



1 **Hidden behind the scene – high diversity, low connectivity of deep-sea Amphipoda in the**
2 **polymetallic nodule fields in the central East Pacific**

3 Anna M. Jażdżewska¹, Karolina Biniek¹, Pedro Martínez Arbizu², Annemiek Vink³

4 ¹Department of Invertebrate Zoology and Hydrobiology, Faculty of Biology and Environmental
5 Protection, University of Lodz, 12/16 Banacha st., 90–237 Lodz, Poland,

6 ²Senckenberg am Meer, Department German Center for Marine Biodiversity Research (DZMB),
7 Südstrand 44, D-26382 Wilhelmshaven, Germany

8 ³Federal Institute for Geosciences and Natural Resources, Marine Geology, 30655, Hannover, Germany

9 **Corresponding author:** Anna Jażdżewska (anna.jazdzewska@biol.uni.lodz.pl)

10 AMJ ORCID: 0000-0003-2529-0641

11 KB ORCID: 0009-0007-2984-9322

12 PMA ORCID: 0000-0002-0891-1154

13 AV ORCID: 0000-0002-5178-9721

14

15 **Keywords**

16 Clarion-Clipperton Zone, abyssal, DNA barcoding, species richness, species distribution, deep-
17 sea mining

18

19 **ABSTRACT**

20 The Clarion-Clipperton Zone (CCZ) situated in the central east Pacific holds major portions of
21 manganese nodule deposits and is therefore subject to intense exploration for future deep-sea
22 mining. However, mining rises multiple concerns. Among others about its direct or indirect
23 impact on abyssal environment. The more, proper evaluation of deep-sea protection plans to be
24 applied there is still hindered by insufficient knowledge of the abyssal fauna diversity and their
25 assemblages.

26 Amphipoda are speciose and abundant in all marine habitats and were proven to be important
27 food source for higher trophic levels. These crustaceans are brooders with no planktonic larval
28 stage, so migration of species depend only on swimming capacities of adult animals. In the CCZ
29 macrofauna-sized and relatively mobile, epibenthic Amphipoda, not collected with commonly
30 used box corers or multicorers, remained unstudied. The present work aims to fill in this gap in
31 knowledge by: 1) characterizing the diversity of amphipods across the CCZ and one Area of



32 Particular Environmental Interest (APEI), 2) studying the amphipod species distribution and
33 assemblages as well as 3) inferring the connectivity between eastern and western parts of the
34 CCZ and other deep-sea regions.

35 The material was collected with epibenthic sledge from three contractor zones (UK, Singapore,
36 Germany) and from APEI-06. Specimens were sorted into families and preferably morphospecies
37 and subjected to cytochrome *c* oxidase subunit I gene (COI) barcoding.

38 Within total of 708 individuals 23 known and one undescribed families were identified. The
39 barcoding allowed to obtain 581 sequences that were ascribed to 207 Molecular Operational
40 Taxonomic Units (MOTUs). The families that dominated barcoded material in terms of
41 abundance and species richness were: Pardaliscidae (123 ind., 44 MOTUs), Eusiridae (122 ind.,
42 32 MOTUs) and Synopiidae (74 ind., 31 MOTUs). Only 17 known species were identified;
43 another 48 are considered as new to science. Almost 70% of MOTUs were singletons or
44 doubletons and 186 MOTUs were unique for the present study. The remaining 21 taxa appeared
45 to be broadly distributed. Among the MOTUs recorded only in the CCZ majority was distributed
46 within 400 km distance. Just six species reached or exceeded 1000 km distance of occurrence.

47 Additionally only one species was shared between contractor areas and the studied APEI that was
48 supposed to be preserved area representative for the mining zones. The cluster analysis of the
49 fauna collected during three expeditions to the German contractor areas revealed almost no
50 similarity between the West and East part of the CCZ. Additionally, within the eastern part of the
51 studied region geographic proximity appeared to have no influence on station clustering.

52 The study confirmed low abundance and high species richness of deep-sea amphipod fauna of the
53 CCZ and their highly variable assemblages. Moreover, it has shown weak connectivity between
54 eastern and western parts of the region as well as between the contractor zones, APEIs and other
55 deep-sea regions of the World. All the above suggest high vulnerability of the assemblages
56 studied and, with the present state of knowledge, weak representativeness of Areas of Particular
57 Environmental Interest for the mining zones. In order to better understand the deep-sea
58 biodiversity and develop Regional Environmental Management Plans for the whole CCZ area
59 two main recommendations are to be made: 1) to conduct more intensive sampling program of
60 APEIs parallel to monitoring studies of contractor zones with the goal to better assess their
61 usefulness as reservoirs of biodiversity for the mining areas, 2) to include the epibenthic sledge

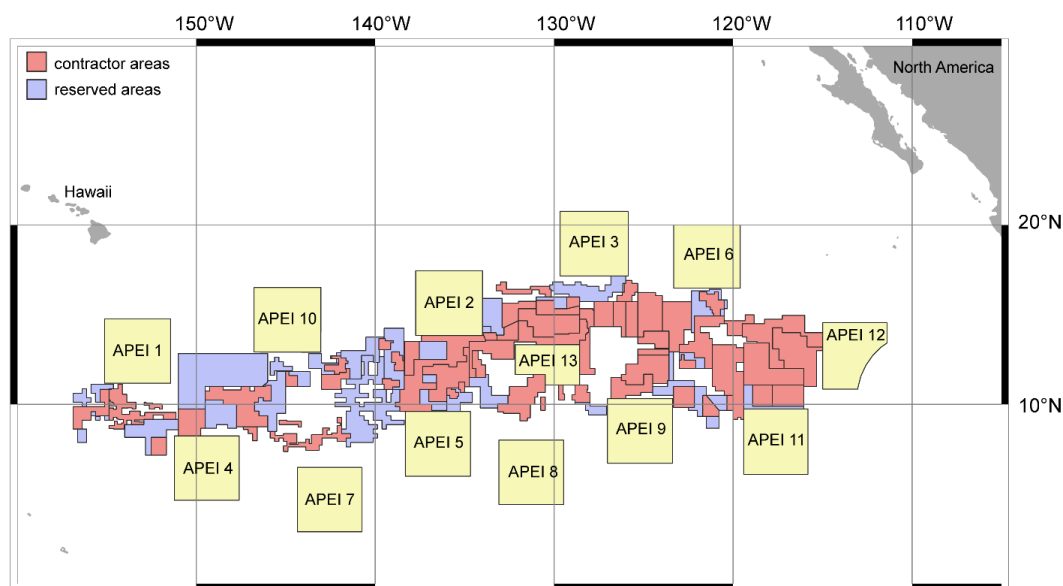


62 among standard gears used for sampling in order to avoid missing important part of deep-sea
63 fauna in the monitoring studies.

64

65 **1 INTRODUCTION**

66 The development of high- and green-tech technologies requires growing amount of rare-earth-
67 element metals (Hein et al., 2013). Being finite resource with on-land deposits often limited or
68 not accessible (McLellan et al., 2016) alternative sources of such elements have been
69 investigated. The Clarion-Clipperton Zone (CCZ) situated between the Clarion and the
70 Clipperton Fracture Zones in the central east Pacific and covering approximately 6 million km²
71 (Wiklund et al., 2019) holds major portions of manganese nodule deposits and is therefore
72 subject to intense exploration for future deep-sea mining activities (ISA, 2010). At present, the
73 International Seabed Authority (ISA) has issued nineteen nodule exploration licenses within the
74 CCZ (<https://www.isa.org.jm/exploration-contracts/polymetallic-nodules/>). Promising from the
75 industry point of view, the deep-sea mining rises multiple concerns including technical,
76 environmental, financial, and legal issues (IISD, 2019). The impact on the deep-sea environment
77 may be direct (the removal of nodules, and the destruction of the seabed during nodules
78 collection) or indirect (the generation of sediment plumes, the discharge of mine tailings)
79 (Rolinski et al., 2001; Sharma et al., 2001; Washburn et al., 2021b) and has already been proved
80 as having serious consequences on the deep-sea communities (Jones et al., 2017; Miljutin et al.,
81 2011; Lefaible et al. 2023; 2024). To reduce the concerns about the irreversible impact of deep-
82 sea mining activities the conservation planning has been conducted and resulted in CCZ-
83 Environmental Management Plan (CCZ-EMP) (ISA 2012). One of the achievements of that plan
84 was the establishment of the network of nine, Areas of Particular Environmental Interest (APEI)
85 (ISA, 2012) that was later updated by addition of four new APEIs in 2021 (ISA, 2021) (Fig. 1).



86

87 Figure 1. Exploration contract areas for polymetallic nodules in the Clarion-Clipperton Zone,
88 central equatorial Pacific basin, APEI – Area of Particular Environmental Interest.

89

90 However, all but one APEIs are situated outside the main area prospected for deep-sea mining
91 and initial studies suggested that the present network of APEIs may not sufficiently reflect
92 environmental conditions as well as faunal communities of the mining zones (Błażewicz et al.,
93 2019; McQuaid et al., 2020; Washburn et al., 2021a, b; Uhlenkott et al., 2023). The more, almost
94 half of the protected areas lack any biodiversity information (see Fig. 1 in Rabone et al., 2023).
95 Among further challenges associated with proper evaluation of deep-sea protection plans is still
96 insufficient knowledge of the abyssal fauna diversity and their assemblages (e.g. Washburn et al.,
97 2021b; Uhlenkott et al., 2023). Numerous studies have been conducted for megafauna (available
98 to be studied also with the use of underwater camera allowing to screen larger parts of the ocean
99 seafloor) (e.g. Uhlenkott et al., 2023) or scavengers easily collected in large numbers with baited
100 traps (e.g. Patel et al., 2020; Bribiesca-Contreras et al., 2021; Mohrbeck et al., 2021). Also
101 meiofauna that is traditionally sampled with multi-corers (MUC) is well represented in the
102 studies of CCZ assemblages (e.g. Hauquier et al., 2019; Tong et al., 2022; Lefaible et al., 2024)
103 as is also the case for sediment dwelling, less mobile macrofaunal taxa like Polychaeta or
104 Tanaidacea collected with box cores (e.g. De Smet et al., 2017; Błażewicz et al., 2019; Pasotti et



105 al., 2021; Washburn et al., 2021b). In contrary, moderately large (macrofauna-sized) and
106 relatively mobile, epibenthic animals, like representatives of the crustacean order Amphipoda, are
107 often not well represented in such studies. They are too small to be recorded with underwater
108 camera, too large to be collected with MUC and too fast or swept away during sampling with box
109 corer. Nevertheless, when sampled with gear designed to collect epi- and suprabenthic taxa,
110 namely epibenthic sledge (EBS), amphipods appear to constitute diverse and sometimes abundant
111 component of deep-sea macrofaunal communities (Frutos et al., 2017; Brandt et al., 2025) so they
112 deserve profound studies.

113 Amphipoda represented in the present moment by 10,740 described species (Horton et al.,
114 2025a), are speciose and abundant in all marine habitats from coastal waters till the deepest
115 trenches. In the deep sea amphipod diversity and abundance is known to be high in the bathyal,
116 decreasing towards abyssal and hadal depths where they are surpassed in diversity by another
117 order, the Isopoda (e.g. Frutos et al., 2017; Jamieson et al. 2011). Amphipods, belonging to the
118 superorder Peracarida, are brooders with no planktonic larval stage, so migration of species
119 depend only on swimming capacities of adult animals. The order comprises truly pelagic species
120 (mainly representatives of the suborder Hyperiidia) but the majority of amphipods are associated
121 with benthic realm living either in the sediment or being the part of suprabenthic communities
122 (Lowry and Myers, 2017; Brix et al., 2018). The crustaceans of this group were also proven to be
123 important food source for higher trophic levels (Dauby et al., 200; Preciado et al., 2017).

124 Since the proposal of the DNA barcoding concept by Hebert et al., (2003) the use of molecular
125 methods in species recognition has become broadly applied and often supplements morphological
126 taxonomy (e.g. Hubert and Hanner, 2015; Seefeldt et al., 2017). Within marine and deep sea
127 Peracarida this technique, when associated with morphological studies, often revealed hidden
128 diversity, including the recognition of cryptic or pseudocryptic species (e.g. Bober et al., 2018;
129 Havermans, 2016; Verheyen et al., 2016).

130 The studies of Amphipoda in the CCZ conducted by now concerned mainly the scavengers and
131 gave insights into the diversity and molecular connectivity of representatives of this trophic guild
132 including confirmation of cosmopolitan distribution of some species (e.g. Patel et al., 2020;
133 Bribiesca-Contreras et al., 2021; Jążdżewska et al., 2021; Mohrbeck et al., 2021). Contrariwise,
134 the non-scavenging amphipods were never studied in details.

