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# DNA Barcoding of Scavenging Amphipod Communities at Active and Inactive Hydrothermal Vents in the Indian Ocean

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Hydrothermal vent areas have drawn increasing interest since they were discovered in 1977. Because of chemoautotrophic bacteria, they possess high abundances of vent endemic species as well as many non-vent species around the fields. During the survey conducted by the Bundesanstalt für Geowissenschaften und Rohstoffe (Federal Institute for Geosciences and Natural Resources, BGR) to identify inactive polymetallic sulfide deposits along Central and Southeast Indian Ridges, the INDEX project studied the scavenging amphipod community at three newly discovered hydrothermal fields. A sample consisting of 463 representatives of Amphipoda (Malacostraca: Crustacea) was collected by means of baited traps in active and inactive vents of three different sites and subsequently studied by both morphological and genetic methods. Molecular methods included the analysis of two mitochondrial (cytochrome *c* oxidase subunit I [COI] and 16S rRNA) and one nuclear (18S rRNA) genes. By six delimitation methods, 22 molecular operational taxonomic units (MOTUs) belonging to 12 genera and 10 families were defined. The existence of potential species complexes was noted for the representatives of the genus *Paralicella*. The inactive site, where 19 species were found, showed higher species richness than did the active one, where only 10 taxa were recorded. Seven genera, *Ambasiopsis*, *Cleonardo*, *Eurythenes*, *Parandania*, *Pseudonesimus*, *Tectovalopsis*, and *Valettiopsis*, were observed only at inactive sites, whereas *Haptocallisoma*, was collected exclusively at active ones. The species *Abyssorchomene distinctus* (Birstein and Vinogradov, 1960), *Hirondellea brevicaudata* Chevreux, 1910, and *Hirondellea guyoti* Barnard and Ingram, 1990, have been previously reported from vent sites in the Atlantic or Pacific oceans. The present study provides the first report of *Eurythenes magellanicus* (H. Milne Edwards, 1848) and five other already described species in the Indian Ocean. The addition of 356 sequences strongly increases the number of amphipod barcodes

in reference databases and provides for the first time COI barcodes for *Cleonardo neuvillei* Chevreux, 1908, *Haptocallisoma abyssi* (Oldevig, 1959), *Hirondellea guyoti*, *Tectovalopsis fusilus* Barnard and Ingram, 1990, and the genera *Haptocallisoma*, *Pseudonesimus*, and *Valettiopsis*.

**Keywords: Indian Ocean, hydrothermal vent, barcoding, genetic diversity, Amphipoda, abyssal, deep sea, baited trap**

## INTRODUCTION

Much less is known about hydrothermal vents and the deep sea in general than about terrestrial and shallow-water ecosystems. The first hydrothermal vent was discovered in 1977 along the Galapagos Rift (Lonsdale, 1977). Over the past 50 years the study of hydrothermal vents has progressed, but most studied vents are located in the Pacific and Atlantic oceans (German and Von Damm, 2006). The first vent fields on the Central Indian Ridge were discovered in 2000 and 2001 (Gamo et al., 2001; Hashimoto et al., 2001; Van Dover et al., 2001). Compared to that of Atlantic and Pacific vents, the fauna of the Indian Ocean vents is underexplored (Ingole and Koslow, 2005; Nakamura et al., 2012).

Discovery of the first hydrothermal vent field changed the view of primary production in the world's oceans fundamentally (De Busserolles et al., 2009). At hydrothermal vent fields chemoautotrophic bacteria use inorganic substances such as ferrous iron, hydrogen sulfides, and methane for primary production (De Busserolles et al., 2009). They occur free living or in symbiosis with eukaryotic species. Because this food source is independent of primary production in the photic zone, many endemic species are found in these fields, but non-vent species also occur in higher abundance around the vents than in the rest of the deep sea (Podowski et al., 2009).

Kato et al. (2010) revealed that abundance, diversity, and activity of microbial communities within sulfide structures of inactive vents are higher than or comparable to those of active vents. Inactive vent fields consist of polymetallic sulfides, like active vents, but without any detectable emissions (Van Dover, 2011) and are located within or close to active vent fields. Inactive fields are often inhabited by a mix of general deep-sea species, inactive vent species, and a reduced number of vent species, which are found in low abundances (Erickson et al., 2009; Levin et al., 2009; Collins et al., 2012; Boschen et al., 2016).

The high food availability at active and inactive vent fields leads to the presence of specific scavenging megafaunal species (Gerdes et al., 2019a). In this benthic deep-sea environment food is a limiting factor, and scavengers play an important role in recycling the organic carbon reaching the ocean floor and providing it as food for higher trophic levels. Marine scavengers are found throughout all phyla and habitats (King et al., 2007). They include some fishes and many invertebrate taxa like ophiuroids, asteroids, holothurians, decapods, isopods, and amphipods. Scavenging amphipods have been collected in great numbers by means of baited traps (Perrone et al., 2002; Jamieson et al., 2009; Gallo et al., 2015) and include mainly the representatives of Lysianassoidea, from the genera *Abyssorchomene*, *Anonyx*, *Cyclocaris*, *Cyphocaris*, *Eurythenes*, *Hirondellea*, *Orchomene*, *Orchomenella*, and the alicelloid *Paralicella* (Shulenberger and Hessler, 1974; Sainte-Marie, 1986; Christiansen, 1996; Legeżyńska et al., 2000; Blankenship and Levin, 2007; Duffy et al., 2016).

Deep-sea scavenging amphipods are adapted to endure the extreme conditions and limitations, using chemosensory appendages to detect and take their bait (Tamburri and Barry, 1999). As one limiting factor in deep-sea environment is food, some scavenging amphipods complement their necrophagy with detritivory, carnivory, and even cannibalism (Blankenship and Levin, 2007; Jamieson et al., 2010; Havermans and Smetacek, 2018). Another recent finding of cellulase in one scavenging species, *Hirondellea gigas* (Birstein and Vinogradov, 1955), suggests that it may digest wood debris, although no wood particles were recorded in its digestive tract (Kobayashi et al., 2012). Other vent amphipod species, also well-adapted to their environment, feed on the microbes present there.

*Ventiella sulfuris* Barnard and Ingram, 1990, is the most abundant amphipod species at the Eastern Pacific Rise vent fields. It lives in symbiosis with microbial communities inhabiting its midgut and hindgut and is known to be vent endemic (Corbari et al., 2012). Another vent endemic amphipod species, *Dulichiopsis dianae* Corbari and Sorbe, 2017, was detected at hydrothermal vents along the Mid-Atlantic Ridge (Corbari and Sorbe, 2017), but several amphipods are reported to be caught at or near hydrothermal vent systems all over the world (Barnard and Ingram, 1990; Desbruyères et al., 2006; Bellan-Santini, 2007; Larsen, 2007) that are not necessarily vent endemic (*We use "endemic" to refer to species occurring within a biotope and not within a geographical region; as per Wolff, 2005).

Baited traps were deployed during INDEX 2018, providing the opportunity to examine scavenging amphipods at the vent fields in the Indian Ocean. The objectives of the present study were: (1) to identify scavenging amphipod species at the Southeast and Central Indian ridges and (2) to determine whether the distribution pattern of recorded species is associated with the hydrothermal activity.

## MATERIALS AND METHODS

### Sampling

Our study was part of the INDEX 2018 expedition on Dutch RV *Pelagia*. The Southeast and the Central Indian Ridges are located in the Indian Ocean about 1,400 km southeast of the island of Mauritius. At each of three newly discovered vent fields, an amphipod trap was placed by the Canadian Remotely

Operated Vehicle (ROV) *ROPOS*. It was deployed three times by the manipulators and placed traps at one inactive (AT1: on the Southeast Indian Ridge) and two active areas (AT2: on the Southeast Indian Ridge; AT3: on the Central Indian Ridge). The distance between AT1 and AT2 was approximately 342 km, that between AT2 and AT3, 210 km (**Table 1** and **Figure 1**).

To attract the scavenging fauna, fish and cat food were enclosed in a net (40 μm mesh size) inside each trap. After 7–29 h the trap was recovered by the ROV (**Table 1**). On shipboard, the larger individuals were hand-picked, and the smaller ones, including the sediment were, passed through a 40-μm sieve, and all samples preserved in 96% undenatured ethanol.

## Morphological Analyses

All intact specimens were studied morphologically. Badly damaged specimens were not counted and processed. The material is stored at the German Centre for Marine Biodiversity Research (DZMB) in Wilhelmshaven.

In the first step all amphipods were identified by means of a Leica M 125 stereomicroscope and the relevant literature (Chevreux, 1889; Shulenberger and Barnard, 1976; Lincoln, 1979; Barnard and Ingram, 1990; Barnard and Karaman, 1991; Bousfield and Hendrycks, 1995; Berge and Vader, 2001; Hendrycks, 2007; Lowry and De Broyer, 2008; Lowry and Stoddart, 2010; Lowry and Kilgallen, 2014; d'Udekem d'Acoz and Havermans, 2015; Horton and Thurston, 2015; Kilgallen and Lowry, 2015). For purposes of the morphological work, one representative of each recognized morphospecies was dissected and all appendages were mounted on permanent slides with polyvinyl-lactophenol containing lignin pink.

Undescribed or unknown species were named according to the nomenclature rules of Sigovini et al. (2016). Because of taxonomic problems, specimens of the genus *Paralicella* were divided into two morphological groups, and "sp. group" identifier was added to the name. In addition, a unique species code was given to the species where the species-level identity was not known or the species was new to science (Horton et al., 2021). This species code is a standardized code in the INDEX project and combines storage, year of publication, and a serial number.

## Photographing and Confocal Imaging

The 279 specimen used for molecular analysis were photographed with a Leica M 125 stereomicroscope equipped with a Leica MC 170 HD camera. In addition, one representative of each morphospecies [excluding the largest species, *Eurythenes magellanicus* (H. Milne Edwards, 1848)] was chosen for confocal laser scanning microscopy (**Supplementary Table 1**). The specimens were stained with a 1:1 solution of acid fuchsin and Congo red overnight according to procedures adapted from Michels and Büntzow (2010), then temporarily mounted on an objective slide with glycerine and self-adhesive reinforcement rings to support the coverslip (Michels and Büntzow, 2010). For larger specimens, double-sided tape pieces and some drops of Karo® light corn syrup were mounted between slide and coverslip (Brix et al., 2018).

