



Same (sea) bed different dreams: Biological community structure of the Haima seep reveals distinct biogeographic affinities

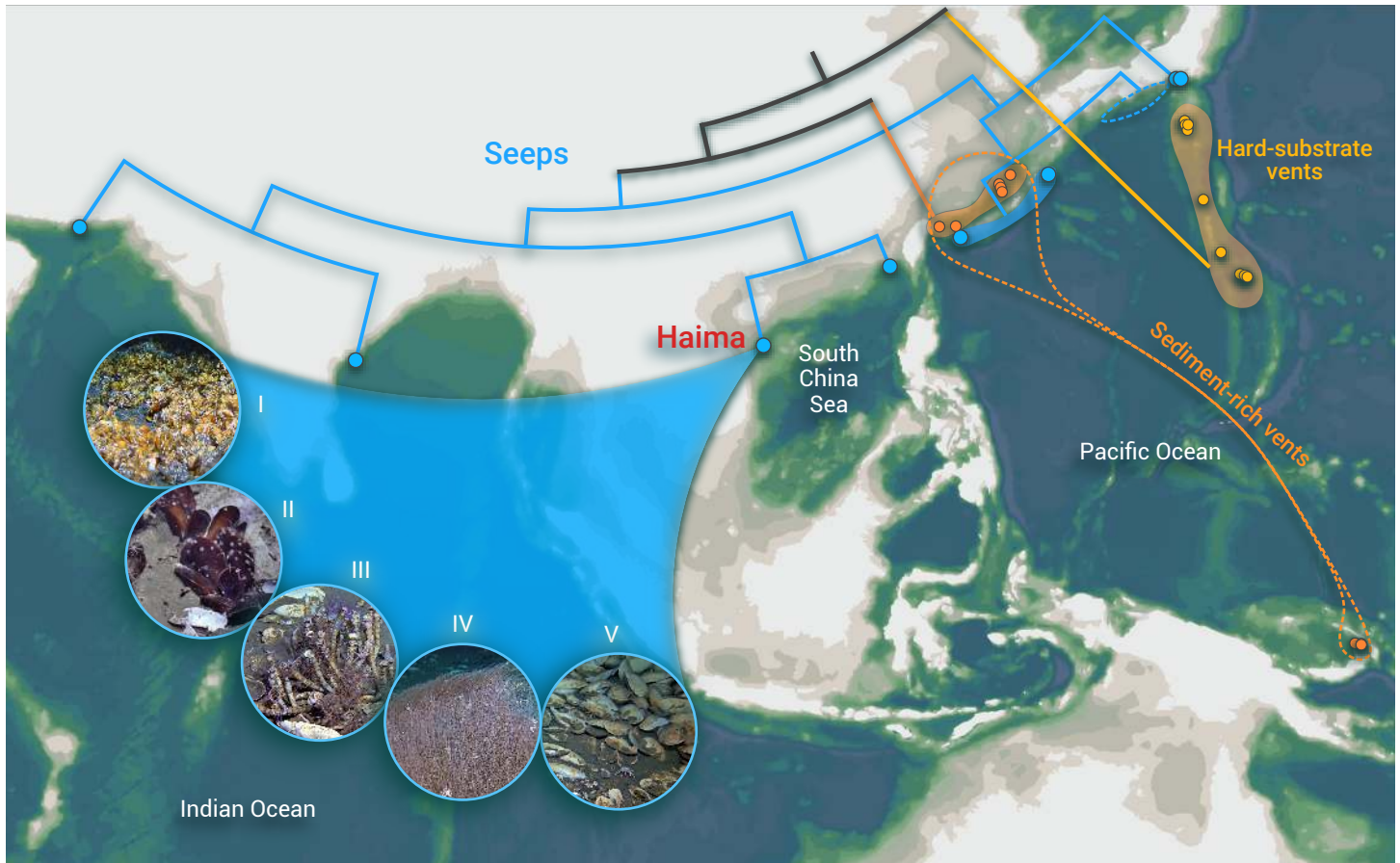
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GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- A total of 65 macrofauna were found at the Haima seep, and 35 of them appear endemic to this area.
- Sediment-rich vents are in intermediate habitats between seeps and hard-substrate vents.
- Seep communities in the South China Sea and the North Indian Ocean are closely related.
- The rich and endemic biodiversity at Haima calls for conservation measures.



Same (sea) bed different dreams: Biological community structure of the Haima seep reveals distinct biogeographic affinities

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Deep-sea chemosynthetic communities, including hydrothermal vents and cold seeps, harbour hundreds of endemic species currently threatened by deep-sea mining and hydrocarbon extraction. The South China Sea (SCS), a semi-enclosed marginal sea with two well-investigated active seeps (Haima in the west and Site F in the east), provides an opportunity to understand the biogeography of chemosynthetic ecosystems. Here, we conducted extensive field surveys using Remotely Operated Vehicles (ROVs) and collected specimens for morphological observations, molecular barcoding, phylogenetic analysis, and stable isotope analysis. Cluster analyses were applied to reveal the community structure of vents and seeps in the Western Pacific and Northern Indian Ocean. A total of 65 species covering seven phyla and 14 classes have been identified from Haima, doubling the number of species reported previously. Among them, 35 species are currently known only from Haima. Stable isotope analysis shows a typical chemosynthesis-based biological community. Community structure analysis at the genus level clustered Haima with Site F, though the species compositions and dominance of two seeps are fairly distinct. Between the two active seeps in the SCS, the higher species richness and endemism at Haima are potentially due to multiple factors, including the unique environmental factors there and the geographic isolation in the northwestern corner of the SCS. Moreover, a similarity in community compositions at the genus level between seeps in the SCS and North Indian Ocean was revealed, potentially mediated by the Early Pliocene opening of the Indonesian islands and the strong westward Indonesia Throughflow. Given the ongoing gas hydrate exploration activities in the SCS, our results will contribute to establishing a global network of marine protected areas for chemosynthetic-based ecosystems. The rich and endemic biodiversity at Haima calls for policymakers to formulate regulations to conserve the unique biodiversity there.

INTRODUCTION

Fueled by chemolithoautotrophic microbial production, chemosynthetic communities of deep-sea hydrothermal vents and cold/methane seeps flourish in various tectonic settings in the global ocean.^{1,2} With hundreds of hydrothermal vents and cold seeps discovered so far, and additional hundreds remain inferred,³ these island-like 'oases' are natural laboratories to study ancient and contemporary connectivity of deep-sea macrofauna, as well as how they have been impacted by historical tectonic activities and ocean currents.⁴ The larvae of many species in the chemosynthetic communities can either drift in the surface layer⁵ or disperse in the deep water,⁶ where ocean currents could drive them afield resulting in long-distance dispersal.⁵ Hydrothermal vent fields are most common on mid-ocean ridges, and the predominant larval dispersal pathway among different vents in this geological setting is most likely driven by currents along the ridges, therefore, historical tectonic activity is an important factor in shaping the community structure and faunal biogeography of global vent fields as we see today.^{7,8} Population connectivity at the species level reflects larval dispersal as a cumulative effect of physical ocean currents⁹ and larval dispersal barriers.¹⁰

In comparison, inter-species level genetic divergence and community compositions typically result from major geological events and tectonic repositioning.¹¹

The abundance of vents and seeps in the Northwest Pacific, together with the Northern Indian Ocean, enable us to characterize the process of speciation as well as the community connectivity,¹² especially as some species inhabit both habitat types in the Indo-West Pacific.¹³ In the South China Sea (SCS), a semi-enclosed marginal sea in the Northwest Pacific, three active methane seeps have been discovered (Figure 1A), including Site F¹⁴ (also known as Formosa Ridge or Jiaolong Ridge), the Yam Seep at Four-Way Closure Ridge^{15,16} in the northeastern SCS off Taiwan Island, and the Haima seep in the northwest off Hainan Island.¹⁷ Among them, the seep community of Yam Seep is poorly characterized,¹⁸ with records of only three bathymodioline mussels, two described ones including *Gigantidas platifrons* (formerly "*Bathymodiolus*" *platifrons*) and *G. securiformis* (formerly "*Bathymodiolus*" *securiformis*) as well as an undescribed species (i.e., *Bathymodiolus* sp. 1 *seusu* Kuo et al., 2019).¹⁹ Due to the geographical proximity of the Yam Seep and Site F (only 53 km apart), the macrofauna compositions at the Yam Seep is likely more similar to Site F than to Haima, although more expeditions are required in the future to verify this.²⁰ In contrast, the seep communities at Haima and Site F, approximately 1150 km apart, are fairly distinct. For instance, the dominant species of bathymodioline mussels at Haima is *G. haimaensis* whereas that at Site F is *G. platifrons*; *Archivesica marissinica*, the dominant species of vesicomyid clam at Haima, has not been found at Site F.²⁰⁻²² In the Indian Ocean, two active seep areas, the Makran seep off Pakistan²³ and a seep off India^{24,25} have been discovered, however, very little is known about their species composition.

As the first discovered active methane seep in the SCS, Site F has been extensively investigated with respect to the macrofaunal diversity.^{20,26,27} There have been several studies on the species compositions of the Haima seep,²⁸⁻³⁰ however, many species at this site have not been identified to the species level, and some species were incorrectly identified, with few molecular barcodes available to support their species identification. In addition, several species at Haima have recently been described or taxonomically revised.^{21,31-37} As such, we aimed to (1) revise and update the species list of the Haima seep via a combination of morphological observation and molecular barcoding, (2) revise the genus-level species list at the seeps off India and Pakistan based on published photographs and online video, (3) compare the macrofauna compositions of the SCS seeps (mainly Haima and Site F) with those in the Western Pacific and the Indian Ocean, and thereafter (4) investigate the biogeographic affinity of Haima and Site F within an Indo-West Pacific context.