135 The present study aims to:



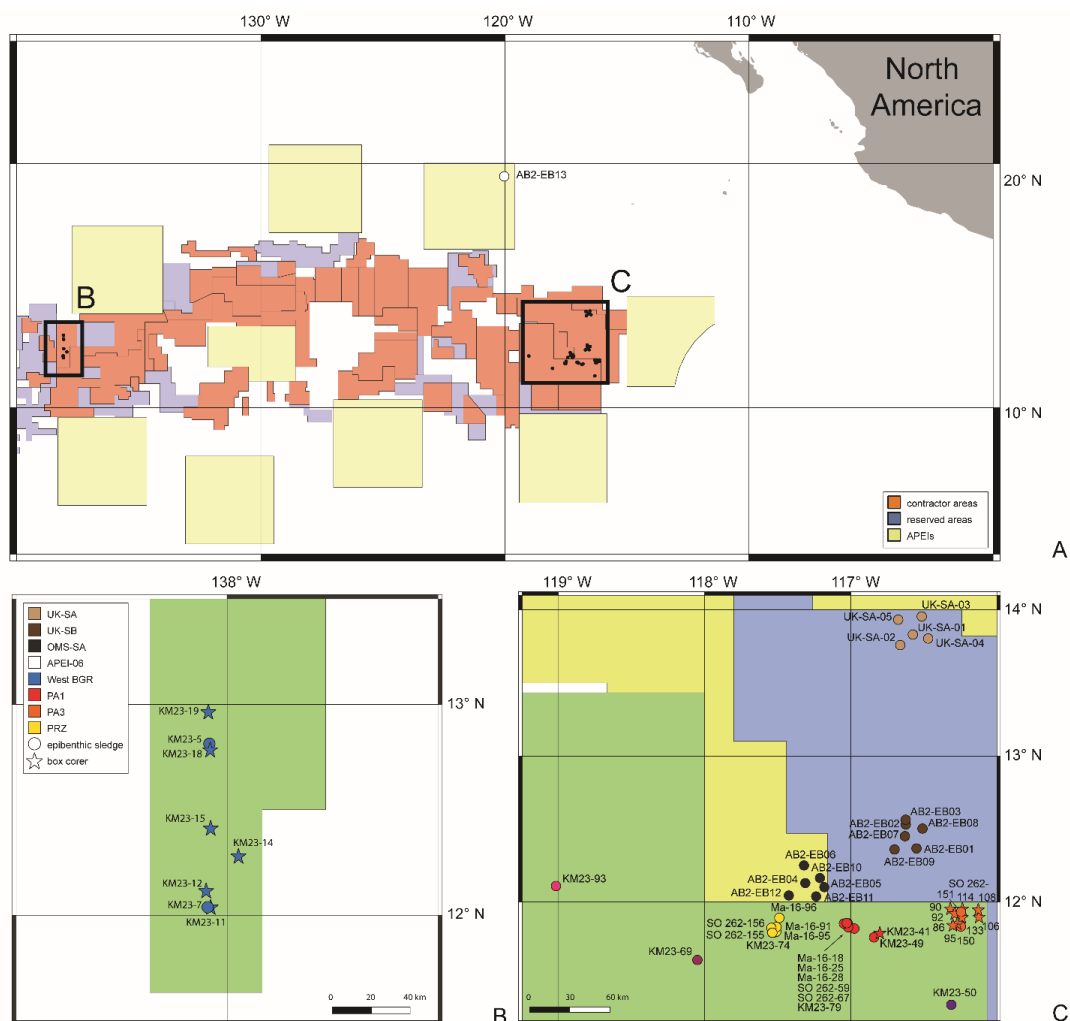
136 - characterize the morphological and molecular diversity of Amphipoda across three contractor
137 zones of CCZ and one Area of Particular Environmental Interest,
138 - study the amphipod species distribution and assemblages in the area,
139 - infer the connectivity between eastern and western part of the CCZ as well as with other deep-
140 sea regions.
141 As such the research fills in the gap in knowledge of important part of the abyssal communities
142 and brings insights into monitoring and protection plans of the zone prospected for deep-sea
143 mining.

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145 **2 MATERIAL AND METHODS**

146 The material for present study was collected during five scientific deep-sea cruises (Fig. 2, Table
147 1). The ABYSSLINE-1 (ABYSSal baseLINE project) expedition, on board the R/V *Melville*, was
148 conducted in 2013 (Smith et al., 2013), while the ABYSSLINE-2 (ABYSSal baseLINE project)
149 expedition, on board the R/V *Thompson*, in 2015 (Smith et al., 2015) and collected samples from
150 the UKSR License Area (UK Seabed Resources Ltd, United Kingdom), OMS License Area
151 (Ocean Mineral Singapore Pte. Ltd.) as well as from APEI-06. The other three expeditions,
152 MANGAN 2016, MANGAN 2018 and MANGAN 2023 sampled the German License Area
153 (BGR—Bundesanstalt für Geowissenschaften und Rohstoffe) on board the R/V *Kilo Moana*
154 (MANGAN 2016 and 2023) and R/V *Sonne* (MANGAN 2018) (Rühlemann et al., 2017, 2023;
155 Rühlemann, 2018).

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158 Figure 2. Localization of sampling stations. A – general view of the study area with indication of
159 the locality of station from APEI-06 (APEI – Area of Particular Environmental Interest), B –
160 close up of the West BGR area, C – close up of the eastern part of CCZ (East BGR, OMS and
161 UKSR contractor areas).

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166 Table 1. Station data including sampling gear information (EBS – epibenthic sledge, BC – box
 167 corer), total number of individuals collected, the number of individuals used for barcoding as
 168 well as number of sequences obtained. NA – no data available.

| Station | Area | Latitude (N) | Longitude (W) | Depth [m] | Collection date | Gear | Total no of ind. | No of ind. for barcoding | No of seq. obtained | Sequencing success | Notes |
|--------------------|----------------------|---------------------------|-----------------------------|-----------|-----------------|------|------------------|--------------------------|---------------------|--------------------|---|
| ABYSSLINE-1 | | | | | | | | | | | |
| UK-SA-01 | UKSR (UK1-Stratum A) | 13° 49.86' | 116° 34.5' | 4597 | 09-Oct-2013 | EBS | NA | 18 | 6 | 33 | No information about the total abundance of Amphipoda in the sample available, no vouchers available, not used for diversity studies. |
| UK-SA-02 | UKSR (UK1-Stratum A) | 13° 45.54' | 116° 39.66' | 4070 | 12-Oct-2013 | EBS | NA | 2 | 2 | 100 | |
| UK-SA-03 | UKSR (UK1-Stratum A) | 13° 57.3' | 116° 30.84' | 4143 | 13-Oct-2013 | EBS | NA | 2 | 1 | 50 | |
| UK-SA-04 | UKSR (UK1-Stratum A) | 13° 48.24' | 116° 28.2' | 4128 | 17-Oct-2013 | EBS | NA | 25 | 11 | 44 | |
| UK-SA-05 | UKSR (UK1-Stratum A) | 13° 55.98' | 116° 40.5' | 4196 | 19-Oct-2013 | EBS | NA | 5 | 3 | 60 | |
| ABYSSLINE-2 | | | | | | | | | | | |
| AB2-EB01 | UKSR (UK1-Stratum B) | 12° 22.02' | 116° 33' | 4209 | 18-Feb-2015 | EBS | NA | 7 | 6 | 86 | No information about the total abundance of Amphipoda in the sample available, not used for diversity studies. |
| AB2-EB02 | UKSR (UK1-Stratum B) | 12° 31.86' | 116° 37.38' | 4219 | 20-Feb-2015 | EBS | NA | 7 | 5 | 71 | |
| AB2-EB03 | UKSR (UK1-Stratum B) | 12° 33.78' | 116° 37.5' | 4219 | 23-Feb-2015 | EBS | NA | 7 | 7 | 100 | |
| AB2-EB04 | OMS (OMS-Stratum A) | 12° 7.8' | 117° 18.66' | 4111 | 25-Feb-2015 | EBS | NA | 7 | 6 | 86 | |
| AB2-EB05 | OMS (OMS-Stratum A) | 12° 6.12' | 117° 10.86' | 4100 | 26-Feb-2015 | EBS | NA | 7 | 5 | 71 | |
| AB2-EB06 | OMS (OMS-Stratum A) | 12° 15.06' | 117° 19.2' | 4137 | 01-Mar-2015 | EBS | NA | 7 | 7 | 100 | |
| AB2-EB07 | UKSR (UK1-Stratum B) | 12° 27.06' | 116° 37.8' | 4145 | 02-Mar-2015 | EBS | NA | 7 | 1 | 14 | |
| AB2-EB08 | UKSR (UK1-Stratum B) | 12° 30.18' | 116° 30.54' | 4244 | 04-Mar-2015 | EBS | NA | 7 | 2 | 29 | |
| AB2-EB09 | UKSR (UK1-Stratum B) | 12° 21.6' | 116° 42' | 4170 | 10-Mar-2015 | EBS | NA | 8 | 5 | 62 | |
| AB2-EB10 | OMS (OMS-Stratum A) | 12° 9.84' | 117° 12.66' | 4047 | 11-Mar-2015 | EBS | NA | 8 | 2 | 25 | |
| AB2-EB11 | OMS (OMS-Stratum A) | 12° 2.28' | 117° 14.22' | 4097 | 14-Mar-2015 | EBS | NA | 8 | 4 | 50 | |
| AB2-EB12 | OMS (OMS-Stratum A) | 12° 2.7' | 117° 25.44' | 4223 | 16-Mar-2015 | EBS | NA | 8 | 7 | 87 | |
| AB2-EB13 | APEI-06 | 19° 27.9' | 120° 1.5' | 4026 | 20-Mar-2015 | EBS | NA | 8 | 6 | 75 | |
| MANGAN 2016 | | | | | | | | | | | |
| Ma-16-18 | BGR-PA1 | 11° 51.372' - 11° 51.662' | 117° 01.535' - 117° 00.482' | 4132-4123 | 28-Apr-2016 | EBS | 8 | 8 | 8 | 100 | |
| Ma-16-25 | BGR-PA1 | 11° 49.143' - 11° 49.975' | 116° 58.492' - 116° 57.797' | 4107-4101 | 29-Apr-2016 | EBS | 15 | 15 | 15 | 100 | |
| Ma-16-28 | BGR-PA1 | 11° 49.654' - 11° 49.902' | 117° 00.299' - 116° 59.174' | 4143-4133 | 01-May-2016 | EBS | 11 | 11 | 11 | 100 | |
| Ma-16-91 | BGR-PRZ | 11° 49.792' - 11° 49.842' | 117° 30.458' - 117° 29.208' | 4344-4344 | 09-May-2016 | EBS | 24 | 24 | 22 | 92 | |



| | | | | | | | | | | | |
|--------------------|------------------|------------------------------|--------------------------------|---------------|-------------|-------------------|-----|----|----|-----|---|
| Ma-16-95 | BGR-PRZ | 11° 47.862' - 11° 47.152' | 117° 30.639' - 117° 29.490' | 4356- 4359 | 09-May-2016 | EBS | 28 | 26 | 24 | 86 | 2 badly damaged individuals not used for barcoding |
| Ma-16-96 | BGR-PRZ | 11° 53.492' - 11° 53.791' | 117° 29.357' - 117° 28.209' | 4193- 4256 | 10-May-2016 | EBS | 6 | 6 | 5 | 83 | |
| MANGAN 2018 | | | | | | | | | | | |
| SO 262-59 | BGR-PA1 | 11° 49.720' - 11° 50.055' | 117° 01.080' - 116° 59.530' | 4097- 4128 | 22-Apr-2018 | EBS | 10 | 10 | 10 | 100 | |
| SO 262-67 | BGR-PA1 | 11° 51.190' - 11° 51.621' | 117° 02.830' - 117° 00.804' | 4131- 4131 | 24-Apr-2018 | EBS | 6 | 6 | 5 | 83 | |
| SO 262-150 | BGR-PA3 | 11° 50.009' - 11° 49.978' | 116° 14.780' - 116° 13.316' | 4074- 4095 | 07-May-2018 | EBS | 28 | 27 | 26 | 96 | 1 ind. of Hyperideae not used for barcoding |
| SO 262-151 | BGR-PA3 | 11° 55.986' - 11° 55.992' | 116° 14.706' - 116° 13.320' | 4150- 4151 | 08-May-2018 | EBS | 5 | 5 | 5 | 100 | |
| SO 262-155 | BGR-PRZ | 11° 47.436' - 11° 47.677' | 117° 32.213' - 117° 30.910' | 4352- 4351 | 09-May-2018 | EBS | 46 | 45 | 39 | 87 | 1 ind. of Hyperideae not used for barcoding |
| SO 262-156 | BGR-PRZ | 11° 49.381' - 11° 49.752' | 117° 32.663' - 117° 30.760' | 4340- 4340 | 09-May-2018 | EBS | 23 | 22 | 21 | 95 | 1 ind. of Hyperideae not used for barcoding |
| SO 262-86 | BGR-PA3 | 11° 50.584' | 116° 17.514' | 4181 | 29-Apr-2018 | BC, 0-3 cm layer | 1 | 1 | 1 | 100 | |
| SO 262-90 | BGR-PA3 | 11° 57.039' | 116° 19.096' | 4157 | 29-Apr-2018 | BC, 0-3 cm layer | 2 | 2 | 1 | 50 | |
| SO 262-92 | BGR-PA3 | 11° 55.449' | 116° 17.439' | 4172 | 30-Apr-2018 | BC, 0-3 cm layer | 2 | 2 | 2 | 100 | |
| SO 262-95 | BGR-PA3 | 11° 50.636' | 116° 16.112' | 4165 | 30-Apr-2018 | BC, 0-3 cm layer | 1 | 1 | 1 | 100 | |
| SO 262-106 | BGR-PA3 | 11° 53.825' | 116° 07.305' | 4171 | 02-May-2018 | BC, 3-5 cm layer | 1 | 1 | 1 | 100 | |
| SO 262-108 | BGR-PA3 | 11° 56.986' | 116° 07.628' | 4207 | 02-May-2018 | BC, 0-3 cm layer | 1 | 1 | 1 | 100 | |
| SO 262-114 | BGR-PA3 | 11° 57.017' | 116° 14.157' | 4175 | 03-May-2018 | BC, 0-3 cm layer | 1 | 1 | 1 | 100 | |
| SO 262-133 | BGR-PA3 | 11° 53.833' | 116° 14.262' | 4107 | 05-May-2018 | BC, surface water | 1 | 1 | 1 | 100 | |
| MANGAN 2023 | | | | | | | | | | | |
| KM23-5 | West BGR | 12°48.7751' - 12°50.0513' | 138°05.043' - 138°05.1315' | 4996- 5020 | 16-Apr-2023 | EBS | 14 | 14 | 14 | 100 | |
| KM23-7 | West BGR | 12°02.2976' - 12°03.5888' | 138°05.4932' - 138°05.7092' | 5109- 5139 | 17-Apr-2023 | EBS | 30 | 29 | 24 | 83 | Additional 1 badly damaged individual of Oedicerotidae not used for barcoding |
| KM23-49 | BGR WA3 (PA1) | 11°45.4904' - 11°46.3035' | 116°50.4565' - 116°49.3268' | 4150- 4173 | 30-Apr-2023 | EBS | 52 | 51 | 37 | 72 | 1 ind. of Hyperideae not used for barcoding |
| KM23-50 | BGR WA4 | 11°17.7919' - 11°18.5445' | 116°18.8626' - 116°17.6747' | 4185- 4182 | 01-May-2023 | EBS | 111 | 99 | 78 | 79 | 11 ind. of Hyperideae and 1 ind. of Oxycephalidae? not used for barcoding |
| KM23-69 | BGR WA5 | 11°36.252' - 11°37.1050' | 118°02.981' - 118°01.2511' | 4368- 4356 | 04-May-2023 | EBS | 83 | 83 | 77 | 93 | |
| KM23-74 | BGR WA6 (PRZ) | 11°47.6444' - 11°48.0414' | 117°30.9650' - 117°29.5413' | 4360- 4364 | 06-May-2023 | EBS | 26 | 24 | 24 | 100 | 2 ind. of Hyperideae not used for barcoding |
| KM23-79 | BGR WA2 (PA1) | 11°51.3560' - 11°51.7516' | 117°01.2662' - 116°59.8924' | 4126- 4128 | 07-May-2023 | EBS | 16 | 16 | 14 | 87 | |
| KM23-93 | BGR WA7 | 12°06.612' - 12°07.3536' | 119°01.072' - 118°59.8503' | 4381- 4430 | 10-May-2023 | EBS | 16 | 16 | 15 | 94 | |
| KM23-11 | West BGR | 12°02.298' | 138°05.423' | 5105 | 18-Apr-2023 | BC | 6 | 6 | 6 | 100 | |
| KM23-12 | West BGR | 12°06.8609' | 138°06.0754' | 5173 | 19-Apr-2023 | BC | 1 | 1 | 1 | 100 | |
| KM23-14 | West BGR | 12°16.6825' | 137°56.7545' | 4976 | 19-Apr-2023 | BC | 1 | 0 | x | x | 1 ind. of Hyperideae not used for barcoding |



