

1 **Phylogenetic relationships among hadal amphipods of the Superfamily Lysianassoidea:**
2 **Implications for taxonomy and biogeography**

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8 **Highlights:**

- 9 • Phylogenetic relationships among hadal Lysianassoidea amphipods, based on mitochondrial
10 and nuclear DNA sequence variation, showed an incongruence between molecular
11 phylogeny and classification based on morphological characters
- 12 • Some of the Lysianassoidea taxa do not form monophyletic clades at the family, genus and
13 species levels
- 14 • Cryptic species-level diversity is shown in two genera (*Eurythenes* and *Paralicella*)
- 15 • *Hirondellea dubia* has a greater geographical range than previously considered
- 16 • The Lysianassoidea includes species with an abyssal cosmopolitan distribution, and species
17 found only in trenches that show bathymetric partitioning

18 **Abstract**

19 Amphipods of the superfamily Lysianassoidea are ubiquitous at hadal depths (>6000 m) and
20 therefore are an ideal model group for investigating levels of endemism and the drivers of speciation
21 in deep ocean trenches. The taxonomic classification of hadal amphipods is typically based on
22 conventional morphological traits but it has been suggested that convergent evolution, phenotypic
23 plasticity, intra-specific variability and ontogenetic variation may obscure the ability to robustly

24 diagnose taxa and define species. Here we use phylogenetic analysis of DNA sequence variation at
25 two mitochondrial (COI and 16S rDNA) and one nuclear (18S rDNA) regions at to examine the
26 evolutionary relationships among 25 putative amphipod species representing 14 genera and 11
27 families that were sampled from across seven hadal trenches. We identify several instances where
28 species, genera and families do not resolve monophyletic clades, highlighting incongruence between
29 the current taxonomic classification and the molecular phylogeny for this group. Our data also help
30 extend and resolve the known biogeographic distributions for the different species, such as
31 identifying the co-occurrence of *Hirondellea dubia* and *Hirondellea gigas* in the Mariana trench.

32 **Keywords:** Lysianassoidea, Amphipoda, Hadal zone, deep sea, phylogeny

33 **1. Introduction**

34 The hadal zone is the deepest part of the ocean, extending from 6000m to c. 11000m. It is
35 comprised of 37 trench systems, primarily located around the Pacific Rim, that are formed at
36 tectonic subduction zones (Jamieson et al., 2010). Despite representing less than 2% of the marine
37 benthic habitat, bathymetrically the hadal zone accounts for the deepest 45% and yet the trenches
38 remain some of the most poorly explored and least understood marine ecosystems on Earth
39 (Jamieson and Fujii, 2011; Jamieson, 2015). The hadal zone differs from the littoral, bathyal and
40 abyssal zones in that it is formed from a disjunct cluster of habitats rather than a spatio-bathymetric
41 continuum. Most trenches lack adjoining corridors of sufficient depth to provide any connection, and
42 thus are analogous with both high altitude mountain ecosystems, albeit inverted, and hydrothermal
43 vent systems which are linear spans of distinctive habitat with large intervening abyssal plains.

44

45 This level of geographic isolation, coupled with potent selection pressures that promote local
46 adaptation, has meant that hadal trenches have traditionally been considered centres of high
47 species endemism (Beliaev, 1989; Wolff, 1970, 1960). Such a perception, however, is difficult to

48 reconcile with the ubiquity of some key cosmopolitan taxa that are found across the abyssal plains
49 and in different trenches, and even more so with the presence of the same putative species in
50 geographically distinct trenches but which are apparently absent from the adjoining abyssal regions
51 (France and Kocher, 1996; Eustace et al., 2013; Fujii et al., 2013; Jamieson, 2015).

52 Understanding the phylogenetic relationships among hadal taxa within and between trenches will
53 resolve the extent of endemism within individual trenches and identify drivers of speciation. A major
54 challenge within hadal biology is in disentangling environmental and ecological effects of purely
55 depth-related trends versus the environmental and ecological conditions unique to each individual
56 trench (Jamieson and Fujii, 2011). It is difficult to ascertain how speciation is affected by conditions
57 which are associated with depth (i.e. hydrostatic pressure) relative to drivers which are trench
58 specific (e.g. topography and food supply).

59 Amphipods of the superfamily Lysianassoidea represent an important model group for examining
60 such issues given their ubiquity within trenches (Jamieson et al., 2010), their key role in hadal food
61 webs (Blankenship and Levin, 2007) and the ease at which they can be sampled in sufficient
62 numbers and diversity using simple baited traps (Blankenship et al., 2006; Fujii et al., 2013; Hessler
63 et al., 1978).

64 The taxonomic classification of the Lysianassoidea has traditionally been based upon morphological
65 variation associated with trophic adaptations including mouthparts and gnathopods (Bousfield and
66 Shih, 1994). However, multiple authors have highlighted discrepancies between morphological and
67 phylogenetic relationships such that classification is not necessarily robust (e.g. Corrigan et al., 2014;
68 Havermans et al., 2010). At the highest level, the monophyly of the superfamily Lysianassoidea has
69 been questioned as a consequence of taxonomic instability associated with the use of morphological
70 characters (Corrigan et al., 2014; Havermans et al., 2010). Within the superfamily there are 23
71 individual families of which many are monotypic and in several cases it is unclear why certain taxa
72 have been grouped together as a family. The orchomenid genus complex is a notable example where

73 revisions based on different morphological characters have resulted in several taxa being reassigned
74 at the family, supergenus, genus and subgenus level (De Broyer, 1983, 1984, 1985a,b; Barnard,
75 1969). The study of De Broyer et al. (2007) splits five genera into two families; *Falklandia*,
76 *Pseudorchomene*, *Orchomenella* and *Orchomenyx* into the Lysianassidae (Tryphosinae) and
77 *Abyssorchomene* into the Uristidae. However, this still does not marry with molecular analyses that
78 suggest that there are four main phylogenetic clusters (Havermans et al., 2010) and that
79 *Abyssorchomene* and *Orchomenella* do not form reciprocally monophyletic groups. The most recent
80 revision of *Abyssorchomene* (d'Udekem d'Acoz and Havermans 2012) has reclassified *A. plebs* and *A.*
81 *rossi* as *Pseudorchomene plebs* and *P. rossi* but this revision has not reclassified all species of the
82 orchomenid complex. It has been suggested that convergent evolution of morphological characters
83 has obscured the ability to confidently diagnose respective groups (Corrigan et al., 2014; Havermans
84 et al., 2010).

