frontiers | Frontiers in Marine Science

ORIGINAL RESEARCH
published: 22 July 2022
doi: 10.3389/fmars.2022.920327

Community Characteristics and Genetic Diversity of Macrobenthos in Haima Cold Seep

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Knowledge about community structure and genetic diversity can help assess the potential for change in the loss and restoration of biodiversity, thereby facilitating effective management and ecosystem protection. Macroinvertebrate communities are an important biotic component of deep sea cold seep ecosystems. As Haima cold seep is increasingly being assessed for its potential gas hydrate mineral wealth, knowledge of community characteristics and genetic diversity of macrobenthos is needed to anticipate the potential impacts on biodiversity. In this study, we examined species diversity and community structure at five sites in the Haima cold seep using a remote-operated vehicle (ROV) for *in situ* surveying. The results identified 12 macrobenthic species from 5 phyla and 12 families. The macrobenthos community could be divided into two communities (H1 and H2 = mussel bed community, and H3 and H4 = vesicomyid clams community) based on CLUSTER and NMDS analyses. *Gigantidas haimaensis* (Mollusca), *Branchipolynoe pettiboneae* (Annelida), and *Histampica haimaensis* (Echinodermata) were most dominant within their respective phyla, with values of the dominance of 0.160, 0.021 and 0.114, respectively. The genetic diversity of these three typical macrobenthic species in the Haima cold seep was evaluated using the mitochondrial cytochrome c oxidase subunit I (*COI*) gene, haplotype, and nucleotide diversity values were 0.651 to 0.912 and 0.00148 to 0.00812, respectively, representing high haplotype diversity but low nucleotide diversity. Finally, mitochondrial concatenated dataset (MCD) sequences from three mitochondrial genes (*ATP6*, *COI*, and *NAD4*) and 294,734 genome-wide single nucleotide polymorphisms (SNPs) from restriction site-associated DNA-sequencing (RAD-seq) data were obtained from 60 individuals from two sites (H1 and H2), providing deep insight into the genetic diversity and structure of *G. haimaensis*, the engineer species in Haima cold seep. No significant genetic differentiation between *G. haimaensis* in H1 and H2 was detected based on MCD sequences. Nevertheless, when using SNP datasets, a small but clear genetic subdivision between *G. haimaensis* in the two sites as revealed by STRUCTURE and principal component analysis (PCA). The results comprehensively illuminate macrobenthos biodiversity in the Haima cold seep ecosystem and provide a baseline against which population dynamics may be assessed in the future.

Keywords: macrobenthos, *Gigantidas haimaensis*, genetic diversity, mitochondrial gene, RAD-seq, Haima cold seep, deep sea, chaotic genetic patchiness

OPEN ACCESS

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Specialty section:
This article was submitted to Deep-Sea Environments and Ecology, a section of the journal Frontiers in Marine Science

Received: *14 April 2022*
Accepted: *23 June 2022*
Published: *22 July 2022*

Citation:
Yao G, Zhang H, Xiong P, Jia H, Shi Y and He M (2022) Community Characteristics and Genetic Diversity of Macrobenthos in Haima Cold Seep. Front. Mar. Sci. 9:920327. doi: 10.3389/fmars.2022.920327

INTRODUCTION

Cold seeps previously believed to support minimal life are generally situated in the deep sea, with extremely high levels of toxic compounds and exceptionally high pressure (Yang et al., 2012). Unlike shallow-water ecosystems, which are based on photosynthesis, cold seep ecosystems rely on chemosynthetic production, in which chemotrophic bacteria perform oxidative reduction reactions to generate energy for carbon fixation (Feng et al., 2018). The high chemosynthetic primary production enables these unique ecosystems to develop high biomass in deep-water ecosystems (Ramirez-Llodra et al., 2010). Knowledge about these unique deep-sea chemotrophic ecosystems could expand our understanding of Earth's biodiversity (Vrijenhoek, 2010; Baco et al., 2016).

Macroinvertebrate communities are important biotic components of aquatic ecosystems, and a sound understanding of the genetic diversity and community structure of macroinvertebrates can provide a basis for assessing community changes following anthropogenic or natural disturbances (Hunter and Halanych, 2008). In particular, genetic diversity is associated with population fitness and is central to many conservation challenges such as species response to environmental changes, ecosystem recovery, and the viability of recently endangered populations (Frankham, 2010; Romiguier et al., 2014). Loss of genetic diversity can result in inbreeding depression, which can trigger a vortex of extinction, even in seemingly healthy populations, especially in populations with low effective sizes that are prone to genetic drift (Tanaka, 2000). Although maintenance of genetic diversity has been recognised as three essential components in conservation biology, genetic diversity is greatly ignored in management and policy (Laikre et al., 2010). In deep-sea ecosystems, patchy distribution of low-quality food, low faunal abundance, and low temperatures slow biological processes (McClain et al., 2012; Jones et al., 2017), making deep-sea species vulnerable to environmental change (Miyazaki et al., 2013; Breusing et al., 2015). By monitoring community structure and genetic diversity in deep-sea ecosystems, we can assess the potential for change in the loss and restoration of diversity and ecosystem resilience. However, investigating these remote ecosystems is challenging due to technical and financial limitations. As a result, information about the community structure of macrobenthos and genetic diversity in deep-sea ecosystems remains scarce (Breusing et al., 2015; Thaler et al., 2017).