RESULTS AND DISCUSSION

Macrofaunal assemblages at Haima

The macrofaunal assemblages at the Haima seep can be generally grouped into five types with different dominant species (Supplementary Figure S1 and Table 1) as follows. Type I: bathymodioline mussel beds dominated by *Gigantidas haimaensis* along with a variety of other macrofauna,

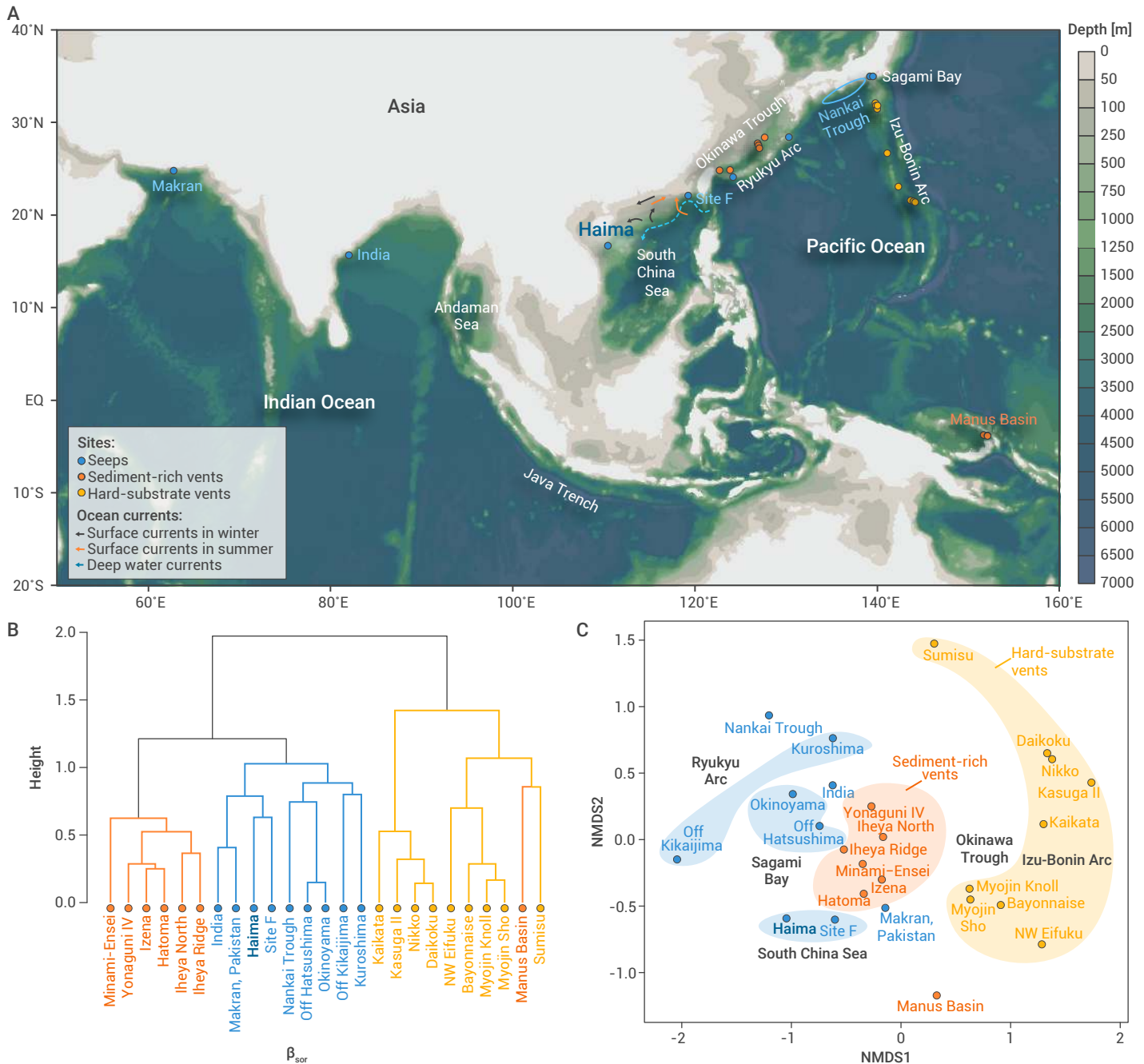


Figure 1. The distributions of vents and seeps in Indo-West Pacific and their biological community structures (A) A map showing 25 vents and seeps in the Indo-West Pacific with 145 fauna examined in this study. The map was drawn using Ocean Data View (ODV) v.5.0 (<https://odv.awi.de>). Colour coding represents the water depth (unit: m); ocean currents were drawn according to a previous study.⁵⁸ Black, surface current in winter; orange, surface current in summer; and blue, deep water current. (B) Cluster analyses of 16 vents and nine seeps on the same community composition data. Seeps, sediment-rich vents and hard-substrate vents are labelled in blue, orange and yellow, respectively;³⁹ (C) Two-dimensional non-metric multidimensional scaling (nMDS) plot of inter-field Soresen Index based on the genus-level community compositions of 16 vents and nine seeps summarized in [Supplementary Table S1](#). Seeps, sediment-rich vents and hard-substrate vents are labelled in blue, orange and yellow, respectively.

such as the occasional occurrence of another bathymodioline mussel *Gigantidas platifrons* (Figure S1a), the holothurian *Chiridota hydrothermica* (Figure S1b), free-living polynoid scale worms (Figure S1c), the alvinocaridid shrimps *Alvinocaris longirostris* and *A. kexueae* (Figure S1d), the patellogastropod limpet *Bathyaecmaea lactea* (Figure S1e indicated by white arrows) and the *Leptochiton* chitons (Figure S1e indicated by a black arrow) mainly living on mussel shells, the provannid snail *Provanna clathrata* (Figure S1f indicated by white arrows), the coioidean whelk *Phymorhynchus buccinoides*, and brittle stars (Figure S1g, h) dominated by *Histampica haimaensis*; Type II: bathymodioline mussel communities of "*Bathymodiolus*" *adoloides* (Figure S1i), occasionally with chitons living on their shells (Figure S1i indi-

cated by a white arrow); Type III: vestimentiferan tubeworm clusters of *Paraescarpia echinospica*, along with the co-occurrence of the sea anemone *Actinernus elongatus*, the holothurian *C. hydrothermica* and brittle stars, as well as the shrimp *Alvinocaris longirostris* (Figure S1j, k); Type IV: dense tubeworm aggregation of *Sclerolinum annulatum* (formerly *S. annulatus*³⁴), together with other macrofauna such as the mudpuppy squat lobsters *Munidopsis* spp. and the brittle star *Ophiophthalmus serratus* (Figure S1l); and Type V: vesicomid clams of *Archivesica marissinica*, along with the holothurian *Chiridota hydrothermica*, the propeamussid scallop *Catillopecten margaritatus* (Figure S1m, n indicated by white arrows), and the awning clam *Acharax* sp. (Figure S1m indicated by a black arrow).

Table 1. Taxa present at the Haima seep. (1) †, species only known from Haima so far; (2) #, species with barcode sequences (accession numbers in Supplementary Table S1). (3) *, only empty shells were collected.

Clade	Class	Family	Species	Clade	Class	Family	Species
Annelida	Polychaeta	Amphinomidae	<i>Eurythoe complanata</i> #	Mollusca	Bivalvia	Lucinidae	<i>Lucinoma</i> sp. *†
	Polychaeta	Capitellidae	<i>Notomastus</i> sp. †, #		Bivalvia	Lucinidae	<i>Myrtea</i> sp. *†
	Polychaeta	Hesionidae	<i>Sirsoe</i> sp. †		Bivalvia	Mytilidae	" <i>Bathymodiolus</i> " <i>aduloides</i> #
	Polychaeta	Maldanidae	<i>Nicomache</i> sp. †, #		Bivalvia	Mytilidae	<i>Gigantidas haimaensis</i> †, #
	Polychaeta	Nereididae	<i>Nereis</i> sp. †		Bivalvia	Mytilidae	<i>Gigantidas platifrons</i> #
	Polychaeta	Polynoidae	<i>Branchipolynoe pettiboneae</i> #		Bivalvia	Mytilidae	<i>Nypamodiolus samadiae</i> †, #
	Polychaeta	Polynoidae	<i>Branchinotogluma</i> sp.		Bivalvia	Propeamussiidae	<i>Catillopecten margaritatus</i> †
	Polychaeta	Siboglinidae	<i>Paraescarpia echinospica</i> #		Bivalvia	Solemyidae	<i>Acharax</i> sp. †, #
	Polychaeta	Siboglinidae	<i>Sclerolinum annulatum</i> †, #		Bivalvia	Thyasiridae	<i>Conchocele</i> cf. <i>bisecta</i> *
Bryozoa	Indet.	†	Bivalvia	Thyasiridae	<i>Thyasira</i> sp. †, #		
Cnidaria	Anthozoa	Actinernidae	<i>Actinernus elongatus</i> #	Bivalvia	Vesicomysidae	<i>Archivesica marissinica</i> †, #	
	Anthozoa	Hormathiidae	Indet. †, #	Bivalvia	Vesicomysidae	Indet. †, #	
Crustacea	Malacostraca	Alicellidae	<i>Paralicella</i> sp. †	Bivalvia	Yoldiidae	<i>Yoldiella</i> sp. †, #	
	Malacostraca	Alvinocarididae	<i>Alvinocaris longirostris</i> #	Caudofoveata	Indet. 1	†, #	
	Malacostraca	Alvinocarididae	<i>Alvinocaris kexueae</i> #	Caudofoveata	Indet. 2	†	
	Malacostraca	Cirolanidae	<i>Bathynomus jamesi</i>	Cephalopoda	Cranchiidae	<i>Helicocranchia pfefferi</i>	
	Malacostraca	Eurytheneidae	<i>Eurythenes maldoror</i>	Gastropoda	Buccinidae	<i>Phaenomenella samadiae</i> #	
	Malacostraca	Lithodidae	<i>Neolithodes brodiei</i>	Gastropoda	Desbruyeresiidae	<i>Desbruyeresia costata</i> #	
	Malacostraca	Lithodidae	<i>Paralomis</i> sp. †	Gastropoda	Fissurellidae	<i>Puncturella</i> cf. <i>parvinobilis</i> #	
	Malacostraca	Munidopsidae	<i>Munidopsis</i> cf. <i>bairdii</i>	Gastropoda	Muricidae	<i>Scabrotrophon scitulus</i> #	
	Malacostraca	Munidopsidae	<i>Munidopsis lauensis</i>	Gastropoda	Neolepetopsidae	<i>Paralepetopsis polita</i> †, #	
	Malacostraca	Munidopsidae	<i>Munidopsis verrilli</i>	Gastropoda	Pectinodontidae	<i>Bathyacmaea lactea</i> #	
	Malacostraca	Munidopsidae	<i>Munidopsis pilosa</i>	Gastropoda	Provannidae	<i>Provanna clathrata</i> #	
	Malacostraca	Munidopsidae	<i>Shinkaia crosnieri</i>	Gastropoda	Provannidae	<i>Provanna fenestrata</i> *	
	Malacostraca	Nematocarcinidae	<i>Nematocarcinus</i> sp. †	Gastropoda	Provannidae	<i>Provanna subglabra</i> #	
	Malacostraca	Parapaguridae	<i>Sympagurus</i> sp. †, #	Gastropoda	Raphitomidae	<i>Phymorhynchus buccinoides</i> #	
	Malacostraca	Uristidae	<i>Ichnopus</i> sp. †	Gastropoda	Raphitomidae	<i>Phymorhynchus</i> sp. †, #	
	Echinodermata	Echinoidea	Indet.	†, #	Gastropoda	Skeneidae	<i>Iheyaspira?</i> sp. †
Holothuroidea		Chiridotidae	<i>Chiridota hydrothermica</i> #	Polyplacophora	Leptochitonidae	<i>Leptochiton</i> sp. †, #	
Holothuroidea		Synallactidae	<i>Paelopatides</i> sp. †	Polyplacophora	Ischnochitonidae	<i>Thermochiton xui</i> †, #	
Ophiuroidea		Amphiuridae	<i>Amphioplus</i> sp. †, #	Solenogastres	Indet.	†, #	
Ophiuroidea		Ophiothamnidae	<i>Histampica haimaensis</i> †, #	Platyhelminthes	Polycladida	Discocelidae	Indet. †, #
Ophiuroidea		Ophiacanthidae	<i>Ophiophthalmus serratus</i> #				