85 There is also some debate about whether current classification accurately resolves true species-level
86 diversity (Havermans et al., 2011). This issue is potentially exacerbated in hadal trenches given
87 largely unknown communities of amphipods. This is well illustrated in *Eurythenes gryllus*, which has
88 a global distribution and a bathymetric range of 184 – 8000m thus spanning the entire bathyal,
89 abyssal and hadal zones (Fujii et al., 2013). Initial phylogenetic analyses based on 16S ribosomal DNA
90 identified genetic homogeneity between locations within the same depth zone over oceanic scales,
91 but genetically divergent, cryptic taxa distributed at different depths more locally (France and
92 Kocher, 1996). Subsequent analyses involving both nuclear and mitochondrial DNA polymorphism
93 identified nine putative species-level clades for Arctic, Atlantic, Pacific and Southern Ocean samples
94 (Havermans et al., 2013). Clearly there is greater ecological and genetic diversity than a single
95 species description would suggest, and understanding how hadal samples relate to abyssal and
96 bathyal equivalents will shed further light on the extent of cryptic diversity.

97 Conversely, in other species such as those in the genus *Paralicella* it is unclear whether the
98 morphological differences that define the different species reflect morphological plasticity
99 associated with instar development (Barnard and Shulenberger, 1976) and whether the currently
100 described species, particularly *P. tenuipes* and *P. caperesca*, should be collapsed into a single group.
101 The similar situation arises in the genus *Uristes*. Blankenship et al. (2006) documented a new species
102 of *Uristes* from the Tonga trench that was later classified as *Uristes chastaini* (as cited in Blankenship
103 and Levin, 2009). However *Uristes chastaini* is a *nomen nudum* and the species is no longer thought
104 to belong to *Uristes*, but rather to a new genus within the subfamily Tryphosinae (Lysianassidae;
105 M.H. Thurston and T. Horton pers. comm.). It is unclear how robust the morphological traits that
106 define this classification are, or if they instead reflect ontogenetic variability.

107 The difficulty in accurately defining species through traditional taxonomic approaches further
108 complicates the ability to assess species biogeography. A confirmation of the true number of
109 reciprocally monophyletic operational taxonomic units found within different trenches is a pre-
110 requisite for assessing the extent of local endemism and the respective levels of species diversity in
111 the different trench systems. Current data suggest that there are considerable differences in species
112 diversity across trenches (Fujii et al., 2013). For example, the Peru-Chile Trench has higher levels of
113 species diversity and local endemics than other trenches around the Pacific Rim (Fujii et al., 2013).
114 This coincides both with the Peru-Chile Trench being the geologically youngest trench and also the
115 most eutrophic given its proximity to the South American continental landmass. Molecular analyses
116 are required to verify the unique nature of certain amphipod communities to then hypothesise
117 which processes are responsible for speciation.

118 Here we provide an overview of the molecular taxonomic relationships among 25 putative species of
119 lysianassoid amphipods identified using classical morphological analysis. Different species were
120 sampled from across seven hadal trenches around the Pacific Rim and surrounding abyssal regions.
121 We use sequence variation at the mitochondrial 16S ribosomal DNA, cytochrome oxidase I and

122 nuclear 18S ribosomal DNA regions to examine whether the currently defined classifications at
123 several levels within the taxonomic hierarchy reflect monophyletic groupings in a molecular
124 phylogeny, and from that characterise the amphipod communities within different trenches.

125 **2. Materials and Methods**

126 **2.1. Sample Collection**

127 Amphipods were collected over the course of seven sampling campaigns: In 2007 to the Kermadec
128 and Tonga trenches (Cruise SO197), the Japan Trench (Cruise KH0703) and the Mariana Trench
129 (KR0716); in 2009 to the Izu-Bonin Trench (Cruise KT0902) and the Kermadec Trench (Cruise
130 KAH0910); in 2010 to the Peru-Chile Trench (Cruise SO209); in 2011 to the Kermadec Trench (Cruise
131 KAH1109); in 2012 to the Kermadec Trench (Cruise KAH1202); and in 2013 to the Kermadec trench
132 (Cruise KAH1301) and the New Hebrides Trench and South Fiji Basin (Cruise KAH1310) (Table 1). In
133 all cases an autonomous, full ocean depth rated lander vehicle (Jamieson et al., 2009) was deployed
134 to the sea floor for up to eight hours, incorporating small funnel traps (30 cm length x 6 cm diameter
135 with a trap opening of approximately 2.5 cm) baited with approximately 100 g of mackerel or tuna.
136 Upon recovery of the lander, amphipods were transferred immediately to 99% ethanol prior to
137 morphological identification in a shore-based laboratory (National Institute for Water and
138 Atmospheric Research, New Zealand or latterly the Australian Museum).

139 **2.2. DNA Extraction and PCR Amplification**

140 Total genomic DNA was extracted from either the sixth pereopod or whole body of individual
141 specimens using a standard phenol-chloroform approach. PCR amplification of part of the
142 mitochondrial 16S rRNA gene, part of the cytochrome c oxidase subunit I (COI) and separate 5' and
143 3' portions of the nuclear 18S rRNA gene was carried out using universal primers: AMPH1 (France
144 and Kocher, 1996) and 'Drosophila-type' 16SBr (Palumbi et al., 1991), LCO1490 and HCO12198
145 (Folmer et al., 1994) and 18SF and 18SR (Englisch et al., 2003), respectively. The PCR reaction mixes

146 contained 0.2mM each dNTPs, 2.5mM MgCl₂, 0.5μM each primer, 0.5U of *Taq* DNA polymerase
147 (Bioline), 10-40ng DNA template in 1x NH₄ buffer (Bioline) in a total reaction volume of 20μl. PCR
148 conditions for 16S amplicons were: initial denaturation at 94°C for 1 min, followed by 35 cycles of
149 denaturation at 94°C for 30 s, annealing 50°C for 30 s, extension at 72°C for 30 s before a final
150 elongation step at 72°C for 1 min. The PCR conditions for COI amplicons were: initial denaturation at
151 94°C for 1 min, followed by 30 cycles of denaturation at 94°C for 45 s, annealing 45°C for 45 s,
152 extension at 72°C for 45 s before a final elongation step at 72°C for 1 min. PCR conditions for 18S
153 amplicons were: initial denaturation at 94°C for 1 min, followed by 30 cycles of denaturation at 94°C
154 for 45 s, annealing 55°C for 45 s, extension at 72°C for 45 s before a final elongation step at 72°C for
155 1 min.

156 PCR products were purified enzymatically using ExoSAP-IT® (USB, Cleveland, OH, USA) as described
157 in Bell (2008) and quantified by direct comparison with lambda DNA size standards on a 1% TBE
158 agarose gel. Sequencing was undertaken with an ABI 3730xl automated DNA sequencer (MWG
159 Eurofins Ebersberg, Germany) using the same PCR primers as used in the original PCR.

160 **2.3. Phylogenetic analyses**

161 Electropherograms were viewed in MEGA v.6.0.5 (Tamura et al., 2013) and primer sequences and
162 any ambiguous bases were trimmed. Nucleotide alignments were made using webPRANK (Löytynoja
163 and Goldman, 2010) and confirmed by eye. All indels were removed from the analysis. Sequence
164 identity was confirmed using NCBI BLASTn (Altschul et al., 1990). All COI sequences were translated
165 to their equivalent amino acid sequence in NCBI BLASTx to confirm the absence of stop codons.