| | | | | | | | | | | | |
|---------|---------------|-------------|--------------|------|-------------|----|---|---|---|-----|---|
| KM23-15 | West BGR | 12°24.5724' | 138°04.6848' | 5033 | 19-Apr-2023 | BC | 2 | 0 | x | x | 2 ind. of Hyperiidea not used for barcoding |
| KM23-18 | West BGR | 12°47.3146' | 138°04.8688' | 4940 | 20-Apr-2023 | BC | 2 | 1 | 1 | 100 | 1 ind. of Hyperiidea not used for barcoding |
| KM23-19 | West BGR | 12°57.7304' | 138°05.3714' | 5009 | 20-Apr-2023 | BC | 2 | 2 | 2 | 100 | |
| KM23-41 | BGR WA3 (PA1) | 11°47.1427' | 116°48.0835' | 4169 | 29-Apr-2023 | BC | 3 | 3 | 2 | 67 | |

169

170 2.1 Sample collection and processing

171 The samples were collected using a Brenke-type epibenthic sledge (EBS, Brandt and Barthel,
 172 1995; Brenke, 2005). The deployment protocol followed Brenke (2005). Upon recovery, samples
 173 were passed through 300 µm and either sorted out immediately and preserved in 80% ethanol
 174 kept at -20°C, or immediately transferred into chilled (-20°C) 96% ethanol. In the second case,
 175 the sorting by stereomicroscope was carried out after 48 h storage in a -20°C freezer (Riehl et al.,
 176 2014). A few individuals collected with box corer during MANGAN 2018 and MANGAN 2023
 177 expeditions were added.

178

179 2.2 Morphological study

180 From ABYSSLINE-1 collection only a set of sequences was available, in case of ABYSSLINE-2
 181 a representation of amphipods (6-7 per station) was given to the authors of the study. In contrast
 182 all individuals collected with EBS during MANGAN 2016, 2018 and 2023 were studied.
 183 Specimens were initially examined using a Leica M125 stereomicroscope, sorted into families
 184 and preferably morphospecies (groups that form phenotypic clusters without overlap between
 185 them) and later subjected to cytochrome *c* oxidase subunit I gene (COI) barcoding (details given
 186 below). Present analysis considers only non-pelagic species even though the latter are
 187 occasionally retrieved using EBS. As a result, the representatives of the suborder Hyperiidea,
 188 even if sometimes recorded in the samples were not studied further. After specification of the
 189 Molecular Operational Taxonomic Units (MOTUs) representatives of each of them were again
 190 studied morphologically (in certain cases including preparation of microscopic slides) to provide
 191 as detailed taxonomic identification as possible. The taxa from amphipod suborders other than
 192 Hyperiidea that only after the barcoding appeared to belong to pelagic taxa were excluded
 193 (although their barcodes were used for delimitation of taxa and are published).

194



195 **2.3 Molecular investigation**

196 All individuals that possessed at least head were used for the total genomic DNA extraction. The
197 extraction was made from one pleopod (if the posterior part of the body was missing the last
198 remaining leg).

199 The DNA extraction of individuals collected during all expeditions apart from MANGAN 2023
200 was performed using 100 µl InstaGene Matrix (BIO-RAD). Digestion was carried out at 56°C for
201 40 min. The extraction of DNA from individuals from MANGAN 2023 was carried out using a
202 mixture of 112,5 µl pure H₂O with 0.011 g Chelex (Sigma-Aldrich Co.) and 7,5 µl proteinase K.
203 The digestion at 55 °C lasted for 6 h.

204 The DNA barcoding fragment of COI (658 bp) was amplified using universal LCO1490
205 (GGTCAACAAATCATAAAGATATTGG) and HCO2198
206 (TAAACTTCAGGGTGACCAAAAAATCA) primer pair (Folmer et al., 1994; collection from
207 ABYSSLINE-1) or the degenerate LCO1490-JJ (CHACWAAYCATAAAGATATYGG) and
208 HCO2198-JJ (AWACTTCVGGRTGVCCAAARAATCA) primer pair (Astrin and Stüben, 2008;
209 collections from ABYSSLINE-2, MANGAN 2016, MANGAN 2018). The polymerase chain
210 reaction was performed with AccuStart II PCR SuperMix (Quantabio) and the reaction conditions
211 following Hou et al. (2007) (Table 2). Sequencing was done bi-directionally in Macrogen Inc.
212 (the Netherlands) on an Applied Biosystems 3730xl capillary sequencer. Electropherograms were
213 viewed in Geneious 10.2.6 and primer sequences and ambiguous positions were trimmed.

214 The COI barcodes of Amphipoda from MANGAN 2023 were obtained with Oxford Nanopore
215 Technologies sequencing (ONT). The marker was amplified using a primer mix containing
216 LCO1490-JJ and HCO2198-JJ primers and a tagging sequence specific to each sample, which
217 consisted of a 9 nt (Srivathsan et al., 2023). Samples were amplified in batches, in sets consisting
218 of three 96-well plates. Each sample consisted of 6.5 µl of Dream Taq PCR Mastermix (Thermo
219 Scientific), 2µl of primers mix, 1 µl of matrix DNA and 3.5 µl of H₂O to reach 13 µl of final
220 volume. The PCR conditions were modified from Hou et al. 2007 (Table 2).

221

222 Table 2. PCR conditions.

| Step | For Sanger sequencing (Hou et al. 2007) | | | For ONT sequencing (Hou et al. 2007 modified) | | |
|----------------------|--|-------------|-----------------|--|-------------|-----------------|
| | Temp [°C] | Time [s] | No of cycles | Temp [°C] | Time [s] | No of cycles |
| Initial denaturation | 95 | 300 | | 95 | 300 | |



| | | | | | | |
|-----------------|----|-----|----|----|-----|----|
| Denaturation | 95 | 30 | 5 | 95 | 50 | 5 |
| Annealing | 45 | 90 | | 45 | 50 | |
| Extension | 72 | 60 | | 72 | 60 | |
| Denaturation | 95 | 30 | 35 | 95 | 50 | 35 |
| Annealing | 51 | 90 | | 51 | 50 | |
| Extension | 72 | 60 | | 72 | 60 | |
| Final extension | 72 | 300 | | 72 | 120 | |

223

224 Amplicons in a volume of 5 μ l each were pooled and purified according to the Sera-Mag
 225 protocol. In the next step, concentration of DNA in the sample using a Qubit 4 fluorometer (Thermo
 226 Fisher Scientific) was measured and the Qubit dsDNA BR Assay Kit reagent (Thermo Fisher
 227 Scientific). Purified DNA was stored at -20°C until the next steps were initiated. Sequencing was
 228 performed according to the Ligation Sequencing Amplicons V14 (SQK-LSK114) protocol (Oxford
 229 Nanopore Sequencing 2024). The procedure included editing the ends of barcode sequences,
 230 attaching an adapter protein, and loading and running the reaction plate (Flongle). Sequencing took
 231 about 24 hours.

232 The obtained data were then subjected to a decoding process (basecallig). This step involves
 233 assigning voltage changes to the corresponding nucleotides to obtain a complete sequence (Wang
 234 et al., 2021). It was followed by demultiplexing using the ONTbarcoder tool. This process involves
 235 assigning the obtained sequences to a specific sample based on the tagging sequences used earlier.
 236 In addition, ONTbarcoder evaluates the quality of the obtained reads, creating a consensus barcode
 237 sequence (Srivathsan et al., 2021).

238 Sequences were blasted using default parameters on NCBI BLASTn and translated into amino
 239 acid sequences to confirm that no stop codons were present. All sequences were deposited in
 240 GenBank with the accession numbers: PQ734211 – PQ734784. Relevant voucher information,
 241 taxonomic classifications and sequences are deposited in the data set “DS-AMPHICZ” in the
 242 Barcode of Life Data System (BOLD) (dx.doi.org/10.5883/DS-AMPHICZ)
 243 (www.boldsystems.org) (Ratnasingham and Hebert, 2007).

244 All sequences were aligned with the MAFFT v.7.308 algorithm (Katoh et al., 2002; Katoh and
 245 Standley, 2013) in Geneious 10.2.6, resulting in a 614 bp alignment and subsequently three
 246 molecular species delimitation methods were applied to reveal the Molecular Operational
 247 Taxonomic Units (MOTUs). Two methods were distance-based: Barcode Index Number (BIN)
 248 System (Ratnasingham and Hebert, 2013), and the Assemble Species by Automatic Partitioning



249 (ASAP) (Puillandre et al., 2021) and were supplemented by one tree-based phylogenetic
250 approach using Generalized Mixed Yule Coalescent (GMYC) model-based method (Pons et al.,
251 2006), according to Monaghan et al. (2009).

252 The Barcode Index Number (BIN) System (Ratnasingham and Hebert, 2013) in BOLD compares
253 newly submitted sequences with the sequences already available. They are clustered according to
254 their molecular divergence using distance-based algorithms (single linkage clustering followed
255 by Markov clustering) that aim at finding discontinuities between Operational Taxonomic Units
256 (OTU). Each OTU receives a unique and specific code (BIN), either already available or new if
257 submitted sequences do not cluster with already-known BINs.

258 The Assemble Species by Automatic Partitioning (ASAP) (Puillandre et al., 2021) is a method
259 that uses pairwise genetic distances to assemble individuals into groups and proposes species
260 partitioning ranked according to a scoring system. For the purpose of the present study the first
261 two partitions characterized by the lowest ASAP-scores were taken into account.

262 The GMYC method defines MOTUs through identification of the switch from intraspecific
263 branching patterns (coalescent) to interspecific species branching patterns (Yule process) on a
264 phylogenetic tree. Because for GMYC an ultrametric tree is required, as an input, a Bayesian tree
265 was reconstructed in BEAST 2.6.3 (Bouckaert et al., 2019). The site model was set up with
266 bModelTest (Bouckaert and Drummond, 2017). The tree prior was set to Birth-Death following
267 Bayes factors. Two runs of Markov chain Monte Carlo (MCMC) were performed each 1 M
268 generations-long, sampled every 1,000 generations. Runs were examined for convergence in
269 Tracer 1.7 (Rambaut et al., 2018). The final tree was summarised with TreeAnnotator 2.6.3 with
270 25% burn-in, all being part of BEAST 2.6.3 package. The Bayesian tree was uploaded into the R
271 4.0.5 (R Core Team 2021) software package ‘SPLITS’ (Species Limits by Threshold Statistics)
272 (Ezard et al., 2009) and analyzed using the single threshold model.

273 To visualize the delimitation results Neighbour-Joining (NJ) trees of all sequences divided into
274 seven different family groups (details of each alignment provided in the Supplementary Material
275 S1) were built based on the uncorrected *p*-distance matrix, with both transitions and transversions
276 included and partial deletion chosen (Saitou and Nei 1987) in MEGA v.11.0.13 (Tamura et al.,
277 2021). Node support was inferred with a bootstrap analysis (1000 replicates) (Felsenstein, 1985).

278 The lineages were collapsed according to their BINs assignment. The COI sequence of



279 freshwater isopod from the family Asellidae, *Asellus aquaticus* (Linnaeus, 1758) (GenBank
280 accession number MN810583; Raupach et al., 2022), was used as an outgroup.
281 In case of taxa represented in our dataset by five or more sequences (and at least two haplotypes)
282 the molecular divergence of COI haplotypes (with all ambiguous positions excluded) was
283 presented as Median Joining Network generated using PopART 1.7 (Bandelt et al., 1999).

284

285 **2.4 Species distribution**

286 The distribution of each recognized MOTU within the CCZ was checked. Additional check was
287 done to infer which MOTUs were already recorded elsewhere in the World. To infer the range
288 sizes of the MOTUs recorded pairwise distances between all stations were calculated with QGIS
289 3.36 (QGIS, 2021) using standard distance plugin. For each MOTU that was recorded at more
290 than one station the largest distance between the stations where they occurred was noted (given in
291 values rounded to full kilometres).