The scanning was performed with a Leica TCS SP5 equipped with a Leica DM5000 B upright microscope and three visible-light lasers (DPSS 10 mW 561 nm; HeNe 10 mW 633 nm; Ar 100 mW 458 nm, 476 nm, 488 nm, and 514 nm), combined with the software LAS AF 2.2.1. Leica Application Suite Advanced Fluorescence (Kihara and Rocha, 2013) at the DZMB in Wilhelmshaven. To obtain the images, we used objective HCX PL APO CS 10.0 × 0.40 DRY UV and 561 nm excitation wavelength with 80% acousto-optic tuneable filter. Series of stacks were created with a resolution of 2,048 × 2,048 pixels. The final images were obtained by means of maximum projection. Finally, the individual images were merged in Adobe® Photoshop® 21.1.3 and edited for contrast and brightness.

## Molecular Studies

For each recognized morphospecies a representation of one to ten individuals was chosen for DNA barcoding. In cases where taxa caused morphological identification problems the number of analyzed specimens was increased or all specimens were used. For 279 specimens genomic DNA was extracted from one pleopod or pereopod (for smaller specimens), which was removed and treated with 30 μl of CHELEX (BIO-RAD Insta Gene Matrix) for 20 min at 56°C and 10 min at 99°C. For some samples the additional purification of the chelex extract was performed with columns (E.Z.N.A.® Mollusc DNA Kit, NucleoSpin® Tissue) according to the manufacturer's protocol.

Polymerase chain reactions of the mitochondrial cytochrome *c* oxidase subunit I (COI) and mitochondrial ribosomal large subunit (16S) were performed with amphipod-specific primers (**Table 2**) and protocols provided by their authors (Costa et al., 2009; Lörz et al., 2018). The fragment of the COI gene was amplified for all chosen individuals, whereas 16S and the nuclear small ribosomal subunit (18S) was amplified on a subset of specimens. For 18S the universal primer set 18SE and 18SL (**Table 2**) was used, and the polymerase chain reaction conditions

**TABLE 1 |** Deployed amphipod traps with station ID, coordinates, ridge (Central Indian Ridge = CIR, Southeast Indian Ridge = SEIR), hydrothermal activity, collection depth, the deployment time of the trap on the seafloor and the measured environmental parameters (temperature, salinity, and pH-value).

| Trap No. | Station ID | Coordinates | Ridge | Hydrothermal activity | Sampled depth (m) | Time on bottom (h) | Temperature (°C) | Salinity (psu) | pH |
|---|---|---|---|---|---|---|---|---|---|
| AT1 | I18_067RO_AT1 | 27° 39′ S, 73° 53′ E | SEIR | inactive | 2,508 | 29 | 1.79 | 34.72 | 3.10 |
| AT2 | I18_075RO_AT1 | 27° 15′ S, 72° 43′ E | SEIR | active | 2,919 | 7 | 1.71 | 34.73 | 3.10 |
| AT3 | I18_099RO_AT1 | 25° 28′ S, 69° 56′ E | CIR | active | 2,629 | 22 | 2.0 | 34.69 | 3.09 |

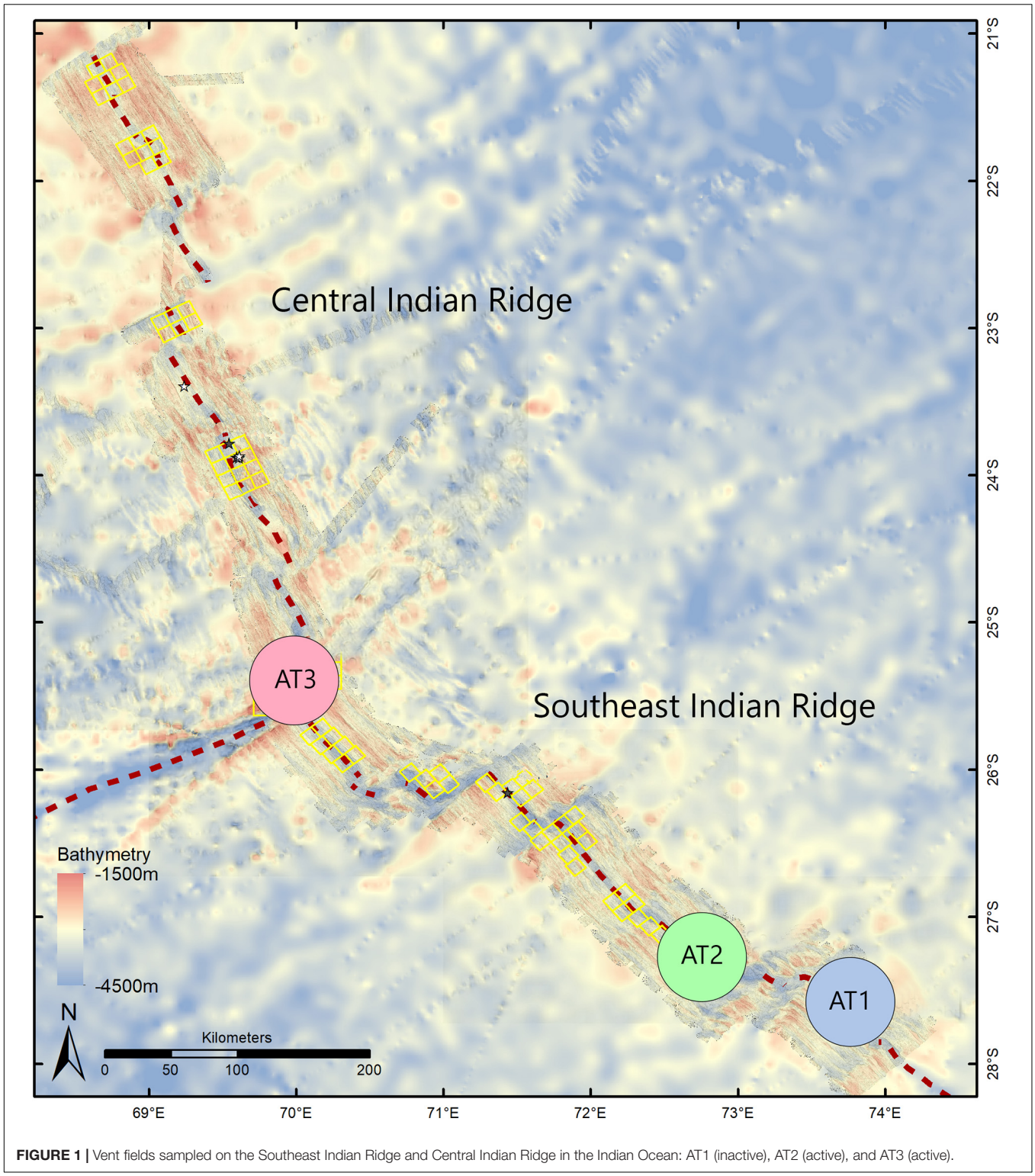


**FIGURE 1 |** Vent fields sampled on the Southeast Indian Ridge and Central Indian Ridge in the Indian Ocean: AT1 (inactive), AT2 (active), and AT3 (active).

were as follows: initial denaturation at 94°C for 3 min; 45 cycles of 30 s at 94°C, 45 s at an annealing temperature of 55°C, and 1 min at 72°C; final elongation for 3 min at 72°C. To all primers M13 tails were added to provide defined nucleotide sequences for sequencing (**Table 2**). All amplified products were purified with Exo-SAP-IT®. Afterwards they were sequenced by Macrogen Inc., The Netherlands. In order to assemble long DNA fragments of the 18S gene, we sequenced amplified fragments with intermediate primers synthetized by Macrogen Inc. (**Table 2**) in addition.

**TABLE 2 |** Primers used in the present study.

| Gene | Name | Sequence 5′–3′ | Direction | References |
|---|---|---|---|---|
| COI | UCOIR | ACWAAYCAYAAAGAYATYGG | Forward | Costa et al., 2009 |
| | UCOIF | TAWACTTCDGGRTGRCCRAAAAAYCA | Reverse | Costa et al., 2009 |
| 16S | 16SFt_amp | GCRGTATIYTRACYGTGCTAAGG | Forward | Lörz et al., 2018 |
| | 16SRt_amp2 | CTGGCTTAAACCGRTYTGAACTC | Reverse | Lörz et al., 2018 |
| 18S | 18SE | CTGGTTGATCCTGCCAGT | Forward | Hillis and Dixon, 1991 |
| | 18SL | CACCTACGGAAACCTTGTTACGACTT | Reverse | Hamby and Zimmer, 1988 |
| | F-566 | CAGCAGCCGCGGTAATTCC | Forward, intermediate | Hadziavdic et al., 2014 |
| | R-1200 | CCCGTGTTGAGTCAAATTAAGC | Reverse, intermediate | Hadziavdic et al., 2014 |
| M13 | M13-FP | TGTAAAACGACGGCCAGT | Forward | Schuelke, 2000 |
| | M13R-pUC | CAGGAAACAGCTATGAC | Reverse | Messing, 1983 |

*Each primer was added by the universal M13 tail (M13-FP, M13R-pUC).*

The resulting sequences were edited with Geneious Prime® 2020.1.2 (Kearse et al., 2012) as a check for ambiguities and errors. All edited sequences were aligned and trimmed with MAFFT v7.450 (Katoh et al., 2002) alignment in Geneious by the automatic algorithm. Afterward, similarity analyses with Blastn (Altschul et al., 1990) search against GenBank and the Barcode of Life Data System (BOLD, Ratnasingham and Hebert, 2007) were performed. Short sequences and sequences with bad quality were not included in the analysis. All sequences were deposited in GenBank with the accession numbers COI, MZ197178–MZ197435; 16S, MZ197436–MZ197490; and 18S, MZ197491–MZ197533. Relevant voucher information, pictures, taxonomic classifications, and sequences were deposited in the dataset "DS-INMAC01" in BOLD (Ratnasingham and Hebert, 2007)[1] (doi: 10.5883/DS-INMAC01) (GenBank accession numbers: **Supplementary Table 1**).

## Species Delimitation and Phylogenetic Analyses

To infer the number of species in the present study, we delimited them according to their morphology by grouping them to morphospecies and by analyzing genetic distances of molecular taxonomic units (MOTUs). The MOTUs were delimited on COI sequences by five methods–three distance-based and two tree-based.