166 The optimal evolutionary model for each dataset was identified by jModelTest 2.1.6 (Darriba et al.,
167 2012) using both the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC).
168 Both AIC and BIC identified the same best-fit models: the general time-reversible substitution model
169 (GTR+I+G) for COI and 18S rDNA, and the Hasegawa, Kishino and Yano model (HKY+G) for 16S rDNA.

170 The phylogenetic relationships between individuals were ascertained from concatenated sequences
171 and individual locus data. In all cases topologies were inferred using both maximum-likelihood and
172 Bayesian approaches using PAUP* v.4.0b8 (Swofford, 2002) and either Mr. Bayes v.3.2.3 (Ronquist
173 and Huelsenbeck, 2003) or *BEAST (Drummond et al., 2012), respectively. Maximum likelihood
174 analyses were conducted using a heuristic search with the starting tree obtained by neighbour-
175 joining (NJ) and using tree-bisection-reconnection (TBR) branch swapping, and 10 random tree
176 replicates using the model of sequence evolution estimated by jModelTest but with the parameters
177 estimated by PAUP*. The stability of nodes was assessed from bootstrap support (Felsenstein, 1985)
178 based upon 10,000 iterations. Each Bayesian analysis was run for 50,000,000 generations sampling
179 500,000 trees (every 100 generations) using the model of sequence evolution estimated by
180 jModelTest but with the parameters estimated by Mr. Bayes or *BEAST. The first 150,000 trees were
181 discarded as burn-in where the partition frequencies among the remaining trees give the posterior
182 probabilities to provide an estimate of clade credibility. Trees were visualised using FigTree v1.4.2
183 (Rambaut, 2012), and annotated using Inkscape 0.48.

184 Species delimitation for both *Eurythenes* spp. (at the 16S locus) and *Paralicella* spp. (using a
185 concatenated mtDNA dataset) was undertaken using a Bayesian Poisson Tree Processes (bPTP)
186 model to infer putative species boundaries using speciation or branching events in terms of number
187 of substitutions (Zhang et al., 2013). Each Bayesian analysis was run for 500,000 MCMC generations.
188 Outputs were analysed using Tracer v1.6 (Rambaut et al., 2014) to check mixing, chose a suitable
189 burn-in and examine trends to ensure convergence.

190 Haplotype networks were constructed using the TCS method in PopART v1.7 (Leigh and Bryant,
191 2015) for both *Eurythenes* and *Paralicella* genera .

192 **3. Results**

193 A total of 24 different putative species were identified with high confidence using morphological
194 characters, with 90 individuals being sampled from eight trenches. Across the gene amplicons, a
195 total of 260 unambiguous base pairs (bp) were resolved for the 16S rRNA gene, 624bp for COI,
196 742bp for the 5' end of the 18S rRNA gene, and 727bp at the 3' end (combined amplicon length was
197 2353bp). Not all individuals were sequenced across all genes given some species yielded DNA of
198 poor quality which precluded the amplification of large amplicons, or hampered sequencing across
199 stretches of high GC-content and repetitive areas. GenBank accession numbers are provided in Table
200 1.

201 Subsequently we present both topologies based upon a concatenated data set (total 1626bp: 16S
202 260bp, COI 624bp and 18S 742bp) for 18 key species (Figure 1) and 16S data alone for 24 species to
203 maximise taxonomic coverage (Figure 2). Individual gene topologies for 16S, COI and 18S, as well as
204 a combined mtDNA topology are also presented in the supplementary materials (Supplementary
205 Figures 1-4). Any lack of congruence among topologies that may affect overall interpretation is
206 highlighted in the text in each instance.

207 A coalescent Bayesian tree of 18 key species, based on a concatenated dataset is given in Figure 1.
208 The phylogeny shows the superfamily Lysianassoidea to be monophyletic however this is not the
209 case when the phylogeny is based solely on the 16S locus (Supplementary Figure 5). In the 16S
210 phylogeny, to be monophyletic *Bathycallisoma (Scopelocheirus) schellenbergi* would need to be
211 evolutionarily closer to the rest of the Lysianassoidea superfamily than *Lanceola* sp. which is
212 considered part of a different superfamily (the Lanceolidae). The evolutionary distance between
213 *Bathycallisoma (Scopelocheirus) schellenbergi* and the remainder of the Lysianassoidea at the 16S
214 locus is sufficiently large that it affects the overall resolution of the phylogeny causing instability in
215 the internal topology where the overall phylogeny is altered and, as such, *Bathycallisoma*
216 (*Scopelocheirus) schellenbergi* has been removed from the final 16S phylogeny (Figure 2).

217 Notwithstanding there is still a level of distinction *between Bathycallisoma (Scopelocheirus)*
218 *schellenbergi* and *Bathycallisoma schellenbergi* that resolves them as two distinct species (Figure 1).

219 The relative relationships of the species show variance between the concatenated dataset and the
220 16S gene tree. Given the species coverage in the concatenated dataset incongruence is shown at the
221 family and genus level.

222 The Alicellidae family supposedly consists of the genera *Alicella*, *Paralicella* and *Tectoalopsis* (Lowry
223 and Broyer, 2008). Whilst each genus does form a monophyletic clade there are several
224 discrepancies between the concatenated phylogeny and the 16S gene tree. In the 16S phylogeny
225 *Alicella* is a sister taxa to *Tectoalopsis* but in the concatenated dataset *Cyclocaris* is the sister taxa to
226 *Tectoalopsis*. Both datasets suggest that *Alicella*, *Tectoalopsis* and *Cyclocaris* form a distinct clade.
227 Also, in the 16S phylogeny *Paralicella* is monophyletic and a sister taxa to the Hirondelleidae but in
228 the concatenated dataset forms a clade with *Valettietta* which is part of the Valettiopsidae family.
229 Neither phylogeny suggests that *Alicella*, *Paralicella* or *Tectoalopsis* form a distinct monophyletic
230 family therefore the Alicellidae is not monophyletic.

231 Another point of contention between the 16S phylogeny and the concatenated dataset is the
232 relative placement of the Eurytheneidae family. Figure 1 shows *Eurythenes* and *Bathycallisoma* to be
233 sister genera but this is not the case in Figure 2 where *Bathycallisoma* is shown to be ancestral to a
234 polyphyletic *Eurythenes*. *Eurythenes* being placed sister to *Bathycallisoma* in the concatenated
235 dataset is more statistically supported than its placement in the 16S phylogeny but this still disagrees
236 with the findings of Corrigan et al. (2014) that suggests that *Eurythenes* is sister to a group
237 containing *Paralicella* and *Stephonyx*, and as such is more closely related to *Paralicella* than
238 *Cyclocaris*. Our concatenated phylogeny suggests that *Paralicella* is more evolutionary close to
239 *Cyclocaris* than to *Eurythenes*.

