **E.** Lateral teeth of radula (Paratype 7, NHMUK 20190554). Scale bars: **A, C** = 1 mm; **B** = 200 μm; **D** = 50 μm, **E** = 25 μm.
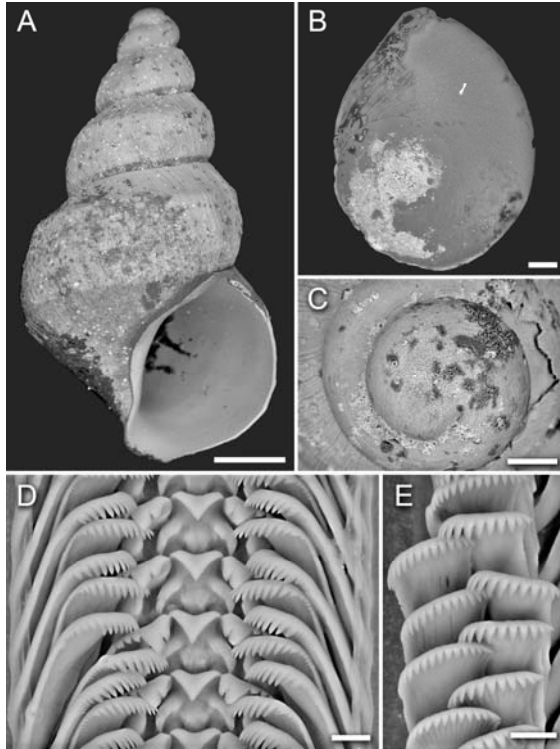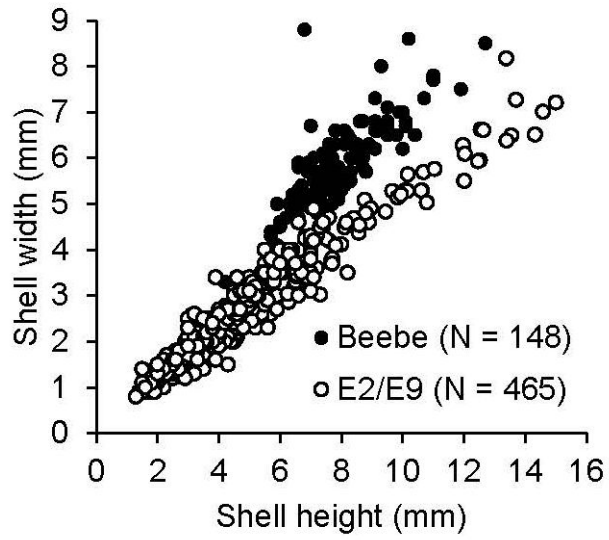
**Figure 6.** *Provanna cooki* sp. nov., egg capsule (NHMUK 20190556). Left, single egg capsule with juveniles visible; Right, multiple egg capsules. Scale bar = 1 mm.
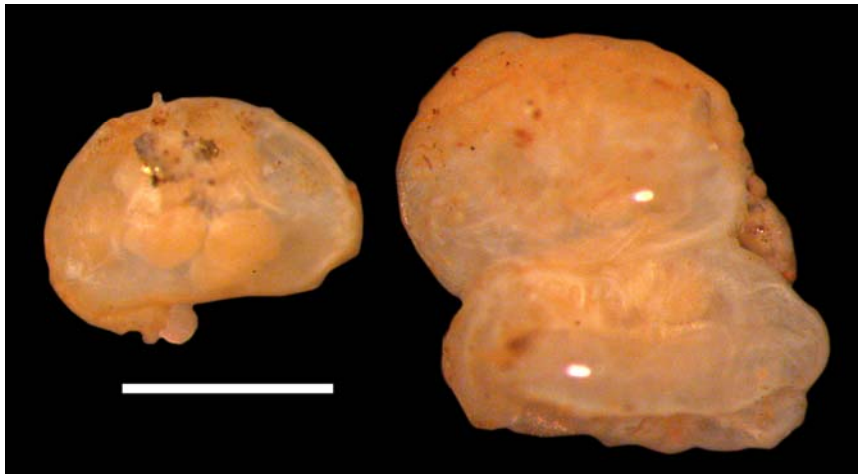
**Figure 7.** *Provanna beebei* sp. nov. and *P. cooki* sp. nov., variation of shell length with shell width. *N*, number of individuals measured.

