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Cold Seep Communities in the Sea of Japan Are Species-Poor and Dominated by a New Species of Provannid Snail

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ABSTRACT

The Sea of Japan is a semi-enclosed marginal sea in the northwestern Pacific whose bathyal communities exhibit a depauperate species diversity due to anoxic events during the last glacial maximum. Cold seeps associated with chemosynthetic ecosystems were discovered in this region over three decades ago, yet their fauna have not been characterised. Here, we surveyed three bathyal seeps in the northeastern Sea of Japan off Okushiri Island, Hokkaido, to reveal their faunal composition and environmental conditions. We documented only eight macrofaunal species across all sites, of which six are well-known ambient fauna, with just two gastropod species in the genera *Provanna* and *Hyalogyrina* being seep endemics. Dissolved oxygen (DO) concentrations were consistently reduced on the seep bacterial mats, while the temperature remained similar to the ambient seawater. The *Provanna* species was new to science and is named *P. cocytus* sp. nov., characterised by a smooth shell with very convex whorls and a nearly holostomous aperture and a radula with a truncated central tooth cusp and the lateral teeth lacking a buttress. Phylogenetic reconstruction using the mitochondrial cytochrome c subunit I (COI) gene recovered *P. cocytus* sp. nov. as sister to all other congeners, indicating this genus did not recolonise the Sea of Japan after the last glacial maximum from seep or vent sources. The low species richness at seeps likely represents a combined effect of shallow connections limiting dispersal and impacts of the very cold Japan Sea Proper Water to larval settlement. Our results contribute to the understanding of the biodiversity in the Sea of Japan and close a major knowledge gap in the biogeography of chemosynthesis-based ecosystems across the western Pacific.

1 | Introduction

Chemosynthetic ecosystems such as hydrothermal vents and hydrocarbon seeps are 'oases' in the deep sea harbouring an unusually high biomass, supported by bacterial primary production [1, 2]. These systems are environmentally challenging and are typically dominated by a mostly endemic, specially adapted fauna found nowhere else [3]. Vents and seeps are sustained by the input of geofluids and are thus mostly distributed along plate boundaries [4]. Waters around Japan, where four plates—including the Eurasian and North American continental plates and the Pacific and Philippine Sea oceanic plates—converge, are therefore exceptionally rich in vent and seep sites [4, 5]. Previous research has illuminated the faunal diversity in many of these sites, particularly those located in the Okinawa Trough [6], the Izu-Ogasawara Arc [7, 8], and the Nankai Trough [9]. Cold seeps in the Sea of Japan, however, remain largely unknown in terms of their faunal diversity.

The Sea of Japan (Figure 1A) is a semi-enclosed marginal sea formed by the Japanese Archipelago being pulled apart from Eurasia around 20 Ma (million years) ago [10]. Despite having a maximum depth of around 3800 m, only four very shallow passages less than 140 m depth connect the Sea of Japan to surrounding seas, making it an exceptionally isolated body of water [11]. The deep water in the Sea of Japan below the ~500 m depth thermocline is exceptionally cold and uniform with a narrow temperature range between 0.0°C and 0.6°C with high oxygen concentrations, known as the Japan Sea Proper Water [12]. Although the Japan Sea Proper Water is currently well-oxygenated [11], it has seen numerous environmental events leading to the depletion of dissolved oxygen (DO) since its formation, most notably a massive input of freshwater from continental Eurasia between 20 and 27 ka (thousand years) ago that led to a complete anoxification and a great reduction in salinity [13]. Except for a few species that apparently persisted in refugia between 100 and 400 m depth, most deep-water fauna are thought to have been eliminated during this anoxic event. The current bathyal assemblage therefore consists largely of opportunistic recolonisers that entered the basin after the end of the last glacial maximum (19 ka ago) [14, 15]. As only very shallow connections to the surrounding oceans exist, these recolonising taxa are primarily shallow-dispersing species from the northern Pacific that were able to adapt rapidly to the very cold temperatures and high hydrostatic pressure [16]. The combined effects of historical anoxia, restricted deep-water exchange, and strong environmental filtering have resulted in the bathyal Sea of Japan being a very species-poor region, with just about 30 species of invertebrate animals in total known from depths below 2000 m despite considerable sampling efforts [15].

Cold seeps with associated bacterial mats and animals have been reported from two regions of the Sea of Japan; the northeastern part off Okushiri Island, Hokkaido (Figure 1B) [17–19], and the southeastern part off Joetsu, Niigata Prefecture [20] (Figure 1A). At least three active seeps thought to be associated with subduction are known off Okushiri Island, including the northwestern slope of the Okushiri Ridge ("NW Okushiri" hereafter, 44°14.21'N, 139°6.05'E, ~3100 m depth), the eastern slope of the Okushiri Ridge ("E Okushiri" hereafter; 42°50.88'N, 139°25.33'E, ~2700 m depth), and one located at the west of the Matsumae Plateau ("W Matsumae" hereafter; 41°16.85'N, 138°31.44'E, ~3350 m depth) [19, 21]. Both NW Okushiri and E Okushiri seeps were associated with earthquake-linked fissures where geofluids emerge, while the W Matsumae site was associated with chimney-like structures presumed to be carbonates [21]. The NW Okushiri seep is the only site with some information on the faunal community published in the form of in situ photographs and preliminary identifications [17], suggesting a dominance by abyssochrysoidean snails in the genus *Provanna* typically associated with chemosynthetic habitats [5]. The Off Joetsu seep (37°32.00'N, 137°55.00'E, ~1000 m deep) is characterised by methane hydrate deposits overlaid by dense empty shells of the cleftclams *Conchocele* and *Thyasira* [20, 22, 23] while *Provanna* snails have also been recovered from dredges [5]; however, no published data exist for the overall faunal composition.

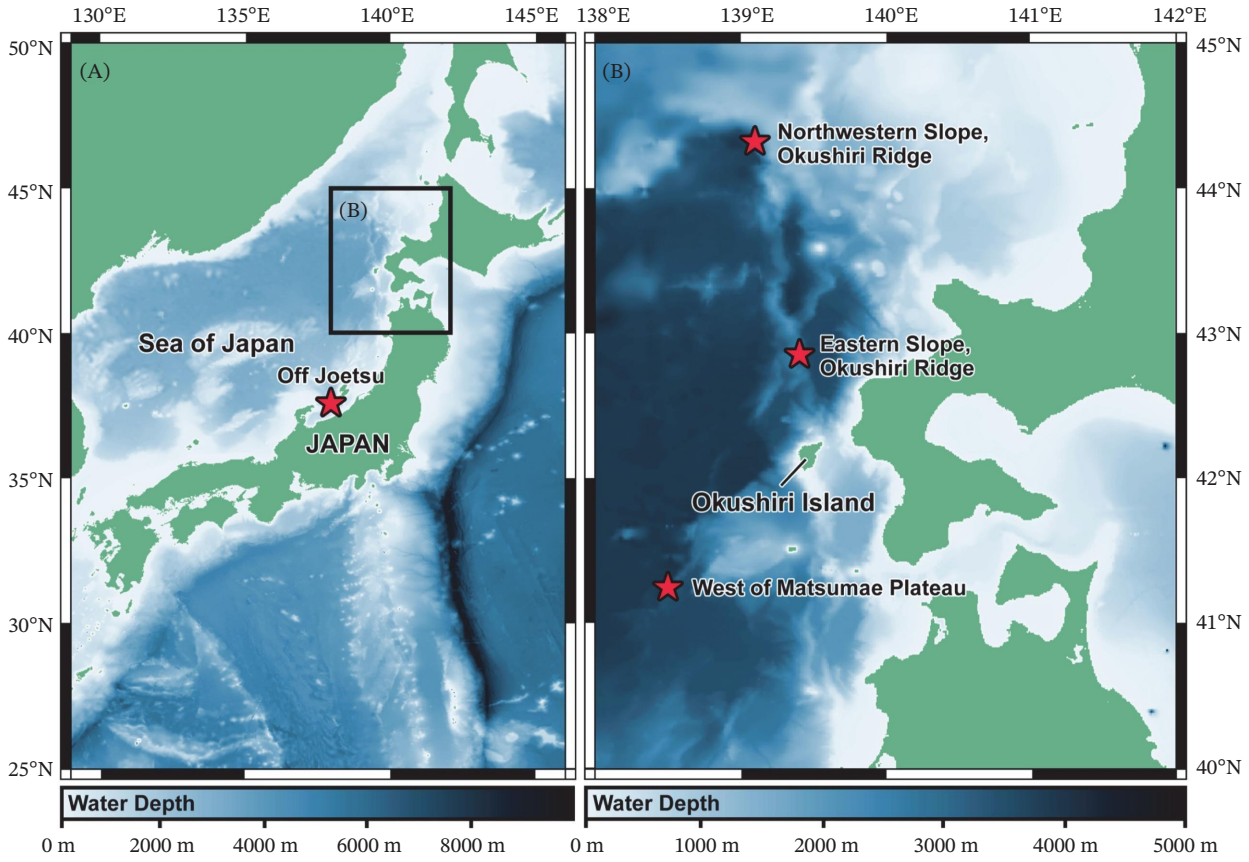

FIGURE 1 | Maps showing the locations of relevant cold seep sites in the Sea of Japan. (A) An overview of central Japan showing the general location of the Sea of Japan. (B) Enlarged map of the northeastern Sea of Japan showing the three cold seeps off Okushiri Island visited in this study. Equidistant cylindrical projection, bathymetry generated using publicly available data from GEBCO (General Bathymetric Chart of the Oceans).