Haima cold seep, discovered in the South China Sea in 2015 (Liang et al., 2017), covers an area of 618 km^2, including an active cold seep area of 350 km^2 (Zhao et al., 2020). However, huge amounts of gas hydrates providing a potentially vital energy resource have been detected in the shallow sediments of the Haima cold seep (Fang et al., 2019), making the area a potential target of gas hydrates extractions, which threatens deep-sea ecosystems. Therefore, there is an urgent need for efficient biodiversity surveys to inform conservation. Studies on biodiversity of some deep-sea species in Haima cold seep have been reported (Sun et al., 2018; Dong et al., 2021; Ke et al., 2022); however, knowledge on community characteristics and genetic diversity of macrobenthos in Haima cold seep remains limited. As Haima cold seep is increasingly being assessed for its gas hydrate mineral wealth, baseline data on the biodiversity of benthic invertebrates is needed for effective management and ecosystem protection (Everett and Park, 2018). In the present work, remote operated vehicle (ROV) *in situ* surveying, mitochondrial DNA fragment analysis of dehydrogenase subunit 4 (*NAD4*), ATP synthase F0 subunit 6 (*ATP6*), and cytochrome c oxidase submit I (*COI*), and restriction site-associated DNA sequencing (RAD-seq) were used to comprehensively characterise genetic diversity and composition of the macrobenthos communities in Haima cold seep. The results provide a valuable baseline biodiversity assessment prior to gas hydrates extractions.

MATERIALS AND METHODS

Sample Collection and Species Identification

Five sites were selected in Haima cold seep (**Figure 1**). During cruise HYDZ6-202005 of the *Haiyang 6* research vessel of the Guangzhou Marine Geological Survey, specimens were collected using the ROV *Haima* on September 1-6, 2020. Specimens were sampled using a 30 cm diameter, and 50 cm length sampling net manipulated by mechanical arms and photographed and videotaped using high-definition underwater cameras during the dive. The sampling net was held perpendicular to the sea floor when sampling and moved in parallel until the macrobenthos reached the bottom of the sampler. Megabenthos (e.g., *Neolithodes brodiei*) that a sampler cannot capture was directly grabbed using the mechanical arms or qualitatively analysed based on video records. The collected species were photographed immediately after being transported to the deck, then placed in liquid nitrogen and stored at -80°C. Morphological characteristics were used to preliminarily identify species for collected organisms according to relevant references (Zhao et al., 2020; Dong et al., 2021), and DNA barcodes were used for further species identification. The description of the species identified was given in supplementary electronic material.

Species Diversity and Community Structure of Macrobenthos in Haima Cold Seep

Species richness was estimated based on species presence/absence data (Marshall and Stepien, 2020). The percentage of species richness detected was calculated as a number of species collected at a given locality divided by the total number of species collected at the Haima cold seep. The relative abundances of species were calculated from species count data. Shannon-wiener (Shannon, 1948), Pielou (Pielou, 1966), and Margalef (Margalef, 1968) indices were used to assess biodiversity. The dominant value (Y) was used to calculate species dominance in Haima cold seep using the followed formula:

$$Y = fi' pi, pi = ni/N \quad (1)$$

"N" is the total number of samples collected, "pi" is the proportion of species "i" within the total number of samples

collected, and "fi" is the frequency of occurrence of species "i" at sampling points. Nonmetric multidimensional scaling (NMDS) and CLUSTER analyses were used to visualise the community structure of macrobenthos patterns using Primer 7 (https://www.primer-e.com/). Site H5 was removed from the analysis since this station has no fauna.

Mitochondrial Gene (COI, NAD4 and ATP6) Amplification

The type species from each phylum in Haima cold seep were chosen by the dominance values for analysis of population genetic diversity, which were *G. haimaensis* (Mollusca), *Branchipolynoe pettiboneae* (Annelida) and *Histampica haimaensis* (Echinodermata) Representative Cnidaria and Arthropoda species were absent due to insufficient samples. A total of 60 specimens (30 each for H1 and H2) of *G. haimaensis*, 14 specimens of *B. pettiboneae*, and 16 specimens of *H. haimaensis* (including 32 pre-existing *H. haimaensis COI* sequences downloaded from GenBank) were used for subsequent genetic diversity analysis based on *COI*.

Primers HCO2198 and LCO1490 (Vrijenhoek, 1994) were used to amplify *COI*. Total genomic DNA was extracted using an EasyPure Marine Animal Genomic DNA Kit (Transgen, Beijing, China) following the manufacturer's instructions. PCR amplification was carried out in a volume of 30 μL containing 1 μL of DNA template (100-200 ng/μL), 0.5 μL of each primer (10 μM), 13 μL of ddH_2O, and 15 μL of SuperMix (Transgen). Thermal cycling included an initial denaturation for 5 min at 94°C, followed by 34 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 52°C, and extending for 30 s at 72°C, followed by a final extension at 72°C for 10 min. PCR products were separated by 1% agarose gel electrophoresis, stained with ethidium bromide, then sequenced directionally (BGI, Shenzhen, China). Raw sequences were verified by visualising sequence peaks and assembled using the NASTAR Lasergene package. Sequence alignment was performed using MEGA v6.0 with default settings for sequence variation analysis (complete deletion), including parsimony informative sites and segregating sites (Tamura et al., 2011).