240 For the *Abyssorchomene* genus there is congruence between our 16S and concatenated datasets,
241 which are also consistent with the phylogeny constructed by Corrigan et al. (2014). *Abyssorchomene*
242 and *Orchomenella* are currently classified as separate genera belonging to different families
243 (Uristidae and Lysianassidae, respectively). However, they have been shown not to form reciprocally
244 monophyletic clades with *Orchomenella* being situated within the *Abyssorchomene* clade in every
245 instance. The relative placement of this clade in relation to the rest of the Lysianassoidea is also
246 uncertain. Both our concatenated phylogeny and that in Corrigan et al. (2014) shows the
247 *Abyssorchomene* to be the most ancestral clade of the Lysianassoidea which is not shown in our 16S
248 phylogeny - although this placement in the 16S is poorly supported.

249 Across the phylogeny further apparent anomalies involve: 1) Two individuals classified as *Hirondellea*
250 *wagneri* from the Peru-Chile trench fall out with the main *Hirondellea* clade that contains five other
251 reciprocally monophyletic *Hirondellea* species from across six trenches; 2) An individual positively
252 identified as *Valettietta gracilis* from the New Hebrides trench did not fall as a sister taxa to
253 *Valettietta anacantha*, and as such *Valettietta* is not a monophyletic genus; 3) Individuals from the
254 Tonga trench that have been tentatively classified as a novel species of *Uristes* (Blankenship et al.,
255 2006) clearly fall within the monophyletic *Hirondellea* clade.

256 We identified examples of potentially overlooked hadal amphipod diversity. A sample from the
257 Kermadec trench was catalogued as a hitherto unknown *Hirondellea* species. This fell within the
258 *Hirondellea* clade, but did not associate with any of the other *Hirondellea* species catalogued from
259 this location. The original sample was identified to genus based upon morphological characters with
260 a high level of confidence.

261 Several putative species were also shown not to be monophyletic groupings. Figure 3a is a
262 mitochondrial concatenated phylogeny focused upon the relationships between individuals
263 identified as *Paralicella tenuipes* and *Paralicella caperesca*, with the topology rooted through
264 *Hirondellea dubia*. The phylogeny shows that the morphological characteristics used to distinguish

265 between *P. tenuipes* and *P. caperesca* are not sufficiently robust to ensure consistent and accurate
266 identification. This is further highlighted by a species-delimitation analysis which suggests the
267 phylogeny may actually represent up to four species. The concatenated dataset for *Paralicella* spp.
268 (comprising 13 unique sequences each consisting of 257 variable positions of which 203 were
269 parsimony-informative) was also used to construct a haplotype network (Supplementary Figure 6)
270 which also showed the same pattern of groupings as the species-delimitation analysis.

271 The relationship between *Abyssorchomene* spp. and *Orchomenella gerulicorbis* based on a
272 mitochondrial concatenated phylogeny is shown in Figure 3b. The phylogeny shows that
273 *Abyssorchomene* is paraphyletic due to the inclusion of *O. gerulicorbis* in the clade and this is
274 consistent with our concatenated phylogeny (Figure 1), 16S phylogeny (Figure 2) and the phylogeny
275 constructed in Corrigan et al (2014). For both Figures 3a and 3b the likelihood of the resolved
276 topologies is significantly greater than any topology constrained to be reciprocally monophyletic for
277 the individual species (Shimodaira-Hasegawa test; $p < 0.05$). Moreover, these patterns are consistent
278 with all our individual gene trees (Supplementary Figures 1-4).

279 Four amphipod individuals were collected from the Izu-Bonin trench labelled Unidentified Amphipod
280 1-4 (Figure 2) that had morphological characteristics similar to the *Tryphosella* genus but they also
281 exhibited characteristics not previously associated with *Tryphosella* and, as such, the specimens
282 could not be positively identified to any previously described *Tryphosella* species with a high degree
283 of confidence. Furthermore, *Tryphosella* is noted as being a genus into which many species have been
284 placed due to a lack of affinity with other genera, so it is difficult to ascertain what species are truly
285 *Tryphosella* (Lowry and Stoddart, 2011). It has been hypothesised that they perhaps represent two
286 novel species within a novel genus (N.M. Kilgallen, pers. comm.). The DNA sequence data however,
287 is difficult to reconcile with this contention (Figure 2). The four individuals do form two distinct
288 groups though these do not correspond to the two supposed species separated based on
289 morphological differences. Moreover, one of these groups is indeed a sister group to *Tryphosella*

290 (Unidentified amphipod 1 and 2) but the other is completely distinct with an evolutionary separation
291 at a level similar to differences between families (Unidentified amphipod 3 and 4).

292 The only differences that occur between gene trees and concatenated datasets are associated with
293 relative placement of taxa. For example, *Hirondellea* is sister to the *Abyssorchomene* and
294 *Orchomenella* clade in the 18S dataset (Supplementary Figure 3), but is sister to *Paralicella* in 16S
295 (Supplementary Figure 1), sister to no clades in the fully concatenated dataset (Figure 1), and is
296 polyphyletic in both the COI and mtDNA concatenated phylogenies (Supplementary Figure 2 and 4,
297 respectively).

298 Species-delimitation analyses demarked four separate species of *Eurythenes* from the Peru-Chile
299 trench at the 16S locus (Figure 4). These are *Eurythenes* sp. Hadal, Abyssal-major, Abyssal-minor and
300 Bathyal. The relationships between these four *Eurythenes* species with the nine putative species-
301 level clades previously identified by Havermans et al. (2013) using 16S rDNA sequences is shown in
302 Figure 4. Three groups (Hadal, Abyssal-major and Bathyal) form monophyletic clades that are distinct
303 from any lineage previously described. A third group (Abyssal-minor) was placed with a Brazilian
304 abyssal group (Eg4) identified in Havermans et al. (2013) which has recently been described as
305 *Eurythenes magellanicus* (d'Udekem d'Acoz and Havermans, 2015). While this phylogeny is only
306 supported with the Bayesian posterior probability our sequences have a 100% identity to those in
307 the Eg4 group. One of our unique *Eurythenes* clades (Hadal) represents the only *Eurythenes* from
308 hadal depths and from the branch lengths shown in Figure 4 is one of the most highly divergent
309 forms. Repeating a species delimitation analysis across the 16S dataset identified eleven putative
310 species boundaries with a high degree of confidence (Table 2) among the *Eurythenes* clades. There is
311 no obvious grouping of the species by latitude, longitude or trench but species are separated into
312 bathyal, abyssal and hadal depths.

313 The single gene database for *Eurythenes* spp. is comprised of 18 unique sequences each consisting of
314 93 positions of which 57 were parsimony-informative. The *Eurythenes* spp. haplotype network
315 shows the same pattern of grouping as the species delimitation analyses (Supplementary Figure 7).