Therefore, the species composition at cold seeps in the Sea of Japan remains a gap in understanding both the biodiversity patterns in this marginal sea as well as the biogeography of chemosynthetic ecosystems around Japan and in the western Pacific. Here, we revisited the three cold seeps off Okushiri Island to characterise their faunal diversity for the first time. Furthermore, the *Provanna* species dominating these seeps [5, 17] was found to be new to science and is described herein.

2 | Materials and Methods

2.1 | Seafloor Observations and Sampling

Three hydrocarbon seeps in the northeastern Sea of Japan (Figure 1) were explored using the human-occupied vehicle (HOV) *Shinkai 6500* during the R/V *Yokosuka* cruise YK25-14C, including NW Okushiri (dive #1873; August 30, 2025), E Okushiri (dive #1874; August 31, 2025), and W Matsumae (dive #1875; September 3, 2025). Names of the seep sites used herein follow the review and list of chemosynthetic ecosystems around Japan in Fujikura et al. [5]. Seafloor images were taken using the *Shinkai 6500*'s digital still camera (Olympus E-PL6, 16 megapixels) or the high-definition science video camera (SONY FCB-H11, 1920 × 1080 pixels), both mounted on a pan-and-tilt stage outside the submersible hull.

The water temperature and DO concentrations were measured every second using a handheld stand-alone RINKO III optical sensor (JFE Advantech). At the seep site, the sensor was held using the submersible's manipulator over the bacterial mat (~1–2 cm above the seafloor) for 2 min prior to biological sampling to generate 120 data points. Prior to measurements at the seep, the sensor was also held for 2 min in ambient seafloor without seepage influence at the same depth in order to capture the background environmental conditions for comparison with the seep data. Animals on and around bacteria mats on active seeps were sampled using a suction sampler mounted on the *Shinkai 6500*.

2.2 | Morphological Examination

Upon recovery of the *Shinkai 6500* to the research vessel, animal samples were immediately moved into a cold room set to 4°C. Macrofaunal specimens were retrieved by sieving using pre-chilled seawater on a 0.5 mm sieve. The sieved samples were sorted and identified morphologically under an Olympus SZX7 dissecting stereomicroscope. Photographs of the live animals were taken using an EOS 5Ds R digital single-lens reflex camera equipped with an EF 100 mm F2.8L Macro IS USM lens. Multiple photographs of each specimen were taken and focus-stacked using Adobe Photoshop CC. Animal specimens were preserved in either 99% ethanol or 10% seawater-buffered formalin.

For the type material of the *Provanna* snail, photographs were taken after preservation using a Keyence VHX-S770E digital microscope with the automatic image stacking function. Shell size measurements, including shell length (SL) and shell width (SW), were taken using digital Vernier callipers and rounded to the nearest 0.1 mm. For the examination of gross external anatomy, the shell of both male and female specimens was dissolved in 0.1 M hydrochloric acid, and the periostracum was peeled manually using fine tweezers under a stereo microscope (Olympus SZX9). Morphological terminology of *Provanna* snails used herein follows Warén and Bouchet [24] as well as Betters and Cordes [25].

2.3 | Scanning Electron Microscopy (SEM)

The radula ribbon of a *Provanna* snail was extracted by dissecting out the radula sac using fine tweezers, and then the surrounding soft tissue was dissolved using a 10% commercial bleach solution. The cleaned radula ribbon was washed twice using fresh MilliQ water and then again with 99% ethanol before mounting on aluminium stubs using conductive carbon tape. The operculum from an adult specimen was dissected out and then cleaned and mounted using the same methods. For the protoconch, we examined the apex of specimens from all three seep localities, and with the exception of two juvenile specimens from E Okushiri, the protoconch had been corroded away in all specimens. Some specimens did retain the apex under a layer of black mineral deposit, but peeling back this mineral layer revealed that the surface sculpture of the protoconch had already been lost. Therefore, a juvenile specimen from E Okushiri was selected for protoconch examination, with the shell lightly washed in 10% commercial bleach to remove the periostracum and other deposits and then washed twice in MilliQ water before mounting onto an aluminium stub. Observations were conducted using a Hitachi TM-3000 tabletop SEM without coating, at an acceleration voltage of 15 kV.

2.4 | DNA Barcoding

Pieces of the foot tissue from the *Provanna* snail were used for genomic DNA extraction using the DNeasy Blood & Tissue Kit (QIAGEN), and then 1 μL of the extraction was purified using GeneReleaser (BioVenture, San Carlos, CA, USA), both following the manufacturers' standard protocols. The barcoding fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified by polymerase chain reaction using the universal primer pair LCO1490 (5′–GGTCAACAAATCATAAAGATATTGG–3′) and HCO2198 (5′–TAAACTTCAGGGTGACCAAAAAATCA–3′) [26], following published protocols for *Provanna* [27, 28]. The successful products were sent for bidirectional Sanger sequencing using the same primers through the commercial service by FASMAC Corporation (Kanagawa, Japan). The COI sequences obtained were deposited in NCBI GenBank under the accession numbers PX626439-PX626441.

2.5 | Molecular Analyses

The closest match to the newly obtained COI sequence of the Sea of Japan *Provanna* species among publicly available data was searched using the NCBI BLAST (Basic Local Alignment Search Tool, https://blast.ncbi.nlm.nih.gov/). The uncorrected pairwise distances between sequences were also calculated using the BLAST tool.

Available COI sequences of gastropods in the superfamily Abyssochrysoidea (which includes *Provanna*) were downloaded from NCBI GenBank and aligned using Geneious Prime 2026.0 (https://www.geneious.com/) into a 530 bp alignment for downstream analyses. Following a previous study [27] on the same genus, we included four non-abyssochrysoid caenogastropods

as the outgroup, including two cerithiids, *Cerithium zonatum* (Wood, 1828) and *Bittiolum varium* (Pfeiffer, 1840), and two littorinids, *Littorina littorea* (Linnaeus, 1758) and *Echinolittorina vidua* (Gould, 1859).

For phylogenetic reconstruction, PartitionFinder v. 2.1.1 [29] was used to select nucleotide substitution models per codon position using the Bayesian information criterion, resulting in HKY + I + G selected for the first and second codon positions and GTR + I + G for the third codon position. Phylogenetic reconstruction was carried out in MrBayes v3.2 [30] by Bayesian inference, with Markov chain Monte Carlo chains run for 1 million generations while topologies were being sampled every 100 generations. Tracer v1.7 [31] was used to check for convergence after discarding the first 25% of the trees obtained as 'burn-in.'

2.6 | Specimen Repositories

Type specimens were deposited in two public museums, the National Museum of Nature and Science, Tsukuba (NSMT), Japan, and the Senckenberg Research Institute and Natural History Museum, Frankfurt (SMF), Germany.

3 | Results

3.1 | Seafloor Observations and Faunal Composition

At NW Okushiri (Figure 2A–D), the ambient seafloor (0.33°C, SD = 0.01; DO = 6.72 mL/L, SD = 0.00) comprised mudstones overlaid with a layer of sediment and was extensively covered by a turf of polychaete tubes made by the ampharetid worm *Ampharete* sp. (Figure 3C). The ampharetid tubes were densely colonised by the glass scallop *Delectopecten vancouverensis* (Whiteaves, 1893) (Figure 3E), which is known to selectively attach to these worm tubes by byssal threads in the bathyal Sea of Japan [34]. On the sediment surface, aggregations of the burrowing sea anemone *Edwardsia sojabio* Sanamyan & Sanamyan, 2013 [35] (Figure 3B) were common. The munnopsid isopod *Eurycope spinifrons* Gurjanova, 1933 (Figure 3D) appeared to be abundant on the sediment throughout the entire dive, while the polynoid scale worm *Harmothoe derjugini* (Annenkova, 1937) was occasionally seen (Table 1).

Eventually, we successfully located the active seepage site at NW Okushiri (Figure 2A,B; 44°14.2064'N, 139°6.0480'E, 3086 m depth) [17, 18]. The extent of seepage we observed was limited to a linear extension of about 20 m along a fissure on the seafloor, marked by white bacteria mats. The ampharetid turf with glass scallops attached as well as *Edwardsia sojabio* anemone aggregations (Figure 2C) occurred right up to the edge of the bacterial mat (Figure 2B), but were not found on the mat. On the contrary, *Eurycope spinifrons* isopods and *Harmothoe derjugini* scale worms were observed to intrude into the bacterial mats (Figure 2D). The dominant species on the active bacterial mat was an abyssochrysoidean snail in the genus *Provanna* (Figure 3F), which co-occurred with the heterobranch snail *Hyalogyrina* cf. *umbellifera* [36] (Figure 3H). Both species were strictly bound to the bacterial mat, although *Provanna* was nearly two orders of magnitude more abundant than *Hyalogyrina*—among the many hundreds of *Provanna* specimens collected, we only found about 10 specimens of *Hyalogyrina*. Compared to background values, the environmental variables on the bacterial mat showed a slight drop in DO while the temperature remained the same (0.33°C, SD = 0.00; DO = 6.68 mL/L, SD = 0.03).