G. haimaensis is the most important, iconic, and engineer species in the Haima cold seep ecosystem (Liang et al., 2017; Xu et al., 2019). Dense mussel beds generate a highly complex habitat for many deep-sea species to inhabit. Thus, genetic diversity and structure were further assessed using three mitochondrial genes (*COI*, *ATP6*, and *NAD4*). Sixty (60) specimens of *G. haimaensis* from H1 (30 individuals) and H2 (30 individuals) were sampled. *ATP6* (Xu et al., 2019) and *NAD4* (Bielawski and Gold, 1996) were amplified using primers as previously described. The procedure for amplification was the same as that described above. Aligned sequences were removed and trimmed simultaneously. Finally, manual assembly yielded a mitochondrial concatenated dataset (MCD, *ATP6+COI+NAD4*) which was used for subsequent analysis.

RAD-Seq for SNP Calling

The above samples were subjected to RAD-seq, and the library was created according to an established method (Etter et al., 2012) using 0.1–1 μg of genomic DNA from each sample. DNA was digested using the restriction enzyme *Eco*RI, Solexa P1 Adapter, and Solexa P2 Adapter were ligated to the digested products, and libraries were sequenced on an Illumina platform to obtain 150 bp paired-end reads. To ensure that reads were reliable and without artificial bias in subsequent analyses, raw data (raw reads) in FASTQ format were first processed through a series of quality-control (QC) procedures. QC standards were as follows: (1) reads with ≥10% unidentified nucleotides (Ns) were removed; (2) reads with >50% of bases having a Phred quality<5 were removed; (3) reads with >10 nt aligned to the adapter were removed, allowing ≤10% mismatches. Finally, we obtained high-quality reads (average Q20 = 94.52% and average Q30 = 87.5%). The error rate reads were calculated using in-house Perl scripts made by Novogene Co., Ltd (Beijing, China;Setting:-r 150 -N 0.1 -q 33 -L 5 -p 0.5). Next, RAD tags were extracted from the draft genome (Sun et al., 2017), which served as a reference for downstream alignment and SNP discovery. Burrows-Wheeler Aligner (BWA) software was used to align clean reads from each individual against the reference genome (settings = mem–t – 4–k

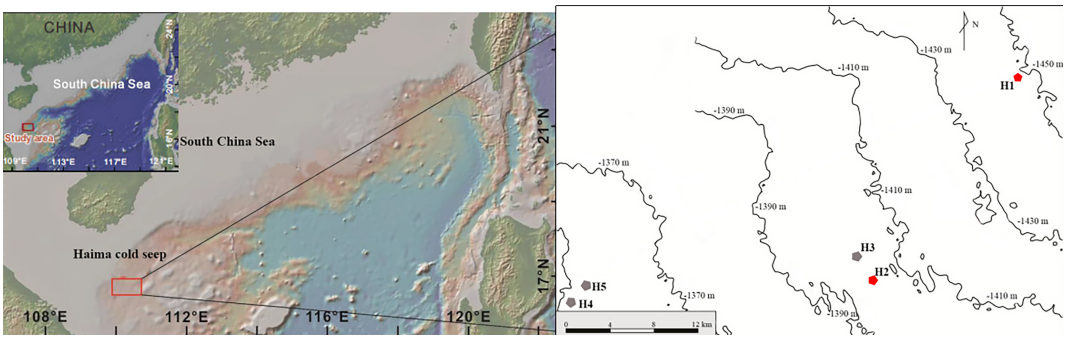

FIGURE 1 | Locations for sample collection of *G. haimaensis*. Five investigating sampling sites were set, only H1 and H2 that were marked by red hexagon were found to have *G. haimaensis* populations. H3, H4 and H5 in which no mussel population were marked by grey hexagon.

32–M) (Li & Durbin, 2009). Alignment files were converted to bamfiles using SAMtools software (settings = rmdup). After alignment, SNP calling was performed using a conservative Bayesian approach as implemented in SAMtools (Li et al., 2009). To improve the accuracy of SNP genotyping, additional filters were applied using (1) coverage depth >3 and (2) global minor allele frequency (MAF) ≥0.05.

Genetic Diversity and Population Dynamics Analyses of the Three Representative Macrobenthic Species Based on COI

Genetic diversity, including haplotype diversity (*Hd*), the number of haplotypes (*H*), and nucleotide diversity (π), were estimated using DNasp5.10 (Librado and Rozas, 2009). Population demography was examined using two different approaches based on *COI* sequences. Demographic history was investigated by mismatch distributions using DnaSP5.10 (Librado and Rozas, 2009), and *Tajima's D* (Tajima, 1989) and *Fu's Fs* (Fu, 1997) tests were conducted to examine the neutrality of sequences with Arlequin 3.5 (Shen et al., 2016).

Genetic Diversity and Structure Analyses of *G. haimaensis* based on MCD Sequences and SNPs

The genetic diversity index of *G. haimaensis* based on MCD was calculated using the method described above, including *Hd*, *H* and π. Expected heterozygosity (*He*), the effective number of alleles (*Ne*), and observed heterozygosity (*Ho*) were calculated using genome-wide SNPs. To examine the genealogical relationships among mitochondrial DNA (mtDNA) haplotypes, haplotype network analysis based on *COI*, *NAD4*, and *ATP6* was performed using Network 4.6 (Bandelt et al., 1999). Genetic divergence (*Fst*) of *G. haimaensis* between H1 and H2 was examined based on MCD sequences using Arlequin 3.5 (Excoffier and Lischer, 2010). The population structure of *G. haimaensis* for H1 and H2 was examined using the Bayesian algorithm implemented in STRUCTURE 2.3.4 (Pritchard et al., 2000) based on the SNP dataset. The optimal value of K was estimated using both Puechmaille's method (Puechmaille, 2016) and Evanno's ΔK method (Evanno et al., 2005) as implemented in STRUCTURE SELECTOR (Li and Liu, 2018). FST values between the two populations of *G.haimaensis* based on SNPs were calculated using Arlequin, with 10,000 permutations to determine significance. Principle component analysis (PCA) was performed to explore population division.