316 **4. Discussion**

317 **4.1. Recommendations for taxonomic revision**

318 The salient finding of this study is a discordance between morphological-based classification and
319 molecular-based phylogeny in the lysianassooid amphipods. This is apparent at the level of the family,
320 genus and species. Higher level taxonomic classifications in amphipods have traditionally been more
321 unstable than their lower level equivalents (Bousfield and Shih, 1994) and the incongruence seen
322 here in the Lysianassoidea echoes the work of Havermans et al, (2010) and Corrigan et al, (2014)
323 which have also highlighted a discordance between morphological taxonomy and phylogenetics
324 data. Our analysis also revealed some plasticity among different gene trees resolved from nuclear
325 and mitochondrial data. Combined, this highlights some of the difficulties associated with producing
326 a definitive phylogeny for deep-sea amphipods, and argues that any attempt requires a considerable
327 body of molecular data from across multiple loci, proper knowledge of the extent of phenotypic
328 plasticity in morphological traits and descriptions of samples that have not been damaged during
329 sampling.

330 A consistent feature across individual gene trees and the concatenated data sets was that key
331 taxonomic groups failed to form reciprocally monophyletic clades. This indicates several issues
332 associated with classification that require consideration and revision which are described below and
333 summarised in Table 3.

334 The Scopelocheiridae is the potentially most problematic family as it does not form a monophyletic
335 group with the Lysianassoidea superfamily at the 16S locus but does in a concatenated dataset. In
336 the 16S genealogy *Bathycallisoma (Scopelocheirus) schellenbergi* is shown to be more ancestral to

337 the Lysianassoidea than *Lanceola* sp. which forms part of a different superfamily (Lanceolidae).
338 Whether this is their true evolutionary relationship or a consequence of incomplete lineage sorting is
339 difficult to ascertain. Moreover, the ability to accurately determine the true evolutionary history of
340 the Scopelocheiridae is also difficult to confirm given that the overall divergence between
341 *Bathycallisoma schellenbergi* and *Bathycallisoma (Scopelocheirus) schellenbergi* is largely driven by
342 the diversity at the 16S locus. It is noteworthy that *Scopelocheirus schellenbergi* has previously been
343 suggested to be synonymous with *Bathycallisoma schellenbergi* based upon morphological similarity
344 (Barnard, 1964; Dahl, 1979) and this has recently been revised by Kilgallen and Lowry (2015)
345 whereby *Scopelocheirus* has been collapsed into *Bathycallisoma*. This revision cannot be reconciled
346 with our phylogeny given that *Bathycallisoma* and *Bathycallisoma (Scopelocheirus)* form two very
347 distinct groupings, the former within the main Lysianassoidea grouping whereas the latter is further
348 removed at the 16S locus, and in the total concatenated phylogeny they still have an evolutionary
349 distance between them that separates them to species level. Interestingly, Dahl (1959) distinguishes
350 '*Bathycallisoma schellenbergi*' from Schellenberg's '*Scopelocheirus schellenbergi*' based on
351 morphological differences in an individual from the Kermadec trench and the genetic data presented
352 here show differences between a *B. schellenbergi* individual and a *B. (S.) schellenbergi* from the
353 Kermadec trench suggesting there are indeed two species present.

354 The Alicellidae family have been described as comprising of the genera *Apotectonia*, *Diatectonia*,
355 *Transtectonia*, *Alicella*, *Paralicella* and *Tectoalopsis* (Lowry and De Broyer, 2008). Here we present
356 molecular data on *Alicella*, *Paralicella* and *Tectoalopsis* which shows these three genera are
357 reciprocally monophyletic, but do not group as a monophyletic family given that *Valettietta* is sister
358 to *Paralicella*, and *Cyclocaris* is sister to *Tectoalopsis* in the concatenated dataset, and *Paralicella* is
359 sister to *Hirondellea* in the 16S dataset. Taxonomic revision should aim to correctly delimit genera in
360 the Alicellidae. This will require more molecular data since the most robust phylogeny can be
361 difficult to ascertain given the instability of internal topologies and poor node support. Discordance
362 at higher taxonomic levels can be attributed to short internal branches united with proportionally

363 long terminal branches which is often indicative of taxa which have undergone ancient rapid
364 speciation (Donoghue and Sanderson, 1992; Macdonald III et al., 2005). This is consistent with the
365 findings of Corrigan et al. (2014) that show that amphipods from the Atlantic abyssal plain
366 underwent an adaptive radiation during the Eocene-Oligocene transition when deep sea habitat
367 formed and provided new ecological niches and the opportunity for adaptive radiation.

368 The genus *Paralicella* itself also requires taxonomic scrutiny at the species level. Currently *Paralicella*
369 has six described species with *P. tenuipes* and *P. caperesca* being the most commonly recovered
370 from abyssal and hadal depths. The descriptions of *P. tenuipes* and *P. caperesca* have been debated
371 with concerns raised over the morphological characteristics used to differentiate them and
372 whether they actually reflect morphological plasticity associated with instar developmental stages
373 (Barnard and Shulenberger, 1976). Here we demonstrate that the current taxonomic descriptions
374 are insufficient to consistently identify species. The phylogeny also suggests that there are more
375 species than previously appreciated with the species delimitation analysis of the 16S locus
376 indicating there may be up to four separate species.

377 Within the phylogeny, *Orchomenella* and *Abyssorchomene* are shown to form a distinct but mixed
378 clade. This has been shown previously using specimens of different species within these genera,
379 from different geographical locations at bathyal and abyssal depths (Corrigan et al, 2013; Havermans
380 et al, 2010). An individual of *Abyssorchomene musculosus* was also shown to have the same
381 sequence as several *A. distinctus* individuals. The *Abyssorchomene* genus requires further revision at
382 both lower and higher taxonomic levels to ensure appropriate species-level delimitation and to
383 address the remaining polyphyly at the genus and family level. Both *Orchomenella* and
384 *Abyssorchomene* are often difficult to taxonomically identify using morphological characteristics so
385 this complex would benefit from the addition of molecular data for identification purposes.

386 Inconsistencies at the genus-level have also been uncovered across the 16S phylogeny. A *Valettettia*
387 *gracilis* individual that was identified with high confidence using morphological characters does not

388 form a monophyletic group with the remainder of the *Valettettia* clade. This is also the case with
389 *Hirondellea wagneri* which does not fall into the remainder of the *Hirondellea* clade. Furthermore,
390 the putatively identified *Uristes* sp. nov. does not appear to be either *Uristes* or *Tryphosella*, but
391 instead is positioned within the *Hirondellea* clade. Such anomalies likely reflect the plastic nature of
392 the morphological traits being used to assemble species in a particular genus.