292 Thanks to very high sequencing success associated with samples collected during three
293 MANGAN expeditions that allowed to ascribe more than 80% of individuals caught to separate
294 MOTUs, their composition in each haul was used in community analyses. The similarities
295 between samples were calculated using the Bray–Curtis similarity formula on relative
296 abundances of the MOTUs in each sample. Due to the fact that the difference between the most
297 and the least abundant species was more than 20 times, and to increase the importance of
298 singletons species which constituted 50% of all taxa, square root transformation of data was used.
299 Hierarchical agglomerative clustering was performed using the group average method.
300 Each assemblage discriminated was characterized using taxon richness (number of BINs),
301 rarefaction (ES(50)), diversity (Shannon index) and evenness (Pielou index) indices (Magurran,
302 2004).

303 All figures were adjusted for publication in Adobe Illustrator CS6.

304

305 **3 RESULTS**

306 Within the collection of 708 individuals 23 known and one undescribed families were identified
307 (Table 3). The latter is presently in the description process (Horton et al., 2025e). Some
308 representatives of the superfamilies Isaeoidea, Photoidea and Aoroidea were not identified further
309 and are presented as “Corophioidea indet.”, whereas unidentified specimens of one of the three



310 families: Lysianassidae, Tryphosidae and Uristidae are left as “Lysianassoidea indet.”. The COI
 311 gene analysis allowed to obtain 581 sequences that gave overall sequencing success at the level
 312 of 82%. The families that dominated barcoded material were Pardaliscidae (123 ind.) and
 313 Eusiridae (122 ind.). The other families that contributed largely to the material were: Synopiidae
 314 (74 ind.), Phoxocephalidae (70 ind.) and Oedicerotidae (51 ind.).

315 Table 3. Number of individuals studied (No of ind.), COI sequences, Barcode Index Numbers
 316 (BINs), consensus number of Molecular Operational Taxonomic Units (MOTUs) based on
 317 different delimitation methods recognized in each family as well as the reference to the figure
 318 with NJ tree and MJN. Systematic division based on Myers and Lowry (2017), available online in
 319 World Amphipoda Database (Horton et al., 2025a).

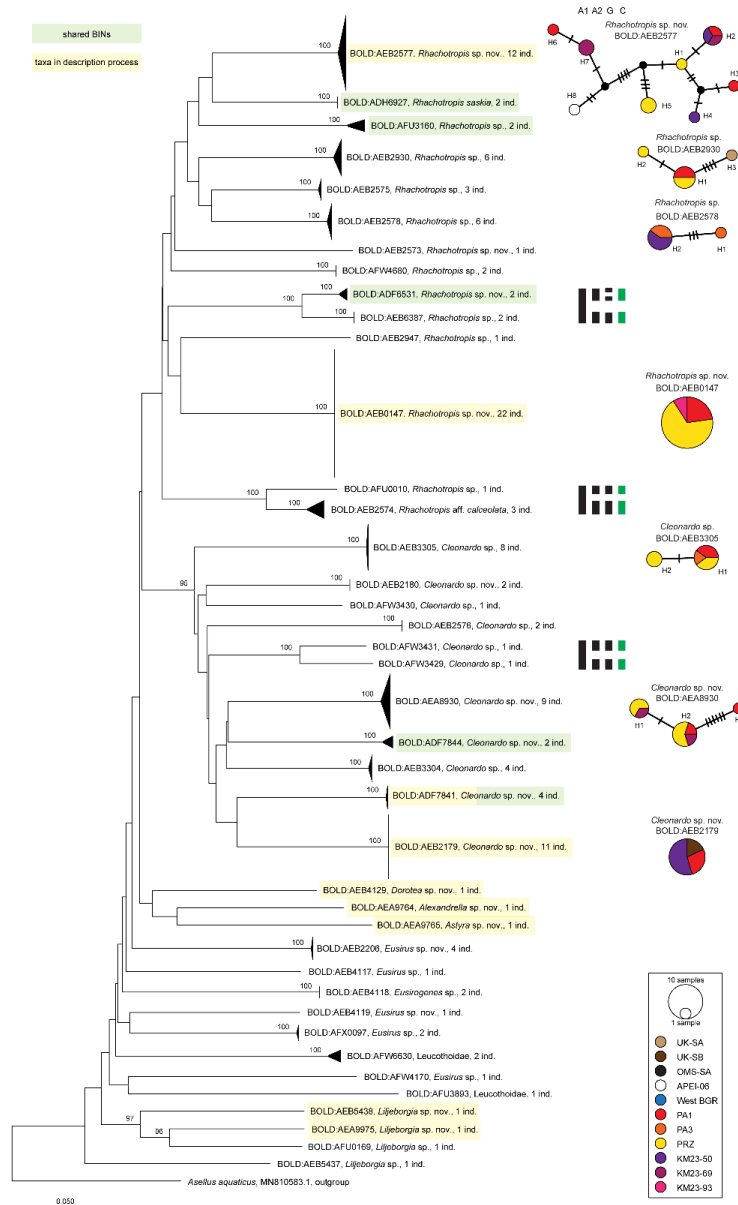
| | Suborder | Parvorder | Superfamily | Taxon | COI | BINs | MOTUs | tree & MJN |
|-----|------------------|----------------------------------|------------------------------------|-----------------|------------|------------|------------|------------|
| 1. | Amphilocheia | Amphilochidira | Iphimedioidea | Stilipedidae | 2 | 2 | 2 | Fig. 3 |
| 2. | | | Leucothoidea | Leucothoidae | 3 | 2 | 2 | |
| 3. | | Eusiridira | Eusiroidea | Eusiridae | 122 | 32 | 32 | |
| 4. | | | Liljeborgioidea | Liljeborgiidae | 4 | 4 | 4 | |
| 5. | | Haustoriidira | Haustorioidea | Phoxocephalidae | 70 | 14 | 14 | Fig. 4 |
| 6. | | Lysianassidira | Alicelloidea | Alicellidae | 1 | 1 | 1 | Fig. 5 |
| 7. | | | | Valettiopsidae | 1 | 1 | 1 | |
| 8. | | | | Vemanidae | 1 | 1 | 1 | |
| 9. | | | Lysianassoidea | Cyphocarididae | 4 | 2 | 2 | |
| 10. | | | | Hirondeidae | 2 | 1 | 1 | |
| 11. | | | | Tryphosidae | 23 | 6 | 6 | |
| 12. | | | | Uristidae | 7 | 6 | 6 | |
| 13. | | | Lysianassoidea indet. ^b | 16 | 6 | 6 | | |
| 14. | | Stegocephaloidea | Stegocephalidae | 9 | 5 | 5 | | |
| 15. | | Oedicerotidira | Oedicerotoidea | Oedicerotidae | 51 | 22 | 21 | Fig. 6 |
| 16. | | Synopiidira | Dexaminoidea | Lepechinellidae | 5 | 3 | 3 | Fig. 7 |
| 17. | | | | Melphidippidae | 2 | 1 | 1 | |
| 18. | | | | Pardaliscidae | 123 | 44 | 44 | |
| 19. | | | Synopioidea | Ampeliscidae | 13 | 3 | 3 | Fig. 8 |
| 20. | | Synopiidae | | 74 | 31 | 31 | | |
| 21. | Hyperioptidea | Hyperioptidira | Hyperioptioidea | Hyperioptidae | 7 | 4 | 4 | Fig. 9 |
| 22. | Senticaudata | Caprellidira | Caprelloidea | Dulichidae | 1 | 1 | 1 | |
| 23. | | Corophiidira | Aorioidea | Unciolidae | 2 | 1 | 1 | |
| 24. | | Corophioidea indet. ^a | | | 19 | 5 | 5 | |
| 25. | Hadziidira | Calliopoidea | Calliopiidae | 2 | 1 | 1 | | |
| 26. | | | | Fam. nov. | 12 | 5 | 5 | |
| 27. | Amphipoda indet. | | | | 5 | 4 | 4 | |
| | | | | Total | 581 | 208 | 207 | |



320 The delimitation methods identified from 184 (ASAP with the lowest ASAP-score, threshold
321 distance – 0.085925) up to 270 (GMYC) MOTUs (Figs 3–9). Although large variation in absolute
322 numbers the differences in the number of recognized MOTUs concerned a small number of taxa
323 and in 17 cases indicated obvious oversplitting of single units. The consensus delimitation
324 identified 207 MOTUs that are congruent with BINs ascription with an exception of one unit
325 within Oedicerotidae, namely two BINs of *Bathymedon* sp. (BOLD:AEA9456 and
326 BOLD:AGC2705) that according to all other methods constitute one MOTU. The *p*-distance
327 between these two BINs is 0.025 but only two sequences are available so it is impossible to infer
328 the intraspecific variation in this case. There are additional six cases where consensus
329 delimitation shall be treated with caution: two within the families: Phoxocephalidae (Fig. 4) and
330 Synopiidae (Fig. 8), one within Pardaliscidae (Fig. 7) as well as within undescribed family of
331 Senticaudata (Fig. 9). In all presented cases additional genes and preferably more individuals
332 shall be studied to help with final delimitation of these taxa.

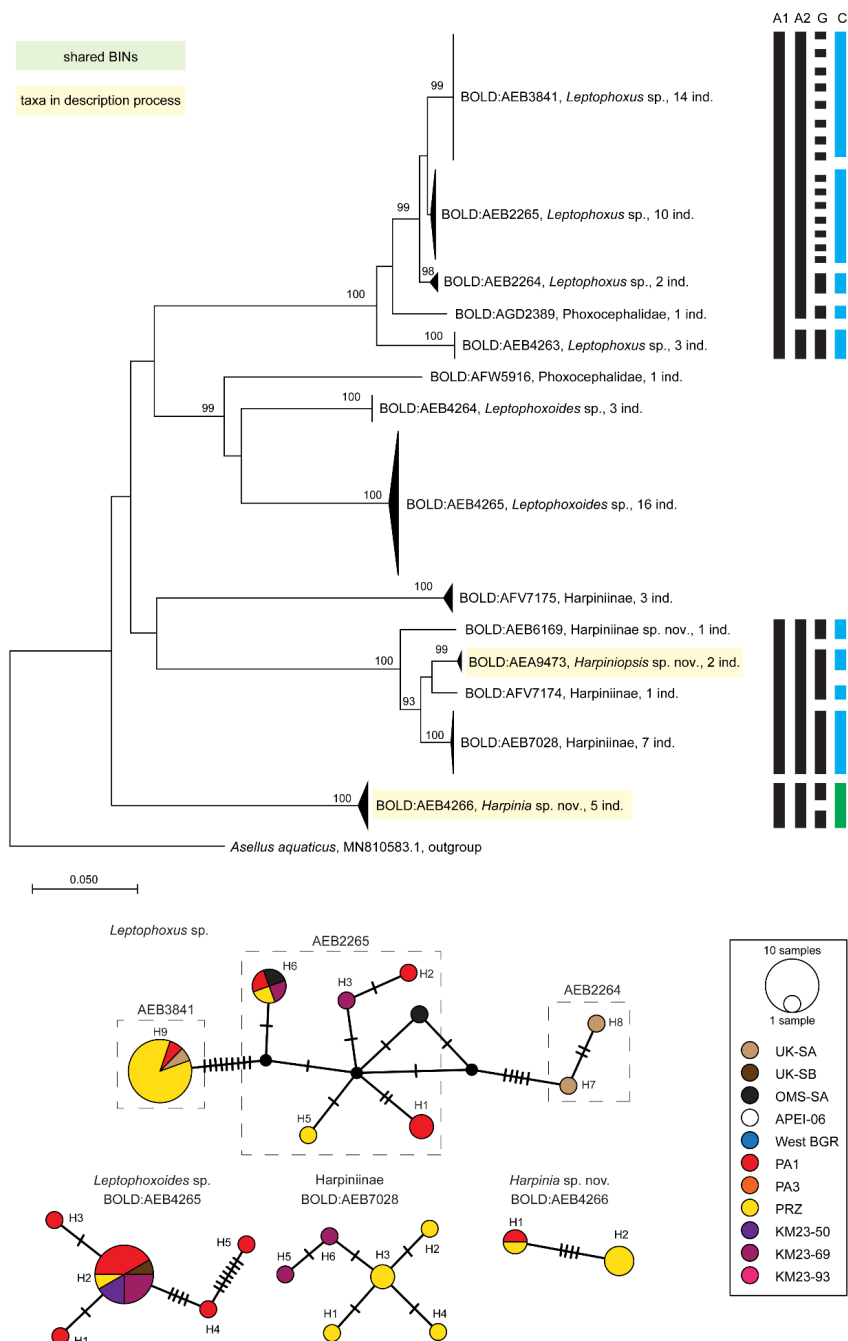
333 The family with the largest number of identified taxa was Pardaliscidae (44 MOTUs), followed
334 by Eusiridae (32 MOTUs) and Synopiidae (31 MOTUs). The most species rich genus was
335 *Rhachotropis* (Eusiridae) where 14 MOTUs were identified. It was followed by *Cleonardo*
336 (Eusiridae) represented by 11 MOTUs and *Syrrhoe* (Synopiidae) with 9 MOTUs recognized.
337 *Caleidoscopsis* (8 MOTUs) and *Pardaliscopsis* (6 MOTUs) were the two genera within
338 Pardaliscidae with the highest number of taxa delimited. Other recognized genera were
339 represented by smaller number of species but large part of the material (particularly from
340 MANGAN 2023 expedition) was identified only to the family level.