Fst *vs* heterozygosity analysis was implemented to identify the outliers loci derived from habitat selection. A locus with heterozygosity and an FST value above the 99.95th percentile of the related distribution is considered an outlier (Bovo et al., 2020). FST and heterozygosity of H1 and H2 were calculated with vcftools v 0.1.14 and the script (Setting:fst-window-size: 10,000; fst-window-step: 5,000). The top 0.05 Hp and top 0.05 Fst of the two sites of *G. haimaensis* were intersected, and then the intersection of the two populations was combined for deduplicating. Finally, the outliers loci were filtered using VCFtools.

RESULTS

Species Diversity and Community Structure of Macrobenthos in Haima Cold Seep

Twelve macrobenthic species from five phyla and 12 families were identified. Two of 12 macrofauna species were identified for the first time in Haima cold seep (**Table S1**). Regarding relative abundance, Mollusca (41.95%), Annelida (32.33%) and Echinodermata (15.36%) were the most represented phyla. The most abundant species was *G. haimaensis* (30.09%),

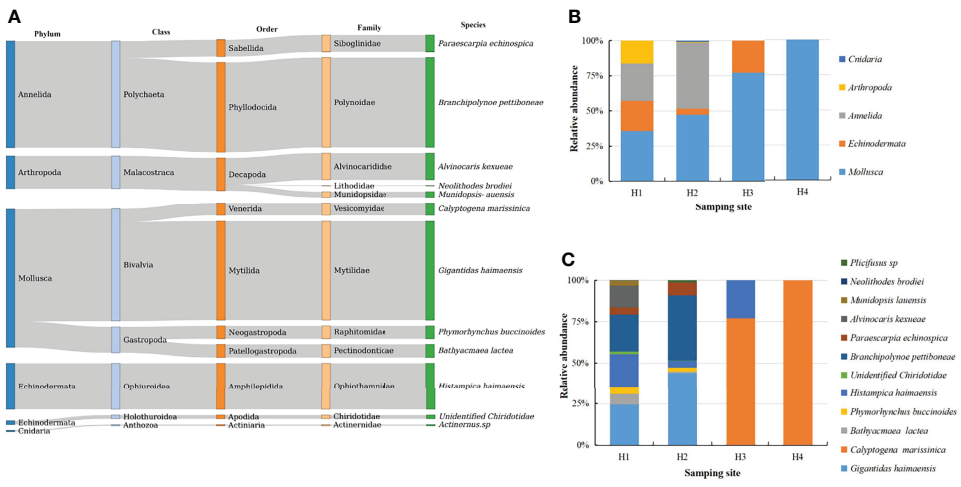

FIGURE 2 | Relative abundance of epibenthic macrofauna in Haima cold seep at each taxonomic level **(A)**, at the phylum level **(B)** and species level **(C)** in every sampling site.

followed by *B. pettiboneae* (27.22%) and *Histampica haimaensis* (14.23%; **Figure 2**). According to the values of the dominance of macrobenthos in Haima cold seep, *G. haimaensis* (0.160), *B. pettiboneae* (0.145) and *Histampica haimaensis* (0.114) were the most represented members of Mollusca, Annelida and Echinodermata, respectively (**Table S2**).

Regarding community structure, 10, 9, 2 and 1 species were identified at sites H1, H2, H3 and H4, respectively (**Table S1**). The highest species richness, species abundance and biomass were observed in site H1 (S = 10, D = 1.45, H = 1.906) and H2 (S = 9, D = 1.421, H = 1.267) (**Table 1**). The macrobenthos community could be divided into two communities (H1 and H2 = mussel bed community, and H3 and H4 = vesicomyid clams community) based on CLUSTER and NMDS analyses (**Figure S1**).

TABLE 1 | Indexes of the biodiversity analysis of the macrobenthos community in Haima cold seep.

Sampling site	*S*	*D'*	*J'*	*H'*
H1	10	1.457	0.8277	1.906
H2	9	1.421	0.5767	1.267
H3	2	0.2813	0.7755	0.5375
H4	1	0	0.776	0
Average	4.4	0.7898	0.7390	0.7421

S, Total species; D', Species richness (Margalef); J', Pielou's evenness' H', Shannon-Wiener index.

Genetic Diversity of the Three Representative Macrobenthos Species

Based on *COI* sequences, the haplotype diversity, the number of haplotypes, and nucleotide diversity of the three representative macrobenthic species are presented in **Table 2**. For *G. haimaensis*, 55 *COI* sequences (633 bp) were successfully amplified, along with 13 segregating sites (2.05%), four of which were parsimony informative, and 16 haplotypes were detected. The nucleotide and haplotype diversity values of *G. haimaensis* were 0.651 and 0.00148, respectively. For *B. pettiboneae*, *COI* sequences (624 bp) were successfully amplified, along with 25 segregating sites (4.00%), eight of which were parsimony informative, and eight haplotypes were detected. The nucleotide diversity and haplotype diversity values of *B. pettiboneae* was 0.00812 and 0.912, respectively. For *H. haimaensis*, 48 *COI* sequences (528 bp) were obtained, along with 11 segregating sites (2.08%), six of which were parsimony informative, and 11 haplotypes were detected. The nucleotide diversity and haplotype diversity values of *B. pettiboneae* were 0.00214 and 0.748, respectively.