Community structure

Community structure analysis performed at the genus level uncovered that the vent and seep macrofauna within the Indo-West Pacific form three clusters, which mainly correspond to the habitat types rather than geographic regions (Figure 1B; Supplementary Table S2). This result indicates species compositions of vent fields and seep areas tend to be distinct, which is in agreement with previous research showing that few chemosynthesis-associated macrofauna are capable of inhabiting both habitats.³⁸ The results also showed that the sediment-rich vent communities (represented mainly by vent communities at the Okinawa Trough) are in the intermediate position between seep communities and hard-substrate vent communities lacking sediment cover (represented by the Izu-Bonin vent communities), which is in line with the former network analysis suggesting that the sediment-rich vent communities may serve as an important connection between seep and vent macrofauna.³⁹ Although the species compositions of Haima and Site F exhibit considerable differences, the result of community structure analysis at the genus level still illustrated they are most closely related, with lower similarities than that between the two seeps off India and Pakistan (Figure 1B, C). In addition, it is intriguing that the genus-level community compositions of these two seeps is more similar to the two seeps in the Northern Indian Ocean, rather than other vent fields and methane seeps in the northwestern Pacific (Figure 1B), indicating ancient connections in-between.

Updated species list, molecular barcodes and molecular phylogeny of bathymodioline mussels

A total of 65 morphospecies covering seven phyla and 14 classes have been discovered from the Haima seep, doubling the number of species previously reported. Mollusca is the most dominant group containing 31 species, followed by Crustacea containing 15 species, and Annelida containing 9 species (Figure 2; Table 1). The complete species list and their gene accession numbers are summarized in Supplementary Table S1.

Of these, only 12 species are known to occur at both Haima and Site F, including five molluscs (i.e., "*Bathymodiolus*" *adoloides*, *Bathymacmaea lactea*, *Gigantidas platifrons*, *Provanna subglabra*, and *Phymorhynchus buccinoides*), three annelids (i.e., *Branchipolynoe pettiboneae* which lives inside the mussel mantle cavity, the free-living scale worm *Branchinotogluma* sp., and *Paraescarpia echinospica*), three crustaceans (i.e., *Alvinocararis longirostris*, *Paralomis* sp., and *Shinkaia crosnieri*), and the brittle star *H. haimaensis*. Outside Haima, the alvinocaridid shrimp *A. kexueae* has only been reported from hydrothermal vents in the Manus Basin.⁴⁰ Moreover, some species shared between Haima and Site F differed in relative abundance. For instance, *G. platifrons* was the dominant bathymodioline mussel at Site F; by contrast, only a few *G. platifrons* were collected at Haima where the mussel beds were dominated by *G. haimaensis*.²² The patellogastropod limpets mainly living on mussel shells at Haima were exclusively *B. lactea*; but at Site F the dominant species was *Bathymacmaea nipponica* which co-occurred with a few *B. lactea*.^{41,42} Clusters of the vestimentiferan tubeworm *Paraescarpia echinospica* were found from at least three localities at Haima;²⁹ nevertheless, only one individual of this species was found at Site F.²⁰ Brittle stars were highly abundant at Haima, but they were scarce at Site F.³⁷

At least 35 of the species found at Haima (~54%) appear to be endemic to this site, but only seven of them have been named, including the vesicomid clam *Archivesica marissinica*,²¹ the glass scallop *Catillopecten margaritatus*,⁴³ the bathymodioline mussels *Gigantidas haimaensis*²² and *Nypamodiolus samadiae*,³⁵ the limpet *Paralepetopsis polita*,⁴⁴ the chiton *Thermochiton xui*,³⁶ the brittle star *Histampica haimaensis*,³⁷ and the tubeworm *Sclerolinum annulatum*.³⁴ Many others are also almost certainly undescribed species. For instance, another vesicomid clam discovered at Haima ("Vesicomidae indet. Haima") did not fall into any clades representing described genera in the sub-family Pliocardiinae which usually harbour endosymbionts, among species with comparable genetic data (Figure 3). The hypertrophied gill tissue plus the dark red hemolymph indicate that it houses endosymbionts, agreeing with placing it in Pliocardiinae. Phylogenetic reconstructions have played a major role in reshaping the taxonomy of Vesicomidae,⁴⁵⁻⁴⁷ and the unique position of this undescribed clam indicates it most likely belongs to an undescribed genus. It is also noted that many genera in Pliocardiinae likely require taxonomic revision as they appear to be paraphyletic (Figure 3). This vesi-

comid was misidentified as *Lucinoma* sp. in former studies,^{28,30} corrected herein.

The protobranch clam *Yoldiella* sp. (*Malletia* sp. in the same former studies^{28,30}) was identified based on shell morphology (Figure 2Q), though molecular phylogenetic analyses based on 18S rRNA + 28S rRNA + H3 has indicated paraphyly of many genera in the superfamily Nuculanoidea, including *Yoldiella* and *Malletia* (Supplementary Figure S2), as has been previously shown in other studies.⁴⁸ Some other molluscs are also likely new, including the chiton *Leptochiton* (Figure 2K; Supplementary Figure S3), the lucinid clam *Lucinoma* (though only empty shells were collected) (Figure 2L), the awning clam *Acharax* (Figure 2M), another conoid snail *Phymorhynchus*, the thyasirid clam *Thyasira*⁴⁹ (Figure 2R), the glass scallop *Catillopecten* (Figure 2T), and the *Paralepetopsis* limpet (Figure 2V). We also discovered three infaunal worm-molluscs, two belonging to the class Caudofoveata (Figure 2I) and another to the class Solenogastres (Figure 2J), both previously unreported from Western Pacific seeps or vents. However, due to the paucity of molecular sequences within these two classes, it is currently difficult to unambiguously assign these three species to any genus (Supplementary Figure S4, 5). Probable new species unique to Haima are also common in other phyla. For instance, the capitellid worm *Notomastus* sp. (Figure 2A) was found to be morphologically distinct from other described species. A flatworm tentatively assigned to Discocelidae based on molecular phylogenetic analyses (Supplementary Figure S6) is also likely new. Both capitellids and flatworms are rare in deep-sea chemosynthetic environments, with flatworms only reported from the Kairei vent field in the Indian Ocean.⁵⁰ These results collectively suggest a high species diversity and also endemism at Haima.

Even though some species are not new species, their partial mitochondrial cytochrome *c* oxidase subunit I (COI) sequence shows a marginal similarity (i.e., below 98%) with those of the same species. For instance, *Provanna clathrata* (Figure 2Y) exhibits a 97.9% pairwise identity with specimens collected from the Okinawa Trough vents,⁵¹ suggesting a population-level differentiation. Another *Phymorhynchus* sp. is distinct from *P. buccinoides* based on morphological characteristics, and COI sequence shows a 97.8% similarity to *Phymorhynchus* sp. PHY03-Kilo_Moana collected from the Lau Basin.⁵²

Bathymodioline mussels have been suggested to be a good model in studying the biogeography of chemosynthetic ecosystems including vents and seeps because 1) they are foundation species at global vent and seep ecosystems,^{2,53} 2) most species have DNA/RNA sequences available in public databases, and 3) a few fossil records are also available for the reconstruction of time trees to understand their evolutionary history. With the newly generated transcriptome of *Gigantidas horikoshii* and the assembled transcriptomes published previously (Supplementary Table S3), a genome-level phylogeny of bathymodioline mussels with outgroups including three species of modioline mussels was reconstructed based on 5700 OGs with a total alignment of 1,458,741 amino acids. The topology of the resultant ML tree (Figure 4) is consistent with that reported previously⁵³ but with maximum bootstrap support. The closest relative of *G. haimaensis* was inferred to be *G. platifrons* (Figure 4). Calibrated by four available fossil records, the divergent time of *G. haimaensis* and *G. platifrons* was estimated to be late Miocene [i.e. 5.36 Ma, with a 95% highest posterior density (HPD) ranging from 2.70 Ma to 7.19 Ma]. The occasional occurrence of *G. platifrons* at Haima and the absence of *G. haimaensis* at Site F imply a weak westward gene flow from Site F to Haima, and a lack of gene flow from Haima to Site F. Nevertheless, more detailed studies are warranted in the future to better disentangle the causes of their historical divergence and speciation process.

The Kuroshio current is one of the major driving forces shaping the larval dispersal and species compositions around the eastern part of the SCS (including Site F) and vent fields in the Okinawa Trough.³³ Nevertheless, the Kuroshio current does not invade far deep into the SCS which is a semi-enclosed marginal sea since the uplift of Taiwan island in the late Miocene^{54,55} (Figure 1A); this could be a possible reason that shape the difference between Haima and Site F. In addition, the special faunal compositions at Haima seep could be the potentially unique local environmental factors (such as regional turbidity¹⁷) at Haima, though the exact factors require future studies.