393 **4.2. Biogeographic patterns**

394 The Scopelocheiridae have recently undergone revision (Kilgallen and Lowry, 2015) with the
395 collapsing of the genus of *Scopelocheirus* into the junior synonym of *Bathycallisoma*. However we
396 have shown there is sufficient evolutionary distance between the specimens to determine them as
397 separate species. Both individuals of *Bathycallisoma* and *Bathycallisoma (Scopelocheirus)* have been
398 sampled from the Kermadec trench at the same depth range suggesting an overlap in resource use
399 by the two species. It is also worth noting that individuals of *B. (S.) schellenbergi* show genetically
400 similar COI sequences from Puerto-Rico, Kermadec and New Hebrides trenches. It is unclear how the
401 distribution of *Bathycallisoma (Scopelocheirus) schellenbergi* stretches from the SW Pacific
402 (Kermadec Trench) to the Atlantic (Puerto-Rico Trench) without being found in the intervening SE
403 Pacific (Peru-Chile Trench) but it is outside the scope of this paper to make further comments on the
404 ecology of *B. (S.) schellenbergi*.

405 The distribution patterns of the lysianassoids are key for determining their evolutionary and
406 ecological histories. All the families in the Lysianassoidea investigated here are found across all of
407 the trenches explored. In this study, however, at the species level there can be geographically
408 distinct patterns of distribution. For example, species such as *Alicella gigantea* and *Eurythenes*
409 *gryllus* have been shown to have cosmopolitan distributions (France and Kocher, 1996; Jamieson et
410 al., 2013). *Eurythenes* has also been shown to exhibit bathymetric stratification that may be due to
411 cryptic speciation (Havermans et al., 2013) and although data is more limited for *Alicella gigantea*
412 the data that is available shows very little differentiation between individuals located in the

413 Kermadec trench, New Hebrides trench or the Central North Pacific (Jamieson et al., 2013)
414 suggesting that only *A. gigantea* is truly cosmopolitan although this assertion would benefit from
415 further investigation. Similarly, *Paralicella* spp. has shown to form two major groups in our mtDNA
416 concatenated phylogeny where the first group consists of individuals from the Japan, Mariana and
417 Peru-Chile trenches and the second group from Kermadec, New Hebrides, Mariana and Peru-Chile
418 trenches. This suggests a degree of ecological structure in the most northerly (Japan) and southerly
419 (New Hebrides and Kermadec) trenches with mixture occurring in the mid-Pacific (Mariana and Peru-
420 Chile). Also, differing patterns of trench association have also been shown for other genera that have
421 an abyssal distribution: 1) *Valettietta anacantha* shows differentiation between individuals located
422 in the New Hebrides and Kermadec trenches, and the Mariana trench and 2) *Abyssorchomene* shows
423 differentiation where *A. distinctus* is found in both the Peru-Chile and New Hebrides trenches
424 whereas *A. chevreuxi* is only found in the Peru-Chile trench, one unknown *Abyssorchomene* species
425 is found only in the New Hebrides trench and another unknown *Abyssorchomene* species is found
426 only in the Mariana trench. While geographic isolation of hadal trenches have often been considered
427 conducive to endemism the homogeneity of the abyssal plains would suggest gene flow would be
428 less restricted in abyssal species due to the lack of physical barriers to gene flow. The differentiation
429 of distribution patterns described here suggests that the driver of speciation at abyssal depths
430 cannot solely be geographical distance. It is more likely that a combination of habitat-specific and
431 species-specific factors influence speciation such as: water chemistry, sediment type, water
432 temperature, nutritional input, species community structure, locomotory ability or dispersal method
433 (Dawson and Hamner, 2008; Ricklefs, 2004).

434 *Hirondellea* is a genus that has been well documented within hadal depths. However, the
435 distribution of different *Hirondellea* species across trenches is not entirely clear. Within the Pacific it
436 was believed that *H. sonne* and *H. thurstoni* are endemic to the SE trenches (Peru-Chile Trench), *H.*
437 *gigas* is endemic to NW trenches (e.g. Mariana and Izu-Bonin trenches) (Eustace et al., 2013; France,
438 1993) and that *H. dubia* is endemic to the SW trenches (e.g. Kermadec and Tonga trenches)

439 (Blankenship et al., 2006; Jamieson et al., 2011). Here we show that *Hirondellea dubia* are also
440 located in the vicinity of the Mariana Trench which was previously believed to only be inhabited by
441 *H. gigas*. *H. dubia* from the New Hebrides Trench also showed higher affinity to those in the Mariana
442 Trench than to those in the Kermadec and Tonga trenches despite being geographically closer,
443 suggesting that bathymetric partitioning (in this case, the Kermadec forearc) is a greater barrier to
444 gene flow than horizontal distance. Also, the individuals from the Mariana region were sampled at
445 the abyssal depth of 5469 m which is shallower than the previous distribution limit of 6000 m set in
446 the Kermadec Trench (Jamieson et al., 2011).

447 Species richness of hadal assemblages might also be underestimated by current taxonomic
448 approaches for species identification. For example, amphipods from the Izu-Bonin trench which
449 displayed morphology similar to the *Tryphosella* genus, but which could not be identified to any
450 previously described species, were classified as two distinct new species (N.M. Kilgallen, pers.
451 comm.). These were shown to form two distinct groups in a molecular phylogeny, however, one
452 group (Unidentified amphipod 1 and 2) was placed as a sister taxa to *Tryphosella* but the other
453 (Unidentified amphipod 3 and 4) is far removed from the *Tryphosella* clade. This highlights how
454 phylogenetically divergent morphologically similar species can be, and hence the potential for
455 cryptic species diversity.

456 Cryptic diversity has previously been identified for *Eurythenes gryllus* (Havermans et al., 2013). Their
457 previous analysis of *Eurythenes* involved individuals from 24 locations across the Arctic, Pacific,
458 Atlantic and Southern Oceans where nine putative species-level clades were identified (Havermans
459 et al., 2013). The addition of two abyssal, one hadal and one bathyal group from the Peru-Chile
460 trench in this study increases this to eleven species. In addition to the genetic divergence between
461 individuals located at bathyal and abyssal depths we resolve a further distinction with hadal
462 samples. This reinforces the suggestion that bathymetry is more influential on *Eurythenes* speciation
463 than geographical distance (France and Kocher, 1996; Havermans et al., 2013). Since both bathyal

464 and abyssal clades are characterised by a widespread geographic distribution, the analyses of
465 additional hadal morphotypes from other trenches would allow us to further test this hypothesis of
466 speciation influenced by bathymetry.

467 **5. Conclusions**

468 Here we have provided a phylogenetic analysis of the Lysianassoidea which has highlighted several
469 problematic issues in the taxonomic classification of the superfamily alongside informing a better
470 understanding of the biogeographical distributions of key hadal species. Traditional taxonomic
471 analysis of amphipods has focused on morphological variation which has been shown, here and in
472 previous studies, to both under- and overestimate species richness. The use of molecular data allows
473 for more robust analysis into the classification of species and provides a barcoding tool for the
474 correct species identification of unidentified individuals. Combining classical approaches with
475 molecular data will inform an understanding of how morphological variation reflects taxonomic
476 relationships alongside phenotypic plasticity and ontogenetic variation. In turn this provides a
477 framework for building an understanding of the eco-evolutionary drivers of variation seen in the
478 largely unknown hadal zone.