To provide insight into the demographic history of the three representative macrobenthos species, *Fu's Fs* and mismatch distribution were inferred. Both *Fu's Fs* and Tajima's *D* values were found to be negative and significant for *G. haimaensis* and *H. haimaensis* populations in Haima cold seep (p<0.05), and all mismatch distribution shapes were clearly unimodal, apart from the *B. pettiboneae* population in Haima cold seep (**Figure 3**).

Genetic Diversity and Structure of *G. haimaensis* Based on MCD Sequences and SNPs

Based on MCD sequences, the haplotype diversity, the number of haplotypes, and nucleotide diversity for the two groups of *G. haimaensis* are presented in **Table 3**. A total of 41 haplotypes were identified from 51 *G. haimaensis* individuals (**Table S3**). The *Hd* value for H1 and H2 was 0.988 and 0.970, respectively. The π value was 0.00191 and 0.00179. Genetic diversity analysis of *G. haimaensis* detected a similar level of nucleotide and haplotype diversity between the two sites. The network exhibited a star-like shape, with the most frequent haplotypes shared between the two groups in the centre, surrounded by private and low-frequency haplotypes. No clade of haplotypes was revealed in haplotype networks constructed using a median-joining network (MJN) approach (**Figure 4**). The *Fst* between the two sites was 0.00745 (p >0.05), indicating no significant genetic differentiation between the two sites for *G. haimaensis* (**Table 4**).

RAD-seq yielded ~953 million reads in total, with a mean of 15.88 million reads per individual. The average mapping rate for each individual was 95.53%, with an average depth per individual of 14.00 (**Table S4**). The high mapping rate and sequencing depth indicated that the raw data could be used for subsequent SNP analysis. After genotyping and strict filtering, 294,734 SNPs were identified. Genetic diversity analysis of *G. haimaensis* based on SNPs detected a similar level between H1 and H2, with *Na*, *Ne*, *Ho* and *He* values of 2.000, 1.429, 0.304 and 0.271, respectively (**Table 4**). The pairwise *Fst* value calculated based on the entire set of 294,734 SNPs was 0.0106 (p<0.05). STRUCTURE analyses (i.e., optimal K = 2) revealed a small but clear differentiation between the two sites (**Figure 5**; **Figure S3**). The PCA results also detected this minor genetic subdivision of population genetic structure (the two minor separated clusters in the **Figure 6**). To test whether the genetic differentiation is not driven by a few outlier loci derived from habitat selection, Fst *vs* heterozygosity analyses were implemented. A total of 475 SNPs in 50 regions were eliminated, and the result showed that, with a few exceptions, individuals from two groups (H1 and H2) form distinct two clades (**Figure S4**). In addition, PCA at different allele coverage depths (dp=3 *vs*. dp=5) was also compared to determine whether the allele coverage depth might have affected the results. The result agrees with our findings, with identical

TABLE 2 | DNA polymorphism and neutrality tests values for the three representative macrobenthos species in Haima cold seep based on *COI* sequences.

Phylum	Species	SequenceLength	*N*	*H*	*Hd*	π	Tajima's *D*	Fu's *Fs*
Mollusca	*Gigantidas haimaensis*	633 bp	55	16	0.651	0.00148	-1.98414*	-14.75081**
Annelida	*Branchipolynoe pettiboneae*	624 bp	14	8	0.912	0.00812	-1.50963	-0.3153
Echinodermata	*Histampica haimaensis*	528 bp	48	11	0.748	0.00214	-1.59342*	-6.12204*

*N, sample size; H, number of haplotypes; Hd, haplotype diversity; π, nucleotide diversity; *p< 0.05; **p< 0.001.*

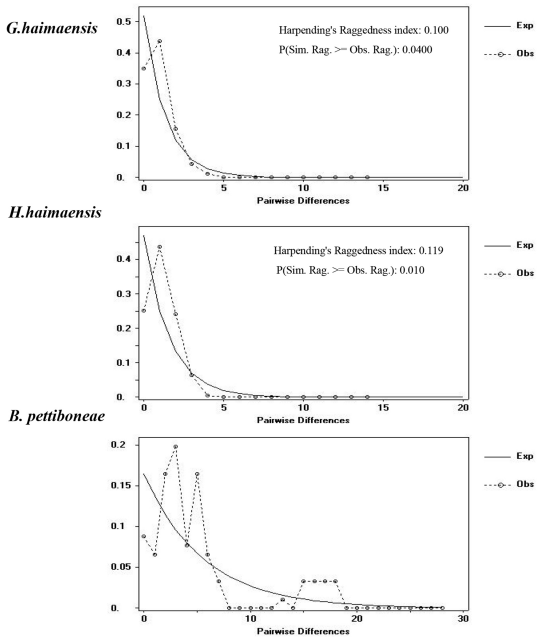

FIGURE 3 | Observed (broken lines) and expected (solid lines) mismatch distribution for three representative macrobenthos species basing on *COI* sequence. X-axis = pairwise differences and Y-axis = frequency.

results (**Figure S5**). The new results (Fst *vs* heterozygosity and dp3 *vs*. dp5) showed that the genetic differentiation is not driven by a few outlier loci and is affected by allele coverage depth, which is in close agreement with our conclusion

TABLE 3 | Summary genetic statistics of each local group of *G. haimaensis* based on MCD sequences.

group	*N*	*H*	*Hd*	π
H1	26	23	0.988	0.00191
H2	25	19	0.970	0.00179
H1+H2	51	41	0.980	0.00186

N, sample size; H, number of haplotypes; Hd, haplotype diversity; π, nucleotide diversity.