Figure 2. A photo plate showing the dominant macrofauna at the Haima seep (A) *Notomastus* sp.; (B) *Nicomache* sp.; (C) *Sclerolinum annulatum*; (D) *Paraescarpia echinospica*; (E) *Alvinocaris kexueae*; (F) *Munidopsis* sp.; (G) *Ophiophthalmus serratus*; (H) *Histampica haimaensis*; (I) Caudofoveata sp. Haima; (J) *Solenogastres* indet. Haima; (K) *Leptochiton* sp.; (L) *Lucinoma* sp.; (M) *Acharax* sp.; (N) "*Bathymodiolus*" *aduloides*; (O) *Nypamodiolus samadiae*; (P) *Gigantidas haimaensis*; (Q) *Yoldiella* sp.; (R) *Thyasira* sp.; (S) *Conchocele* cf. *bisecta*; (T) *Catillopecten margaritatus*; (U) *Bathyacmaea lactea*; (V) *Paralepetopsis polita*; (W) *Puncturella* cf. *parvinobilis*; (X) *Desbruyersia costata*; (Y) *Provanna clathrata*; (Z) *Iheyaspira?* sp. The scale bar in white: 1 cm; the scale bar in blue: 1 mm.

Table 2. A species list identified based on morphological features at the seeps off India and Makran seep off Pakistan. ^{23-25,66,88}

Clade	Class	Genus/Species	Indian	Source	Pakistan	Source*
Annelida	Polychaeta	<i>Alaysia</i>	+	Figure 3k ²⁵		
	Polychaeta	<i>Amphisamytha</i>			+	8:03 ⁶⁶
	Polychaeta	<i>Branchinotogluma</i>			+	8:03 ⁶⁶
	Polychaeta	<i>Branchipolynoe</i>	+	Figure 3m ²⁵		
	Polychaeta	<i>Lamellibrachia</i>	+	²⁴	+	5:24 ⁶⁶
	Polychaeta	Siboglinidae indet.			+	6:22 ⁶⁶
Crustacea	Malacostraca	<i>Alvinocaris</i>			+	5:38 ⁶⁶
	Malacostraca	<i>Ashinkailepas</i>			+	Figure 78 ¹⁶
	Malacostraca	<i>Leucolepas</i>	+	Figure 3q ²⁵	+	Figure 113 ¹⁶
	Malacostraca	<i>Munidopsis</i>	+	Figure 3n ²⁵	+	Figure 55 ¹⁶
	Malacostraca	<i>Paralomis</i>			+	7:16 ⁶⁶
	Malacostraca	<i>Shinkaia</i>	+	Figure 3o ²⁵	+	Figure 84 ¹⁶
Mollusca	Bivalvia	<i>Acharax</i>	+	Figure 3e ²⁵	+	⁶⁸
	Bivalvia	<i>Calyptogena makranensis</i>	+	Figure 3b,c ²⁵	+	Figure 85 ¹⁶
	Bivalvia	<i>Catillopecten</i>	+	Figure 3f ²⁵		
	Bivalvia	<i>Conchocele</i>	+	Figure 3d ²⁵		
	Bivalvia	<i>Gigantidas</i> *	+	Figure 3a ²⁵	+	Figure 84 ¹⁶
	Gastropoda	<i>Bathyaemaea</i>	+	Figure 3m ⁸⁸	+	Figure 55 ¹⁶
	Gastropoda	<i>Phymorhynchus</i>	+	Figure 3i ²⁵		
	Gastropoda	<i>Provanna</i>	+	Figure 3o ⁴⁵	+	5:30 ⁶⁶

Notes: *the COI sequences of *Gigantidas* from these two sites have a 98.4% similarity and were therefore treated as the same species. * The time shows the appearance time of the target species in the online video.

Trophic structure

Stable isotopes were used to reveal the trophic levels of 23 species inhabiting Haima. In general, our results are in agreement with those of the former studies^{30,56,57} but with more species and tissues examined (Figure 5). Overall, the non-symbiotic tissue (such as foot or digestive gland) exhibits a higher $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values than the symbiotic tissue (such as gill and trophosome) of the same species, indicating the dependence of these animals on the symbionts for nutrition. The bathymodioline mussel *Gigantidas haimaensis* exhibited the lowest $\delta^{13}\text{C}$ value (-54.3‰), since it harbours methane-oxidizing bacteria (MOB) in its gill.^{22,58} A number of species were suggested to harbour sulphur-oxidizing bacteria (SOB),^{34,35,59,60} including *Acharax* sp., *Archivesica marissinica*, "*Bathymodiolus*" *adoloides*, *Nypamodiolus samadiae*, *Paraescarpia echinospica*, *Sclerolinum annulatum*, and *Vesicomysidae* indet. Haima. Their $\delta^{13}\text{C}$ values (range: -40.4‰ to -29.7‰) were all higher than that of *G. haimaensis*, suggesting a different carbon source than methane. Their nitrogen sources are likely to be different since the $\delta^{15}\text{N}$ values vary dramatically (range: -2.6‰ to 4.8‰). SOB use CO_2 for carbon fixation via Calvin-Benson-Bassham (CBB) cycle as indicated by the symbiont genomes of *A. marissinica*⁵⁹ and *P. echinospica*.⁶¹ More detailed features on the carbon and nitrogen sources warrant 1) additional symbiont genome sequencing, 2) extensive profiling of the stable isotope data from the ambient environments, such as seawater, pore water from the sediments, among others, a strategy applied in other studies⁶² and 3) trace element analyses.⁶³ For $\delta^{34}\text{S}$, the species that harbour SOB have an overall lower $\delta^{34}\text{S}$ values compared to *G. haimaensis* with MOB and other species, with *Vesicomysidae* indet. Haima exhibiting the lowest $\delta^{34}\text{S}$ value (-0.6‰). In the former analyses,^{56,57} the sulphur source of *G. platifrons* (closest-related species to *G. haimaensis*) was suggested to be seawater sulfate (though from different sources), and in species harbouring SOB, the sulfur source was thought to be sulfide derived from isotopically light sulfate-dependent anaer-

obic oxidation of methane. The brittle stars (three species in total) and the sea anemone *Actinernus elongatus* are likely suspension-feeders²⁸ which are widely distributed in different sites of the Haima seep, and exhibit overall high trophic levels among the 23 species examined.

The Indian and Pakistan seep communities

The following 14 genera are known at both the SCS and northern Indian Ocean seeps: *Acharax*, *Alvinocaris*, *Amphisamytha*, *Bathyaemaea*, *Branchinotogluma*, *Branchipolynoe*, *Catillopecten*, *Conchocele*, *Gigantidas*, *Munidopsis*, *Paralomis*, *Phymorhynchus*, *Provanna*, and *Shinkaia*, though they are likely different at the species level (Supplementary Table S2). For the molluscan genera (eight in total) from the Northern Indian Ocean seeps, all of them were also found at the SCS seeps. There are also some genera that differ between the northern Indian Ocean seeps and SCS seeps, for instance, the two siboglinid worms *Alaysia* and *Lamellibrachia*, one unidentified siboglinid, and the two neolepadid barnacles *Ashinkailepas* and *Leucolepas* from the northern Indian Ocean seeps. The Indian and Pakistan seep communities are similar⁶⁴ (Figure 1B). Although no detailed faunal information is available at the seep off Pakistan, except one description of the vesicomysid clam *Calyptogena makranensis*,⁶⁵ photographs suggest that it is dominated by the *Shinkaia* squat lobsters and bathymodioline mussels.^{23,66} It is possible that SCS and northern Indian Ocean seeps (plus any further undiscovered seeps between or around them) form a biogeographic region distinct from other western Pacific chemosynthetic communities.

Currently, only three molecular sequences of species from the seep off Pakistan are available, a COI sequence of an undescribed *Gigantidas* mussel⁶⁷ and two 18S rRNA sequences of the *Acharax* awning clam.⁶⁸ There is only one COI sequence of *Gigantidas* mussel from the Indian seep. We reconstructed a phylogeny of bathymodioline mussels, including those from seeps off India and Pakistan based on the partial COI gene. The two India and

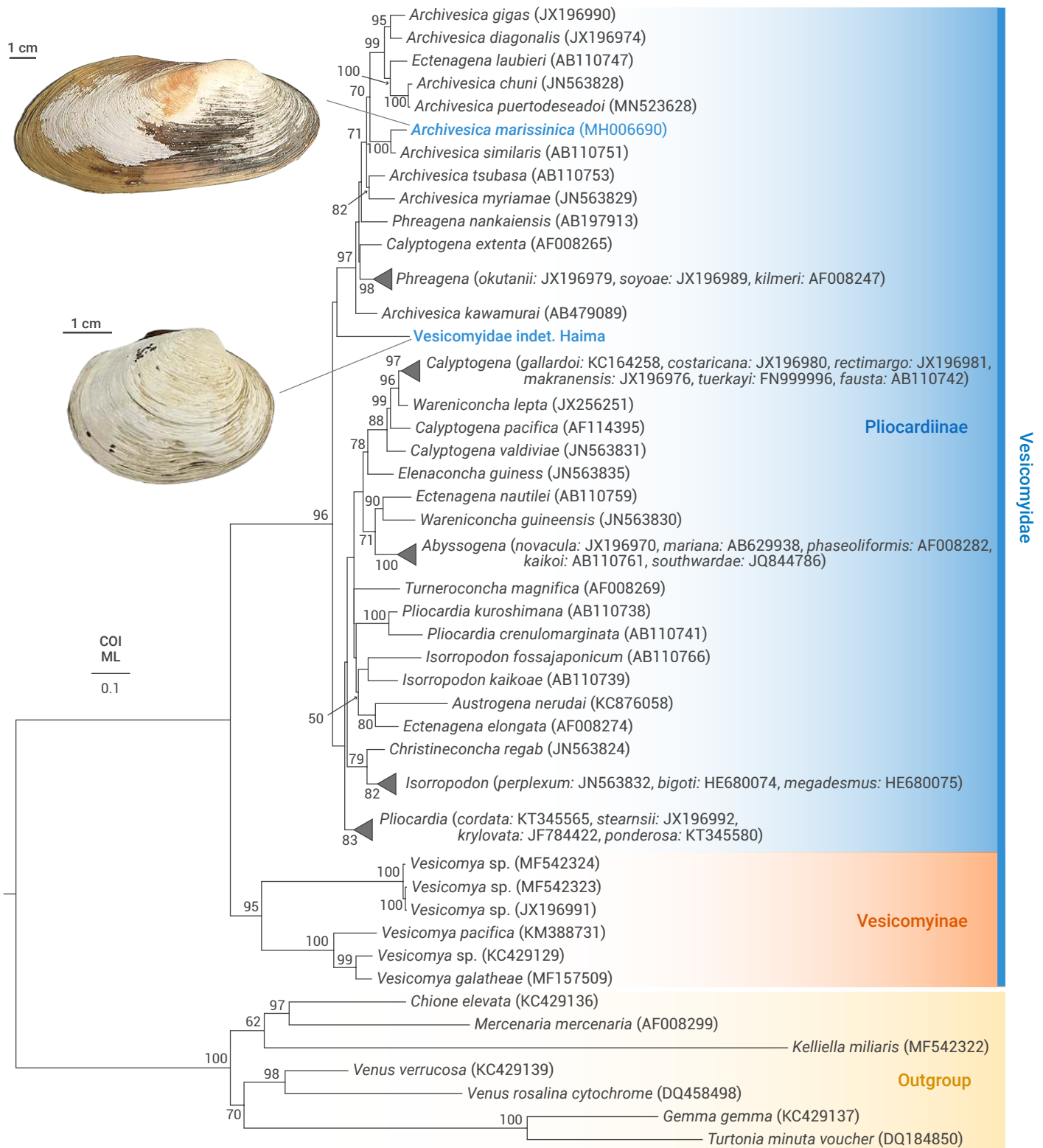


Figure 3. A maximum likelihood phylogeny of Vesicomiyidae based on partial COI gene The two vesicomiyid clams found at Haima were labelled in bold.