**Figure 8.** *Provanna beebei* sp. nov. (**A, B**) and *P. cooki* sp. nov. (**C−I**), variation of shell length. **A.** Beebe Chimlets. **B.** Anemone Field. **C.** E2 Vent Field (all). **D.** E9 Vent Field (all). **E.** E2 Dog's Head. **F.** E9 Marsh Tower. **G.** E2 Deep Castle. **H.** E9 SW Field. **I.** E2 Diffuse. *N*, number of individuals measured. E2 was dominated by the smooth form of *P. cooki* and only the ribbed from was collected in E9.
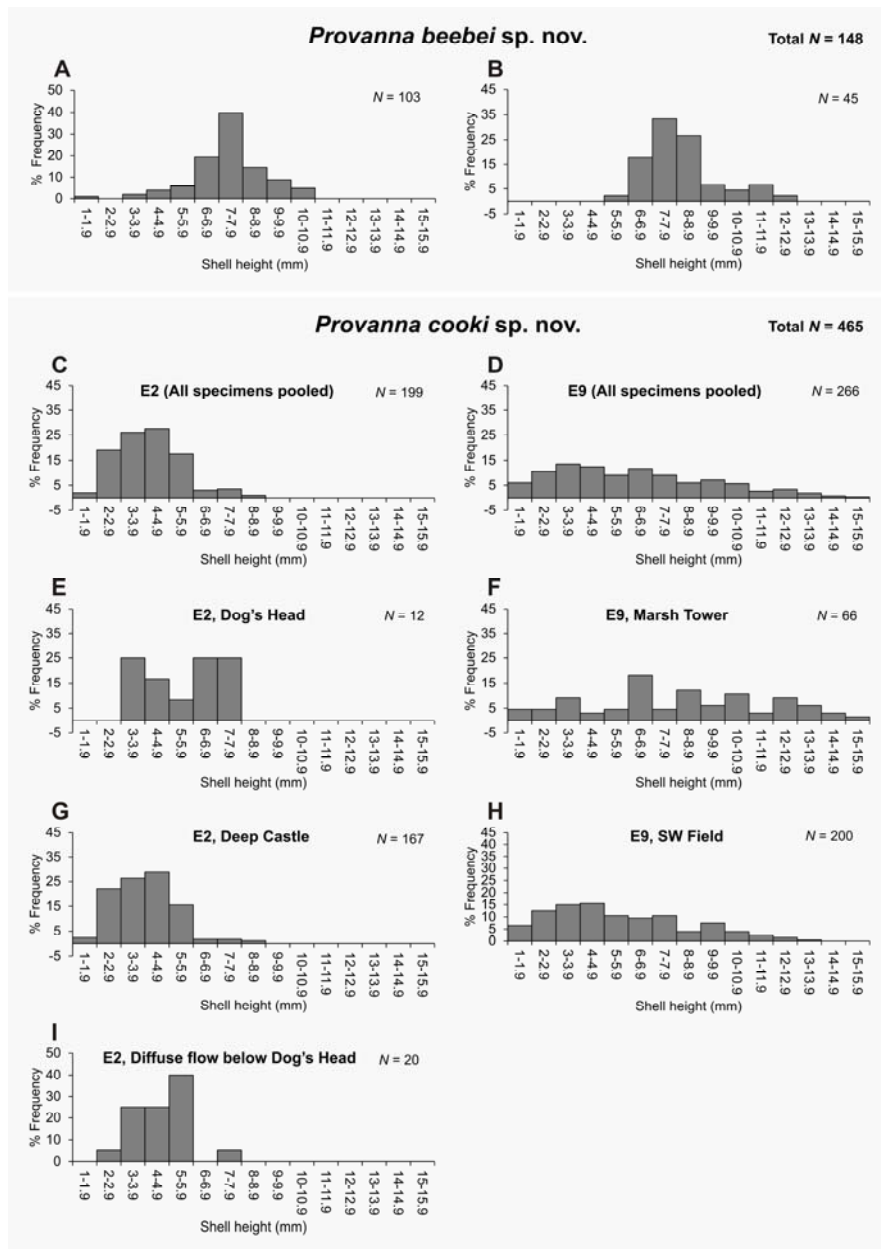
**Figure 9.** Phylogenetic reconstruction of *Provanna* within Abyssochrysoidea, using mtCOI sequences (530 bp), showing Bayesian topology. Support scores (in %) are shown to the left of each node: Bayesian posterior probabilities above line, ML bootstrap values in italics below; where ML values are missing the topology of the ML tree differed.