341



342

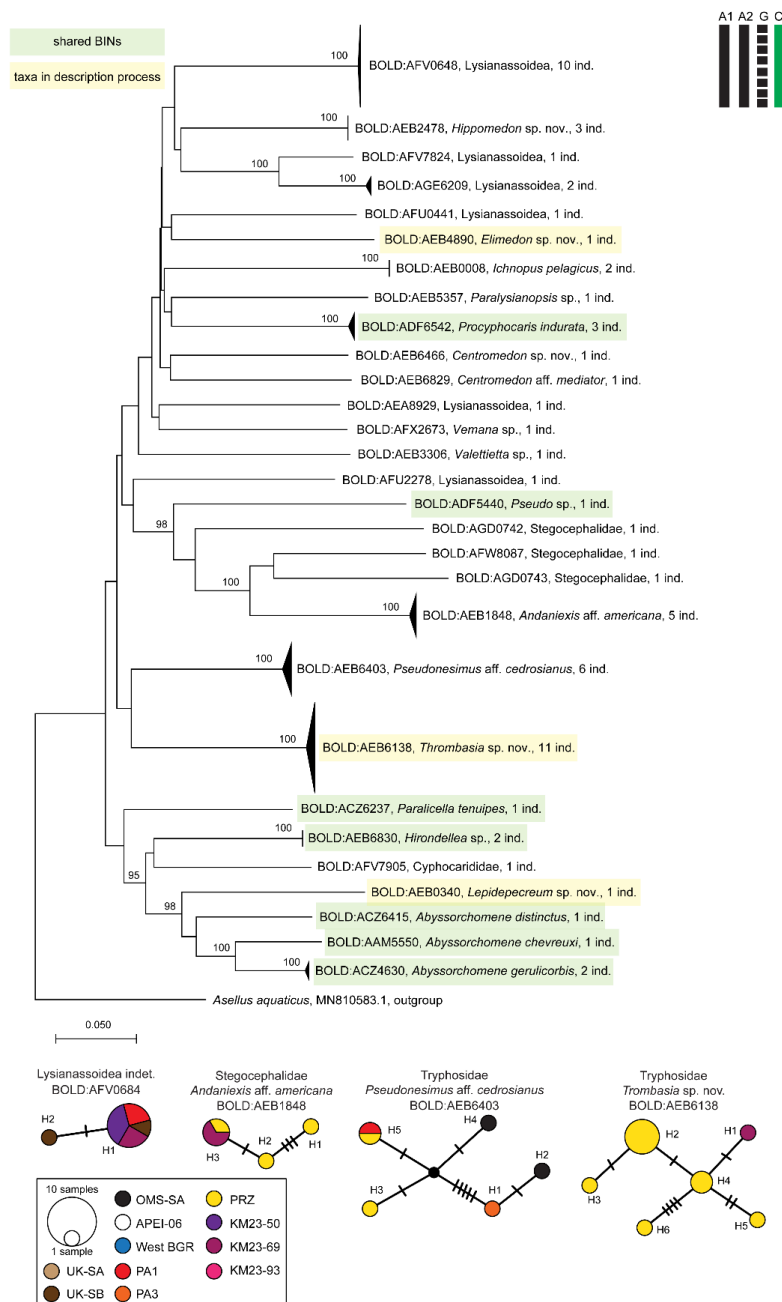
343 Figure 3. Neighbour-joining (NJ) tree of COI sequences of amphipods belonging to the families Eusiridae,
344 Leucothoidae, Liljeborgiidae, and Stilipedidae (parvorders Amphilochidira and Eusiridira). The distances
345 were calculated with p-distance. Triangles indicate the relative number of individuals studied (height) and
346 sequence divergence (width). The numbers in front of the nodes indicate bootstrap support (1000
347 replicates, only values higher than 50% are presented). The vertical bars represent species delimitations
348 taxonomies obtained from different species delimitation methods (A1: BIN, A2: ASAP, G: GMYC, C:
349 consensus). Only the cases where incongruence between different delimitation methods were observed are
350 shown. Note that this tree is not the reconstruction of evolutionary history of presented taxa.



351

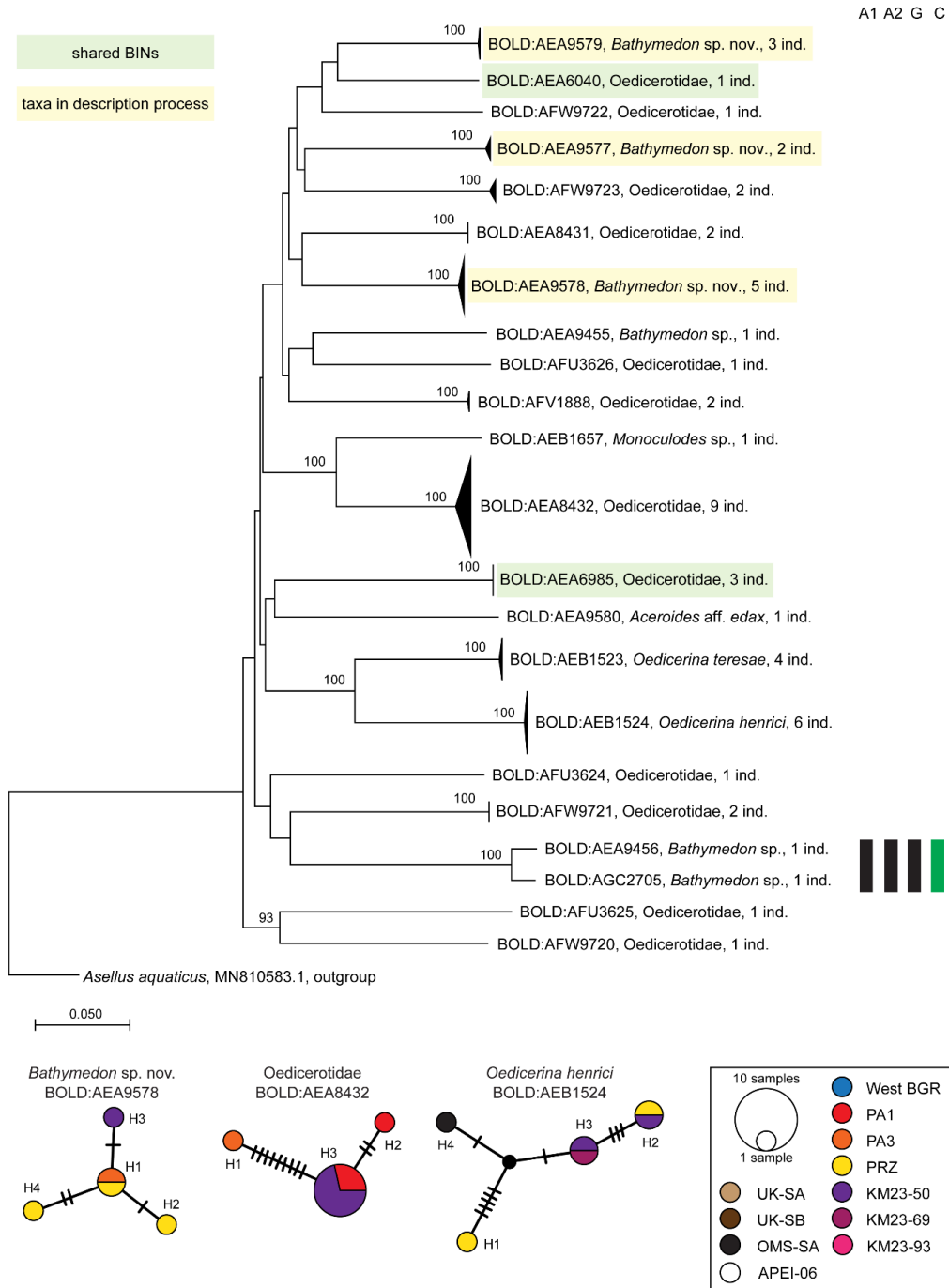
352 Figure 4. Neighbour-joining (NJ) tree of COI sequences of amphipods belonging to the family

353 Phoxocephalidae. Details about the calculation and presentation of the tree as in Fig. 3



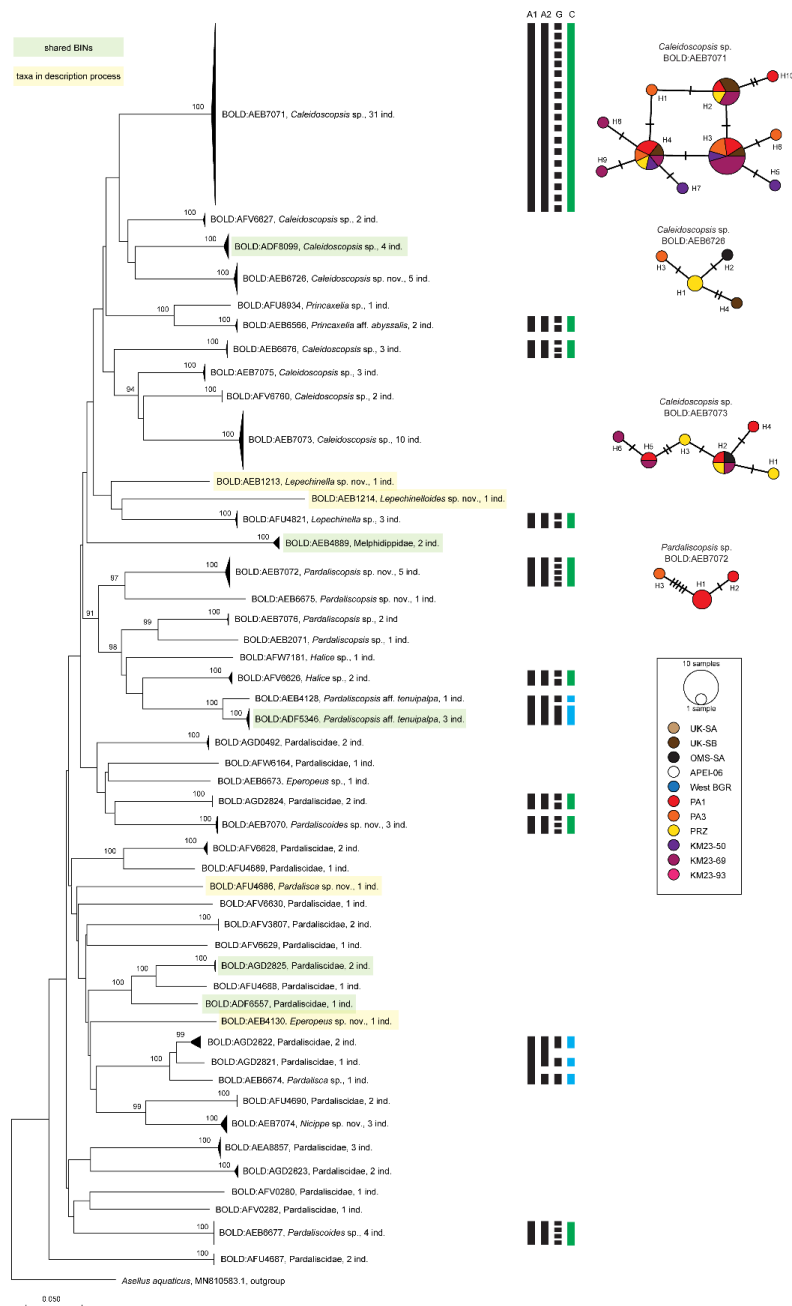
354

355 Figure 5. Neighbour-joining (NJ) tree of COI sequences of amphipods belonging to the parvorder
356 Lysianassidira (for families see Table 3). Details about the calculation and presentation of the tree
357 as in Fig. 3.



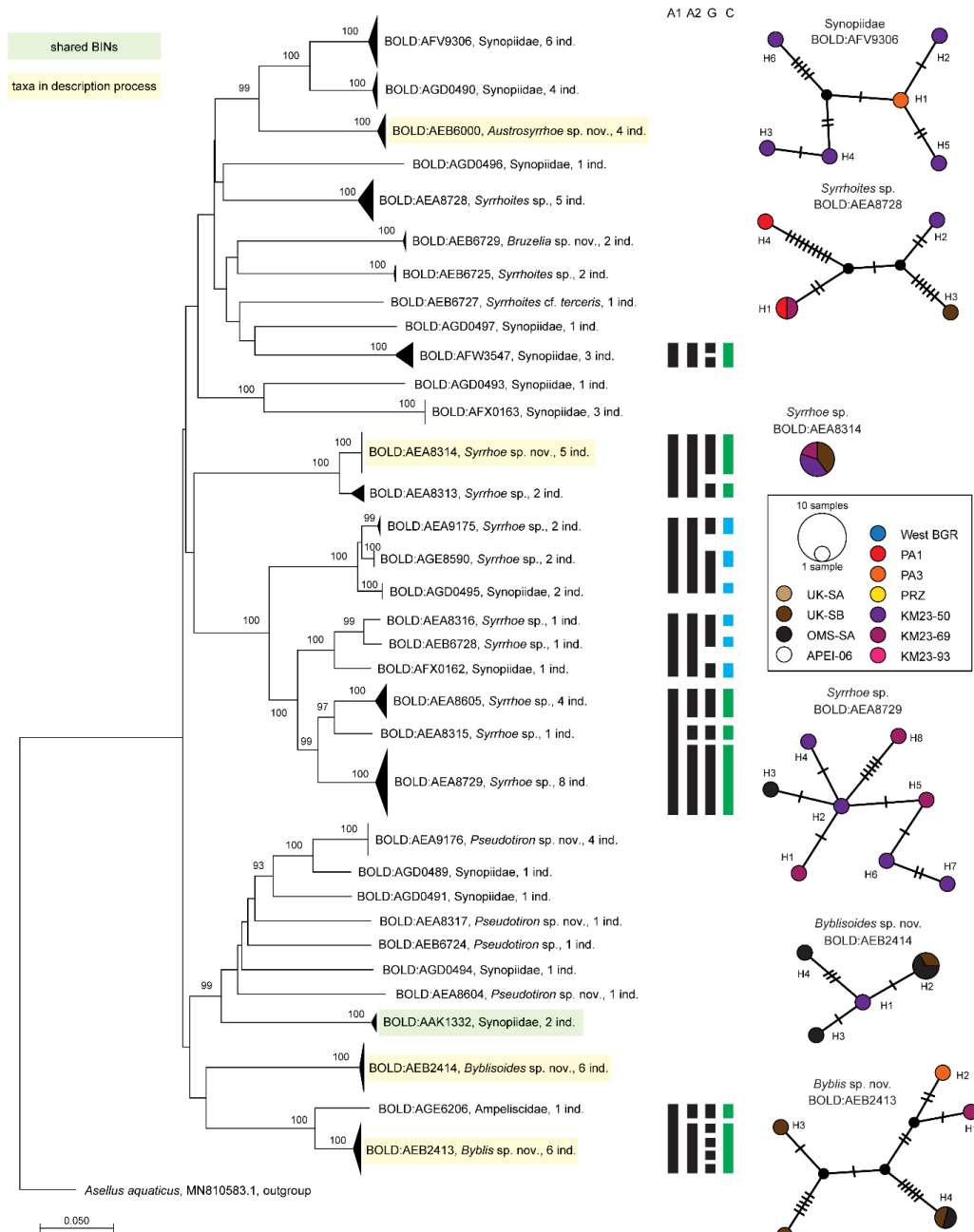
358

359 Figure 6. Neighbour-joining (NJ) tree of COI sequences of amphipods belonging to the family
 360 Oedicerotidae. Details about the calculation and presentation of the tree as in Fig. 3.