Pakistan seep *Gigantidas* mussels (Genbank accession No. ON964868 and KU597624) clustered together with a sequence similarity of 98.4%, indicating that they are the same species but with substantial inter-population differentiation (Supplementary Figure S7). Besides, these two mussels were clustered with those of *G. platifrons*, *G. haimaensis*, *G. childressi* and *G. mauritanicus* with a high bootstrap supports (i.e., 97), indicating close phylogenetic relationships among them. For *Acharax*, the two 18S rRNA sequences from the seep off Pakistan formed one clade, while the *Acharax* from Haima

showed over 99% similarity to another *Acharax* collected from the Java Trench off Indonesia.⁶⁸ Nevertheless, it should be noted that the 18S rRNA gene is highly conserved across metazoans, therefore, it is difficult to use this gene for species delimitation.

A possible hypothesis for the Indian Ocean affinity of SCS is through the connection of the Andaman Sea area perhaps at a geological time when the sea level was higher than the present level and the westward Indonesian Throughflow was stronger.⁶⁹ A persistent land bridge was present between

the SCS and Indian Ocean until the Early Pliocene.⁷⁰ Therefore, this through-flow may have facilitated species dispersal from the western Pacific towards Indian Ocean since then, leading to the establishment of genera like *Shinkaia* at the Indian Ocean seeps. This hypothesis can only be tested when genetic data from more species in the Indian Ocean become available for comparison. At least at the genus level, seven molluscan genera were found to be shared among the Pleistocene and Pliocene fossil seeps in Leyte, Philippines, and the SCS seeps as well as the northern Indian Ocean seeps, including *Archarax*, *Archivesica*, *Bathymodiolus*/*Gigantidas*, *Conchocele*, *Desbruyeresia*, *Lucinoma*, and *Provanna*,⁷¹ indicating part of the current SCS and the Northern Indian Ocean seep fauna potentially originated from the Philippines. We therefore call for further investigations on the species compositions of the chemosynthetic communities in the Andaman Sea and the Java Trench,^{68,72} which would not only enhance our understanding of genetic connectivity between these two oceans but also help delineate the biogeography of global chemosynthetic ecosystems.

CONCLUSION

Collectively, through in-depth field observation, morphological investigation, together with molecular barcoding, community structures, phylogenetic analyses, and stable isotope characterization, we present the most complete community compositions of the Haima seep up to date. Our community structure analysis indicated a relatively similar structure between Haima and Site F at the genus level, yet there are substantial differences in both species compositions and the relative abundance of dominant species between them. Among the 65 species identified at Haima, 35 have only been found at this seep. The stable isotope analysis revealed a complexity of trophic levels, with the primary producers being symbiotic MOB or SOB. These results revealed a very high species diversity and species endemism at Haima compared with those at other vents and seeps including Site F in the Indo-West Pacific region. The northern continental slope of SCS has been proposed as an ideal location for future gas hydrate extraction due to the presence of a major deposit.⁷³ Trials targeting commercial extraction of this energy resource have been conducted.⁷⁴ However, it is unclear whether species diversity and abundance would be jeopardized during the mining process, though this is likely.⁷⁵ Considering the unique community structure at Haima compared to Site F, this study will serve as an essential baseline for future environmental impact assessments under the mining scenarios and also for guiding conservation actions.

METHODS

Benthic sample collection, identification, barcoding, and phylogenetic analyses

Samples of macrofauna were collected from the Haima seep in May 2019, September 2020, and September 2022 using the ROVs *Haima*, *Haima2*, and *Pioneer*, respectively onboard the research vessels (R/Vs) *Haiyang 6* of Guangzhou Marine Geological Survey (Guangzhou, China) and *Xiangyanghai 01* of the First Institute of Oceanography, Ministry of Natural Resources (MNR) (Qingdao, China). Two sites, namely Site 01 (16°43.937' N 110°27.681' E, depth 1385 m) and Site 02 (16°54.033' N 110°28.460' E, depth 1428 m), were extensively sampled by using a suction sampler mounted on the ROVs, a hand net, or a box corer. Once the samples were recovered onboard the R/Vs, they were firstly identified based on the morphological features. Meanwhile, the samples were either preserved in 95% ethanol or immediately stored at -80°C in deep freezers for later dissection and genomic DNA extraction.

Genomic DNA was extracted from the muscle tissue of each sample using the CTAB method following a formerly published protocol.⁵⁸ The following primer pairs were used to amplify the genes for molecular barcoding: LC01490 and HCO2198 for the mitochondrial cytochrome c oxidase subunit I (COI) gene,⁷⁶ F19⁷⁷ and R1843⁷⁸ for the nuclear 18S rRNA gene, D1RF and D3CaR⁷⁹ for the nuclear 28S rRNA gene, and H3F and H3R for the histone H3 gene.⁸⁰ Gene amplifications were performed using a 96-well Veriti Thermal Cycler (Applied Biosystems, Carlsbad, USA). The purified PCR products were then bi-directionally sequenced on an ABI PRISM® 3730xl DNA Analyzer (Applied Biosystems, Foster, USA) with the same primers as for PCRs and analysed using the DNASTAR Lasergene package (DNASTAR Inc., USA).

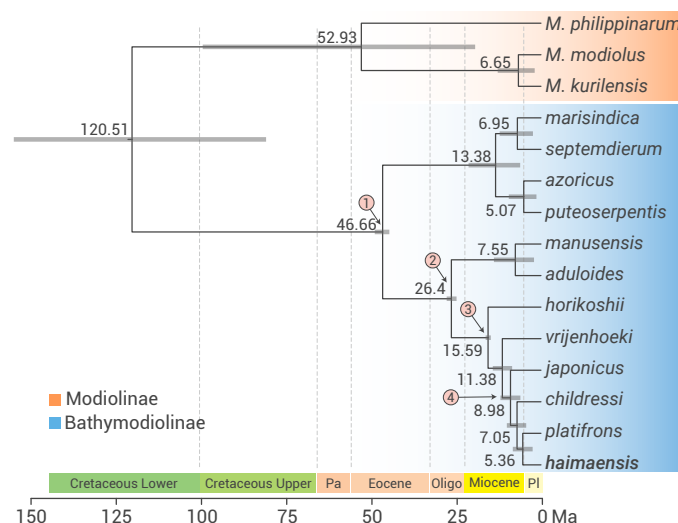


Figure 4. A time-calibrated phylogeny showing the estimated divergent time among bathymodioline species Three fossil constraints were used to calibrate the tree: node 1, soft maximum of 48.6 Ma and hard minimum of 45 Ma; node 2, soft maximum of 27.3 Ma and hard minimum of 25.2 Ma; node 3, soft maximum of 15.9 Ma and hard minimum of 15.1 Ma; and node 4, a hard minimum of 2.58 Ma.

For species without successful amplification in the above four gene fragments, their genomic DNA was paired-end sequenced on an Illumina NovaSeq 6000 Sequencer in Novogene (Beijing, China). The raw sequencing data were filtered using Trimmomatic v.0.38⁸¹ to eliminate adapters and low-quality reads. The resultant high-quality reads were assembled using either SPAdes v.3.15.2⁸² or CLC Genomics Workbench v.7.5 (CLCbio, Arhus, Denmark) under the default settings. Afterward, the four barcoding genes were manually retrieved from the assembly with the BLASTn to search against those of their close relatives retrieved from GenBank with an *E*-value threshold of 1e-20.

The resultant sequences were aligned using MUSCLE,⁸³ trimmed by trimAL v.1.4.1.⁸⁴ and then used for phylogenetic analyses. The maximum-likelihood (ML) approach implemented in IQ-TREE 2 v.2.1.3⁸⁵ was applied, running 1000 ultrafast bootstraps under the best-fitting nucleotide-substitution model determined based on the Bayesian information criterion (BIC) via ModelFinder implemented in IQ-TREE 2.