TABLE 4 | Genetic diversity of *G. haimaensis* among the two sites based on SNPs.

Group_name	*Na*	*Ne*	*Ho*	*He*
H1	1.996	1.430	0.311	0.271
H2	1.993	1.421	0.297	0.266
H1+H2	2.000	1.429	0.304	0.271

Na, number of observed alleles; Ne, effective number of alleles; Ho, observed heterozygosity; He, expected heterozygosity.

(the genetic differentiation between the two mussel beds of *G. haimaensis*).

DISCUSSION

Species Diversity and Community Structure of Macrobenthos in Haima Cold Seep

Knowledge about species diversity is fundamental for biodiversity conservation (Yang et al., 2004). In the present study, we identified 12 macrobenthic species from five phyla, and 12 families in the Haima cold seep surveys. By comparison, Dong et al. (2021) detected 24 macrofauna species in Haima cold seep. This apparent difference in species richness may be explained by differences in sampling effort (Nakajima et al., 2014). Data used in the present study was based on a single dive at each site, while multiple dives were implemented in Dong et al. (2021). Species richness at Haima cold seep is higher than 42 previously reported chemosynthesis-based ecosystems (range 2–38, mean 9.8) in the northwest Pacific (Nakajima et al., 2014). Biodiversity patterns are shaped by energy availability in deep-sea ecosystems (Woolley et al., 2016). The high species richness at Haima cold seep may be attributed to high active seep activities, the foundation for chemotrophic communities. Intensive investigation in recent years could be another reason for the apparent high species richness at Haima cold seep. In addition, our study identified two new macrofauna species for the first time in the Haima cold seep ecosystem, and the new records can expand our understanding of the geographic distributions of some

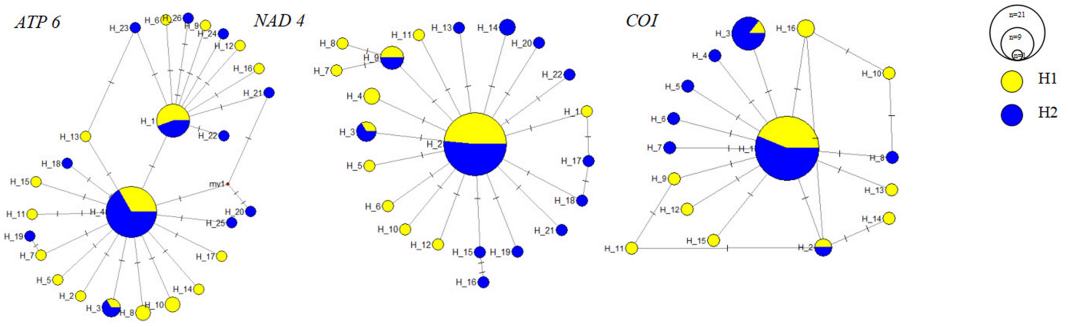

FIGURE 4 | Statistical parsimony network for haplotypes from *G. haimaensis* samples collected at Haima cold seep. Large circles represent a single individual. H1 represented by yellow circles. H2 represented by blue circles.

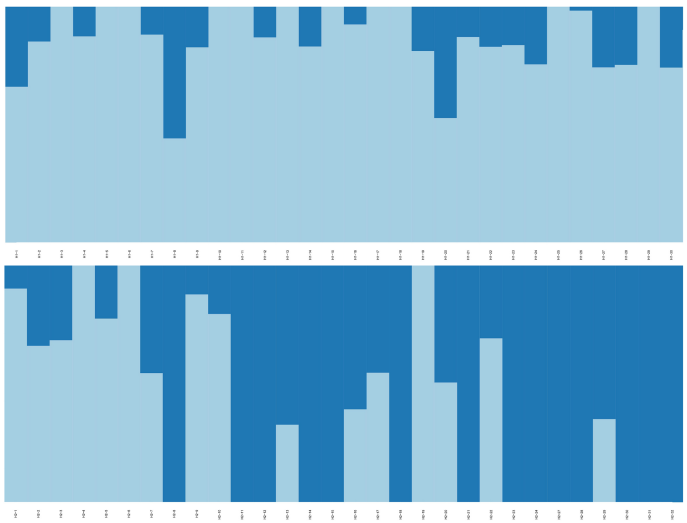

FIGURE 5 | Population genetic structure of *G. haimaensis* at H1 and H2 sites based on the entire set of 294,734 SNPs using STRUCTURE analyses (K=2). Each individual is represented by a single bar, with different colors showing membership fractions of each inferred cluster. H1 is shown above and H2 is shown below.

species. For instance, *A. kexueae* (**Figure S6**) was previously only described from hydrothermal vents in the Manus Basin in the Southwest Pacific (WANG and SHA, 2017). However, its presence in Haima cold seep indicates that *A. kexueae* is not an endemic species because it inhabits both vents and cold seeps.