Because, for some of the delimitation methods, a threshold is mandatory, intra- and interspecific distances for our dataset were first calculated by the Barcode Gap Analysis provided by BOLD (distance model: Kimura 2 Parameter; alignment options: BOLD aligner; ambiguous base/gap handling: pairwise deletion). As a result the threshold value for the species-delimitation methods was set at 0.976 or 97.6% (mean intraspecific divergence is 2.4 after exclusion of the species complex) (**Supplementary Figures 1, 2** and **Supplementary Table 2**), but note that the threshold for separating species within marine Amphipoda reported in the literature ranges from 93% to 97% and is suggested to be family specific (Knox et al., 2012; Tempestini et al., 2018; Mohrbeck et al., 2021).

Automatic Barcode Gap Discovery (ABGD) (Puillandre et al., 2012) is based on computing the threshold distance or "barcoding gap" between inter- and intraspecific variation that leads toward the number of groups. In the present case ABGD analyses were performed with the default settings (Pmin = 0.001, Pmax = 0.1, Steps = 10, X = 1.5, Nb bins = 20) on the ABGD website[2] with JC69 Jukes-Cantor parameter. This parameter reflects the assumption that base frequencies are equal with one substitution rate (Emerson et al., 2001) and gave the clearest barcoding gap within all tested parameters for our data.

Another distance-based method is CD-HIT (Li and Godzik, 2006), a heuristic clustering process that requires defined sequence similarity thresholds. The CD-HIT-EST method was used on the CD-HIT Suite web server at the University of California, San Diego,[3] and analyses were done with default settings and the predefined threshold of 97.6%.

The third method of delimitation that used calculated distances is the Barcode Index Numbers (BINs) system implemented in BOLD (Ratnasingham and Hebert, 2013). It registers each cluster of sequences and assigns a unique and specific code (BIN). An uploaded sequence goes through cluster analyses that try to find discontinuities between the clusters.

In contrast, general mixed Yule coalescence is a method that determines the point of transition from speciation to coalescent branching patterns on an ultrametric tree (Pons et al., 2006; Monaghan et al., 2009). When this method was performed, a Bayesian inference tree was built with BEAST v1.8.3 (Drummond et al., 2012). Yule-coalescent models as implemented in the R package "splits" (Suchard et al., 2018) were used.

Moreover, another method determining the transition from speciation to coalescent branching, the Bayesian Poisson tree process for larger datasets (Zhang et al., 2013) was tested on the web server.[4] As input file the Bayesian tree calculated by BEAST was used. The data were inserted as an unrooted tree, 100,000 MCMC generations, thinning of 100, and 0.1 burn-in.

For the graphic presentation of MOTUs and morphospecies, Bayesian tree analyses were conducted for the COI dataset. The

[1]http://www.boldsystems.org

[2]https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html

[3]http://weizhong-lab.ucsd.edu/cdhit-web-server/cgi-bin/index.cgi

[4]http://species.h-its.org/ptp/

optimal model was identified by the modeltest carried out by MEGA X (Kumar et al., 2018) using both the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC). For the COI dataset the Tamura-Nei (TN93 + G + I) model was the best fitting model. For construction of the tree, the BEAST v1.8.3 package and Yule-coalescent model as implemented in the R package "splits" (Suchard et al., 2018) were applied. The tree was produced and annotated with Bayesian posterior probabilities (PP) with TreeAnnotator in the BEAST v1.8.3 package.

For inference of phylogenetic relationships among recorded species, 16S and 18S rDNA gene fragments from one to three representatives of recognized MOTUs were amplified and added to the COI dataset. The tree of concatenated sequences of all three markers studied (27 individuals) was generated by the software Mr. Bayes (Ronquist and Huelsenbeck, 2003) by means of 15,000,000 generations, 2 runs, 4 chains, and a burn-in of 4,000. Gaps in the alignment were treated as the nucleotide N. Individual models were calculated for each marker: COI, General Time Reversible (GTR + G); 16S, Hasegawa-Kishino-Yano (HKY + G + I); 18S, General Time Reversible (GTR + G). All trees were graphically adjusted with the software Adobe® Photoshop® 21.1.3.

## Community Analyses and Population Connectivity

Rating the species richness was performed by generating the rarefaction curves (Hessler and Sanders, 1967). The individual rarefaction curve was processed by means of Past 4.05 (Hammer et al., 2001). In combination with the Venn diagram, it was adjusted in the software Adobe® Photoshop® 21.1.3.

For analysis of the population connectivity between the two locations, haplotype networks were generated by Population Analysis with Reticulate Trees (PopART).[5] Minimum spanning network (Bandelt et al., 1999) was applied for all MOTUs of the COI dataset. The haplotypes of all MOTUs of the genus *Paralicella* are presented together to demonstrate the differences and similarities between the recognized molecular units. Furthermore, for the COI dataset statistical tests were carried out by means of DnaSP6 (Librado and Rozas, 2009; Rozas et al., 2017) and Arlequin 3.5 (Excoffier and Lischer, 2010) for estimation of the gene diversity. For all populations with sample size $n \geq 4$, haplotype (h) and nucleotide ($\pi$) diversities (Tajima, 1983), Fu's Fs (Fu, 1996), and Tajima's D (Tajima, 1989) were calculated.

Additional diversity analyses on the COI dataset were performed in Arlequin 3.5 (Excoffier and Lischer, 2010). To detect species population differentiation within and among predefined groups, we performed an AMOVA with 1,000 permutations and pairwise differences. Two groups were selected–"active" and "inactive"–from analysis of the COI sequences obtained.

# RESULTS

## Species Delimitation and Identification

Baited traps AT1 and AT2 captured 463 scavenging amphipods, which could be identified morphologically as 18 different morphospecies (**Figures 2**, **3**) (AT1, 364 individuals; AT2, 99 individuals). Trap AT3 captured no amphipods. From 279 individuals used for molecular study, 258 high-quality sequences

[5]http://popart.otago.ac.nz

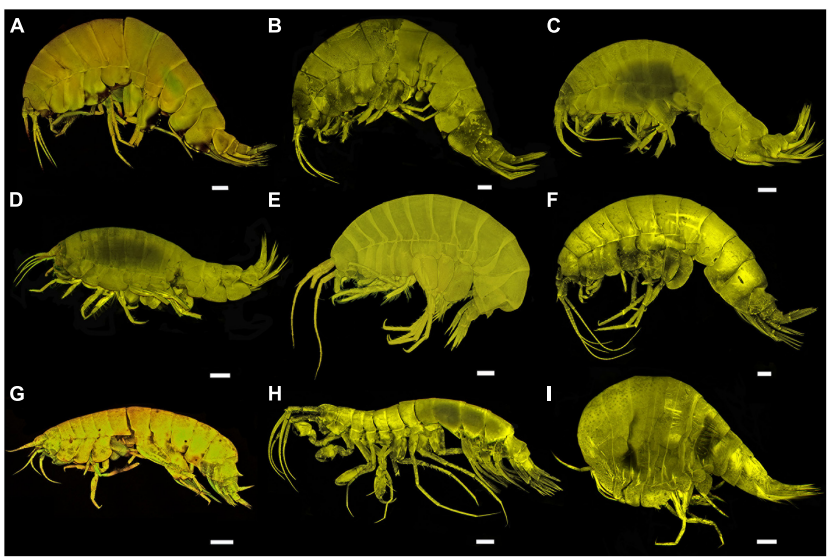


**FIGURE 2 |** Confocal images of all sampled taxa of Alicelloidea, Eusiroidea, and Stegocephaloidea. **(A)** *Paralicella* sp. group 1A DZMB_2021_0085. **(B)** *Paralicella* sp. group 1B DZMB_2021_0086. **(C)** *Paralicella* sp. group 2A DZMB_2021_0087. **(D)** *Paralicella* sp. group 2B DZMB_2021_0088. **(E)** *Tectovalopsis* aff. *diabolus*. **(F)** *Tectovalopsis fusilus*. **(G)** *Valettiopsis* sp. DZMB_2021_0091. **(H)** *Cleonardo neuvillei*. **(I)** *Parandania* sp. **(E,G)**: scale = 500 μm. **(A–D,F,H,I)**: scale = 1 mm.

of COI were obtained (PCR and sequencing success rate: 92.5%) (**Table 3**) (+21 individuals not sequenced +184 individuals checked only morphologically). Fragment lengths ranged from 527 to 658 bp; no indels or stop codons were found.

The molecular species delimitation methods revealed 20 to 29 MOTUs (**Table 3**, **Supplementary Figures 3–5**, and **Supplementary Tables 3–9**). From all the methods combined, consensus clusters were created for conform results of at least three of them. The result in all cases was a consensus cluster except for the genus *Paralicella*. Within this genus two entities were identified on the basis of morphology–*Paralicella* sp. group 1 (presenting morphological similarity to *Paralicella vaporalis* Barnard and Ingram, 1990) and group 2 (showing some similarities with *P. caperesca* Barnard and Shulenberger, 1976)–which were further divided by molecular study. Within the first all delimitation methods recognized two MOTUs, whereas in the second the number of molecular units ranged from three (ABGD, initial partition) to nine (general mixed Yule-coalescence and Bayesian Poisson tree process); in the second case four consensus MOTUs were defined. As a result, 24 MOTUs with 17 clusters and 7 singletons (**Figure 4**) were identified.

All recognized MOTUs were identified to genus level (from a combination of the morphological and molecular identification), and seven units were identified to species level (**Table 3**). For another three MOTUs, affinities with described species were found–*Tectovalopsis* aff. *diabolus* Barnard and Ingram, 1990, *Paracallisoma* aff. *alberti* Chevreux, 1903, and *Pseudonesimus* aff. *abyssi* Chevreux, 1926–whereas, four more recognized units are probably new to science–*Eurythenes* sp. DISCOLL PAP B, *Hirondellea* sp. nov. DZMB_2021_0092, *Ambasiopsis* sp. nov. DZMB_2021_0093, and *Paracallisoma* sp. nov. DZMB_2021_0094.