Community structure

Community structure was characterized using both the non-metric multi-dimensional scaling (nMDS) analysis and the cluster analysis implemented in vegan package in R v.2022.07.2+576.⁸⁶ A Sorensen similarity index was used to calculate the distances based on the presence/absence of macrofauna from methane seeps in the SCS (i.e., Haima and Site F) together with other vent fields and seeps from the surrounding areas in the Pacific (i.e., Manus Basin and Japan) and the Indian Ocean (i.e., India and Pakistan).^{12,20,25,40,87,88} Each vent and seep site with more than five species was used as an independent site in the analysis. Methane seeps in the Nankai Trough were collated into a single mega-site since all sites in this region were poorly sampled. Considering the uncertainties in species identification, particularly for macrofauna inhabiting seeps off India and Pakistan, genus-level presence/absence was scored for most macrofaunal groups. Species in the phyla Brachiopoda, Bryozoa, Chordata, Cnidaria, Echinodermata, and Platyhelminthes were excluded since 1) these groups are poorly known or characterized from chemosynthetic ecosystems, and 2) poor species identification in all the sites examined. Previous vent/seep community studies omitted these poorly identified animal groups as well.^{12,87} Nevertheless, species-level identification was applied to the bathymodioline mussels and vesicomysid clams for most sites (including the seeps off India and Pakistan) based on their available COI barcodes in GenBank.⁶⁷

For comparative purposes, macrofauna from the seep off India were re-identified based on morphological features from available photos.^{24,25,88,89} For example, the snail identified as "Provannidae" is actually *Phymorhynchus*,

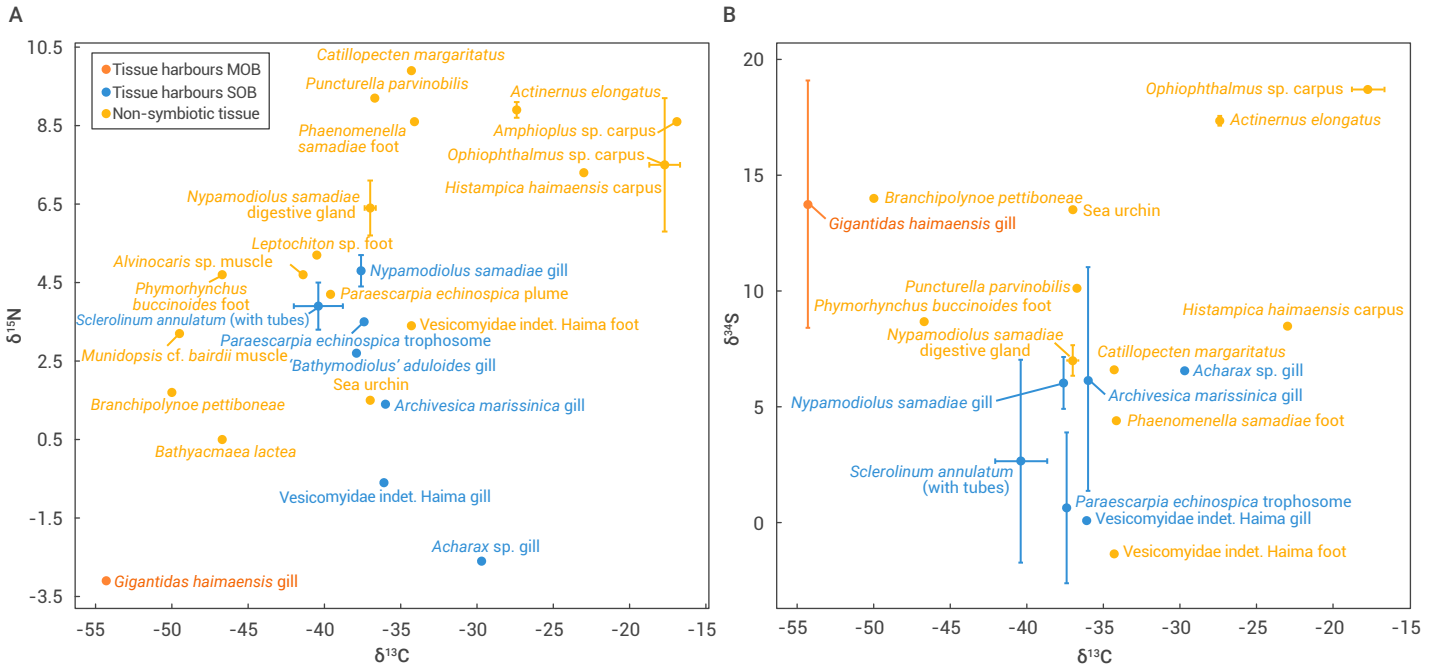


Figure 5. Stable isotope compositions ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$) of 23 macrofauna taxa collected from the Haima seep The dot in orange and in blue indicates that the tissue harbours methane-oxidizing bacteria (MOB) and sulphur-oxidizing bacteria (SOB) symbiont, respectively. (A) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the macrofauna; (B) $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ of the macrofauna. The data for *Archivesica marissinica*, *Gigantidas haimaensis*, and *Paraescarpia echinospica* were retrieved from a former study⁵⁷ and reanalysed herein.

which also occurs at Haima and Site F, and the tubeworm "*Sclerolinum*" is in fact *Alaysia*. The limpet was re-identified as belonging to the genus *Bathyaemaea*, and the "*Neolepas*" is corrected as *Leucolepas*, almost certainly *L. longa* which is widely distributed. The macrofauna from the Makran seep off Pakistan were identified from photos and high-resolution YouTube videos reported previously.^{23,66} The updated species list is provided in Table 2.

Phylogenomics analyses of the bathymodioline mussels

Phylogenomic data were collected from various sources, including the gene models predicted from the genomes,⁵⁸ the available transcriptome sequences from NCBI (with the SRA accession numbers listed in Supplementary Table S2), and the transcriptome of *Gigantidas horikoshii* generated in this study (see below). The low-quality and adapter-contaminated reads were removed using Trimmomatic v.0.38.⁸¹ The clean reads of each species were *de novo* assembled by Trinity v.2.15.0.⁹⁰ The Ion Torrent RNA-Seq data generated for the mussel *Bathymodiolus japonicus* was assembled using SPAdes v3.15.3⁹² with the settings "--rna --iontorrent". The assembled contigs were deduplicated using CD-HIT,⁹¹ translated into proteins using TransDecoder v.5.5.0, and only the "single-best" protein translation per isoform cluster was retained. A second round of CD-HIT was run on the protein sequences with the sequence similarity threshold of 0.8 applied to remove additional potential transcript isoforms. The assembly completeness was assessed using BUSCO v.5.2.2 based on the metazoan dataset.⁹²

Phylogenomic analyses were conducted following a formerly published pipeline,^{93,94} which includes orthologue (OG) identification, alignment, poor alignment trimming, paralogue removal using phylopyrunner, and phylogenetic analyses. For each OG, a taxon-occupancy threshold of 50% was applied. The supermatrix was partitioned, and the phylogenetic tree was constructed using IQ-TREE 2 v.2.1.2⁸⁵ with the setting of MFP which searched for the best model for each partition and 1000 ultrafast bootstraps. The fossil record of *Gigantidas coseli* was used as a calibration point for the divergence of *Gigantidas* from the other bathymodioline lineages.⁵⁵ To meet the requirement of this time-constrain, transcriptome sequencing was also applied to *G. horikoshii*, a specimen collected from the Higashi-Aogashima vent field (32°26.373'N, 139°53.8391'E, 747 m depth) using the deep-submergence vehicle (DSV) *Shinkai 6500* during the dive #1617 of cruise YK22-05 onboard R/V *Yokosuka*. RNA was extracted from the gill tissue of *G. horikoshii* using Trizol, and a eukaryotic type of transcriptome library was

prepared and sequenced on the Illumina Novaseq 6000 platform (Beijing, China) with the paired-end mode. Data cleaning and assembly were applied following the abovementioned pipeline. The resultant ML tree was calibrated with multiple fossil constraints in MCMCTree v.4.10.3.⁹⁵ The root age was set as 110 Ma following the former results.^{53,58} Fossil-based time constraints were applied on the four nodes as illustrated in Figure 4: node 1, soft maximum of 48.6 Ma and hard minimum of 45 Ma, corresponding to the *Vulcanidas? goederti* in the Middle Eocene (45 Ma);⁵³ node 2, soft maximum of 27.3 Ma and hard minimum of 25.2 Ma, corresponding to the *Bathymodiolus heretaunga* in the Early Miocene;⁵³ node 3, and soft maximum of 15.9 Ma and hard minimum of 15.1 Ma, corresponding to *G. coseli* in the Middle Miocene;⁵³ and node 4, a hard minimum of 2.58 Ma, corresponding to the Pliocene record of *Bathymodiolus securiformis* for fossil seeps in the Philippines.⁷¹ The evolutionary model, the total generations, the sampling frequency, and the burn-in was LG+Γ, 10 million, 1000, and the initial 1 million trees, respectively.

Stable isotope analysis

Stable isotope analysis of Carbon (C), Nitrogen (N) and Sulphur (S) of selected macrofaunal species was performed as described previously.³⁴ For species with symbionts, the symbiotic and non-symbiotic tissues were separated for analysis. Each freeze-dried sample was homogenized using a pestle and mortar, and weighed using a Mettler Toledo Microbalance (Mettler Toledo, Columbus, USA) before being sealed into a tin capsule. All the processed samples were then analyzed on a Sercon Integra2 Elemental Analyzer Isotope Ratio Mass Spectrometry (Sercon Instruments, Crewe, UK) either at the Third Institute of Oceanography, MNR (Xiamen, China) or at the University of Hong Kong (Hong Kong, China). Values of stable isotope compositions are permille (‰) using the δ notation relative to the standards, with Vienna Pee Dee Belemnite (VPDB) for C, air N₂ for N, and Vienna-Canyon Diablo Triolite (V-CDT) for S. A precision of $\pm 0.2\text{‰}$ was applied for the determinations of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$.

REFERENCES

- Baker, M.C., Ramirez-Llodra, E.Z., et al. (2010). Biogeography, ecology, and vulnerability of chemosynthetic ecosystems in the deep sea. In *Life in the world's oceans*, pp. 161–182.
- Dubilier, N., Bergin, C., and Lott, C. (2008). Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. *Nat. Rev. Microbiol.* 6, 725–740.