At E Okushiri (Figure 2E–G; Table 1), which was also characterised by sediment-covered mudstones, the overall species composition and distribution pattern were virtually identical with NW Okushiri except the added presence of the thyasirid bivalve *Parathyasira* sp. *sensu* Kamenev, 2013 [33] (Figure 3G) recovered from a suction sample around the bacterial mat [33]. However, the pattern of seepage activity was different here, and instead of running along fissures, we only found three small (about 10 cm × 10 cm) patches of white bacterial mat (42°50.8793'N, 139°25.3299'E, 2679 m depth). Again, these patches were densely colonised by *Provanna* snails (Figure 2G), which were absent from the ambient seafloor. Sorting through the suction sampler material on board recovered two specimens of *Hyalogyrina*, confirming their presence at this site also. Compared to the ambient environment (0.30°C, SD = 0.00; DO = 6.80 mL/L, SD = 0.00), the DO concentration on the bacterial mat was slightly lower while the temperature was similar (0.29°C, SD = 0.00; DO = 6.75 mL/L, SD = 0.00).

The W Matsumae seep (Figure 2H,I) was distinct from the two sites off Okushiri Ridge in that the seepage activity was much more extensive, continuing for a stretch of at least 500 m upslope (41°16.8594'N, 138°31.3900'E, 3389 m depth to 41°16.7983'N, 138°31.4941'E, 3313 m depth). Although the seafloor was also composed of mudstone overlaid by a thin layer of mud, the bacterial mats occurred in larger (often several metres across) patches surrounding brittle, yellowish-white chimney-like structures (Figure 2H) which were previously tentatively identified as 'carbonate chimneys' by Takeuchi et al. [21]. In some cases, the bacterial mats developed into thick, white blobs (Figure 2I) that were not observed at the two Okushiri Ridge sites. At W Matsumae, all conspicuous background fauna such as the ampharetid turfs and glass scallops were absent from the immediate surroundings of bacterial mats. The *Provanna* colonies (Figure 2I) were much denser and more extensive than the two Okushiri Ridge sites. Sorting through the suction sampler material collected from the bacterial mats revealed some tens of *Hyalogyrina* among hundreds of *Provanna* and several specimens of *Eurycope spinifrons* isopods. Compared to the background values (0.34°C, SD = 0.00; DO = 6.76 mL/L, SD = 0.00), the DO concentration on the *Provanna* colony (0.34°C, SD = 0.01; DO = 3.84 mL/L, SD = 2.91) and inside the white blob-like thick bacterial mat (0.35°C, SD = 0.01; DO = 0.80 mL/L, SD = 0.68) were both much lower with high variabilities; the temperatures, on the other hand, did not stray significantly from the background measurement.

3.2 | Systematics

The *Provanna* snail found in association with white bacterial mats in all three Sea of Japan seep sites visited was morphologically distinct from all previously described species [25] and is here described as a new species, *Provanna cocytus* sp. nov.

Subclass CAENOGASTROPODA Cox, 1960

Superfamily ABYSSOCHRYSOIDEA Tomlin, 1927

Family PROVANNIDAE Warén & Bouchet, 1993

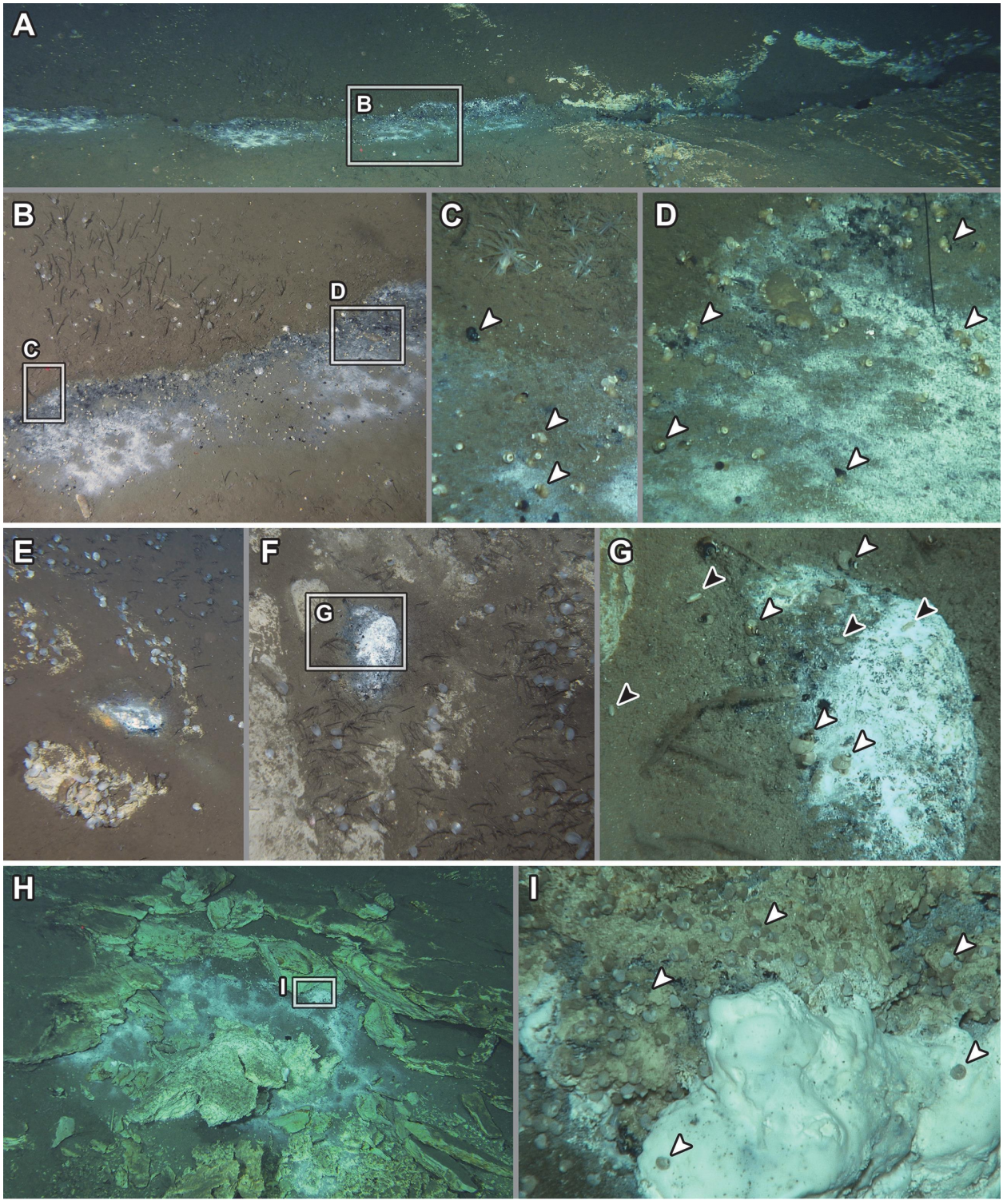

FIGURE 2 | In situ observations of faunal communities in the three Sea of Japan cold seeps visited in this study. (A) NW Okushiri, where seepage and whitish bacterial mats occurred along fissures on the seafloor; (B) a close-up of A showing bacterial mats in close proximity to turfs of *Ampharete* worm tubes and *Delectopecten* scallops; (C) a close-up of B showing *Provanna cocytus* sp. nov. (white arrowheads) aggregating on white bacterial mats and the presence of the sea anemone *Edwardsia sojabio* in close proximity; (D) another close-up of B showing a *Harmothoe* scale worm among *P. cocytus* sp. nov. on bacterial mats. (E,F) E Okushiri, where active seepage was only found in constrained spots of about 10 cm by 10 cm, covered by white bacterial mats; (G) a close-up of F showing *P. cocytus* sp. nov. (white arrowheads) aggregating around bacterial mats, and the munnopsid isopod *Eurycope spinifrons* (black arrowheads) among them. (H) W Matsumae, showing bacterial mats surrounding chimney-like structures; (I) a close-up of H showing *P. cocytus* sp. nov. (white arrowheads) aggregating around thick white bacterial mats.