In addition to the COI dataset, 54 sequences of 16S (PCR and sequencing success rate: 81.8%) and 44 sequences of 18S (PCR and sequencing success rate: 66.7%) were obtained (**Table 3**). The phylogenetic tree containing 27 concatenated sequences represents 16 MOTUs within 4 superfamilies (**Figure 5**). The tree supports the separation of Lysianassoidea and Alicelloidea. Within Lysianassoidea, representatives of different families were grouped together. A different situation can be seen in the case of Alicelloidea, where representatives of two families (Valettiopsidae and Alicellidae) were mixed. The species *Tectovalopsis* aff. *diabolus* and *Paralicella* sp. group 1 formed one cluster with *Valettiopsis* sp. DZMB_2021_0091, whereas the *Paralicella* group 2 was clearly separated with high posterior probabilities.

## Faunistic Composition and Population Connectivity

The 364 individuals collected by trap AT1 at the inactive field formed 19 MOTUs in 11 genera (**Figure 6A**). The 99 captured by trap AT2 at the active field, belonged to 10 MOTUs and five genera. Trap AT3, placed for 22 h close to an active field, captured no amphipods. Only seven MOTUs were captured at both AT1 and AT2. The calculations of the rarefaction curves indicated higher species richness at inactive fields; for active fields the curve approached asymptote and flattens out (**Figure 6B**).

The amphipod assemblage of the inactive site was dominated by *Tectovalopsis fusilus* and *Hirondellea guyoti*, which constituted 60.4% of all individuals collected (**Figure 7**). The remaining 17 MOTUs were represented by similar numbers of individuals. The dominating taxon at the active site was *Paralicella* sp. group 2B DZMB_2021_0088, which alone made up more than 50% of abundance. The other taxa contributing substantially to this assemblage were *Abyssorchomene distinctus*, *Hirondellea* sp. nov. DZMB_2021_0092, *Paracallisoma* sp. nov. DZMB_2021_0094, and *Paralicella* sp. group 1B DZMB_2021_0086. The genera *Eurythenes* and *Tectovalopsis* were collected exclusively at the inactive site.

In the whole area studied, three taxa (*Cleonardo neuvillei*, *Ambasiopsis* sp. nov. DZMB_2021_0093, and *Parandania* sp.) were each represented by a single individual (**Figure 8A**). Among the remaining species, haplotype numbers ranged from 1 to 30 (**Table 4**).

The haplotype diversity (h) was high for AT1 and for the combined data of AT1 and AT2 in *Paralicella* sp. group 2B DZMB_2021_0088 ($h$ = 0.800–0.835), *Eurythenes* sp. DISCOLL PAP B ($h$ = 0.857), *Hirondellea guyoti* ($h$ = 0.709), and *Hirondellea* sp. nov DZMB_2021_0092 ($h$ = 0.782, 0.833), whereas for *Paracallisoma* aff. *alberti* ($h$ = 1.000) only AT1 and, for *Abyssorchomene distinctus* ($h$ = 0.928–0.952), the whole set of data showed high haplotype diversity. Nucleotide diversity ($\pi$) was low for all populations, ranging between $\pi$ = 0.00047 for *Paracallisoma* sp. nov. DZMB_2021_0094 (combined data) and $\pi$ = 0.00674 for *Eurythenes* sp. DISCOLL PAP B (**Table 4**).

The haplotype network for the genus *Paralicella* (**Figure 8B**) indicated a clear separation between the six MOTUs, with a mutation rate of 29 to 103 substitutions. The three individuals constituting MOTU *Paralicella* sp. group 2A DZMB_2021_0087 showed 24 and 27 substitutions among its representatives.

The neutrality and population-expansion tests revealed that, for the populations of *Paralicella* sp. group 2B DZMB_2021_0088, *Tectovalopsis fusilus*, and *Abyssorchomene distinctus*, the Tajima's D values were negative and significant ($p < 0.05$), indicating an excess of rare nucleotides thus an expansion of the population or indicative of a selective sweep. Fu's Fs confirmed this theory with negative values and highly significant $p$-values ($p < 0.001$–$0.0001$). Similarly, evidence for expansion of populations was observed in *Hirondellea* sp. nov. DZMB_2021_0092, *Paracallisoma* aff. *alberti*, and *Paracallisoma* sp. nov. DZMB_2021_0094. The values for Tajima's D and Fu's Fs combined with mainly significant $p$-values (**Table 4**).

Within-population differences in the analysis of molecular variance (AMOVA) accounted for all the variation (100%) for most of the species, and no evidence supported separation into genetically distinct populations (**Table 5**). In addition, negative or near-zero Fst-values indicated that the studied populations were genetically homogeneous, but all $p$-values were not significant, indicating no population structure. The only exception was *Abyssochormene distinctus*, where the variation of the AMOVA within the population was 95.39% and the Fst was positive, but $p$-values were still not significant, indicating no population structure.

**TABLE 3 |** The list of taxa identified, with superfamily, family and genus information, number of sequences for COI, 16S, and 18S, morphological delimitation (M), number of MOTUs based on Automatic Barcode Gap Discovery (Ai, initial, and Ar, recursive, partition), CD-Hit (C), GMYC (G), bPTP (B), and BIN.

| Superfamily | Family | Genus | Species | No. individuals | | Marker | | | Species delimitation methods | | | | | | | BOLD BIN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | AT1 | AT2 | CO1 | 16S | 18S | M | Ai | Ar | C | G | B | BIN | |
| Alicelloidea | Alicellidae | *Paralicella* Chevreux, 1908 | *Paralicella* sp. group 1A DZMB_2021_0085 | 2 | 3 | 5 | 2 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | BOLD:AEF8804 |
| | | | *Paralicella* sp. group 1B DZMB_2021_0086 | 0 | 6 | 6 | 1 | 2 | | 1 | 1 | 1 | 1 | 1 | 1 | BOLD:AEF6691 |
| | | | *Paralicella* sp. group 2A DZMB_2021_0087 | 0 | 3 | 3 | 3 | 2 | | 1 | 1 | 3 | 3 | 3 | 3 | BOLD:AEF9380 BOLD:AEF9381 BOLD:AEF9383 |
| | | | *Paralicella* sp. group 2B DZMB_2021_0088 | 16 | 54 | 63 | 10 | 6 | 1 | 1 | 1 | 1 | 3 | 4 | 1 | BOLD:AEF6635 |
| | | | *Paralicella* sp. group 2C DZMB_2021_0089 | 2 | 1 | 3 | 1 | 0 | | 1 | 1 | 1 | 2 | 2 | 1 | BOLD:AEF6636 |
| | | | *Paralicella* sp. group 2D DZMB_2021_0090 | 1 | 0 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | BOLD:AEF9382 |
| | | *Tectovalopsis* Barnard and Ingram, 1990 | *Tectovalopsis* aff. *diabolus* Barnard and Ingram, 1990 | 3 | 0 | 3 | 3 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | BOLD:AEF9480 |
| | | | *Tectovalopsis fusilus* Barnard and Ingram, 1990 | 131 | 0 | 78 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | BOLD:AEF6344 |
| | Valettiopsidae | *Valettiopsis* Holmes, 1908 | *Valettiopsis* sp. DZMB_2021_0091 | 2 | 0 | 1 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | BOLD:AEF7847 |
| Eusiroidea | Eusiridae | *Cleonardo* Stebbing, 1888 | *Cleonardo neuvillei* Chevreux, 1908 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | BOLD:AEF6468 |
| Lysianassoidea | Eurytheneidae | *Eurythenes* S. I. Smith in Scudder, 1882 | *Eurythenes magellanicus* (H. Milne Edwards, 1848) | 9 | 0 | 8 | 2 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | BOLD:ADD1766 |
| | | | *Eurythenes* sp. DISCOLL PAP B | 8 | 0 | 7 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | BOLD:AEF7086 |
| | Hirondelleidae | *Hirondellea* Chevreux, 1889 | *Hirondellea brevicaudata* Chevreux, 1910 | 6 | 0 | 5 | 3 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | BOLD:AEF6862 |
| | | | *Hirondellea guyoti* Barnard and Ingram, 1990 | 89 | 0 | 11 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | BOLD:AEF7644 |
| | | | *Hirondellea* sp. nov. DZMB_2021_0092 | 22 | 11 | 13 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | BOLD:AEF9394 |
| | Lysianassoidea incertae sedis | *Ambasiopsis* K.H. Barnard, 1931 | *Ambasiopsis* sp. nov. DZMB_2021_0093 | 1 | 0 | 0 | 1 | 1 | 1 | – | – | – | – | – | – | – |
| | Scopelocheiridae | *Haptocallisoma* Horton and Thurston, 2015 | *Haptocallisoma abyssi* (Oldevig, 1959) | 0 | 3 | 3 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | BOLD:ADH7303 |
| | | *Paracallisoma* Chevreux, 1903 | *Paracallisoma* aff. *alberti* Chevreux, 1903 | 10 | 2 | 9 | 4 | 3 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | BOLD:AEF7929 BOLD:AEF9456 |
| | | | *Paracallisoma* sp. nov. DZMB_2021_0094 | 18 | 9 | 13 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | BOLD:AEF8324 |
| | Tryphosidae | *Pseudonesimus* Chevreux, 1926 | *Pseudonesimus* aff. *abyssi* Chevreux, 1926 | 23 | 0 | 6 | 3 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | BOLD:AEF6700 |
| | Uristidae | *Abyssorchomene* De Broyer, 1984 | *Abyssorchomene distinctus* (Birstein and Vinogradov, 1960) | 19 | 7 | 18 | 5 | 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | BOLD:ACZ6415 |
| Stegocephaloidea | Stegocephalidae | *Parandania* Stebbing, 1888 | *Parandania* sp. | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | BOLD:AAF7953 |
| **Total numbers** | **10** | **12** | **22** | **364** | **99** | **258** | **54** | **45** | **18** | **20** | **21** | **23** | **27** | **29** | **24** | |

*Last column provides the BIN code. Shading indicate cases where incongruence between different delimitation methods was noted.*