3. Beaulieu, S.E., and Szafranski, K. (2020). InterRidge global database of active submarine hydrothermal vent fields, Version 3.4. World wide web electronic publication available from <http://vents-data.interridge.org>.
4. Bachraty, C., Legendre, P., and Desbruyères, D. (2009). Biogeographic relationships among deep-sea hydrothermal vent faunas at global scale. *Deep Sea Res. Pt. I* **56**, 1371–1378.
5. Arellano, S.M., Van Gaest, A.L., et al. (2014). Larvae from deep-sea methane seeps disperse in surface waters. *P. Roy. Soc. B-Biol. Sci.* **281**, 20133276.
6. Van Dover, C.L., German, C.R., et al. (2002). Evolution and biogeography of deep-sea vent and seep invertebrates. *Science* **295**, 1253–1257.
7. Tunnicliffe, V., and Mary R. Fowler, C. (1996). Influence of sea-floor spreading on the global hydrothermal vent fauna. *Nature* **379**, 531–533.
8. Moalic, Y., Desbruyères, D., Duarte, C.M., et al. (2011). Biogeography revisited with network theory: retracing the history of hydrothermal vent communities. *Syst. Biol.* **61**, 127–127.
9. Mitarai, S., Watanabe, H., Nakajima, Y., et al. (2016). Quantifying dispersal from hydrothermal vent fields in the western Pacific Ocean. *Proc. Natl. Acad. Sci.* **113**, 2976–2981.
10. Breusing, C., Biastoch, A., Drews, A., et al. (2016). Biophysical and population genetic models predict the presence of phantom stepping stones connecting mid-Atlantic Ridge vent ecosystems. *Curr. Biol.* **26**, 2257–2267.
11. Yang, J.-S., Lu, B., Chen, D.-F., et al. (2013). When did decapods invade hydrothermal vents. Clues from the Western Pacific and Indian Oceans. *Mol. Biol. Evol.* **30**, 305–309.
12. Nakajima, R., Yamakita, T., Watanabe, H., et al. (2014). Species richness and community structure of benthic macrofauna and megafauna in the deep-sea chemosynthetic ecosystems around the Japanese archipelago: an attempt to identify priority areas for conservation. *Divers. Distrib.* **20**, 1160–1172.
13. Watanabe, H., Fujikura, K., Kojima, S., et al. (2010). Japan: Vents and seeps in close proximity. In the vent and seep biota, pp. 379–401.
14. Suess, E., Huang, Y., Wu, N., et al. (2005). South China Sea continental margin: geological methane budget and environmental effects of methane emissions and gas hydrates. *Ifm-geomar, Kiel. R/V Sonne cruise report* **177**.
15. Klauke, I., Berndt, C., Crutchley, G., et al. (2016). Fluid venting and seepage at accretionary ridges: the Four Way Closure Ridge offshore SW Taiwan. *Geo.-Mar. Lett.* **36**, 165–174.
16. Tseng, Y., Römer, M., Lin, S., et al. (2023). Yam Seep at Four-Way Closure Ridge: a prominent active gas seep system at the accretionary wedge SW offshore Taiwan. *Int. J. Earth. Sci.* **112**, 1043–1061.
17. Liang, Q., Hu, Y., Feng, D., et al. (2017). Authigenic carbonates from newly discovered active cold seeps on the northwestern slope of the South China Sea: Constraints on fluid sources, formation environments, and seepage dynamics. *Deep Sea Res. Pt. I* **124**, 31–41.
18. Wang, X., Guan, H., Qiu, J.-W., et al. (2022). Macro-ecology of cold seeps in the South China Sea. *Geogeo.* **1**, 100081.
19. Kuo, M.-Y., Kang, D.-R., Chang, C.-H., et al. (2019). New records of three deep-sea *Bathymodiolus* mussels (Bivalvia: Mytiliida: Mytilidae) from hydrothermal vent and cold seeps in Taiwan. *J. Mar. Sci. Technol.* **27**, 6.
20. Zhao, Y., Xu, T., Law, Y.S., et al. (2020). Ecological characterization of cold-seep epifauna in the South China Sea. *Deep Sea Res. Part I* **163**, 103361.
21. Chen, C., Okutani, T., Liang, Q., and Qiu, J.-W. (2018). A noteworthy new species of the family Vesicomidae from the South China Sea (Bivalvia: Glossoidea). *Venus* **76**, 29–37.
22. Xu, T., Feng, D., Tao, J., and Qiu, J.-W. (2019). A new species of deep-sea mussel (Bivalvia: Mytilidae: *Gigantidas*) from the South China Sea: Morphology, phylogenetic position, and gill-associated microbes. *Deep Sea Res. Pt. I* **146**, 79–90.
23. Bohrmann, G., and Participants, C. (2008). Report and preliminary results of R/V Meteor Cruise M74/3, Fujairah Male, 30 October - 28 November, 2007. Cold seeps of the Makran subduction zone (continental margin of Pakistan).
24. Mazumdar, A., Dewangan, P., Peketi, A., et al. (2021). The first record of the genus *Lamellibrachia* (Siboglinidae) tubeworm along with associated organisms in a chemosynthetic ecosystem from the Indian Ocean: a report from the Cauvery–Mannar Basin. *J. Earth Syst. Sci.* **130**, 94.
25. Mazumdar, A., Dewangan, P., Peketi, A., et al. (2019). The first record of active methane (cold) seep ecosystem associated with shallow methane hydrate from the Indian EEZ. *J. Earth Syst. Sci.* **128**, 18.
26. Li, X. (2017). Taxonomic research on deep-sea macrofauna in the South China Sea using the Chinese deep-sea submersible *Jiaolong*. *Integr. Zool.* **12**, 270–282.
27. Feng, D., Qiu, J.-W., Hu, Y., et al. (2018). Cold seep systems in the South China Sea: an overview. *J. Asian Earth Sci.* **168**, 3–16.
28. Dong, D., Li, X., Yang, M., et al. (2021). Report of epibenthic macrofauna found from Haima cold seeps and adjacent deep-sea habitats, South China Sea. *Mar. Life Sci. Tech.* **3**, 1–12.
29. Xu, H., Du, M., Li, J., et al. (2020). Spatial distribution of seepages and associated biological communities within Haima cold seep field, South China Sea. *J. Sea Res.* **165**, 101957.
30. Ke, Z., Li, R., Chen, Y., et al. (2022). A preliminary study of macrofaunal communities and their carbon and nitrogen stable isotopes in the Haima cold seeps, South China Sea. *Deep Sea Res. Pt. I* **184**, 103774.
31. Thomas, E.A., Liu, R., Amon, D., et al. (2020). *Chiridota heheva*—the cosmopolitan holothurian. *Mar. Biodivers.* **50**, 110.
32. Thomas, E.A., Sigwart, J.D., and Helyar, S.J. (2022). New evidence for a cosmopolitan holothurian species at deep-sea reducing environments. *Mar. Biodivers.* **52**, 63.
33. Xu, T., Sun, J., Watanabe, H.K., et al. (2018). Population genetic structure of the deep-sea mussel *Bathymodiolus platifrons* (Bivalvia: Mytilidae) in the Northwest Pacific. *Evol. Appl.* **11**, 1915–1930.
34. Xu, T., Sun, Y., Wang, Z., et al. (2022). The morphology, mitogenome, phylogenetic position, and symbiotic bacteria of a new species of *Sclerolinum* (Annelida: Siboglinidae) in the South China Sea. *Front. Mar. Sci.* **8**, 793645.
35. Lin, Y.-T., Kiel, S., Xu, T., and Qiu, J.-W. (2022). Phylogenetic placement, morphology and gill-associated bacteria of a new genus and species of deep-sea mussel (Mytilidae: Bathymodiolinae) from the South China Sea. *Deep Sea Res. Pt. I* **190**, 103894.
36. Wang, H., Liu, H., Wang, X., et al. (2022). Stirring the deep, disentangling the complexity: report on the third species of *Thermochiton* (Mollusca: Polyplacophora) from haima cold seeps. *Front. Mar. Sci.* **9**, 889022.
37. Li, Q., Li, Y., Na, J., et al. (2021). Description of a new species of *Histampica* (Ophiuroidea: Ophiothamnidae) from cold seeps in the South China Sea and analysis of its mitochondrial genome. *Deep Sea Res. Pt. I* **178**, 103658.
38. Wolff, T. (2005). Composition and endemism of the deep-sea hydrothermal vent fauna. *Cah. Biol. Mar.* **46**, 97–104.
39. Kiel, S. (2016). A biogeographic network reveals evolutionary links between deep-sea hydrothermal vent and methane seep faunas. *P. Roy. Soc. B-Biol. Sci.* **283**, 2016233.
40. van Audenhaege, L., Fariñas-Bermejo, A., Schultz, T., and Lee Van Dover, C. (2019). An environmental baseline for food webs at deep-sea hydrothermal vents in Manus Basin (Papua New Guinea). *Deep Sea Res. Pt. I* **148**, 88–99.
41. Xu, T., Wang, Y., Sun, J., et al. (2021). Hidden historical habitat-linked population divergence and contemporary gene flow of a deep-sea patellogastropod limpet. *Mol. Biol. Evol.* **38**, 5640–5654.
42. Zhang, S., Zhang, J., and Zhang, S. (2016). A new species of *Bathyacmaea* (Gastropoda: Pectinodontidae) from a methane seep area in the South China Sea. *Nautilus* **130**, 1–4.
43. Lin, Y.-T., Li, Y.-X., Sun, Y., et al. (2023). A new species of the genus *Catillopecten* (Bivalvia: Pectinoidea: Propeamussidae): morphology, mitochondrial genome, and phylogenetic relationship. *Front. Mar. Sci.* **10**, 1168991.
44. Chen, C., Zhong, Z., Qiu, J.-W., and Sun, J. (2023). A new *Paralepetopsis* limpet from a South China Sea seep hints at a paraphyletic Neolepetopsidae. *Zool. Stud.* **62**, 26.
45. Krylova, E.M., Sahling, H., and Borowski, C. (2018). Resolving the status of the families Vesicomidae and Kelliellidae (Bivalvia: Venerida), with notes on their ecology. *J. Mollus. Stud.* **84**, 69–91.
46. Johnson, S.B., Krylova, E.M., Audzijonyte, A., et al. (2017). Phylogeny and origins of chemosynthetic vesicomid clams. *System. Biodivers.* **15**, 346–360.
47. Decker, C., Olu, K., Cunha, R.L., and Arnaud-Haond, S. (2012). Phylogeny and diversification patterns among vesicomid bivalves. *PLoS One* **7**, e33359.
48. Sato, K., Kano, Y., Setiamarga, D.H.E., et al. (2020). Molecular phylogeny of probabranh bivalves and systematic implications of their shell microstructure. *Zool. Scr.* **49**, 458–472.
49. Li, Y., He, X., Lin, Y., et al. (2023). Reduced chemosymbiont genome in the methane seep thyasirid and the cooperated metabolisms in the holobiont under anaerobic sediment. *Mol. Ecol. Resour.* [Doi:10.1111/1755-0998.13846](https://doi.org/10.1111/1755-0998.13846).
50. Van Dover, C.L., Humphris, S.E., Fornari, D., et al. (2001). Biogeography and ecological setting of Indian Ocean hydrothermal vents. *Science* **294**, 818–823.
51. Sasaki, T., Ogura, T., Watanabe, H.K., and Fujikura, K. (2016). Four new species of *Provanna* (Gastropoda: Provannidae) from vents and a seep off Nansei-shoto Area, Northwestern Japan. *Venus* **74**, 1–17.
52. Poitrimol, C., Thiébaud, É., Daguin-Thiébaud, C., et al. (2022). Contrasted phylogeographic patterns of hydrothermal vent gastropods along South West Pacific: Woodlark Basin, a possible contact zone and/or stepping-stone. *PLoS One* **17**, e0275638.
53. Lorion, J., Kiel, S., Faure, B., et al. (2013). Adaptive radiation of chemosymbiotic deep-sea mussels. *P. Roy. Soc. B-Biol. Sci.* **280**, 20131243.
54. Nan, F., Xue, H., Chai, F., et al. (2011). Identification of different types of Kuroshio intrusion into the South China Sea. *Ocean Dyn.* **61**, 1291–1304.
55. Liu, Z., Li, X., Colin, C., and Ge, H. (2010). A high-resolution clay mineralogical record in the northern South China Sea since the Last Glacial Maximum, and its time series provenance analysis. *Chinese Sci. Bull.* **55**, 4058–4068.
56. Feng, D., Cheng, M., Kiel, S., et al. (2015). Using *Bathymodiolus* tissue stable carbon, nitrogen and sulfur isotopes to infer biogeochemical process at a cold seep in the south China Sea. *Deep Sea Res. Pt. I* **104**, 52–59.
57. Feng, D., Peckmann, J., Li, N., et al. (2018). The stable isotope fingerprint of chemosymbiosis in the shell organic matrix of seep-dwelling bivalves. *Chem. Geol.* **479**, 241–250.
58. Sun, J., Zhang, Y., Xu, T., et al. (2017). Adaptation to deep-sea chemosynthetic environments as revealed by mussel genomes. *Nat. Ecol. Evol.* **1**, 0121.
59. Ip, J.C.-H., Xu, T., Sun, J., et al. (2021). Host-endosymbiont genome integration in a deep-sea chemosymbiotic clam. *Mol. Biol. Evol.* **38**, 502–518.