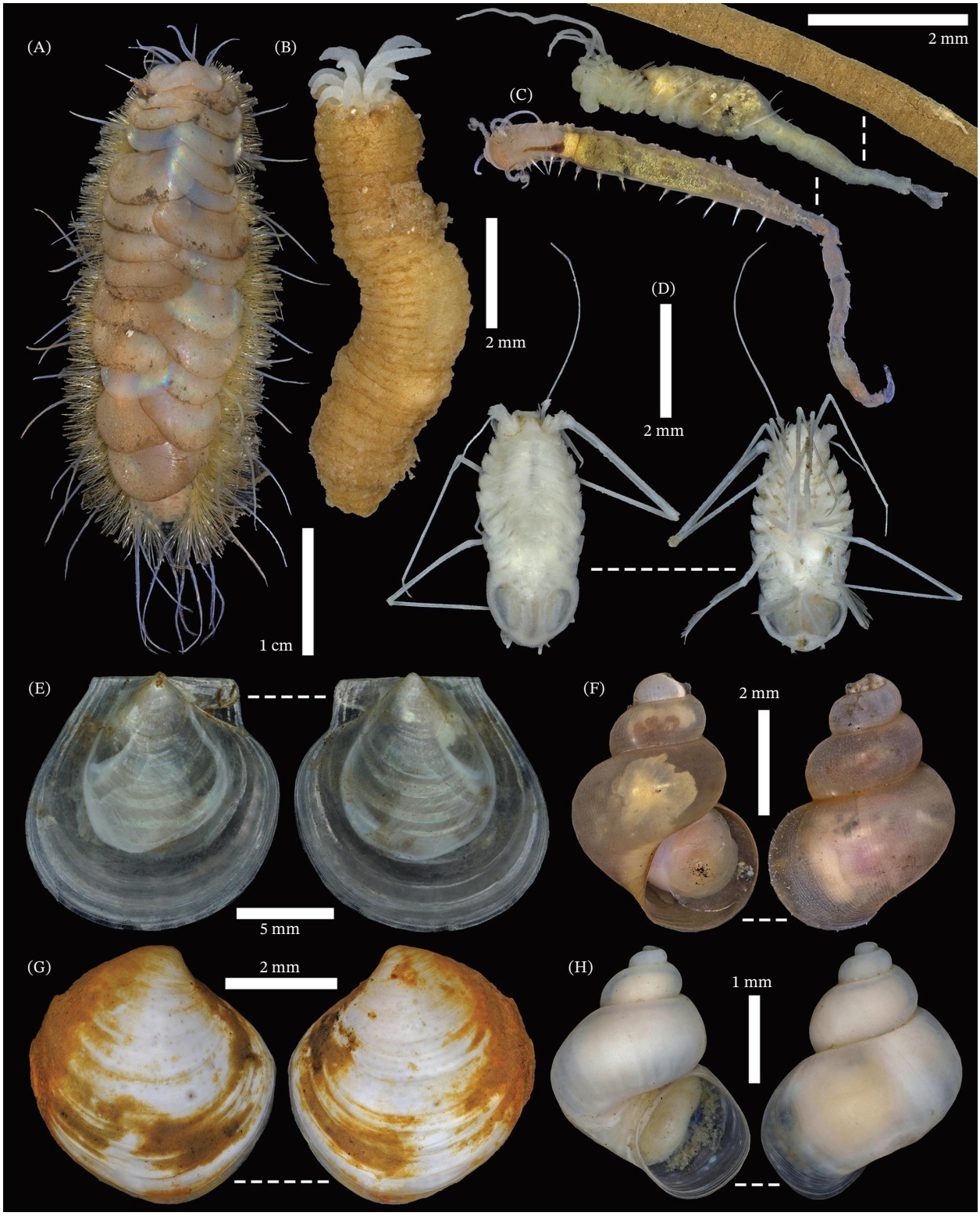

FIGURE 3 | Animals collected from, and around, cold seeps in the Sea of Japan. (A) the polynoid scale worm *Harmothoe derjugini* (not seep-endemic; NW Okushiri); (B) the sea anemone *Edwardsia sojabio* (not seep-endemic; NW Okushiri); (C) the ampharetid worm *Ampharete* sp. *sensu* Alalykina [32] and its muddy tube (not seep-endemic; photographed specimen from NW Okushiri and also found in E Okushiri); (D) the munnopsid isopod *Eurycope spinifrons* (not seep-endemic, photographed specimen from NW Okushiri and also found in E Okushiri and W Matsumae); (E) the glass scallop *Delectopecten vancouverensis* (not seep-endemic, photographed specimen from NW Okushiri and also found in E Okushiri); (F) the abyssochrysoidean snail *Provanna cocytus* sp. nov. (seep-endemic, photographed specimen from NW Okushiri and also found in E Okushiri and W Matsumae); (G) the cleftclam *Parathyasira* sp. *sensu* Kamenev [33] (not seep-endemic, E Okushiri); (H) the heterobranch snail *Hyalogyrina* cf. *umbellifera* (seep-endemic, photographed specimen from NW Okushiri and also found in E Okushiri and W Matsumae).

TABLE 1 | Species recorded from cold seeps in the Sea of Japan, with habitat observations and presence (+) or absence (−) at each site.

Phylum	Taxa	Habitat	NW Okushiri	E Okushiri	W Matsumae
Cnidaria	*Edwardsia sojabio*	Ambient to seep edge	+	+	−
Arthropoda	*Eurycope spinifrons*	Ambient to bacteria mat	+	+	+
Mollusca	*Provanna cocytus* sp. nov.	Only on bacteria mat	+	+	+
Mollusca	*Hyalogyrina* cf. *umbellifera*	Only on bacteria mat	+	+	+
Mollusca	*Parathyasira* sp.	Ambient to seep edge	−	+	−
Mollusca	*Delectopecten vancouverensis*	Ambient to seep edge	+	+	−
Annelida	*Ampharete* sp.	Ambient to seep edge	+	+	−
Annelida	*Harmothoe derjugini*	Ambient to bacteria mat	+	+	−

Provanna Dall, 1918

Type species: *Trichotropis* (*Provanna*) *lomana* Dall, 1918; type by monotypy

Provanna cocytus sp. nov.

(Figures 3F, 4A–F, 5A–D, 6A–D, 7)

'Unidentified gastropods'—Naganuma et al. [17], Figure 3.

3.2.1 | ZooBank Registration

urn:lsid:zoobank.org:act:E9273B0A-1563-455F-9D78-57076E28C390

3.2.2 | Diagnosis

A medium-sized *Provanna* (up to SH 7.5 mm) with a smooth shell lacking in major axial or spiral sculpture except for occasional very weak spiral striations; the aperture is oval and almost holostomous. The whorls are convex and inflated with a globose aperture and a deep, very strongly constricted suture. Radula with a broad central tooth carrying a blunt, truncated cusp. First lateral teeth are truncate and lobate, lacking a buttress; variable in denticle development and carry/bear between 5 and 7 cusps, of which the 3rd or 4th (from the innermost) is the strongest major cusp. Marginal teeth finely serrated into 20–25 cusps.

3.2.3 | Etymology

From Latin *Cōcȳtus* (derived from Ancient Greek *Κωκυτός*, literally meaning 'lamentation'), the ninth and the final Circle of Hell in Dante Alighieri's *Divine Comedy* depicted as a vast and remorseless frozen lake [37]. This is a reference to the very cold habitat of *P. cocytus* sp. nov. in the bottom layer of the Japan Sea Proper Water [12], the coldest known of all deep-sea abyssochrysoidean snails. Even in the Antarctic vents on the East Scotia Ridge, the temperature at diffuse flow sites where *Provanna* live is between 3.5°C and 19°C [38]. Used as a noun in apposition.

3.2.4 | Type Locality

Cold seep on the northwestern slope of the Okushiri Ridge (44°14.2064'N, 139°6.0480'E, 3086 m depth), northeastern Sea of Japan.

Type material. Holotype (NSMT-Mo 79870; Figure 4A), SH 6.3 mm, SW 4.2 mm, 99% ethanol. Paratype #1 (NSMT-Mo 79871; Figure 4B), SH 7.5 mm, SW 6.2 mm, 99% ethanol. Paratype #2 (SMF 383145; Figure 4E), SH 6.1 mm, SW 4.1 mm, 99% ethanol. Paratype #3 (SMF 383146; Figure 4F), SH 6.3 mm, SW 5.1 mm, 99% ethanol. Paratype #4 (NSMT-Mo 79872; Figure 4D), SH 6.5 mm, SW 4.2 mm, 99% ethanol. Paratype #5 (NSMT-Mo 79873; Figure 6), SH 6.8 mm, SW 4.7 mm, 99% ethanol. Paratype #6 (SMF 383147; Figure 4C), SH 7.2 mm, SW 4.1 mm, 99% ethanol. Paratype lot #7 (NSMT-Mo 79874), a growth series of 10 specimens in 99% ethanol. Paratype lot #8 (SMF 383148), a growth series of 10 specimens in 99% ethanol. Paratype lot #9 (NSMT-Mo 79875), a growth series of 10 specimens in 10% buffered formalin. Paratype lot #10 (SMF 383149), a growth series of 10 specimens in 10% buffered formalin. All type material was collected in a single sampling event using a suction sampler mounted on HOV *Shinkai 6500* during dive #1873 (August 30, 2025) on-board R/V *Yokosuka* cruise YK25-14C.

3.2.5 | Other Material Examined

Cold seep on the eastern slope of the Okushiri Ridge (42°50.8793'N, 139°25.3299'E, 2679 m depth), northeastern Sea of Japan, collected by a suction sampler mounted on HOV *Shinkai 6500* during dive #1874 (August 31, 2025) on board R/V *Yokosuka* cruise YK25-14C: 5 specimens in 99% ethanol (NSMT-Mo 79876); 1 juvenile specimen with intact protoconch, periostracum removed for SEM (NSMT-Mo 79877; Figure 5A).