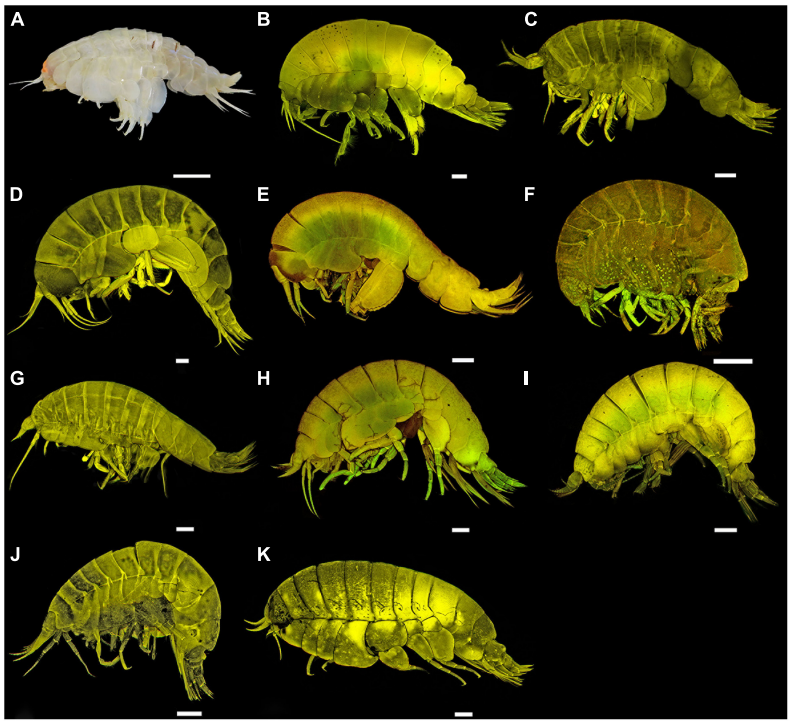


**FIGURE 3 |** Confocal images of all sampled taxa of Lysianassoidea (including one light microscopy image of a large species, **A**). **(A)** *Eurythenes magellanicus*. **(B)** *Eurythenes* sp. DISCOLL PAP B. **(C)** *Hirondellea brevicaudata*. **(D)** *Hirondellea guyoti*. **(E)** *Hirondellea* sp. nov. DZMB_2021_0092. **(F)** *Ambasiopsis* sp. nov DZMB_2021_0093. **(G)** *Haptocallisoma abyssi*. **(H)** *Paracallisoma* aff. *alberti*. **(I)** *Paracallisoma* sp. nov. DZMB_2021_0094. **(J)** *Pseudonesimus* aff. *abyssi*. **(K)** *Abyssorchomene distinctus*. **(A)**: scale = 2 cm. **(B,K)**: scale = 1 mm. **(C–J)**: scale = 500 μm.

# DISCUSSION

## Morphological vs. Molecular Identification of Amphipods

Studying baited-trap samples from hydrothermal vent areas by morphological methods supplemented by DNA barcoding led us to a total of 22 MOTUs belonging to 10 genera and four families. For the MOTUs collected, the morphology agreed with the molecular species delimitation, except for the genus *Paralicella*.

*Paralicella* includes six described species so far (WoRMS Editorial Board, 2021), but taxonomic issues have been mentioned for this genus. Ritchie et al. (2015) reported incongruence between the morphological and molecular identifications of two species within this genus but the discrepancies were not confirmed later (Mohrbeck et al., 2021). In contrast, high mean intraspecific divergences were reported for *Paralicella caperesca* (Jażdżewska et al., 2021; Mohrbeck et al., 2021). Our morphological study of the representatives of *Paralicella* separated them into two different groups, but they were further divided into molecular clades with non-distinct external appearance.

Additional morphological study is required to confirm that these taxa should be treated as cryptic species or to find the morphological characters that will allow separation of the MOTUs within these species complexes. Moreover, individuals within one MOTU of *Paralicella* sp. group 2 (group 2A DZMB_2021_0087) showed high COI sequence divergence (expressed among other evidence by ascription of three different BINs) that may reflect, for example, past population divergence and then subsequent introgression. Because only fragments of two mitochondrial and one nuclear gene loci were sequenced, definite delineation of species within these MOTUs is not possible–ideally data from more than one nuclear gene loci should be obtained.

## Scavenging Community in the Studied Area

The known distribution of the taxa collected is shown in **Table 6**. *Abyssorchomene distinctus* is the only species in our collection that was previously detected in the Indian Ocean. Apart from *Cleonardo neuvillei*, all taxa recorded in the present study were previously caught with baited traps. *Cleonardo* species are considered bathypelagic and carnivorous (Bousfield and Hendrycks, 1995), so the presence of single individual in our samples can be regarded as a by-catch.

The genus *Eurythenes* is very commonly collected with baited traps (Jamieson et al., 2011; Havermans et al., 2013; Narahara-Nakano et al., 2018). Of *Eurythenes magellanicus*, we detected the largest individual (105 mm) reported so far that was still an immature female. The largest previously named representative for that species is a mature female 85 mm collected from the

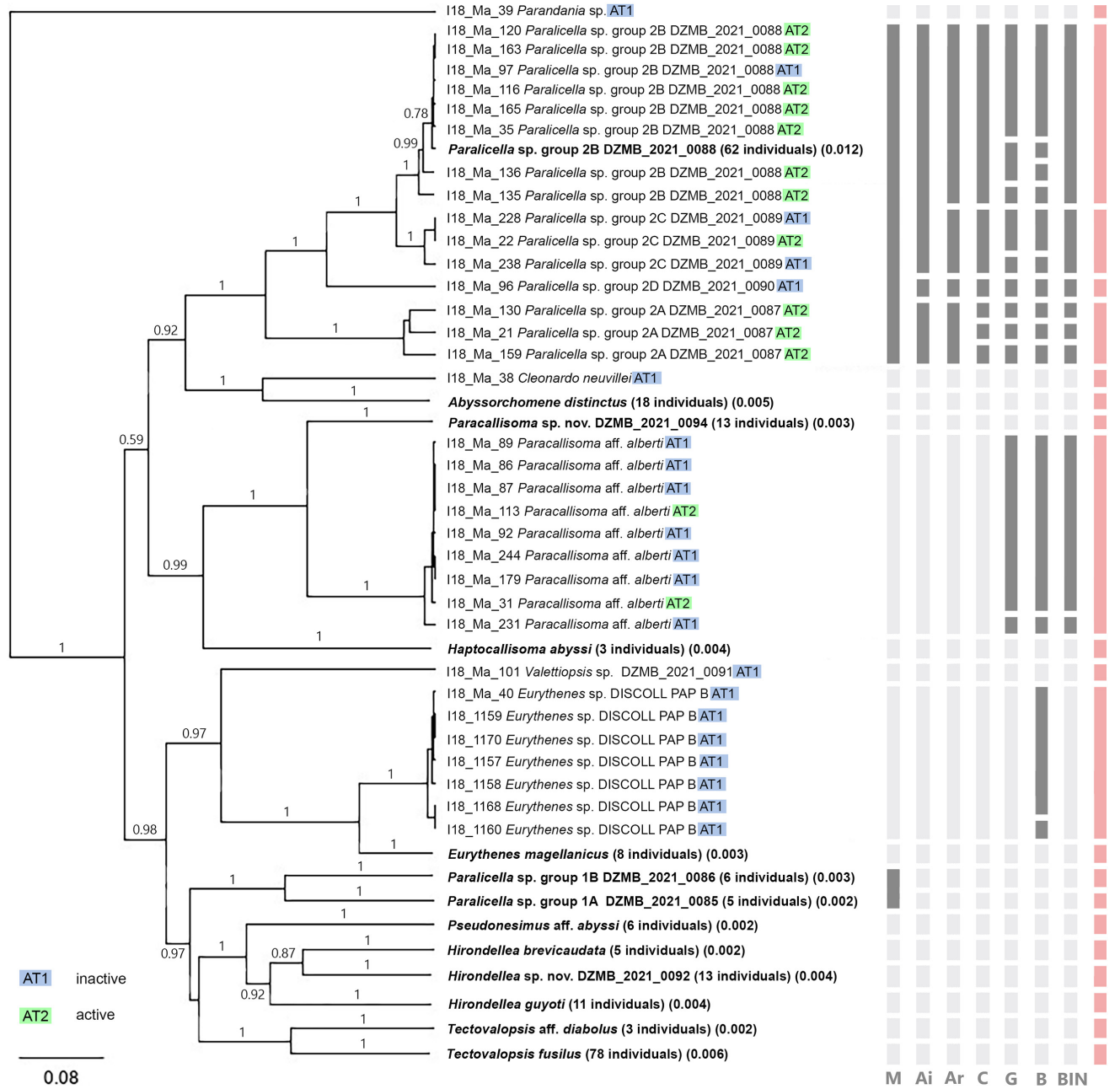


**FIGURE 4 |** Bayesian inference tree for CO1 data (258 individuals). Tree constructed by using Tamura-Nei substitution and Yule-coalescent model. Numbers represent posterior probabilities (only values higher than 40% are presented). Relevant species delimitation result is shown by vertical bars; disagreements are highlighted: M: morphology. Ai: Automatic Barcode Gap Discovery (initial partition). Ar: Automatic Barcode Gap Discovery (recursive partition). C: CD-Hit. G: general mixed Yule-coalescence GMYC. B: bPTP and BIN. The red bar presents the consensus of all delimitation methods. Samples from AT1 (inactive) and AT2 (active) are color-coded; individuals of one MOTU with matching delimitation results are summarized and highlighted in bold.

stomach of a fish off Cape Horn (Stoddart and Lowry, 2004). The deep-sea species belonging to the genus *Hirondellea* are commonly found in baited traps (Jamieson et al., 2011; Ritchie et al., 2015; Duffy et al., 2016) as well as at hydrothermal vent fields (Barnard and Ingram, 1990). *Ambasiopsis*, another genus belonging to Lysianassoidea, has been recorded from the Indian Ocean before, and it was represented by *Ambasiopsis brevipes* Ledoyer, 1986 collected at Banc du Geyser (Ledoyer, 1986). A detailed check of our *Ambasiopsis* material revealed, however, that it is a different species.