60. Sun, Y., Sun, J., Yang, Y., et al. (2021). Genomic signatures supporting the symbiosis and formation of chitinous tube in the deep-sea tubeworm *Paraescarpia echinospica*. *Mol. Biol. Evol.* **38**, 4116–4134.
61. Yang, Y., Sun, J., Sun, Y., et al. (2020). Genomic, transcriptomic, and proteomic insights into the symbiosis of deep-sea tubeworm holobionts. *ISME J.* **14**, 135–150.
62. Becker, E.L., Cordes, E.E., Macko, S.A., et al. (2013). Using stable isotope compositions of animal tissues to infer trophic interactions in Gulf of Mexico lower slope seep communities. *PLoS One* **8**, e74459.
63. Wang, X., Fan, D., Kiel, S., et al. (2022). Archives of short-term fluid flow dynamics and possible influence of human activities at methane seeps: evidence from high-resolution element geochemistry of chemosynthetic bivalve shells. *Front. Mar. Sci.* **9**, 960338.
64. von Rad, U., Berner, U., Delisle, G., et al. (2000). Gas and fluid venting at the Makran accretionary wedge off Pakistan. *Geo-Mar. Lett.* **20**, 10–19.
65. Krylova, E.M., and Sahling, H. (2006). Recent bivalve molluscs of the genus *Calyptogena* (Vesicomysidae). *J. Mollus. Stud.* **72**, 359–395.
66. marumTV (2013). Cold Seeps in the deep sea <https://www.youtube.com/watch?v=QnLA1HyGahU>. YouTube.
67. Assié, A., Borowski, C., van der Heijden, K., et al. (2016). A specific and widespread association between deep-sea Bathymodiolus mussels and a novel family of Epsilonproteobacteria. *Env. Microbiol. Rep.* **8**, 805–813.
68. Neulinger, S., Sahling, H., Süling, J., and Imhoff, J. (2006). Presence of two phylogenetically distinct groups in the deep-sea mussel *Acharax* (Mollusca: Bivalvia: Solemyidae). *Mar. Ecol. Prog. Ser.* **312**, 161–168.
69. Saraswat, R., Singh, D.P., Lea, D.W., et al. (2019). Indonesian throughflow controlled the westward extent of the Indo-Pacific Warm Pool during glacial-interglacial intervals. *Glob. Planet. Change* **183**, 103031.
70. Lohman, D.J., Bruyn, M.d., Page, T., et al. (2011). Biogeography of the Indo-Australian Archipelago. *Annu. Rev. Ecol. Syst.* **42**, 205–226.
71. Kiel, S., Aguilar, Y., and Kase, T. (2020). Mollusks from Pliocene and Pleistocene seep deposits in Leyte, Philippines. *Acta Palaeontol. Pol.* **65**, 589–627.
72. Wiedicke, M., Sahling, H., Delisle, G., et al. (2002). Characteristics of an active vent in the fore-arc basin of the Sunda Arc, Indonesia. *Mar. Geol.* **184**, 121–141.
73. Chen, L., Feng, Y., Okajima, J., et al. (2018). Production behavior and numerical analysis for 2017 methane hydrate extraction test of Shenhu, South China Sea. *J. Nat. Gas Sci. Eng.* **53**, 55–66.
74. Zhang, W., Liang, J., Liang, Q., et al. (2021). Gas hydrate accumulation and occurrence associated with cold seep systems in the Northern South China Sea: an overview. *Geofluids* **2021**, 5571150.
75. Thomas, E.A., Molloy, A., Hanson, N.B., et al. (2021). A global red list for hydrothermal vent molluscs. *Front. Mar. Sci.* **8**, 713022.
76. Folmer, O., Black, M., Hoeh, W., et al. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* **3**, 294–299.
77. Turbeville, J.M., Schulz, J.R., and Raff, R.A. (1994). Deuterostome phylogeny and the sister group of the chordates: evidence from molecules and morphology. *Mol. Biol. Evol.* **11**, 648–655.
78. Elwood, H.J., Olsen, G.J., and Sogin, M.L. (1985). The small-subunit ribosomal RNA gene sequences from the hypotrichous ciliates *Oxytricha nova* and *Stylonychia pustulata*. *Mol. Biol. Evol.* **2**, 399–410.
79. Lenaers, G., Maroteaux, L., Michot, B., and Herzog, M. (1989). Dinoflagellates in evolution. A molecular phylogenetic analysis of large subunit ribosomal RNA. *J. Mol. Evol.* **29**, 40–51.
80. Colgan, D., McLauchlan, A., Wilson, G., et al. (1998). Histone H3 and U2 snRNA DNA sequences and arthropod evolution. *Aust. J. Zool.* **46**, 419–437.
81. Bolger, A.M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120.
82. Pribelski, A., Antipov, D., Meleshko, D., et al. (2020). Using SPAdes *de novo* assembler. *Curr. Protoc. Bioinformatics* **70**, e102.
83. Edgar, R.C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **32**, 1792–1797.
84. Capella-Gutiérrez, S., Silla-Martínez, J.M., and Gabaldón, T. (2009). trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* **25**, 1972–1973.
85. Minh, B.Q., Schmidt, H.A., Chernomor, O., et al. (2020). IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Mol. Biol. Evol.* **37**, 1530–1534.
86. Team, R.C. (2022). R: A Language and Environment for Statistical Computing.
87. Brunner, O., Chen, C., Giguère, T., et al. (2022). Species assemblage networks identify regional connectivity pathways among hydrothermal vents in the Northwest Pacific. *Ecol. Evol.* **12**, e9612.
88. Sangodkar, N., Gonsalves, M.J., and Nazareth, D.R. (2023). Macrofaunal distribution, diversity, and its ecological interaction at the cold seep site of Krishna-Godavari basin, east coast of India. *Microb. Ecol.* **85**, 61–75.
89. Dewangan, P., Sriram, G., Kumar, A., et al. (2020). Widespread occurrence of methane seeps in deep-water regions of Krishna-Godavari basin, Bay of Bengal. *Mar. Pet. Geol.* **124**, 104783.
90. Grabherr, M.G., Haas, B.J., Yassour, M., et al. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat. Biotechnol.* **29**, 644–652.
91. Li, W., and Godzik, A. (2006). Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* **22**, 1658–1659.
92. Seppey, M., Manni, M., and Zdobnov, E.M. (2019). BUSCO: Assessing genome assembly and annotation completeness. In *Gene Prediction: Methods and Protocols*, pp. 227–245.
93. Sun, J., Li, R., Chen, C., et al. (2021). Benchmarking Oxford Nanopore read assemblers for high-quality molluscan genomes. *P. Roy. Soc. B-Biol. Sci.* **376**, 20200160.
94. Liu, X., Sigwart, J.D., and Sun, J. (2022). Phylogenomic analyses shed light on the relationships of chiton superfamilies and shell-eye evolution. *bioRxiv* 2022.2012.2012.520088.
95. dos Reis, M., and Yang, Z. (2011). Approximate likelihood calculation on a phylogeny for bayesian estimation of divergence times. *Mol. Biol. Evol.* **28**, 2161–2172.

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AUTHOR CONTRIBUTIONS

P.-Y.Q. and J.-W.Q. conceived the project. J.S. and C.C. designed the sample collection plans and conducted the species identification for those inhabiting the Indian Ocean seeps. X.H., T.X., G.Y., Y.-X.L., Y.-T.L., Y.L. Y.S. and J.S. collected samples from the Haima seep. X.H., T.X., Z.-Y.Z., X.L. XG and Y.S. performed species identification, DNA extraction, molecular barcoding, and phylogenetic analyses. X.H. and J.S. performed the nMDS and cluster analyses. T.X., Y.T.L. and Y.-X.L. processed the samples for stable isotope analysis. X.H., J.S., T.X., and C.C. drafted the manuscript, with all authors contributing to its revision.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DATA AND CODE AVAILABILITY

Datasets supporting the results are included within the electronic supplementary material. Sequences generated in this study have been deposited in GenBank under the accession No. OQ749906-OQ749936 and OQ921785 for COI, OQ836636-OQ836664 and OQ921802 for 18S rRNA, OQ780403-OQ780425 and OQ921804 for 28S rRNA, and OQ754364-OQ754374 for H3. The raw transcriptomic sequencing data of Gigantidas horikoshii have been deposited in NCBI SRA with the accession number of SRR23924959.

SUPPLEMENTAL INFORMATION

It can be found online at <https://doi.org/10.59717/j.xinn-geo.2023.100019>

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