Cold seep west of Matsumae Plateau (41°16.8478'N, 138°31.4412'E, 3349 m depth), northeastern Sea of Japan, collected by a suction sampler mounted on HOV *Shinkai 6500* during dive #1875 (September 3, 2025) on-board R/V *Yokosuka* cruise YK25-14C: 10 specimens in 99% ethanol (NSMT-Mo 79878); 10 specimens in 10% buffered formalin (NSMT-Mo 79879).

3.2.6 | Description

The shell is thin and translucent, with strongly convex and inflated whorls, up to 7.5 mm in height (Figure 4). The protoconch was corroded away and lost in almost all specimens available for study except two juvenile specimens collected from the E Okushiri seep (Figure 5A). The protoconch is about 0.8 mm in height and 0.65 mm in width, paucispiral and hence

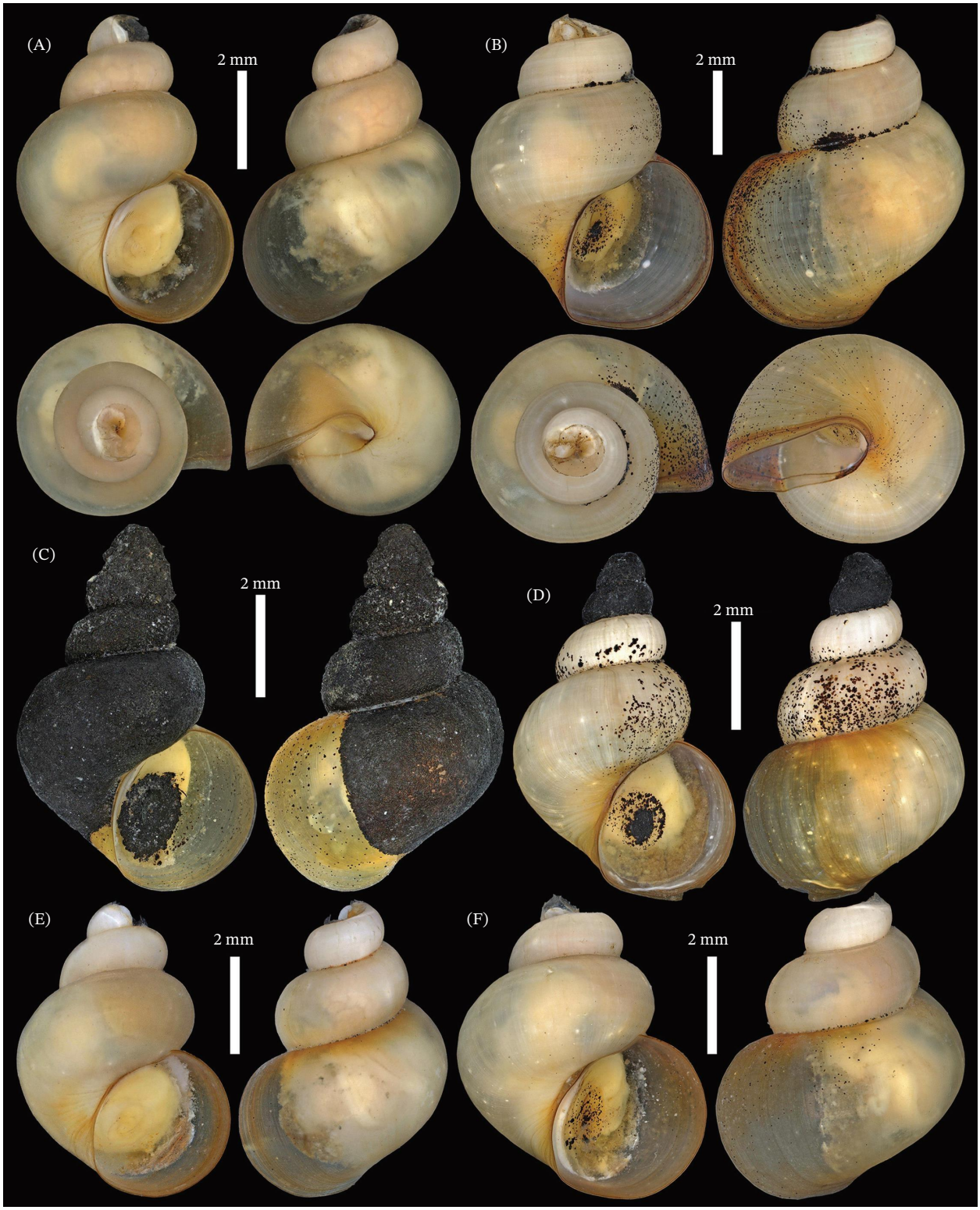

FIGURE 4 | *Provanna cocytus* sp. nov., representative type specimens. (A) Holotype (NSMT-Mo 79870), apertural, abapertural, apical, and basal views; (B) paratype #1 (NSMT-Mo 79871), apertural, abapertural, apical, and basal views; (C) paratype #6 (SMF 383147), apertural and abapertural views; (D) paratype #4 (NSMT-Mo 79872), apertural and abapertural views; (E) paratype #2 (SMF 383145), apertural and abapertural views; (F) paratype #3 (SMF 383146), apertural and abapertural views.

lecithotrophic. The first 0.5 whorls or so of the protoconch are sculptured only by granules arranged as intermittent spiral cords, then these gradually fuse to form about 25 continuous spiral cords. After the first 0.5 whorls of the protoconch, weak axial ribs also start to appear, gradually strengthening and becoming more frequent. The axial ribs become equal in strength to the spiral ribs in the final 0.5 whorls, where the sculpture becomes distinctly cancellate. Transition from the

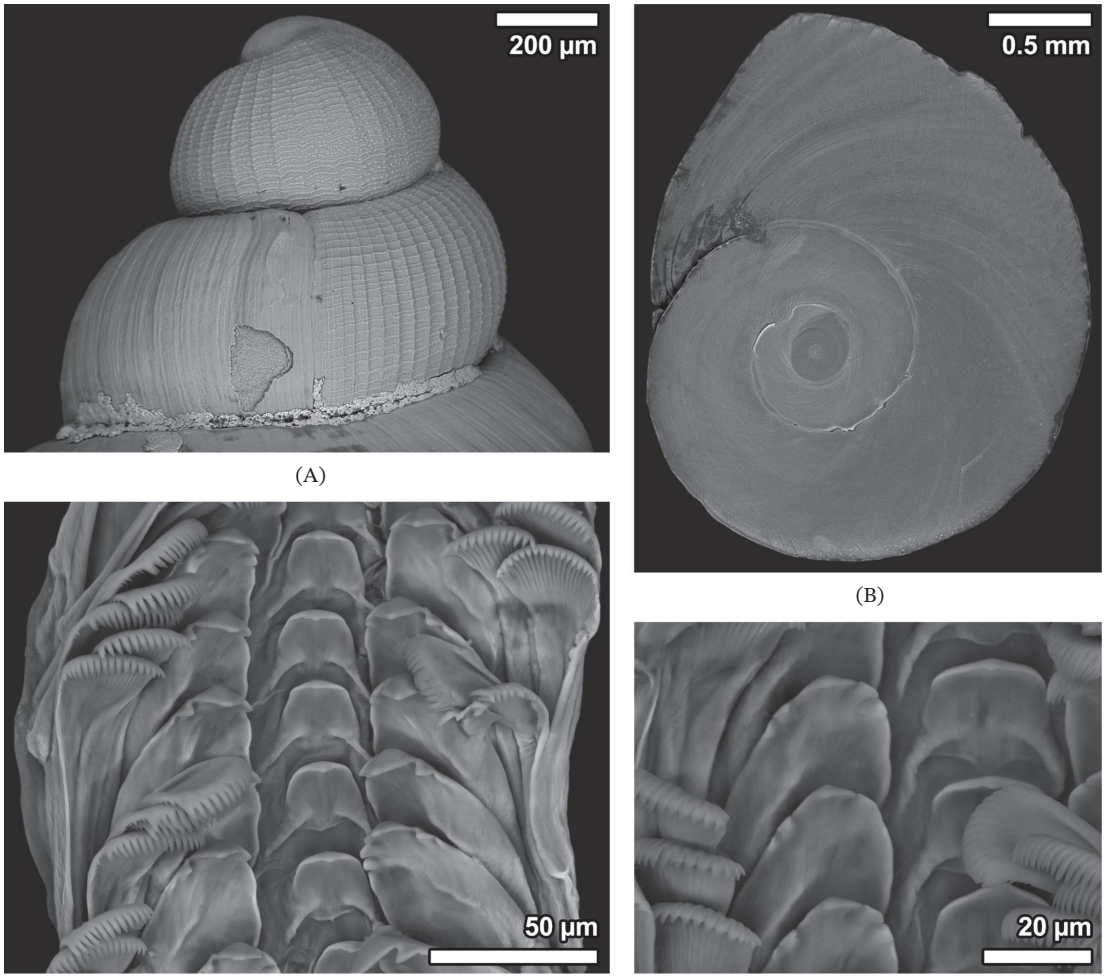

FIGURE 5 | *Provanna cocytus* sp. nov., scanning electron micrographs. (A) Protoconch of a juvenile specimen from the seep on the eastern slope of the Okushiri Ridge (NSMT-Mo 79877); (B) operculum; (C,D) radula.

protoconch to the teleoconch is abrupt but not associated with the formation of a lip.