Some species from the superfamily Alicelloidea were collected as well. The genus *Paralicella* is known from all three oceans, including Antarctic waters (Chevreux, 1908; Shulenberger and Barnard, 1976; De Broyer et al., 2004; Horton and Thurston,

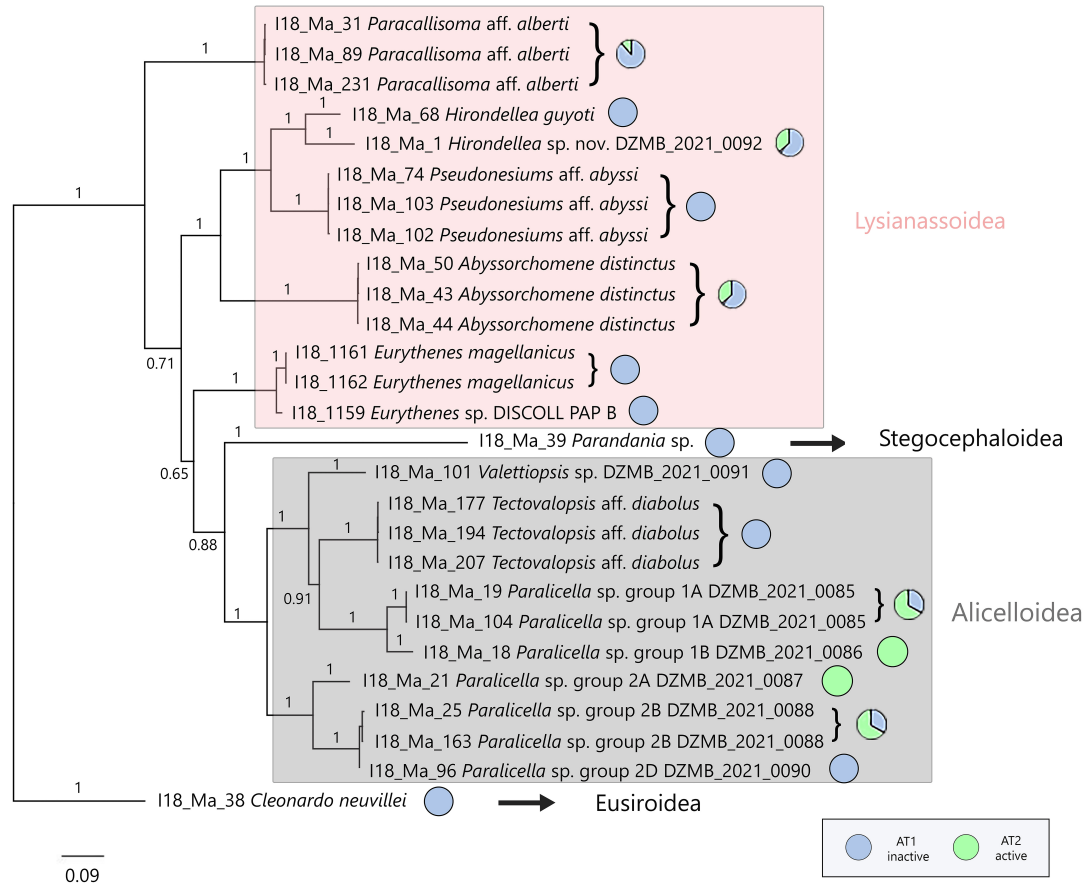


**FIGURE 5 |** Bayesian inference tree construction on the concatenate three-gene dataset (COI, 16S rDNA, 18S rDNA). The distances were calculated with the General Time Reversible model (COI, 18S) and the Hasegawa-Kishino-Yano model (16S). Numbers represent posterior probabilities. Next to the tree information about the relative abundances for the two locations are given. The superfamilies are indicated on the right.

2009; Horton et al., 2020b; Weston et al., 2021; Jażdżewska et al., 2021) and has been collected at hydrothermal vent fields before (Barnard and Ingram, 1990), as has *Valettiopsis* (Juan de Fuca Ridge in the Pacific Ocean) (Tsurumi, 2001). The genus *Parandania* is not only a worldwide distributed genus, but was also sampled at hydrothermal vents (Wang et al., 2019). One species within this genus, *Parandania boecki* is panoceanic and reported from Indian Ocean (De Broyer et al., 2007).

## Population Connectivity and Community Analyses

Our study provides the first known records for scavenging amphipods caught with the help of baited traps at hydrothermal vent fields in the Indian Ocean. Nevertheless, sampling in the deep sea is sometimes challenging, and certain limitations to the study must be mentioned. The first is lack of replicates, second that the active and inactive sites are some distance apart and without adjacent non-vent abyssal controls, and third that the traps were left on the sea floor for different periods of time (**Table 1**). These factors might produce differences within the species composition and abundance. Similarly, differences in abiotic factors at the three traps locations might influence the fauna that approached them.

The distribution of organic matter and the hydrostatic pressure are important in defining the composition of hadal scavenging communities (Wolff, 1959; Beliaev and Brueggeman, 1989). Experimental study has revealed that some amphipod species are not flexible enough to colonize highly disturbed zones, for example glacier melting areas or bottom sections opened after ice shelf collapse because of changes in salinity and intensive sedimentation (Seefeldt et al., 2017). Hydrothermally active areas can also be considered disturbed areas, with irregular abiotic factors like bursting chimneys, hypoxia (Hourdez and Lallier, 2007), high temperatures (Chevaldonné et al., 1991, 1992), and high levels of hydrogen sulfide, methane, ammonia, and heavy metals (Campbell et al., 1988a,b). In comparison, inactive areas offer stable environmental conditions that are more attractive to general deep-sea organisms (Erickson et al., 2009; Levin et al., 2009; Boschen et al., 2016). Similar values of temperature, salinity and pH were observed at all three locations, so these factors cannot explain the complete failure of trap AT3 even though it spent similar time on the bottom as the very successful AT1 trap. Of the three sites AT3

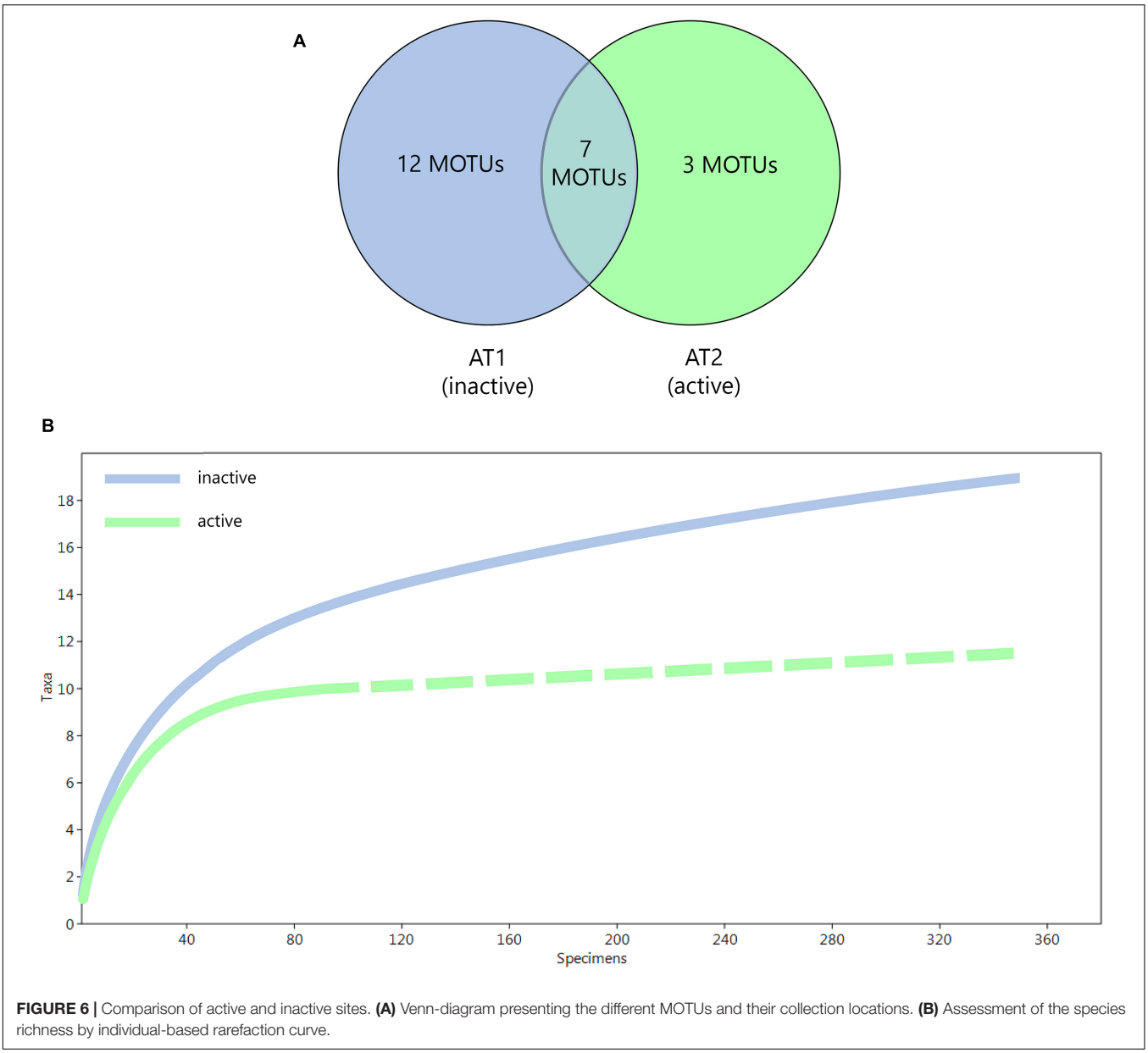


**FIGURE 6 |** Comparison of active and inactive sites. **(A)** Venn-diagram presenting the different MOTUs and their collection locations. **(B)** Assessment of the species richness by individual-based rarefaction curve.

was situated in the closest vicinity of the active vent. The emissions from vents are associated with oxygen depletion in the surrounding waters (Hourdez and Lallier, 2007) while amphipods are regarded as having low tolerance to hypoxia (Modig and Ólafsson, 1998; Wu and Or, 2005; Copilaş-Ciocianu et al., 2020) that may caused their absence there. Unfortunately, the oxygen concentration data are not available for the studied sites, so this issue must remain open question. At the other two traps 463 amphipods were captured, but are these amphipods only occasional visitors to the fields or might they be vent endemic?