361

362 Figure 7. Neighbour-joining (NJ) tree of COI sequences of amphipods belonging to the families
 363 Pardaliscidae, Lepechinellidae, Melphidippidae. Details about the calculation and presentation of
 364 the tree as in Fig. 3.

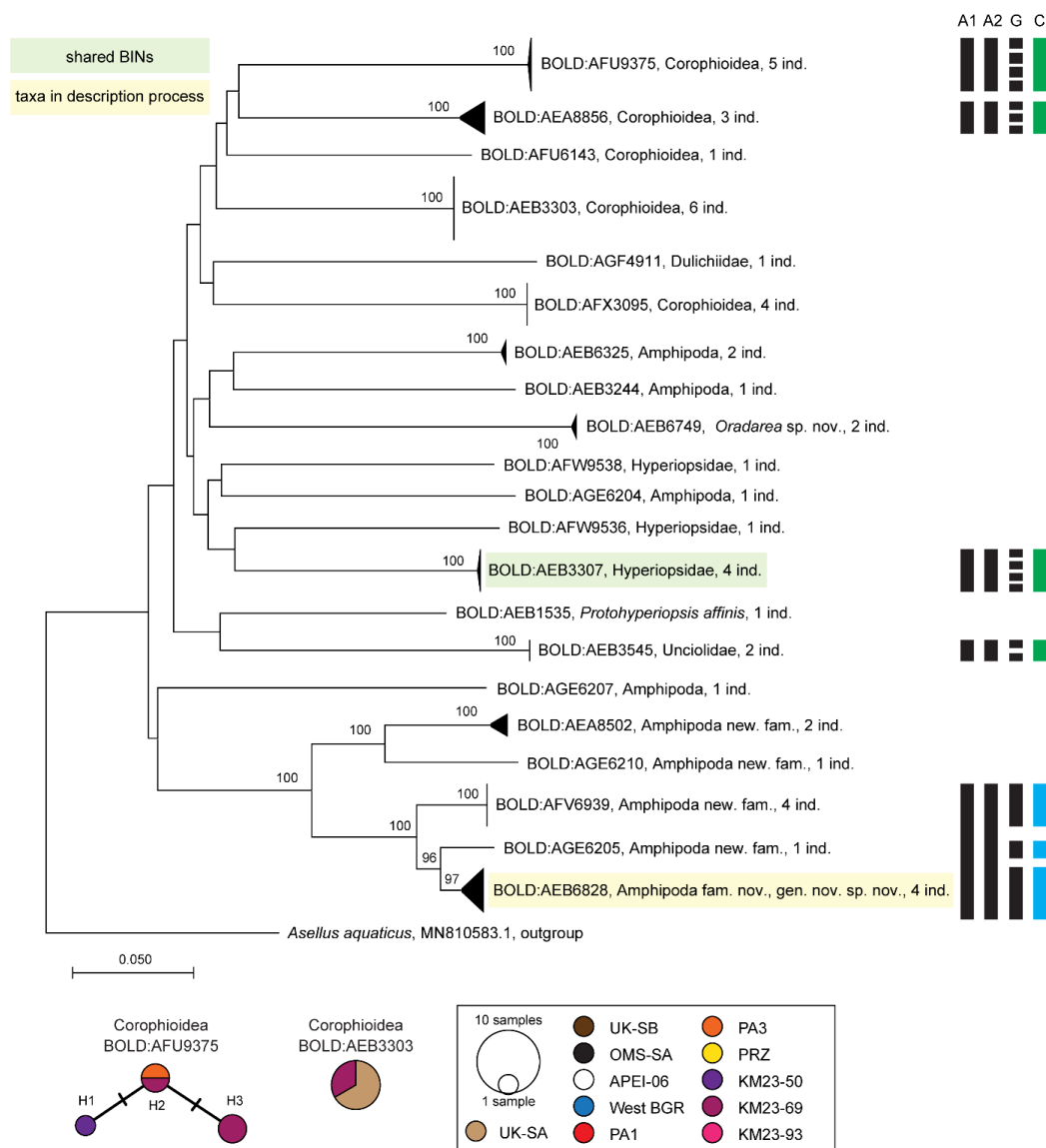


365

366 Figure 8. Neighbour-joining (NJ) tree of COI sequences of amphipods belonging to the families

367 Synopiidae, Ampeliscidae. Details about the calculation and presentation of the tree as in Fig. 3.

368



369

370 Figure 9. Neighbour-joining (NJ) tree of COI sequences of amphipods belonging to the families
 371 Dulichiidae, Callioptidae, Hyperioptidae, Unciolidae, Mirabestidae fam. nov. and unidentified
 372 taxa. Details about the calculation and presentation of the tree as in Fig. 3.

373

374 Combined morphological and molecular identification revealed ten known species: *Rhachotropis*
 375 *saskia* (Eusiridae), *Abyssorchomene chevreuxi*, *A. distinctus*, *A. gerulicorbis*, *Ichnopus pelagicus*



376 (Uristidae), *Paralicella tenuipes* (Alicellidae), *Procyphocaris indurata* (Cyphocarididae),
377 *Oedicerina henrici*, *O. teresae* (Oedicerotidae, described on the basis of presently studied
378 material, Jażdżewska et al., 2022), and *Protohyperiopsis affinis* (Hyperiopsidae). Additional
379 seven taxa were identified as closely resembling known species but with some level of
380 uncertainty: *Rhachotropis* aff. *calceolata* (Eusiridae), *Centromedon* aff. *mediator* (Uristidae),
381 *Pseudonesimus* aff. *cedrosianus* (Tryphosidae), *Andaniexis* aff. *americana* (Stegocephalidae),
382 *Aceroides* aff. *edax* (Oedicerotidae), *Princaxelia* aff. *abyssalis*, *Pardaliscopsis* aff. *tenuipalpa*
383 (*Pardaliscidae*). Final identification was often hindered by the presence of only single
384 representatives of particular taxon and the damage of the material. Apart from known species the
385 study of the material allowed to recognize one undescribed family (Horton et al., 2025e) and at
386 least 48 undescribed species; two of them were already described based on the present material
387 (Jażdżewska et al., 2022), while 27 are in the description process (Andrade and Jażdżewska,
388 2025; Biniek et al., 2025; Horton and Lörz, 2025; Horton et al., 2025b-e; Hughes and Tandberg,
389 2025a, b; Jażdżewska and Horton, in prep, Lörz et al., 2025; Peart and Lörz 2025; Peart and
390 Stewart, 2025; Tandberg and Huges, 2025; Timm et al., 2025; Wróblewski and Jażdżewska,
391 2025).

392 Out of 207 MOTUs recognized as many as 94 were singletons (45% of taxa) while additional 49
393 – doubletons (24% of taxa), only 32 (15%) reached the criterium of being represented by more
394 than five individuals and their haplotypes networks are presented (Figs 3–9). Half of these
395 MOTUs were represented by one to three haplotypes differing in few mutations. Higher diversity
396 of haplotypes was recorded within one MOTU of Eusiridae (*Rhachotropis* sp. nov.,
397 BOLD:AEB2577), three MOTUs of Phoxocephalidae (*Leptophoxus* sp., BOLD:AEB2265,
398 *Leptophoxoides* sp., BOLD:AEB4265, Harpiniinae, BOLD:AEB7028), two MOTUs of
399 Tryphosidae (*Pseudonesimus* aff. *cedrosianus*, BOLD:AEB6403, *Thrombasia* sp. nov.,
400 BOLD:AEB6138), two MOTUs of Oedicerotidae (*Oedicerina henrici*, BOLD:AEB1524,
401 *Bathymedon* sp. nov., BOLD:AEA9578), three MOTUs of Pardaliscidae (*Caleidoscopsis* sp.,
402 BOLD:AEB7071, *Caleidoscopsis* sp., BOLD:AEB6726, *Caleidoscopsis* sp., BOLD:AEB7073),
403 three MOTUs of Synopiidae (*Syrrhoites* sp., BOLD:AEA8728, *Syrrhoe* sp., BOLD:AEA8729,
404 Synopiidae, BOLD:AFV9306), and two of Ampeliscidae (*Byblis* sp. nov., BOLD:AEB2413,
405 *Byblisoides* sp. nov., BOLD:AEB2414) (Figs 3–9).



406 No MOTUs represented by more than five individuals was shared between East and West regions
 407 of the CCZ as well as between West region of CCZ and APEI-06. Only one, *Rhachotropis* sp.
 408 nov. (BOLD:AEB2577) was shared between East region and APEI-06. In a broader scale 187
 409 recognized BINs (and subsequently 186 MOTUs) were unique for the present study, while the
 410 remaining 21 were shared with other studies and belonged to broadly distributed taxa (Table 4).
 411 Table 4. MOTUs represented by Barcode Index Numbers (BIN) shared between present and former
 412 studies with information about their taxonomic affiliation, geographic distribution and ecology. Areas:
 413 UKSR – UK contractor area, OMS – Singapore contractor area, BGRÉ –BGR contractor area East,
 414 BGRW – BGR contractor area West, KKT – Kuril-Kamchatka Trench area, NW Pacific – North West
 415 Pacific, area not specified, NE Pacific – North East Pacific, area not specified. Asterisk indicates cases
 416 where feeding type was confirmed for particular taxon. In case of wide distribution and repeated
 417 ecological studies only representative publications cited.

| Family | BIN | Taxon name | Present study | | Distribution | | Ecology | | |
|-----------------|--------------|---------------------------------------|---------------|------------|--------------------|--|-------------------------------|------------------------------------|---|
| | | | No. ind. | Area | Outside CCZ | References | Feeding type | Mobility | References |
| Alicellidae | BOLD:ACZ6237 | <i>Paralicella tenuipes</i> | 1 | UKSR | Cosmopolitan | Jażdżewska et al. 2021 | scavenger* | highly mobile swimmer | Jażdżewska et al. 2021 |
| Cyphocarididae | BOLD:ADF6542 | <i>Procyphocaris indurata</i> | 3 | BGRE, OMS | KKT | Jażdżewska and Mamos 2019 | unknown | abyssopelagic / epibenthic swimmer | Hendrycks and Conlan 2003 |
| Eusiridae | BOLD:ADF7841 | <i>Cleonardo</i> sp. nov. | 4 | BGRE, OMS | KKT | Jażdżewska and Mamos 2019 | predator | epibenthic swimmer | Bousfield and Hendrycks 1995 |
| Eusiridae | BOLD:ADF7844 | <i>Cleonardo</i> sp. nov. | 2 | BGRE | KKT | Jażdżewska and Mamos 2019 | predator | epibenthic swimmer | Bousfield and Hendrycks 1995 |
| Eusiridae | BOLD:ADF6531 | <i>Rhachotropis</i> sp. | 2 | BGRE | KKT | Jażdżewska and Mamos 2019 | predator | epibenthic swimmer | Bousfield and Hendrycks 1995 |
| Eusiridae | BOLD:ADH6927 | <i>Rhachotropis saskia</i> | 2 | UKSR, BGRW | KKT | Lörz et al. 2018, Jażdżewska and Mamos 2019 | predator* | epibenthic swimmer | Lörz et al. 2018 |
| Eusiridae | BOLD:AFU3160 | <i>Rhachotropis</i> sp. | 2 | BGRW | NE Pacific | Jażdżewska, unpublished | predator | epibenthic swimmer | Lörz et al. 2018 |
| Hirondelleidae | BOLD:AEB6830 | <i>Hirondellea</i> sp. | 2 | BGRE | Indian Ocean | Kniesz et al. 2022 | scavenger* | highly mobile swimmer | Kniesz et al. 2022 |
| Hyperopsidae | BOLD:AEB3307 | Hyperopsidae | 4 | UKSR, BGRÉ | NE Pacific | Jażdżewska, unpublished | unknown | pelagic | Barnard and Karaman 1991 |
| Melphidippidae | BOLD:AEB4889 | Melphidippidae | 2 | BGRE | NE Pacific | Jażdżewska, unpublished | passive suspension feeder | epibenthic, fast swimming | Enequist 1949, De Broyer et al. 2001 |
| Oedicerotidae | BOLD:AEA6040 | Oedicerotidae | 1 | BGRW | NW Pacific | Jażdżewska, unpublished | deposit feeder or carnivorous | sediment dweller | Brix et al. 2018 |
| Oedicerotidae | BOLD:AEA6985 | Oedicerotidae | 3 | BGRW | NW Pacific | Jażdżewska, unpublished | deposit feeder or carnivorous | sediment dweller | Brix et al. 2018 |
| Pardaliscidae | BOLD:ADF8099 | <i>Caleidoscopsis</i> sp. | 4 | BGRW | KKT | Jażdżewska and Mamos 2019 | predator | epibenthic or demersal | Hendrycks and Conlan 2003, Brix et al. 2018 |
| Pardaliscidae | BOLD:ADF5346 | <i>Pardaliscopsis aff. tenuipalpa</i> | 3 | BGRE | KKT | Jażdżewska and Mamos 2019 | predator | epibenthic or demersal | Hendrycks and Conlan 2003, Brix et al. 2018 |
| Pardaliscidae | BOLD:ADF6557 | Pardaliscidae | 1 | BGRW | KKT | Jażdżewska and Mamos 2019 | predator | epibenthic or demersal | Hendrycks and Conlan 2003, Brix et al. 2018 |
| Pardaliscidae | BOLD:AGD2825 | Pardaliscidae | 2 | BGRE | NE Pacific | Jażdżewska, unpublished | predator | epibenthic or demersal | Hendrycks and Conlan 2003, Brix et al. 2018 |
| Stegocephalidae | BOLD:ADF5440 | <i>Pseudo</i> sp. | 1 | BGRE | KKT | Jażdżewska and Mamos 2019 | predator | benthopelagic | Brix et al. 2018 |
| Synopiidae | BOLD:AAK1332 | Synopiidae | 2 | BGRE | Belize | Browne et al. 2007 | unknown | pelagic | Browne et al. 2007 (collected at 0–10 m in the open waters) |
| Uristidae | BOLD:AAM5550 | <i>Abyssorchomene chevreuxi</i> | 1 | BGRE | widely distributed | Havermans et al. 2010, Fujii et al. 2013, Horton et al. 2020 | scavenger* | highly mobile swimmer | Horton et al. 2020 |



| | | | | | | | | | |
|-----------|--------------|-----------------------------------|---|------|--------------------|--|------------|-----------------------|-------------------------------|
| Uristidae | BOLD:ACZ6415 | <i>Abyssochomene distinctus</i> | 1 | BGRE | widely distributed | Kniesz et al. 2022, Dupont et al. 2024, | scavenger* | highly mobile swimmer | Patel et al. 2020 |
| Uristidae | BOLD:ACZ4630 | <i>Abyssochomene gerulicorbis</i> | 2 | BGRE | widely distributed | Barnard and Shulenberger 1976, Horton et al. 2020, | scavenger* | highly mobile swimmer | Barnard and Shulenberger 1976 |