479 **Acknowledgements**

480 We thank the chief scientists, crew and company of the Japanese RV *Hakuho-Maru* (KH0703 and
481 KH0803), the RV *Tansei-Maru* (KT-09-03), the RV *Kairei* (KR0716), the German FS *Sonne* (SO197 and
482 SO 209) and the New Zealand RV *Kaharoa* (KAH0190, KAH1109, KAH1202, KAH1301 and KAH1310).
483 This work was supported by the HADEEP projects, funded by the Nippon Foundation, Japan
484 (2009765188), the Natural Environmental Research Council, UK (NE/E007171/1) and the Total
485 Foundation, France. We acknowledge additional support from the Marine Alliance for Science and
486 Technology for Scotland (MASTS) funded by the Scottish Funding Council (Ref: HR09011) and
487 contributing institutions. Additional sea time was supported by NIWA's 'Impact of Resource Use on

488 Vulnerable Deep-Sea Communities' project (CO1_0906). From NIWA we thank Malcolm Clark, Ashley
489 Rowden, Kareen Schnabel, Sadie Mills for logistical support at the NIWA Invertebrate Collection. We
490 also thank Fredrik Søreide from Promare, USA, for supply of the Puerto-Rico samples, and Dr.
491 Tammy Horton (NOCS, UK) for identifying some of the earlier amphipod samples.

492

493 Table 1. Sample locations, depth and sequence accession numbers for all samples included in the
494 analysis. *Eurythenes gryllus* individuals are labelled as H=hadal, B=bathyal, AMa=Abyssal-major and
495 Ami=Abyssal-minor.

					Accession No.			
	Trench	Depth	Latitude	Longitude	16S	COI	18S-5'	18S-3'
Lysianassoidea								
Alicellidae								
<i>Alicella gigantea</i>	Kermadec	7000m	32°33'S	177°14'W	KP456083	KP713893	---	---
	New Hebrides	4694m	20°56'S	168°28'E	KP456084	KP713894	KP347467	---
	New Hebrides	5180m	20°54'S	168°32'E	KP456085	KP713895	---	---
<i>Paralicella caperesca</i>	Kermadec	6007m	26°43'S	175°11'W	KP456101	KP713924	KP347461	---
	Kermadec	6007m	26°43'S	175°11'W	KP456099	KP713925	---	---
	New Hebrides	2500m	21°13'S	168°14'E	KP456105	KP713921	KP347463	KP347463
	New Hebrides	2500m	21°13'S	168°14'E	KP456097	KP713920	KP347462	KP347462
	Peru-Chile	5329m	04°27'S	81°54'W	KP456108	KP713922	KP347460	KP347460
	Peru-Chile	6173m	07°48'S	81°17'W	KP456107	KP713923	---	---
<i>Paralicella tenuipes</i>	Japan	6945m	40°15'N	144°30'E	KP456113	KP713931	KP347464	KP347464
	Japan	6945m	40°15'N	144°30'E	KP456112	KP713930	KP347465	KP347465
	Kermadec	6007m	26°43'S	175°11'W	KP456104	KP713932	---	---
	Kermadec	6007m	26°43'S	175°11'W	KP456103	KP713933	---	---
	Mariana	5469m	18°49'N	149°50'E	KP456111	KP713929	---	---
	Mariana	5469m	18°49'N	149°50'E	KP456110	KP713928	---	---
	Peru-Chile	6173m	07°48'S	81°17'W	KP456109	---	---	---
	Peru-Chile	6173m	07°48'S	81°17'W	KP347450	KP713934	---	---
	South Fiji Basin	4100m	24°58'S	171°3'E	KP456106	KP713927	---	---
	South Fiji Basin	4100m	24°58'S	171°3'E	KP456098	KP713926	---	---
	<i>Tectoalopsis wegeneri</i>	New Hebrides	2500m	21°13'S	168°14'E	KP456087	KP713945	---
New Hebrides		2500m	21°13'S	168°14'E	KP456086	KP713946	KP347457	KP347457
Cyclocaridae								
<i>Cyclocaris</i> sp.	New Hebrides	4100m	24°58'S	171°3'E	KP456090	KP713899	KT372890	---
	New Hebrides	4100m	24°58'S	171°3'E	KP456091	KP713898	---	---
Cyphocarididae								
<i>Cyphocaris</i> sp.	Peru-Chile	1037m	25°58'S	70°52'W	KP456133	KP713952	---	---
Eurytheneidae								
<i>Eurythenes gryllus</i>	Peru-Chile(H)	7050m	17°25'S	73°37'W	KP456138	KP713955	---	---
	Peru-Chile(H)	7050m	17°25'S	73°37'W	KP456139	KP713956	---	---
	Peru-Chile(AMa)	4602m	06°12'S	81°40'W	KP456140	KP713957	KP347469	KP347469
	Peru-Chile(AMa)	4602m	06°12'S	81°40'W	KP456141	KP713958	---	---
	Peru-Chile(AMi)	4602m	06°12'S	81°40'W	KP456142	---	---	---
	Peru-Chile(AMi)	4602m	06°12'S	81°40'W	KP456143	---	---	---
	Peru-Chile(B)	915m	06°35'S	81°31'W	KP456144	KP713954	---	---
	Peru-Chile(B)	915m	06°35'S	81°31'W	KP456145	---	---	---
Hirondelleidae								
<i>Hirondellea brevicaudata</i>	New Hebrides	6948m	20°38'S	168°36'E	KP456082	KP713900	---	---
<i>Hirondellea dubia</i>	Kermadec	7966m	26°54'S	175°30'W	KP456068	KP713905	---	---
	Kermadec	7966m	26°54'S	175°30'W	KP456067	KP713906	KP347459	KP347459
	Mariana	5469m	18°49'N	149°50'E	KP456069	KP713903	---	---
	Mariana	5469m	18°49'N	149°50'E	KP456070	KP713904	---	---
	New Hebrides	6948m	20°38'S	168°36'E	KP456071	KP713902	---	---
	New Hebrides	6948m	20°38'S	168°36'E	KP456072	KP713901	---	---
	Tonga	8798m	24°08'S	175°10'W	KP456065	KP713908	---	---
	Tonga	8798m	24°08'S	175°10'W	KP456066	KP713907	---	---
<i>Hirondellea gigas</i>	Izu-Bonin	8172m	27°22'N	143°13'E	KP456080	KP713909	---	---
	Izu-Bonin	9316m	27°20'N	143°18'E	KP456079	KP713910	---	---
	Japan	6945m	40°15'N	144°30'E	KP456078	KP713912	KT372891	---
	Japan	6945m	40°15'N	144°30'E	KP456077	KP713911	---	---
<i>Hirondellea sonne</i>	Peru-Chile	7050m	17°25'S	73°37'W	KP456073	---	---	---
	Peru-Chile	7050m	17°25'S	73°37'W	KP456074	---	---	---
<i>Hirondellea</i>	Peru-Chile	7050m	17°25'S	73°37'W	KP456076	---	---	---