At hydrothermal vent fields life depends on the presence of chemoautotrophic microbes. In the Indian Ocean, the microbes support the high abundance of the shrimp *Rimicaris kairei* Watabe and Hashimoto, 2002, which in turn sustains a variety of other vent endemic taxa, including fish species (Gerdes et al., 2021; Thiel et al., 2021). Generally, vent endemic species occur in high abundance around the active fields (Ingole and Koslow, 2005; Thornton et al., 2016), but despite the special food source, typical vent-endemic amphipods have not previously been reported from the Indian Ocean, even though knowledge about the vent fields along the Central and Southeast Indian Ridges has increased during the last decade as a result of the massive sulfide exploration program in the German license area (Gerdes et al., 2019a,b, 2021). The absence up to now of any amphipod records and abundance that is on average lower than those reported in the literature for baited traps (Duffy et al., 2012; Horton et al., 2020b; Patel et al., 2020), we conclude that the presently

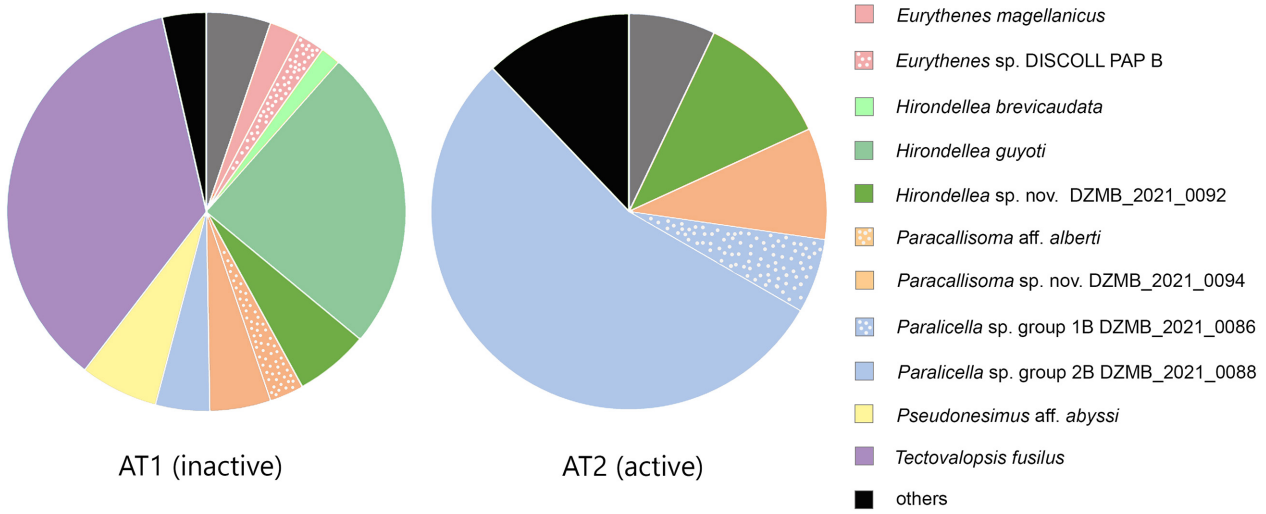


**FIGURE 7 |** Relative abundance of MOTUs at the active and inactive areas. Unnamed MOTUs with $n \leq 3$ are grouped together as "others." More details about numbers of each MOTU can be found in **Table 3**.

studied scavenging amphipod assemblage is probably not vent endemic. The three previously described species (*Hirondellea guyoti*, *Tectovalopsis fusilus*, and *T.* aff. *diabolus*) have so far been recorded exclusively from hydrothermal vent fields (Barnard and Ingram, 1990; Desbruyères et al., 2006), however, and we report four species new to science that have not yet been collected elsewhere. These species do not depend directly on the chemoautotrophic bacteria and primary production in the vent fields, but they may be more resistant to hypoxic conditions than other deep-sea amphipod species and may profit from the primary production that is offered by the vent fields. They may therefore be treated as vent related, but because no baited traps were set in the abyssal plain adjacent to the presently studied area, the presence of these species also outside the hydrothermal vent fields cannot be excluded.

Differences in amphipod assemblages between hydrothermally active and inactive regions can be observed. The inactive site was characterized by higher abundance and species richness (**Table 3** and **Figures 6**, **7**) than the active area. Rarefaction results indicated that the higher diversity at the inactive site would still be the expected result. In addition, the population analyses indicate a significant population expansion of the *Paralicella* sp. group 2B DZMB_2021_0088, *Tectovalopsis fusilus*, and *Abyssorchomene distinctus* (**Table 5**); the other species studied also tended to expand. Expanding populations are a general deep-sea phenomenon particularly in vent-endemic species (Vrijenhoek, 2010; Taylor and Roterman, 2017). Furthermore, the analyses of Tajima's D and Fu's Fs did not reveal clear differences within genetic diversity between populations at active and inactive sites. In addition, AMOVA reinforced this statement by comparing the mitochondrial COI sequences of active and inactive populations for six species from the Southeast Indian Ridge, resulting in a lack of genetic structure, as suggested by negative or non-significant values for Fst. On this basis, we speculate that no "active" or "inactive" vent communities are present. Instead, just one population probably approaches the different fields. The expansion of the populations in the deep sea may be associated with recent bottlenecks, dispersal of random individuals between patchy habitats, or positive selection (Taylor and Roterman, 2017) and, however, signify a large population size.

Different feeding strategies might explain the difference between the traps from active and inactive vents. These differences are not only quantitative (although the quantitative difference should be viewed with caution) but also qualitative, because the active area was dominated by *Paralicella* sp. group 2B DZMB_2021_0088. This genus is known to consist of obligate scavengers (Horton et al., 2020b) that are very successful in locating baits in the deep sea and depend only on this specific kind of food. Horton et al. (2020b) revealed that temporal changes in environmental conditions in the ocean may influence the scavenger community. These changes are reflected in the switch from obligate necrophagous amphipod dominance (*Paralicella*) to a more diverse assemblage with larger numbers of facultative scavengers (*Abyssorchomene* sp., *Eurythenes* spp.). In the present case, the differences in environmental conditions between the active and inactive sites may reflect the temporal changes observed by Horton et al. (2020b). These authors have also observed that the scavenger community changes with the time the bait spends on the bottom, from the dominance of obligate scavengers, to a more diverse assemblage with a higher share of facultative scavengers (Janßen et al., 2000). In our study the trap at the active site spend a shorter amount of time on the bottom, a difference that might have prevented less specialized amphipod species from reaching it, but the rarefaction curve for the active site sample

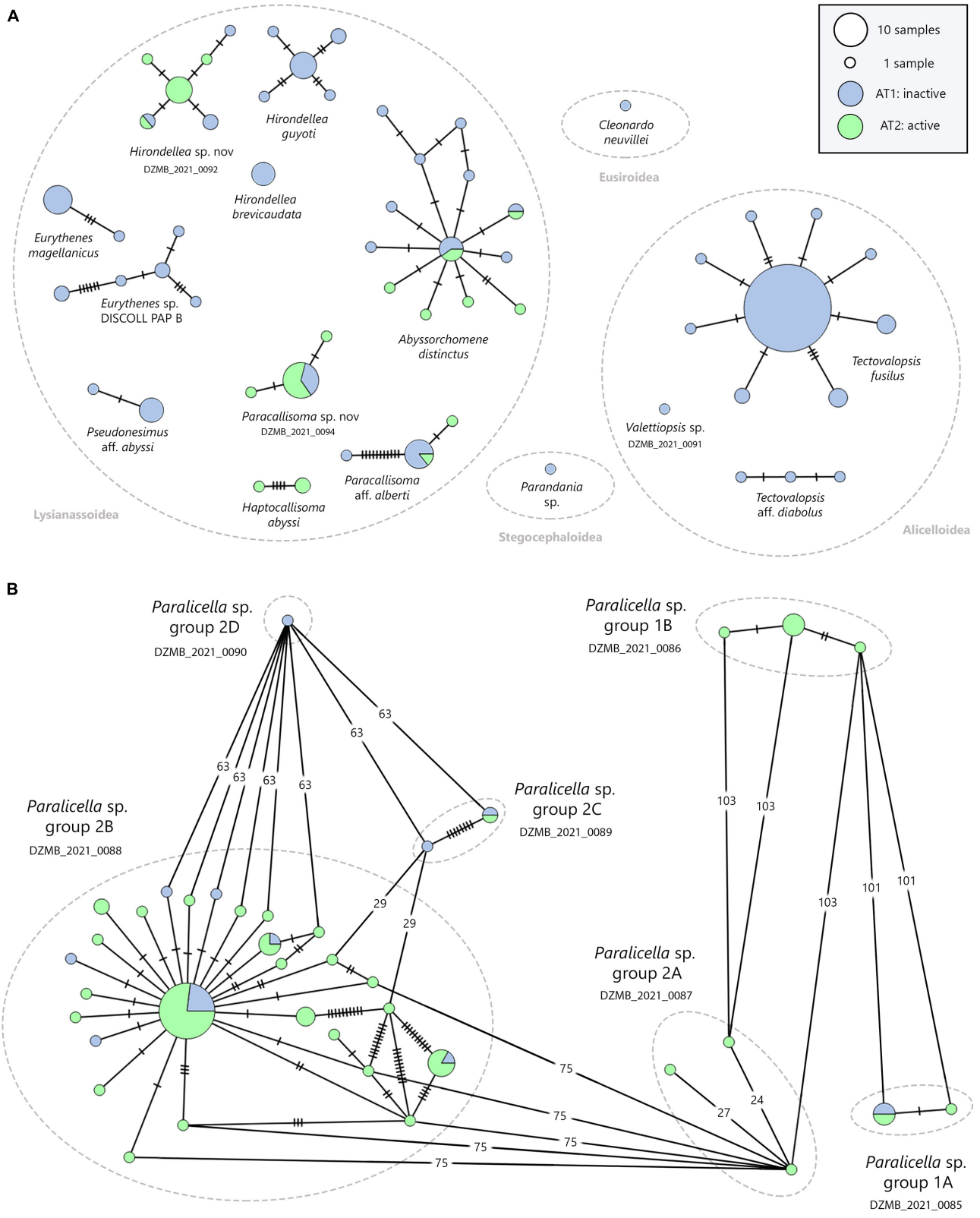


**FIGURE 8 |** Results of the statistical haplotype network analysis based on the COI data-set (258 individuals). Colors refer to the different sampling localities and the size of each circle is proportional to the number of individuals (see legends). Hatch marks show single substitutions. Minimum spanning networks for **(A)** Alicelloidea, Eusiroidea, Lysianassoidea and Stegocephaloidea. **(B)** *Paralicella*; differentiated into six groups. Mutations ($n > 10$) are presented as numbers.