Two clear different kinds of communities (deep sea mussels and vesicomyid clams) were found in Haima cold seep, and the mussel community is much more diversified than the vesicomyid community. The dense mussel beds generate a highly complex habitat for many deep-sea species to inhabit (Vrijenhoek, 2010). Moreover, this may also be related to the fact that seepages are less vigorous for the clam beds, Calyptogena being able to collect sulfides in the sediment with their foot, leaving less chance for the epibenthic fauna to feed and survive. The community structure at Haima cold seep is similar to one of the three currently known active cold seeps (site F) in the South China Sea (SCS) (Feng et al., 2018). Several species are shared between Haima cold seep and site F, including *B. pettiboneae*, *Shinkaia crosnieri* and *B. lactea*, indicating possible community connectivity between the two sites. However, no study has confirmed this hypothesis. Future research should therefore determine whether gene exchange between benthic species. Although some species are shared between Haima cold seep and site F, substantial differences were observed. For example, the engineer species at Haima cold seep is *G. haimaensis*, whereas *G. platifrons* is dominant at site F. Apart from the position of umbones (*G. haimaensis* = subterminal and *G. platifrons* = terminal), the two species are similar in morphology (Xu et al., 2019) and genetics based on the mitochondrial genome (Zhang et al., 2021). However, the difference is that *G. platifrons* inhabiting both vents and cold seeps is widely distributed in the Northwest Pacific, while *G. haimaensis* is endemic to SCS in Northwest Pacific. In addition, *Calyptogena marissinica*, *Ophiophthalmus sp* and *Phymorhynchus buccinoides* are common species at Haima cold seep, but none were found at site F. Characterised by patchy distributions and high heterogeneity, deep-sea cold seeps reportedly harbour highly specialised communities (Teixeira et al., 2013), which might partially explain the finding that macrobenthos in Haima cold seep had a distinct and discernible community structure.

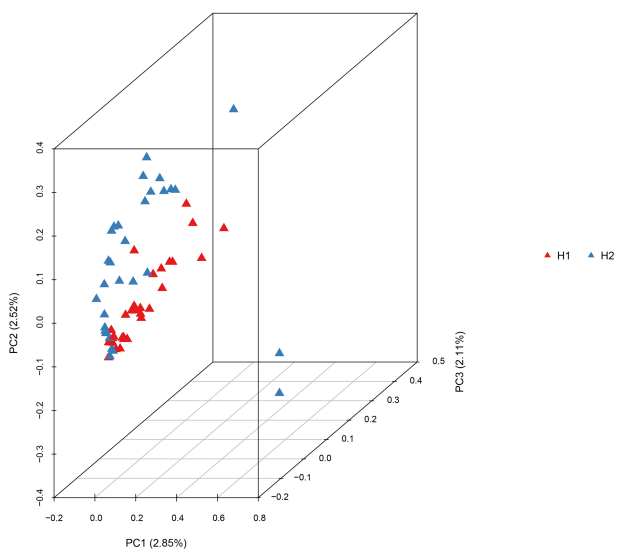

FIGURE 6 | Principal component analysis (PCA) plot of *G. haimaensis* at two sites based on the 294,734 SNPs.

Genetic Diversity of Three Representative Macrobenthic Species

Genetic diversity is closely related to the survivability of populations and long-term adaptability, especially in drastically

and suddenly changing marine environments (Barrett and Schluter, 2008). The present work examined the genetic diversity of three representative macrobenthic species (*G. haimaensis*, *H. haimaensis* and *B. pettiboneae*) in Haima cold seep using mtDNA markers for the first time. Genetic diversity analysis of *G. haimaensis*, *H. haimaensis* and *B. pettiboneae* populations in Haima cold seep detected a similar level of genetic diversity compared with other closely related species populations (Hurtado et al., 2004; Shen et al., 2016). For instance, haplotype diversity and nucleotide diversity of *G. haimaensis* populations were 0.980 and 0.00186, respectively, similar to most other deep-sea mussel species such as *G. platifrons*, for which *Hd* was 0.965 to 0.970 and π was 0.0015 to 0.0021 (Xu et al., 2018). Based on the haplotype and nucleotide diversity results, these three species exhibited low nucleotide diversity but high haplotype diversity (*Hd* >0.5 and π<0.05) (Grant and Bowen, 1998). This trend in genetic diversity was also observed for other deep-sea macrobenthic species populations, and it seems to be shared among macrobenthic communities in deep-sea ecosystems, especially deep-sea mussel communities (**Table S5**). These types of genetic diversity are also common in coastal mussel (eg. *Perna viridis, Mytella charruana* and *Mytilus galloprovincialis. G. haimaensis* population in Haima cold seep exhibits high haplotype diversity, but low nucleotide diversity, which may be explained by the high mutational rate of the mitochondrial gene. Haplotype diversity can increase rapidly in a short period of time by accumulating single nucleotide variations, while nucleotide polymorphism cannot co-occur (Avise et al., 1984). In addition, when a pioneer population with a low effective population size colonizes nascent vents or seeps, the founder effect might also account for the low genetic diversity of the macrobenthos in the Haima cold seep.