The teleoconch shell surface is devoid of any axial sculpture beyond fine and indistinct growth lines (Figure 4). In most specimens the spiral sculpture is also completely lacking on the teleoconch (Figure 4A,E), although some individuals exhibit 8–10 very weak rudimentary spiral striae that lead to a slightly faceted shell profile (Figure 4B,F). Although adult shells have about four teleoconch whorls, the upper spire is typically corroded away leaving only 2.5–3.0 whorls, with the soft parts occupying about two whorls. The apex of such corroded specimens is secondarily sealed with a calcareous plug. The aperture shape is globose and oval, very slightly taller than wide. The anterior siphonal canal is very indistinct. The teleoconch is covered by a layer of very thin and semi-transparent periostracum, which is often in turn overlaid by a layer of sulphide mineral deposit (Figure 4C,D). Specimens bearing this mineral deposit usually retain all teleoconch whorls.

A paucispiral operculum (Figure 5B) of about 3.5 whorls is present, oval in shape with a bluntly pointed tip. The nucleus is eccentric. The operculum is thin and semi-transparent, yellowish-green in colouration.

The radula (Figure 5C,D) is taenioglossate, with the formula 2 + 1 + r + 1 + 2. The central tooth is low trapezoid in shape and wider proximally than distally, with broad, thickened and flat anterior ridges on both sides of the shaft. The cusp of the central tooth is blunt and truncated. The lateral tooth is about twice as broad as the central tooth, with a straight inner edge lacking a lateral posterior buttress. Cusp development on the lateral tooth is quite variable and varies between having 5–7 cusps; in many cases the cusps are barely differentiated. The major cusp is typically the 4th from the inside, but sometimes there is one less inner cusp, leading the major cusp to be the 3rd from the inside. The major cusp shape is truncated and lobate. The inner and outer marginal teeth are similar in size, finely serrated on the distal tip into about 20–25 cusps.

The external anatomy (Figure 6) does not deviate significantly from other species in the genus *Provanna* [24, 39] and is only briefly described here. The animal is gonochoristic. The head is large (Figure 6A,C), with a wide snout and one pair of simple conical cephalic tentacles that are equal-sized in both sexes. Eyes are unpigmented. The foot is large, with the propodium clearly demarcated from the mesopodium by a groove. There is no epipodial tentacle on the foot around the opercular attachment

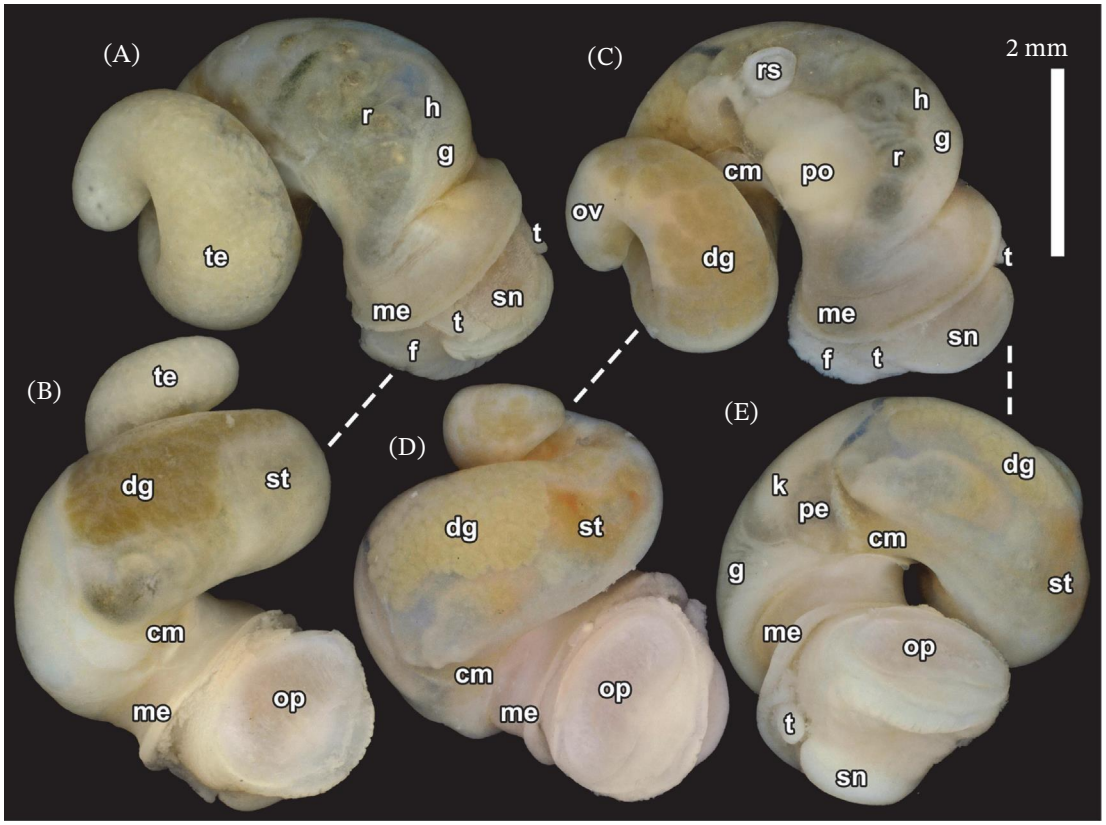

FIGURE 6 | *Provanna cocytus* sp. nov., external anatomy. (A,B) A male individual is shown from two views; (C–E) a female individual is shown from three views. Abbreviations: cm, columellar muscle; dg, digestive gland; f, foot; g, gill; h, hypobranchial gland; k, kidney; me, mantle edge; op, operculum attachment; ov, ovary; po, pallial oviduct; r, rectum; rs, receptacle seminis; sn, snout; st, stomach; t, cephalic tentacle; te, testis.

(Figure 6B,D). The mantle edge is thickened, smooth and lacking in folds or pigments. The pallial cavity (Figure 6A,C,E) extends about 0.4 whorls backwards, the left pallial roof largely occupied by the gill (ctenidium) carrying triangular leaflets. The osphradium is an elongate ridge positioned to the left of the gill and is rather pale in colouration and thus difficult to see externally. Posterior to the gill and the osphradium lie the kidney (nephridium) and the pericardium, positioned close to the anterior part of the columellar muscle. The hypobranchial gland is a narrow ridge on the pallial roof positioned to the right of the gill. Further to the right is a voluminous rectum containing numerous packaged faecal pellets, these are dark greyish-brown in colour and contain fine amorphous material mixed with some dark mineral particles. The rightmost part of the pallial roof is occupied by the gonoduct, in females the whitish pallial oviduct and seminal receptacle can be seen by transparency (Figure 6C). The visceral mass occupies ~1.5 whorls; the anterior half is mostly filled by the digestive gland and the stomach, while the posterior half is taken up by the gonad. The stomach is positioned about 0.5 whorls posterior of the start of the visceral mass, and it is visible through the mantle from the outside.

3.2.7 | Distribution

Known with certainty from three hydrocarbon seep sites in the northeastern Sea of Japan, including the NW Okushiri site, the E Okushiri site, and the W Matsumae site, between 2679 and 3349 m deep. The Off Joetsu methane seep at around 1000 m deep in the southeastern Sea of Japan off Niigata Prefecture, Japan (Figure 1A), has also been reported to be densely populated by a smooth-shelled *Provanna* species [5] and may represent another location for *P. cocytus* sp. nov.

3.2.8 | Remarks

Provanna cocytus sp. nov. joins several other living congeners from the northern Pacific that have a smooth shell surface lacking any significant axial or spiral sculpture [25]. These include *P. abyssalis* Okutani & Fujikura, 2002 [40] from Japan Trench seeps [40], *P. annae* Nekhaev, 2023 [41] from the Piip volcano hot vent in the Bering Sea [41], *P. laevis* Warén & Ponder, 1991 [39] from seeps on both sides of the Pacific [39], *P. subglabra* Sasaki, Ogura, Watanabe & Fujikura, 2016 from vents and seeps of Japan and the South China Sea, *P. lucida* Sasaki, Ogura, Watanabe & Fujikura, 2016 from Okinawa Trough vents, and *P. kuroshimensis* Sasaki, Ogura, Watanabe & Fujikura, 2016 from a Ryukyu Arc seep
[42].

The very thin, translucent shell of *P. cocytus* sp. nov. immediately separates it from *P. laevis*, *P. subglabra* and *P. kuroshimensis*, all of which have sturdy and rigid shells [42]. The oval aperture of *P. cocytus* sp. nov. easily distinguishes it from *P. lucida*, which has a clearly defined siphonal notch. Among all these species, *P. cocytus* sp. nov. has the most inflated whorls with the strongest suture constriction, which helps to distinguish it from *P. abyssalis* and *P. annae* [40, 41]. The weak inflation of whorls in *P. abyssalis* means its shell is much narrower than specimens of *P. cocytus* sp.