**TABLE 4 |** Genetic diversity indices, parameters of demographic history and neutrality and population expansion tests calculated for the COI dataset.

| Species | | n | No. haplotypes | Haplotype diversity (h) | Nucleotide diversity ($\pi$) | Tajima's D | Fu's *Fs* |
|---|---|---|---|---|---|---|---|
| *Paralicella* sp. group 1A DZMB_2021_0085 | AT1 | 2 | 1 | – | – | – | – |
| | AT2 | 3 | 2 | 0.667 | 0.00101 | – | 0.20067 |
| | com | 5 | 2 | 0.400 | 0.00061 | –0.81650 | 0.09021 |
| *Paralicella* sp. group 1B DZMB_2021_0086 | AT2 | 6 | 3 | 0.600 | 0.00203 | –1.29503 | 0.29690 |
| *Paralicella* sp. group 2B DZMB_2021_0088 | AT1 | 15 | 9 | 0.800 | 0.00377 | –1.95478* | –2.10318 |
| | AT2 | 53 | 23 | 0.835 | 0.00560 | –1.95004* | –16.22493*** |
| | com | 68 | 30 | 0.823 | 0.00510 | –2.05811* | –22.31432*** |
| *Tectovalopsis fusilus* | AT1 | 78 | 9 | 0.305 | 0.00077 | –2.09503* | –7.04595*** |
| *Eurythenes magellanicus* | AT1 | 9 | 2 | 0.222 | 0.00122 | –1.44751 | 1.41490 |
| *Eurythenes* sp. DISCOLL PAP B | AT1 | 8 | 5 | 0.857 | 0.00674 | 0.09834 | 0.12110 |
| *Hirondellea brevicaudata* | AT1 | 5 | 1 | – | – | – | – |
| *Hirondellea guyoti* | AT1 | 11 | 5 | 0.709 | 0.00238 | -1.40298 | -0.97174 |
| *Hirondellea* sp. nov. DZMB_2021_0092 | AT1 | 4 | 3 | 0.833 | 0.00329 | -0.06501 | 0.25081 |
| | AT2 | 9 | 4 | 0.583 | 0.00101 | –1.51297 | –1.89165* |
| | com | 13 | 6 | 0.782 | 0.00175 | –1.01207 | –2.69176* |
| *Paracallisoma* aff. *alberti* | AT1 | 7 | 2 | 0.286 | 0.00478 | –1.62257* | 4.56086 |
| | AT2 | 2 | 2 | 1.000 | 0.00152 | – | – |
| | com | 9 | 3 | 0.417 | 0.00405 | –1.87639* | 2.51104 |
| *Paracallisoma* sp. nov. | AT1 | 4 | 1 | – | – | – | – |
| | AT2 | 9 | 3 | 0.417 | 0.00068 | –1.36240 | –1.08110* |
| | com | 13 | 3 | 0.295 | 0.00047 | –1.46801* | –1.40150* |
| *Pseudonesimus* aff. *abyssi* | AT1 | 6 | 2 | 0.333 | 0.00051 | –0.93302 | –0.00275 |
| *Abyssorchomene distinctus* | AT1 | 11 | 9 | 0.945 | 0.00254 | –1.21775 | –7.38058*** |
| | AT2 | 7 | 6 | 0.952 | 0.00261 | –1.52412* | –3.70942** |
| | com | 18 | 13 | 0.928 | 0.00260 | –1.88027* | –11.98916*** |

*Significant P-values are marked with asterisks. < 0.05*, < 0.001**, < 0.0001 ***. Only MOTUs represented by a total n ≥ 4 shown. Combined populations from AT1 and AT2 are presented as "com."*

approached the asymptote, so no addition of new species in this assemblage was predicted.

Nevertheless, in another study the species *Eurythenes gryllus* (Lichtenstein in Mandt, 1822) is reported to be rather an obligate scavenger (Dauby et al., 2001), but as the genus *Eurythenes* has never been collected at hydrothermal vent areas, it seems to avoid areas with active hydrothermal activity. Dauby et al. (2001) also reported one species of *Abyssorchomene* [*A. nodimanus* (Walker, 1903)] as an obligate scavenger, in contrast with Horton et al. (2020b). For *A. distinctus*, which we captured on both active and inactive site in more or less equal numbers, we might conclude it is resistant enough to environmental conditions to feed at the active site and has no preference for habitat. The genus *Hirondellea* has been reported to be either an obligate scavenger (Blankenship and Levin, 2007) or a micropredatory browsing type, so scavenging is just an alternative feeding mode for the species (Dauby et al., 2001). In the stomach of *Hirondellea antarctica* (Schellenberg, 1926) hydrozoans and sea anemones were detected (Dauby et al., 2001). The two described species of *Hirondellea* presently we captured may be assumed to be facultative scavengers avoiding the active vent sites, whereas *Hirondellea* sp. nov. DZMB_2021_0092, collected in both locations, may be an obligate scavenger or may also feed on the vent fauna. Little is known about the dominant species in the inactive site–*Tectovalopsis fusilus*. Similarly to *Paralicella* this genus is regarded as grouping obligate scavengers (Lowry and De Broyer, 2008), but *T. fusilus* has so far been collected only once and is known from single individual, so a final decision cannot be drawn about how variable its food composition is.

Our study leads to the conclusions that the scavenging amphipods at the vent fields are not restricted to the locations studied and that only some of the most resistant species may be able to deal with the difficult conditions at the active area.

**TABLE 5 |** Analysis of molecular variance (AMOVA) based on pairwise difference among haplotypes; significance calculated by 1,000 permutations of the COI dataset.

| Source of variation | Sampling site (active/inactive) = population | | |
|---|---|---|---|
| | | Percentage of variation | FST (*p*-value) |
| *Paralicella* sp. group 1A DZMB_2021_0085 | Among populations | –20.00 | –0.20000 (1.00000) |
| | Within populations | 120.00 | |
| *Paralicella* sp. group 2B DZMB_2021_0088 | Among populations | –3.20 | –0.03200 (1.00000) |
| | Within populations | 103.20 | |
| *Hirondellea* sp. nov. DZMB_2021_0092 | Among populations | –12.78195 | –0.12782 (1.00000) |
| | Within populations | 112.78195 | |
| *Paracallisoma* aff. *alberti* | Among populations | –18.23 | –0.18226 (0.43109) |
| | Within populations | 118.23 | |
| *Paracallisoma* sp. nov. | Among populations | –11.62791 | –0.11628 (1.00000) |
| | Within populations | 111.62791 | |
| *Abyssorchomene distinctus* | Among populations | 4.61522 | 0.04615 (0.11926) |
| | Within populations | 95.38478 | |

*The populations were arranged in active and inactive groups. All p-values are non-significant.*

**TABLE 6 |** Summary of known distribution of presently sampled species indicating the presence at hydrothermal vent areas.

| | Atlantic ocean | Pacific ocean | Hydrothermal vents | References |
|---|---|---|---|---|
| *Tectovalopsis* aff. *diabolus* | Not recorded | East Pacific Rise: 13°N vent site (type locality; only record so far) | | Barnard and Ingram, 1990; Desbruyères et al., 2006 |
| *Tectovalopsis fusilus* | Not recorded | Guerrero, off Punta San Telmo, North East Pacific (type locality) | | Barnard and Ingram, 1990 |
| *Cleonardo neuvillei* | Canary Islands (type locality), only record so far | Not recorded | Not recorded | Chevreux, 1910 |
| *Eurythenes magellanicus* | North and South Atlantic | Cape Horn (type locality), Taiwan, Okinawa Island | Not recorded | Stoddart and Lowry, 2004; Havermans et al., 2013; Havermans, 2016; Narahara-Nakano et al., 2018; Horton et al., 2020a |
| *Eurythenes* sp. DISCOLL PAP B | Porcupine Abyssal Plain, Northeast and Northwest Atlantic | Peru-Chile Trench, South East Pacific | Not recorded | France and Kocher, 1996 (identified as *Eurythenes gryllus* ICE-1); Ritchie et al., 2015 (identified as *Eurythenes* sp. 2 HR-2015); Horton et al., 2020a |
| *Hirondellea brevicaudata* | North Atlantic (type locality) | North of Hawaii, central Pacific | | Chevreux, 1910; Barnard and Ingram, 1990; France, 1993 |
| *Hirondellea guyoti* | Not recorded | Hess Guyot in the North Pacific (type locality), only record so far | | Barnard and Ingram, 1990 |
| *Haptocallisoma abyssi* | Greenland Sea (type locality), North Atlantic | Not recorded | Not recorded | Oldevig, 1959; Horton and Thurston, 2015; Jażdżewska et al., 2018 (erroneously identified as *Scopelocheirus* sp.) |
| *Paracallisoma* aff. *alberti* | Azores (type locality), North Atlantic | Not recorded | Not recorded | Horton and Thurston, 2015 |
| *Pseudonesimus* aff. *abyssi* | Bay of Biscay (type locality), North Atlantic | Cedros Trench, North East Pacific | Not recorded | Chevreux, 1926; Barnard, 1967 |
| *Abyssorchomene distinctus* | South of Palau (type locality), worldwide distributed in the abyss, the only species previously recorded in the Indian Ocean | | Guaymas Basin, East Pacific Rise 13°N | Vinogradov, 1993; Bellan-Santini, 1998; Desbruyères et al., 2006; Jamieson et al., 2011; Ritchie et al., 2015; Duffy et al., 2016; Patel et al., 2020; Weston et al., 2021 |

Reinforcing the patterns observed in our study, we intend to improve our results by increasing the sample size, placing the traps at different distances from the field and setting a standard for the distance between trap and vent field, and the deployment time. Finally, our results show that the amphipod fauna of the ecosystems of the Central and Southeast Indian Ridges are still widely unexplored and that further studies, particularly of environments such as the inactive and active hydrothermal areas, have great potential for the discovery of more scavenging amphipods.

## DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the Barcode of Life Data System (BOLD) as "DS-INMAC01." All sequences were in addition deposited in GenBank with the accession numbers: COI (MZ197178–MZ197435), 16S (MZ197436–MZ197490), and 18S (MZ197491–MZ197533). Additional information about the analyses presented here is included in the **Supplementary Material**.

## AUTHOR CONTRIBUTIONS

TCK was the head of the project. TCK and PMA contributed to the conception and design of the study. AMJ performed the morphological studies and identification of the amphipods. KK did the molecular work, constructed the database, and wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.752360/full#supplementary-material

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