Genetic Structure of *G. haimaensis* Based on Three Mitochondrial Genes and SNPs

A total of 294,734 SNPs and three mitochondrial genes (*ATP6*, *COI*, and *NAD4*) were analysed to understand the genetic structure of *G. haimaensis* in the Haima cold seep. There was no significant genetic differentiation between H1 and H2 sites using the three concatenated mitochondrial genes (MCD). Furthermore, no apparent genetic structure was revealed in haplotype networks or *Fst* values. These results align with a previous study (Xu et al., 2018) based on mitochondrial genes, suggesting high connectivity and lack of population differentiation between deep-sea mussel populations. However, the results of PCA based on 294,734 SNPs detected a genetic subdivision, and analysis by STRUCTURE provided further evidence of genetic differentiation between the H1 and H2 populations of *G. haimaensis*. There may be a lack of demographic connectivity between the H1 and H2 mussel beds. Due to the different types of genetic markers and the number of genetic markers used, there may be discrepancies when assessing SNPs and mitochondrial genes. Analyses of only a few genetic markers can lead to relatively larger confidence intervals for minimal values of Fst, which may result in non-statistical significance between populations (Blanco Bercial and Bucklin, 2016). Discrepancies between results obtained from SNPs and mitochondrial markers have also been reported in genetic structure studies of *G. platifrons* and *S. crosnieri* (Sun et al., 2018; Cheng et al., 2020). Selective sweeps of mitochondrial genes might be more common for organisms living in chemosynthesis-based ecosystems in the deep ocean compared with those in other marine environments (Roterman et al., 2016). The significantly negative *Fu's FS* and *Tajima's D* values for *G. haimaensis* indicate a signature of selective sweeps of mitochondrial genes (Fu, 1997) (Tajima, 1989).

In general, geographic distances have a negative impact on gene exchange between populations (Taguchi et al., 2014). However, in this study, within a geographic distance of only ~18 km, a small but clear genetic subdivision was observed for *G. haimaensis* between the two sites based on STRUCTURE and PCA using the entire dataset of 294,734 SNPs. In contrast, no significant genetic divergence was found between populations of close deep sea mussel *G. platifrons* in the Northwest Pacific, despite a geographic distance of >2,000 km. This scenario is usually referred to as chaotic genetic patchiness (CGP) that patterns of genetic structure that cannot be explained by any clear geographic trend. The driving mechanisms of chaotic genetic patchiness are recognized to be selection, sweepstakes reproductive success, temporal shifts in local population dynamics and collective dispersal (Iannucci et al., 2020). In our study, collective dispersal and sweepstakes reproductive success may be the driving factor for this chaotic genetic patchiness in *G. haimaensis* populations. Collective dispersal indicates any process leading to gene flow by groups of individuals (Yearsley et al., 2013). This type of dispersal may arise from individual dispersal strategies, such as collective larval dispersal by ocean currents. For deep sea mussel larva, only a few larvae can colonize new vents or seeps (Vrijenhoek, 2010). Free-swimming planktotrophic deep sea mussle larvae are expected to have long-distance dispersal potential. Thus, there has been a focus on understanding the genetic connectivity between populations separated by greater distances while ignoring those geographically close populations. Our results indicate that chaotic genetic patchiness is present within deep-sea mussels populations that are geographically close. This scenario may also exist in other deep-sea benthic communities.

Implications for Management Strategies

Assessment of species and genetic diversity can reveal adaptability to environmental disturbance (Vrijenhoek, 2010). Compared with other healthy aquatic ecosystems, the macrobiotic species population in Haima cold seep is characterised by lower nucleotide diversity, suggesting that these populations may be vulnerable to environmental changes when gas hydrates extractions. The possibility of regime changes poses challenges for mitigation programs and environmental management. Therefore, it is

necessary to understand whether such a regime change will have a significant adverse impact, which should influence management. Managers need to understand the magnitude of local changes in populations and community structures in order to anticipate the cumulative effects and environmental impacts of post-disruption regime changes (Thaler et al., 2017).

CONCLUSIONS

Using ROV *in situ* surveys, mitochondrial DNA fragments, and RAD-seq approaches, the species diversity and genetic diversity of macrobenthos in the Haima cold seep were estimated, and 12 macrobenthic species from five phyla and 12 families were identified. The macrobenthos community can be divided into three groups (H1 and H2 = community type 1 and H3 and H4 = community type 2) based on CLUSTER and NMDS analyses. *G. haimaensis* (Mollusca), *Branchipolynoe pettiboneae* (Annelida) and *H. haimaensis* (Echinodermata) had the highest values of dominance (0.160, 0.021 and 0.114, respectively) among members in their respective phyla. All three typical macrobenthic species presented high haplotype diversity but low nucleotide diversity. Mismatch distribution and neutrality analyses suggested that *G. haimaensis* and *H. haimaensis* populations underwent recent population expansions. When using three mitochondrial genes, no significant genetic differentiation was detected between sites H1 and H2 for *G. haimaensis.* Nevertheless, genetic subdivision between the two populations of *G. haimaensis* was evident when using SNP datasets. The results comprehensively illuminate macrobenthos biodiversity in the Haima cold seep ecosystem, and provide a baseline against which population dynamics may be assessed in the future.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are publicly available. The data for the mitochondrial gene sequences can be found here: NCBI, MW384429 to MW384487 for nad4, MW408123 to MW408176for COI, and MW384488 to MW384545 for atp6.The RAD-Seq data can be found here: NCBI, PRJNA837114.

AUTHOR CONTRIBUTIONS

GY performed most of the experiments, analysed data, and wrote the manuscript. MH planned and designed the research. YS, HZ, PX and HJ assisted in the experiment. All authors contributed to the article and approved the submitted version.

FUNDING

This project was supported by the Key Special Project for Introduced Talents Team of Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou) (GML2019ZD0401), the Major Project of Basic and Applied Basic Research of Guangdong Province (2019B030302004), and the Science and Technology Planning Project of Guangdong Province (2020B1212060058).

ACKNOWLEDGMENTS

Data and samples were collected on board of research vessel (R/V) Haiyang 6 of Guangzhou Marine Geological Survey (China), we thank the crew for their help during the cruise. We also want to thank Dr Eric for editing this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.920327/full#supplementary-material

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