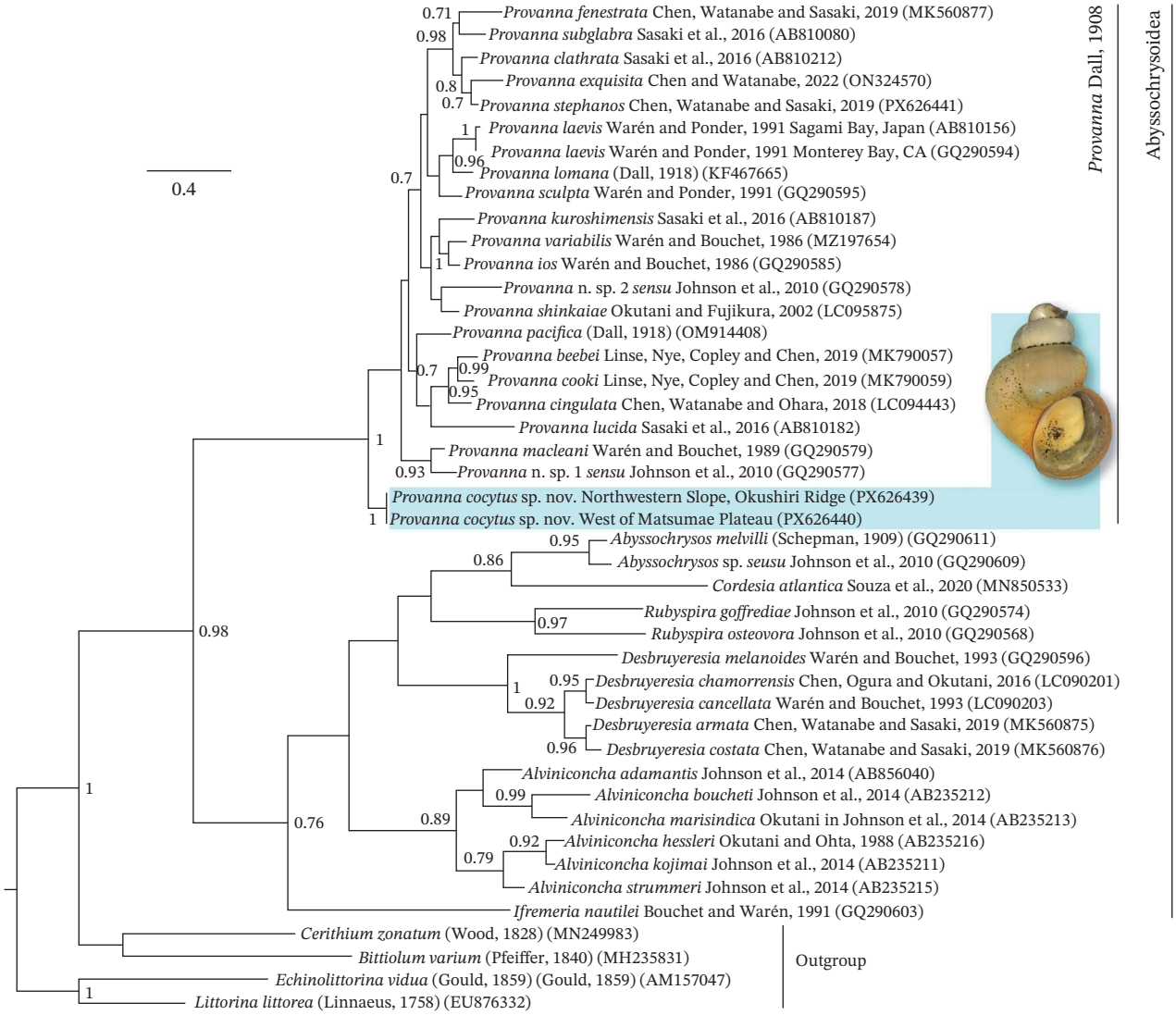

FIGURE 7 | Bayesian phylogenetic reconstruction of the gastropod superfamily Abyssochrysoidea using a 530 bp alignment of the mitochondrial *COI* gene. Node values represent Bayesian posterior probabilities, only values ≥0.7 are shown. The scale bar indicates substitutions per site.

nov. of the same size. The shell of *P. annae* is clearly angulated at both the shoulder and the base, but this is not the case in *P. cocytus* sp. nov; the protoconchs of these two species are also very different in that of *P. annae* lacking in axial ribs. Most importantly, the radula of *P. cocytus* sp. nov. is highly distinct among all smooth-shelled *Provanna* – it has a blunt and truncated central tooth cusp and a lateral tooth where the 3rd or 4th cusp is the strongest and entirely lacking in an angular buttress. All other smooth-shelled living *Provanna* species exhibit long, triangular central tooth cusps and lateral teeth with angulated buttresses [25]. Furthermore, the shell of *P. cocytus* sp. nov. is also similar to a fossil species, *P. urahoroensis* Amano & Jenkins, 2013 [43], from the lower Oligocene Nuibetsu Formation off the east of Hokkaido, Japan [43]. However, in *P. urahoroensis* the shell profile is angulated near the base and is not as strongly inflated as *P. cocytus* sp. nov. Interestingly, a similar, smooth-shelled provannid ('*Provanna*? sp.' of [44]) was also reported from the Miocene of Tsushima Island at the entrance of the Sea of Japan [44].

3.3 | Molecular Support

In our phylogenetic reconstruction of the superfamily Abyssochrysoidea using the mitochondrial COI gene (Figure 7), all extant genera with multiple species included (*Provanna*, *Desbruyeresia*, *Abyssochrysos*, *Alviniconcha*, and *Rubyspira*) were found to be monophyletic with strong to full support (Bayesian posterior probabilities, BPP = 0.89-1). The genus *Provanna* was recovered as a fully supported clade (BPP = 1), which also indicates that the family Provannidae is monophyletic, since *Provanna* is currently the only extant genus recognised in this family [45]. *Provanna cocytus* sp. nov. was recovered as the earliest-diverging lineage within the genus, sister to all other species included in the analysis. A BLAST sequence similarity search using 654 bp of the COI sequence revealed the most similar registered sequence to that of *P. cocytus* sp. nov. to be *P. variabilis* Warén & Bouchet, 1986 [24] (GenBank accession: MZ197654) at a percent identity of 93.36%, followed by

P. fenestrata Chen, Watanabe & Sasaki, 2019 [46] (GenBank accession: MK560877) at 93.32%, and *P. pacifica* (Dall, 1908) at 93.29% (GenBank accession: OP577954). These results support the recognition of *P. cocytus* sp. nov. as a species distinct from all provannids with COI molecular data available.

4 | Discussion

4.1 | Faunal Diversity of Cold Seeps in the Sea of Japan

Collectively, we found eight macrofaunal species in and around the three cold seep sites off Okushiri Island in the Sea of Japan that we surveyed (Figure 3; Table 1). Only E Okushiri exhibited all eight species, while NW Okushiri lacked the thyasirid clam *Parathyasira* sp. *sensu* Kamenev, 2013 [33] and W Matsumae only had three species, including the snails *Provanna cocytus* sp. nov. and *Hyalogyrina* cf. *umbellifera*, plus *Eurycope spinifrons* (Table 1). Our sampling efforts confirmed the species-level identity of the three taxa previously recorded at NW Okushiri by Naganuma et al. [17]: their 'unidentified isopods' were *Eurycope spinifrons*, 'unidentified gastropods' later preliminarily identified as belonging to the genus *Provanna* by Fujikura et al. [5] were *Provanna cocytus* sp. nov., and 'pectinid scallops' were *Delectopecten vancouverensis*. Naganuma et al. [17] also photographed an aeolid nudibranch close to the NW Okushiri seep (Figure 3F, misidentified as 'aspidochirot holothurian'), but they did not consider it to constitute seep-related fauna.

The finding of only eight species, which would be restricted to four if only counting those inhabiting active parts of the seep characterised by bacterial mats (Table 1), shows that the Sea of Japan seeps exhibit an unusually low species richness. These numbers are much lower than the species richness recorded at other western Pacific cold seeps, such as the Off Hatsushima seep in Sagami Bay, Japan, at 40 species [47], the Nankai Trough seeps at 15–29 species [9], the Kuroshima Knoll seep on the Ryukyu Arc at 20 species [48], the South China Sea seeps between 26 and 65 species [49, 50], and serpentinite-hosted seeps on the Mariana Forearc at 15–20 species [51, 52]. Cold seep fauna in the Sea of Japan therefore appears to follow the same trend as the ambient bathyal fauna in having an exceptionally low species diversity than other surrounding marine regions linked to an anoxic event during the last glacial maximum [16]. In addition to shallow connections with the surrounding seas acting as a barrier for species dispersing in deeper waters, the absence of charismatic groups like alvinocaridid shrimps and bathymodioline mussels with planktotrophic surface dispersal may be explained by temperature [53, 54]. Similar to how low water temperature has been cited as a reason for the absence of these groups in Arctic and Antarctic vents [38], the very cold Japan Sea Proper Water may present a physiological barrier that kills dispersing larvae on the way down through the water column or arrests their development.