418

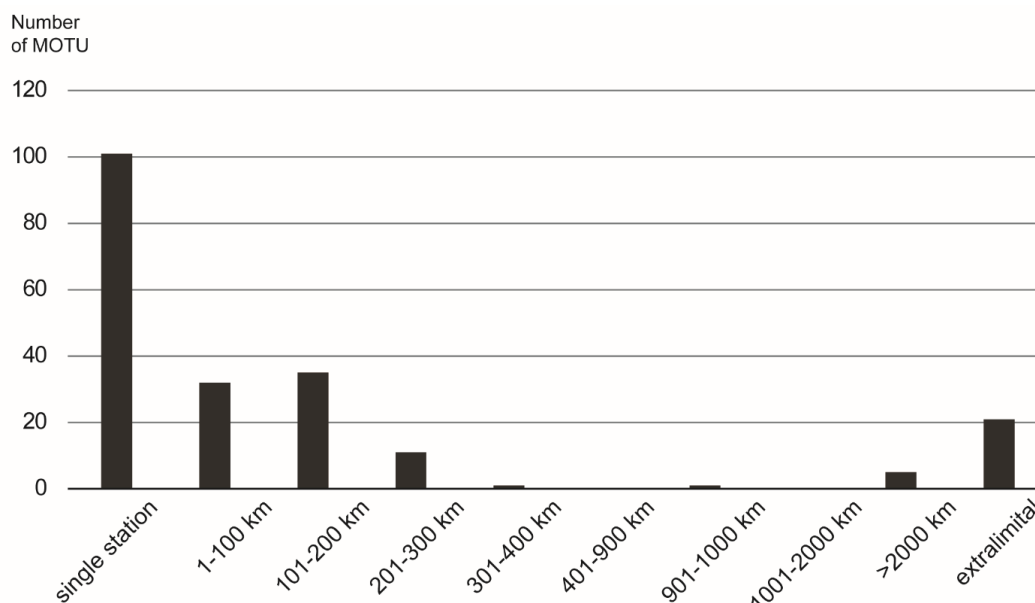
419 Of them nine species were shared between CCZ and the region of Kuril-Kamchatka Trench. Two
 420 MOTUs were shared with NW Pacific and next four with NE Pacific – the sequences of these
 421 taxa from outside CCZ are present in BOLD but they are stored privately, so specific localities
 422 are not available. One species found in CCZ was previously recorded from the Indian Ocean,
 423 another one was already caught in the Caribbean Sea. The remaining four taxa are known from
 424 their wide (sometimes even cosmopolitan) geographic distribution. Among the MOTUs recorded
 425 only in CCZ and sampled at two or more stations 32 were found at localities within 100 km
 426 distance. The next 35 MOTUs were recorded within the range of 101–200 km, followed by
 427 eleven found within 300 km distance and one not exceeding 400 km (its range reached 308 km)
 428 (Fig. 10, Supplementary Table S2). Only one species, *Rhachotropis* sp. nov. (BOLD:AEB2577)
 429 was present within 1000 km distance between the furthest stations, while five: *Cleonardo* sp.
 430 (BOLD:AEB2180), Fam. nov., gen. nov., sp. nov. (BOLD:AEB6828), *Bathymedon* sp. nov.
 431 (BOLD:AEA9579), Oedicerotidae (BOLD:AFW9721) and *Austrosyrrhoe* sp. nov.
 432 (BOLD:AEB6000) were recorded at the stations within more than 2000 km distance (Table 5).

433

434 Table 5. MOTUs represented by Barcode Index Numbers (BIN) found only in CCZ with the largest
 435 distribution ranges with information about their taxonomic affiliation, geographic distribution and
 436 ecology. Areas: APEI-06 – Area of Particular Environmental Interest No. 6, BGRE –BGR contractor area
 437 East, BGRW – BGR contractor area West.

438

| Family | BIN | Taxon name | No. ind. | Area | Distance between furthest stations [km] | Ecology | | |
|---------------|--------------|--------------------------------|----------|---------------|---|--------------------------------|--------------------|------------------------------|
| | | | | | | Feeding type | Mobility | References |
| Eusiridae | BOLD:AEB2577 | <i>Rhachotropis</i> sp. nov. | 12 | APEI-06, BGRE | 988 | predator | epibenthic swimmer | Bousfield and Hendrycks 1995 |
| Eusiridae | BOLD:AEB2180 | <i>Cleonardo</i> sp. | 2 | BGRE, BGRW | 2377 | predator | epibenthic swimmer | Bousfield and Hendrycks 1995 |
| New family | BOLD:AEB6828 | Fam. nov., gen. nov., sp. nov. | 4 | BGRE, BGRW | 2239 | unknown | unknown | unavailable |
| Oedicerotidae | BOLD:AEA9579 | <i>Bathymedon</i> sp. nov. | 3 | BGRE, BGRW | 2292 | deposit feeders or carnivorous | sediment dweller | Brix et al. 2018 |
| Oedicerotidae | BOLD:AFW9721 | Oedicerotidae | 2 | BGRE, BGRW | 2293 | deposit feeders or carnivorous | sediment dweller | Brix et al. 2018 |
| Synopiidae | BOLD:AEB6000 | <i>Austrosyrrhoe</i> sp. nov. | 4 | BGRE, BGRW | 2239 | unknown | epibenthic? | unavailable |

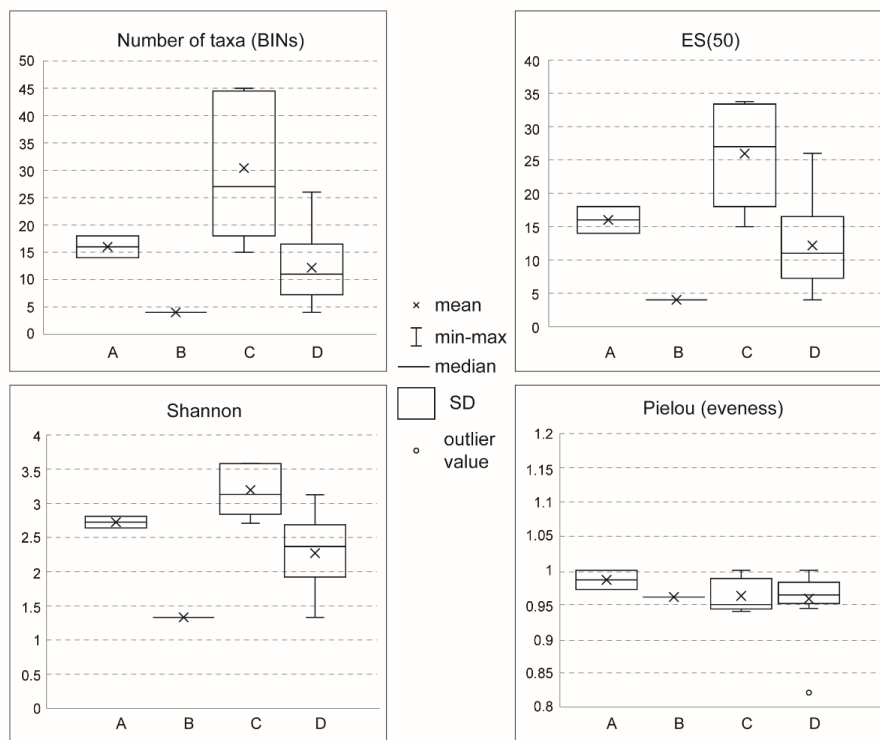
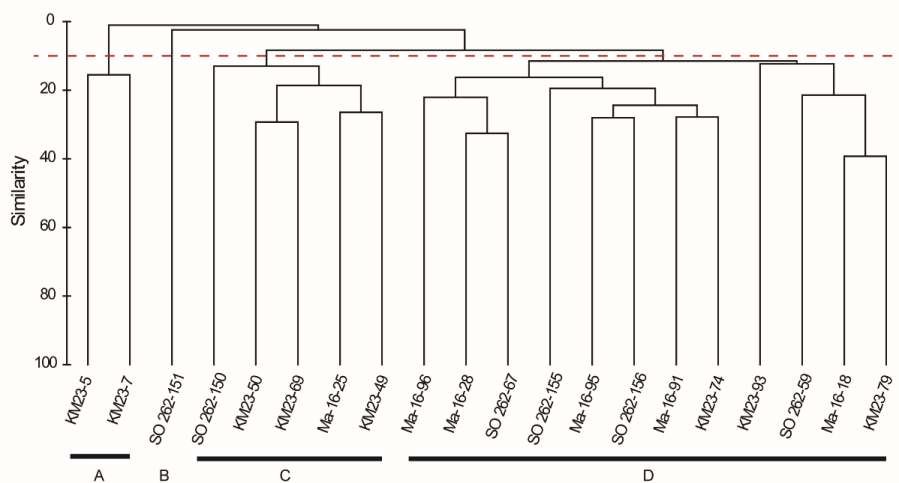


439

440 Figure 10. Number of MOTUs recorded at stations within distribution ranges. Extralimital:
441 MOTUs shared with regions outside CCZ.

442

443 The cluster analysis of the fauna collected during three MANGAN expeditions at the level of
444 10% similarity revealed four groups (A–D, of which one is represented by only single station)
445 (Fig. 11A). In general, the similarities between studied stations were very low and oscillated from
446 almost 0% to ca. 40%. There was almost no similarity between the stations situated in the West
447 part of the CCZ area (group A, West BGR) and the ones from the eastern part of the studied
448 region (groups B, C, D, East BGR). In case of stations from East BGR their geographic proximity
449 does not influence clustering. The diversity indices showed that the single station forming group
450 B is characterized by low values of species richness, ES(50) and Shannon indices (Fig. 11B). In
451 contrary the highest values of these indices were characteristic for the group C. The groups A and
452 D were less species rich and diverse and presented similar mean values of diversity indices. The
453 index of a very high value and low variety for all identified clusters was evenness (Pielou index)
454 with the mean value ranging from 0.961 to 0.986; only one station within group D had value of
455 this index lower than 0.850. Due to low similarity within the groups and very high evenness as
456 well as lack of clear geographic grouping the composition of identified assemblages was not
457 studied further.



458

459 Figure 11. Upper – cluster analysis of stations from BGR contractor area (Bray-Curtis similarity
 460 of square-root transformed relative abundances, group average grouping method), dashed line
 461 placed at the similarity level of 10%. Lower – comparison of diversity measures for each
 462 distinguished group of samples.



463

464 **4 DISCUSSION**

465 **4.1 Low abundance, high species richness**

466 Our study of relatively small amphipod collection (sequences obtained from 581 ind.) revealed
467 high species richness, expressed by identification of as many as 207 MOTUs. This corroborate
468 with studies of Amphipoda conducted in other deep-sea Pacific areas using integrative taxonomy.
469 For example, in the North West Pacific in the Kuril-Kamchatka Trench and adjacent abyssal plain
470 133 MOTUs were identified from the collection of 510 individuals (Jażdżewska and Mamos,
471 2019), while in the Aleutian Trench, adjacent abyss and the abyssal Bering Sea over 150 MOTUs
472 were recorded after study of 450 individuals (pers. obs.). Also in the South West Pacific high
473 deep-sea amphipod species richness was observed (263 MOTUs identified from 953 sequences,
474 Knox et al., 2020). The numerically dominant and the most species rich families in the studied
475 material were: Pardaliscidae, Eusiridae, Synopiidae, Phoxocephalidae and Oedicerotidae that is
476 congruent with their diversity and abundance in the deep sea (e.g. Brix et al., 2018; Jażdżewska
477 and Mamos, 2019). It is important to note however that more profound comparisons of species
478 richness between cited regions are challenged due to different depth zones sampled in each of
479 those studies.