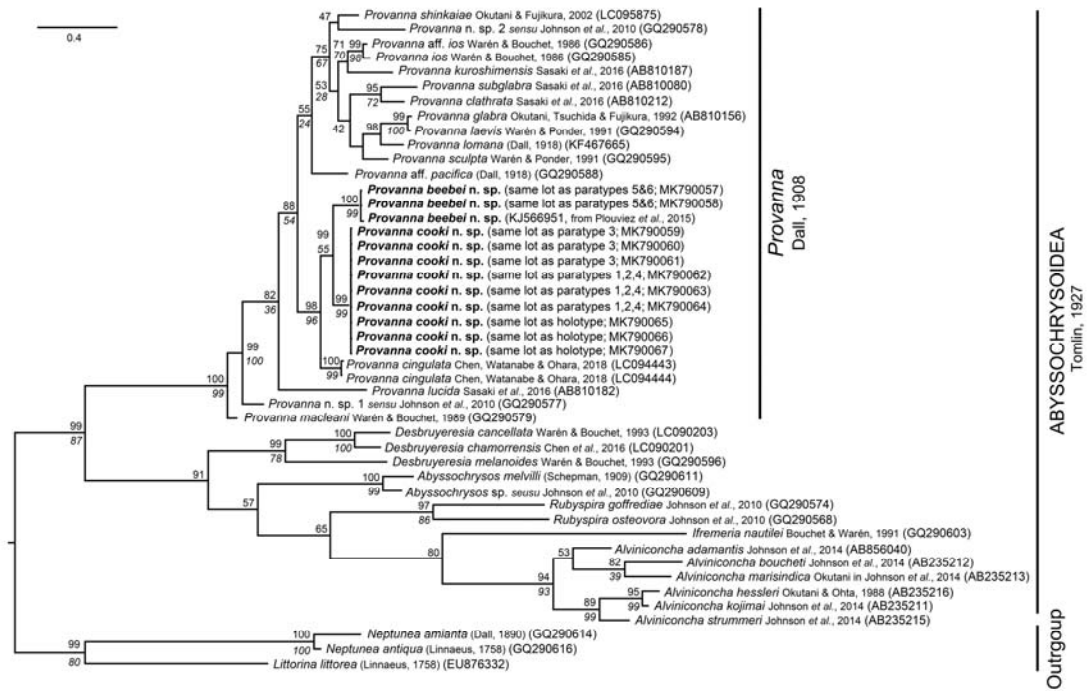
**Table 1.** Provannid gastropods described from the deep sea (confirmed locations and fully described species only).

EPR, East Pacific Rise; ESR, East Scotia Ridge; GoM, Gulf of Mexico; GSC, Galapagos Spreading Centre; JdFR, Juan de Fuca Ridge; MCSC, Mid-Cayman Spreading Centre.

**Table 2.** Variation in shell size (median with inter-quartile range) of *Provanna beebei* sp. nov., Beebe Vent Field, and *P. cooki* sp. nov., E2 and E9 vent fields.

| Species | Location | Shell length (mm) | Shell width (mm) |
|---|---|---|---|
| *P. beebei* sp. nov. | Beebe Chimlets | 7.4 (1.5−10.4) | 5.5 (1.1−8.8) |
| *P. beebei* sp. nov. | Anemone Field | 7.8 (5.9−12.7) | 5.6 (3.2−8.5) |
| *P. cooki* sp. nov. | E2 Dog's Head | 6.1 (3.3−7.7) | 3.1 (1.4−3.8) |
| *P. cooki* sp. nov. | E2 Deep Castle | 3.9 (1.7−8.9) | 2.1 (0.9−4.6) |
| *P. cooki* sp. nov. | E2 Diffuse | 4.5 (2.6−7.2) | 2.2 (1.5−3.6) |
| *P. cooki* sp. nov. | E9 Marsh Tower | 8.1 (1.4−15.0) | 4.3 (0.9−8.2) |
| *P. cooki* sp. nov. | E9 SW Field | 5.0 (1.3−13.2) | 3.0 (0.8−6.2) |