4.2 | Adaptation and Ecology

Among the eight seep-associated species we recorded, only the two snails *Provanna cocytus* sp. nov. and *Hyalogyrina* cf. *umbellifera* found at all three seeps were strictly associated with bacterial mats and are therefore considered endemic to the cold seep environment. This is also supported by the fact that previous extensive sampling efforts using beam trawl and epibenthic sledge did not yield specimens of these species [15]. Both *Provanna* and *Hyalogyrina* are genera restricted to chemosynthetic habitats, including hydrothermal vents, cold seeps, and organic falls [25, 36], and are probably grazers selectively feeding on the white bacterial mats. The thyasirid bivalve *Parathyasira* sp. *sensu* Kamenev, 2013 [33] is a eurybathic species known only from the shelf to lower bathyal depths of the Sea of Japan between about 900–3100 m [33, 55, 56]. Although thyasirids are common constituents of chemosynthetic ecosystems, this species has been collected from many non-seep stations using trawled gear or grab samplers in the Sea of Japan [33] and it is thus unlikely to be seep-endemic. *Parathyasira* species can host chemosymbiotic bacteria, as is known from *P. equalis*, but many such as *P. granulosa* and *P. neozelanica* apparently do not [57]. Even in *P. equalis* the density of symbiotic bacteria and its contributions to host nutrition vary among individuals, and thus the symbiosis is thought to only provide a supplementary source of food in addition to suspension-feeding [57]. *Parathyasira* sp. *sensu* Kamenev, 2013 [33] may also do the same, although the presence of symbiotic bacteria remains to be investigated [33, 56].

The other five species are all well-known species from the bathyal Sea of Japan and constitute background fauna [15] that are likely opportunists taking advantage of the bacterial primary production by living adjacent to, or occasionally intruding, the active seep area when within their physiological tolerance [1]. The DO measurements taken at the bacterial mats were all lower than the ambient values across the three seep sites, and indicate active seepage of an anoxic, reducing geofluid, as previously suggested from fatty acid composition data indicative of chemosynthetic primary production [17, 21]. Compared to the two Okushiri Ridge seeps, the W Matsumae site is not only more extensive in size, spanning over 500 m in length, but apparently also exhibits much greater geofluid output, judging from the much lower DO values recorded (DO = 0.80 mL/L, SD = 0.68) compared to other sites. The water temperature on the bacterial mat was not significantly different from the background environment at all three sites, which agrees with categorising them under cold seeps. Though subduction has been proposed as the driver of geofluid input for all three locations [17, 21], the geochemical background of the geofluid remains to be confirmed.

The absence of all ambient species at W Matsumae except the munnopsid isopod *Eurycope spinifrons* may reflect the physiology of the other species, such as being incapable of tolerating the higher concentration of geofluids indicated by the lower DO concentrations. *Eurycope spinifrons* is the most abundant animal species in the deep basin (>2600 m) of the Sea of Japan [15, 58], and our observations add to the evidence that it is a species with exceptional physiological capacity that likely helped with its extensive colonisation of the bathyal Sea of Japan [59]. It is a detritivore [34] and may ingest bacterial mats at seeps. The scale worm *Harmothoe derjugini* was another species found to intrude the bacterial mats at NW Okushiri and E Okushiri, and as it is a carnivorous species, as indicated by stable isotope evidence [34, 60], it may be feeding on *Provanna* and *Hyalogyrina* snails.

The ampharetid worm *Ampharete* sp. is likely the same species as the one reported by Alalykina [32], collected in abundance from epibenthic sledges between ~2500 and 3500 m deep and especially abundant at 2653 m, which is very close to the depth of E Okushiri. Ampharetids are selective deposit feeders [60] and they may gain

nutritional advantages from living near the bacterial mats and feeding on the bacteria. The glass scallop *Delectopecten vancouverensis* associated with the ampharetid tubes is a very abundant species between the shelf to lower bathyal depths of the Sea of Japan and is one of only three bivalves found below 3000 m depth [33], and the burrowing anemone *Edwardsia sojabio* is also known to be very common on muddy bottoms of the ambient bathyal environment of the Sea of Japan [35]. A previous study showed that both *D. vancouverensis* and *E. sojabio* primarily rely on small planktonic crustaceans [34], and it is unclear whether they can gain additional nutrition from being near seeps. Considering they occurred at similar densities at ambient conditions distant from the seeps, the benefits may be limited, if present. *Delectopecten* is, however, a genus commonly found to encroach on the peripheral areas of cold seeps [9] and hot vents [8] in the western Pacific, and it likely has some tolerance to chemosynthetic environments.

4.3 | Biogeography of Seep-Endemic Species

Our phylogenetic reconstruction of *Provanna* (Figure 7) revealed *P. cocytus* sp. nov. to be the earliest diverging species within the genus and at a distant position compared to all other known congeners described from around Japan [25, 46, 61]. This suggests *Provanna* did not arrive in the Sea of Japan post-last glacial maximum by dispersal from nearby seeps or vents. Given the Sea of Japan was at one time completely anoxic below about 400 m depth [10, 16], *P. cocytus* sp. nov. is unlikely to have persisted there and probably arrived via another route afterwards. One possibility is through a 'stepping stone' of organic falls, particularly wood falls [62]. This is supported by the fossil record of the area starting from the Cretaceous, where wood and plesiosaur falls as well as cold seep fossil assemblages all exhibit similar taxa [44, 63]. Unfortunately, although *Provanna* species have been recorded from wood [25], none of those from around Japan have been named, and their genetic data are currently unavailable for comparison [64]. Another scenario is that *P. cocytus* sp. nov. had an extraordinarily wide bathymetric range for the genus and was not wiped out by the anoxia event [25].

The *Hyalogyrina* species found at the Sea of Japan seeps is morphologically very similar to *H. umbellifera* described from a cold seep at 4808 m in the Aleutian Trench [36] and is here tentatively identified as that species. The Aleutian Trench is rather distant from the Sea of Japan, and the protoconch morphology of *Hyalogyrina* points to lecithotrophic dispersal, hinting that this may represent a cryptic species complex. Molecular data from the type locality (the Aleutian Trench) of *H. umbellifera* are sought in order to compare with the Sea of Japan population and to shed light on their relationship. However, as the diversity at cold seeps in the intermediate Kuril-Kamchatka Trench remains understudied, it is not entirely impossible that *H. umbellifera* represents a widely distributed species limited to cold, bathyal waters in the northern Pacific. Similar distribution patterns have been found in peracarid crustaceans like isopods and amphipods, which are brooders with low theoretical dispersal capabilities [65, 66] and it remains unclear how they disperse across different trench systems.

The Off Joetsu seep in the southeastern Sea of Japan represents the only other cold seep in the Sea of Japan with a known associated faunal community, which has not been fully characterised [5]. Nevertheless, the presence of thyasirid bivalves *Conchocele bisecta* and *Thyasira inadai* as well as a species of *Provanna* have been reported [5, 20, 22], showing that the species composition there is distinct from the three seeps off Okushiri Island surveyed herein. The absence of the two large thyasirids is likely attributable to depth, as the Off Joetsu seep is only around 1000 m deep compared to the sites at 2700–3350 m depth off Okushiri Island. The Okushiri seeps are also too deep to form methane hydrates [23], which may have an impact on faunal distribution. Whether the *Provanna* from Off Joetsu is also *P. cocytus* sp. nov. must also await future comparisons.

5 | Conclusions

We completed the first comprehensive faunal survey of cold seep chemosynthetic ecosystems in the Sea of Japan, filling a long-standing gap in biodiversity knowledge for both the bathyal Sea of Japan and chemosynthetic ecosystems of the western Pacific [4, 5, 15]. With a species richness of only three to eight species per site, the Sea of Japan cold seeps are exceptionally low in species richness compared to other previously studied seeps in the western Pacific [2, 9, 67], indicating the seep systems were equally impacted by the anoxic events before the last glacial period as was the ambient seafloor [15, 16]. The finding of a new *Provanna* species not closely related to any known congeneric species from other parts of Japan indicates *Provanna* did not reach the Sea of Japan by dispersal from nearby seeps or vents and may have instead arrived through organic falls. Numerous additional bottom-simulating reflector signals interpreted as methane hydrate deposits have been detected along the Sea of Japan margin, yet biologically unexplored [23]. Targeted surveys of these sites will yield valuable data for determining how seep faunal composition in the Sea of Japan varies with depth, distance, and geological settings.

Author Contributions

Chong Chen: conceptualisation, methodology, validation, formal analysis, investigation, resources, data curation, writing – original draft, visualisation. **Hiromi Kayama Watanabe**: methodology, validation, investigation, resources, data curation, writing – review and editing. **Shinsuke Kawagucci**: investigation, resources, writing – review and editing, supervision, project administration, funding acquisition.

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Ethics Statement

This work is registered in ZooBank under LSID: urn:lsid:zoobank.org:pub:717AB685-213B-48F2-9B13-74C1B81FBCA6.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

New DNA sequences generated during this study have been deposited in NCBI GenBank under the accession numbers PX626439–PX626441. Specimens examined have been deposited in two public museums: National Museum of Nature and Science, Tsukuba (NSMT), Japan and the Senckenberg Research Institute and Natural History Museum, Frankfurt (SMF), Germany (see main text for museum catalogue numbers). All other data are incorporated into the article main text.

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