480 High species richness in the CCZ was recorded also for other metazoan groups including, among
481 others, Polychatea, Isopoda, Tanaidacea or Echinodermata (e.g. Janssen et al., 2015;
482 Christodoulou et al., 2020; Kaiser et al., 2023; Bonifácio et al., 2024; Błażewicz et al., in review).
483 Recent summary of the known diversity of Metazoa of CCZ revealed 5,578 spp. recorded in the
484 region of which more than 92% are identified as morphospecies with only temporal name
485 ascribed and may be considered as new to science (Rabone et al., 2023). In our study only 48 spp.
486 were confirmed as belonging to species new to science with two already described and 27 in
487 process of description (Jażdżewska et al., 2022; Jażdżewska and Horton, in prep). This
488 constitutes less than 25% of all MOTUs found. However, this relatively low number derives from
489 the fact that large part of the collection from the most recent expedition (MANGAN 2023) was
490 only identified to the family level and further study of recognized MOTUs has not yet been
491 conducted. The final identification of the recognized MOTUs was also often challenged by the
492 presence of individuals that were severely damaged and lacked taxonomically important



493 characters. Nevertheless, possessing their barcodes will allow to identify them in the future when
494 more individuals of given MOTU are collected.

495 Kaiser et al. (2023) summarizing the present knowledge of the diversity of Isopoda from CCZ
496 demonstrated that among 361 MOTUs recorded majority (345 MOTUs) were collected with
497 epibenthic sledge, additional 16 were sampled with box corer. In the case of Tanaidacea the
498 comparison of sampling gears (BC vs. EBS) shown their influence on the taxonomic composition
499 and recognized communities but not on the diversity indices (Błażewicz et al., in review).

500 Although our study was focused on the EBS samples, non-pelagic Amphipoda, present in BC
501 samples from MANGAN 2018 and MANGAN 2023 expeditions were also considered. Similarly
502 to the study of Kaiser et al. (2023) they constituted only a small portion of all animals processed
503 (23 individuals of which 21 sequences were obtained) and were ascribed to 13 MOTUs. This
504 further reaffirm that only parallel usage of box corer and epibenthic sledge during monitoring
505 studies may assure comprehensive evaluation of the biodiversity of CCZ areas.

506

507 **4.2 Highly variable amphipod assemblages in the CCZ area**

508 The study of amphipod assemblages from BGR contractor areas based on discriminated MOTUs
509 revealed great variability of all studied stations, high number of singletons and doubletons in the
510 material and no association between the faunal similarity and geographic proximity. The more
511 there is almost complete dissimilarity between East and West BGR areas. Due to unavailability of
512 the full collection of Amphipoda from other contractor zones (UKSLR and OMS) and the APEI-
513 06 we were not able to characterize the amphipod assemblages of these areas. Nevertheless, out
514 of the six individuals from APEI-06 for which the sequences were obtained, each belonged to
515 different species (with two new to science) and only one of them *Rhachotropis* sp. nov.
516 (BOLD:AEB2577) was shared between CCZ and APEI-06. Low similarity between different
517 contractor areas of the CCZ as well as uncertain representativeness of APEIs towards mining
518 zones was already reported for other macrobenthic groups (e.g. Taboada et al., 2018; Błażewicz
519 et al., 2019, in review; Brix et al., 2020; Bonifácio et al., 2020, 2021; Christodoulou et al., 2020;
520 Washburn et al., 2021a, b; Kaiser et al., 2023). It is worth noting that the cited studies used
521 merged results for each contractor zone, herewith we show that evenness and patchy distribution
522 characteristic for deep-sea fauna (Kaiser et al., 2007) stand at the base of high dissimilarity also
523 within single contractor area. The same phenomenon was recorded for Isopoda and Polychaeta in



524 French and German contractor areas (Janssen et al., 2015). The variability in the faunal
525 assemblages of the study region both in the smaller (within contractor area) and larger (between
526 eastern and western CCZ) scales may derive from various factors. Locally, it may be associated
527 with nodule density that is very heterogenous and may change within a few tens to hundreds of
528 meters (Peukert et al., 2018). However, by now the influence of this factor was proven only for
529 some taxa, like sessile megafauna (Vanreusel et al., 2016) and in case of another peracarid group,
530 Isopoda it was not confirmed (Kaiser et al., 2023). The presence of nodules may directly support
531 the settlement of low mobile, tube building Amphipoda belonging to the infraorder Corophiida by
532 providing hard substrate for constructing their tubes but even more importantly in an indirect way
533 being the bedding for sessile fauna of other groups (cnidarian, sponges). These sedentary macro-
534 or megabenthic taxa are known to provide the substrate for tube-dwelling amphipods in the deep
535 sea (Brandt et al., 2018) as well as for representatives of other infraorders (family
536 Amathillopsidae) that were found clinging on sessile sponges (Lörz and Horton, 2021; Sigwart et
537 al., 2023). The latter were photographically documented also from the APEI-06 (Lörz and
538 Horton, 2021) but not present in the studied samples, while Corophiida were not numerous. In
539 contrary, majority of Amphipoda collected belonged to either moderately or highly mobile
540 epibenthic (families Eusiridae and Pardaliscidae) or to the sediment-dwelling groups (families
541 Oedicerotidae and Phoxocephalidae) (Brix et al., 2018). For these groups the presence or absence
542 of nodules may not be of direct importance, particularly for the sediment-dwelling amphipods for
543 which the type of sediment may play more important role. This determinant was suggested to
544 shape amphipod assemblages in the Cascadia Abyssal Plain in NE Pacific that appeared to be
545 clearly different between two sites separated by only 100 km (Dickinson and Carey, 1978). Here,
546 within East BGR the stations span over ca. 200 km, while the distance between East and West
547 BGR zones is over 2,000 km. The other factors that may influence diversity and the assemblages
548 of CCZ abyssal fauna in the wider scale are depth and food availability. The potential influence
549 of decreasing primary productivity in the surface waters towards West of the CCZ on the animal
550 densities was observed for the whole macrofauna as well as for isopods, polychaetes separately
551 (De Smet et al., 2017; Bonifácio et al., 2020; Washburn et al., 2021b; Kaiser et al., 2023). In the
552 present case due to the semiquantitative character of EBS the densities between samples were not
553 compared and there was no evidence that the two stations situated in the West BGR contractor
554 zone exhibit lower diversity indexes (ES[50] or Shannon Index, Figure 11B). However, their



555 faunal composition distinctness may be associated with the primary production changing in the
556 western direction. Another factor that may play a role here is the depth that is well known as a
557 determinant influencing faunal composition in the deep sea (Rex et al., 2006). The high local
558 variability of nodule coverage and sediment type as well as gradual decrease of depth and of
559 primary productivity in CCZ surface waters towards West combined with still insufficient
560 sampling hinder decision which of the cited factors may play the crucial role in shaping the
561 abyssal communities of the region (see also Kaiser et al., 2023).

562

563 **4.3 Weak connectivity between western and eastern parts of the CCZ as well as other deep-** 564 **sea regions and its implication to conservation programmes**

565 The start of deep-sea mining in the CCZ will have both direct and indirect impact on the abyssal
566 communities and their recovery (Rolinski et al., 2001; Sharma et al., 2001; Washburn et al.,
567 2021b). The estimations suggest that the recovery of the biochemical fluxes in the upper layer of
568 the sediments may last over 200 years (Haffert et al., 2020), while the results of mining
569 experiment in the Peru Basin already showed very slow faunal recovery and serious changes in
570 the composition of communities in the disturbed sites after 26 years (Miljutin et al., 2011; Simon-
571 Lledo et al., 2019). In order to minimise the severe impact that retrieval of nodules serve to the
572 abyssal environment the system of Areas of Particular Environmental Interest that are put aside of
573 any mining operations was adopted by ISA (ISA, 2012) with later modifications (ISA, 2021). All
574 but one of them are situated outside the main zone prospected for deep-sea mining (Fig. 1) and it
575 has been already suggested that they may not fulfil their role (Błażewicz et al., 2019; McQuaid et
576 al., 2020; Washburn et al., 2021a, b; Uhlenkott et al., 2023). It is partly due to highly
577 heterogenous deep-sea environment and multiple factors shaping the faunal communities making
578 them highly variable within short distances. The important role in hindering fast recovery of
579 impacted sites may also have small population sizes of deep-sea invertebrates expressed by the
580 high number of singletons. In our case almost 50% of all MOTUs were collected only once or at
581 a single station (Fig. 10). The small population sizes mean from one hand that in the event of
582 disturbance there are no individuals available to recolonize impacted site in the near future. From
583 the other hand, it can be expected that such species may also be more prone to extinction due to
584 mining operations. Another issue associated with proposal of APEIs that are mostly situated
585 around the CCZ are the distribution ranges of deep-sea species. Our study revealed that majority



586 of species recorded have distribution spanning to 200 km (Figure 10). That means that effective
587 area planned as recovery source needs to be situated in relatively close vicinity of the mining
588 zone. The distances between presently established APEIs in the latitudinal axis are within 500–
589 750 km that seems sufficient for species with larger ranges, but for those with more local
590 distribution such distances may be too large. Although we realize that our sampling was focused
591 mainly on the eastern part of CCZ (with the station located within 300 km radius), still the
592 distribution of large number of species was more restricted and suggests relatively low
593 connectivity within the region. It is worth noting that some species may exhibit extensive ranges
594 but due to their rarity they are rarely collected. If that is the case the disruption due to mining
595 may not influence such species survival, nevertheless it will not allow recovery of impacted place
596 shortly. Important point to consider is also that the movement of small invertebrates within CCZ
597 may be facilitated or prevented by the deep-water bottom currents and local hydrological
598 conditions. These are not fully recognized for the region. However, if the dominating currents in
599 the bottom zones are directed longitudinally (as is the case for the surface ones), the migration of
600 species may be more intensive in the East-West direction than the North-South. In such case the
601 recolonization may be hindered also by the fact that the residual zones (APEIs) are situated North
602 or South from the mining areas. Further studies based on samples collected both in the claim
603 areas but particularly in the APEIs would help in resolving their usefulness as real conservation
604 zones.

605 By now the studies of Amphipoda in the CCZ were focused on very specific ecological group –
606 the scavengers – and listed just 23 amphipod taxa generally widely distributed across different
607 contractor zones and APEIs (Patel et al., 2020; Bribiesca-Contreras et al., 2021; Mohrbeck et al.,
608 2021, Supplementary Table S3). Present study revealed that the non-scavengers community is
609 much more diverse and variable. The striking finding is that only 13% of MOTUs recorded
610 appeared to be widely distributed (MOTUs found at stations at the distance of at least 900 km and
611 those with range spanning out of the CCZ). The more, many of them belong to taxa considered as
612 highly mobile scavengers or predators (Table 4). For three taxa the knowledge is insufficient to
613 make final conclusions about their ecology. Interestingly, four other species recorded in this
614 group belong to the family Oedicerotidae which representatives are recognized as sediment-
615 dwelling deposit feeders or predators (Brix et al., 2018). The question arise how these brooding
616 and less mobile taxa maintain large distributions. The explanation of this phenomenon may be



617 associated with the specific behaviour observed for some shallow-water oedicerotids who migrate
618 into water column for reproduction (Brix et al., 2018). Nothing is known about the mating
619 behaviour of deep-sea species from this family; however, Hendrycks and Conlan (2003) reported
620 one of the species (*Oedicerina denticulata*) from sediment traps set at 50 m above the seafloor.
621 This support the assumption that species of this family probably spend large part of their life in
622 the sediment, but they may also occur in the water column while mating and migrate at larger
623 distances during this period.

624 The above widely distributed species may be considered as the first recolonizers of the
625 disturbed areas. It must be underlined however, that this set of taxa is very small and represent
626 only a small portion of deep-sea functional groups. As a result one can expect that after
627 disturbance event the recovering community will be largely impoverished both in terms of
628 species richness and functional groups. It may lead to general uniformization of deep-sea
629 assemblages and irreversible loss of presently observed specificity of CCZ bottom fauna.

630

631 **5 CONCLUSIONS and RECOMMENDATIONS**

632 Being the first extensive molecular study of the abyssal benthic Amphipoda of Clarion-
633 Clipperton Zone it provides important extension to the barcode library of this group and of the
634 abyssal fauna of the Pacific Ocean, in general. In combination with detailed (although still not
635 finished) morphological identification and description of species new to science (presented in
636 parallel elsewhere) it serves as a baseline for further studies including future monitoring based on
637 eDNA.

638 It is next study showing high diversity and low connectivity of deep-sea faunal groups in the
639 Clarion-Clipperton Zone underlining high specificity of the benthic assemblages of this region.
640 Additionally, with present extent of sampling and knowledge it further supports previous
641 suggestions of moderate or weak representativeness of Areas of Particular Environmental Interest
642 for the mining zones.

643 In the time of discussions about the development of Regional Environmental Management Plans
644 for the whole CCZ area the two main recommendations are to be made:

645 1) to conduct more intensive sampling program of APEIs (of which some are virtually unstudied)
646 parallel to monitoring studies of contractor zones with the goal to better assess their usefulness as
647 reservoirs of biodiversity for the mining areas,



648 2) to include the epibenthic sledge among standard gears used for sampling in order to avoid
649 missing important part of deep-sea fauna in the monitoring studies.

650

651 **Data availability**

652 Datasets supporting this paper can be accessed online through the Barcode of Life Data Systems
653 (boldsystems.org) in the data set “DS-AMPHICZ” (dx.doi.org/10.5883/DS-AMPHICZ). All
654 sequences were also deposited in GenBank with the accession numbers: PQ734211 – PQ734784.

655

656 **Author contributions (CRediT)**

657 Conceptualization – AMJ, PMA

658 Data curation – AMJ

659 Formal analysis – AMJ, KB

660 Funding acquisition – AMJ, PMA, AV

661 Investigation – AMJ, KB

662 Methodology – AMJ

663 Project administration – AMJ, PMA, AV

664 Resources – AMJ, PMA, AV

665 Visualization – AMJ

666 Writing – original draft – AMJ, KB

667 Writing – review & editing – AMJ, KB, PMA, AV

668

669 **Competing interests**

670 authors declare that they have no conflict of interest.

671

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704

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