<i>thurstoni</i>	Peru-Chile	8074m	23°22'S	71°19'W	KP456075	---	---	---
<i>Hirondellea</i>	Peru-Chile	6173m	07°48'S	81°17'W	KP456135	KP713914	KP347468	---
<i>wagneri</i>	Peru-Chile	6173m	07°48'S	81°17'W	KP456134	KP713913	---	---
Lysianassidae								
<i>Orchomenella</i>	Kermadec	6007m	26°43'S	175°11'W	KP456120	KP713919	---	---
<i>gerulicorbis</i>	Kermadec	6007m	26°43'S	175°11'W	KP456119	KP713918	KP347455	KP347455
Scopelocheiridae								
<i>Bathycallisoma</i>	Kermadec	6890m	26°48'S	175°18'W	KP456128	KP713897	KP347453	KP347453
<i>schellenbergi</i>	Kermadec	6890m	26°48'S	175°18'W	KP456129	KP713896	---	---
<i>Bathycallisoma</i>	Kermadec	7884m	32°36'S	177°21'W	KP308148	KP713939	---	---
<i>(Scopelocheirus)</i>	New Hebrides	6000m	20°47'S	168°32'E	KP456060	KP713938	KP347451	KP347451
<i>schellenbergi</i>	New Hebrides	6000m	20°47'S	168°32'E	KP456061	KP713937	KP347452	KP347452
	Puerto-Rico	8300m	19°50'N	66°45'W	---	KP713935	---	---
	Puerto-Rico	8300m	19°50'N	66°45'W	---	KP713936	---	---
Tryphosinae								
<i>Tryphosella</i> sp.	Kermadec	6709m	32°22'S	177°05'W	KP456132	---	---	---
	Peru-Chile	7050m	17°25'S	73°37'W	KP456131	---	---	---
Uristidae								
<i>Abyssorchomene</i>	Peru-Chile	4602m	06°12'S	81°40'W	KP456114	KP713882	KP347454	KP347454
<i>chevreuxi</i>	Peru-Chile	5329m	04°27'S	81°54'W	KP456115	KP713883	---	---
<i>Abyssorchomene</i>	New Hebrides	3400m	21°06'S	168°09'E	KP456123	KP713886	KT372892	---
<i>distinctus</i>	New Hebrides	3400m	21°06'S	168°09'E	KP456124	KP713887	---	---
	Peru-Chile	4602m	06°12'S	81°40'W	KP456121	KP713884	---	---
	Peru-Chile	4602m	06°12'S	81°40'W	KP456122	KP713885	---	---
<i>Abyssorchomene</i>	New Hebrides	3400m	21°06'S	168°09'E	KP456125	KP713888	---	---
<i>musculosus</i>								
<i>Abyssorchomene</i>	New Hebrides	2080m	21°16'S	168°12'E	KP456126	KP713889	---	---
sp.	New Hebrides	2080m	21°16'S	168°12'E	KP456127	KP713890	---	---
	Mariana	5467m	18°49'N	149°50'E	KP456116	KP713891	---	---
	Mariana	5467m	18°49'N	149°50'E	KP456117	KP713892	---	---
<i>Uristes</i> sp. nov.	Tonga	8798m	24°08'S	175°10'W	KP456063	KP713947	KP347458	KP347458
	Tonga	8798m	24°08'S	175°10'W	KP456064	KP713948	---	---
Valettioptidae								
<i>Valettietta</i>	Kermadec	6007m	26°43'S	175°11'W	KP456094	KP713950	KT372893	---
<i>anacantha</i>	Kermadec	6007m	26°43'S	175°11'W	KP456093	---	---	---
	Mariana	5467m	18°49'N	149°50'E	KP456096	KP713949	---	---
	New Hebrides	5350m	20°49'S	168°31'E	KP456095	---	---	---
	New Hebrides	5350m	20°49'S	168°31'E	KP456092	---	---	---
<i>Valettietta</i>	New Hebrides	4694m	20°56'S	168°28'E	KP456130	KP713951	---	---
<i>gracilis</i>								
Lanceoloidea								
Lanceolidae								
<i>Lanceola</i> sp.	Peru-Chile	1037m	25°58'S	70°52'W	KP456062	KP713953	KT372894	---
Miscellaneous								
Unidentified Lysianassoid	Japan	6945m	40°15'N	144°30'E	KP456118	KP713915	KP347456	KP347456
Unidentified Primitive Lysianassoid	Mariana	5467m	18°49'N	149°50'E	KP456100	KP713916	KP347466	KP347466
	Mariana	5467m	18°49'N	149°50'E	KP456102	KP713917	---	---
Unidentified amphipod	Izu-Bonin	8172m	27°22'N	143°13'E	KP456088	KP713941	---	---
	Izu-Bonin	8172m	27°22'N	143°13'E	KP456137	KP713944	---	---
Unidentified amphipod	Izu-Bonin	9316m	27°20'N	143°18'E	KP456089	KP713942	---	---
	Izu-Bonin	9316m	27°20'N	143°18'E	KP456136	KP713943	---	---
Unidentified amphipod	Kermadec	6007m	26°43'S	175°11'W	KP456081	KP713940	---	---

498 Table 2. Output of bPTP species delimitation analysis.

Taxon	No. Seqs	(H)	bPTP Parameters	Delimitation Probabilities T(Max-Likelihood/Heuristic Search)
<i>Eurythenes</i> spp.	24	18	Acceptance rate=0.42501, merge=49783, split=50217, estimated no. species-between 3 and 18, mean=11.21	1=0.937, 2=0.797, 3=0.640, 4=0.471, 5=0.816, 6=0.745, 7=0.636, 8=0.875, 9=0.922, 10=0.968, 11=0.787.
<i>Paralicella</i> spp.	15	13	Acceptance rate=0.28782, merge=50255, split=49745, estimated no. species-between 3 and 12, mean=5.77	1=0.974, 2=0.830, 3=0.859, 4=0.442.

499 Taxon, total number of sequences used (16S for *Eurythenes gryllus* and 16S+COI for *Paralicella* spp.), number of
500 unique haplotypes (H), outcome parameters of bPTP analysis and delimitation results for both maximum-likelihood
501 and a simple heuristic search are shown.

502 Table 3. Summary of taxonomic revisions required.

Family	
Alicellidae	Review the paraphyly of the Alicellidae with the inclusion of <i>Hirondellea</i>
Scopelocheiridae	Reinstate <i>Bathycallisoma</i> as a genus Review <i>Bathycallisoma</i> as a member of the Scopelocheiridae
Uristidae	Review the polyphyly of the Uristidae with <i>Abyssorhomene</i> and <i>Orchomenella</i>
Genera	
Abyssorhomene	Further revise the polyphyly of <i>Abyssorhomene</i> with <i>Orchomenella</i>
Bathycallisoma	Revise the collapse of <i>Scopelocheirus</i> into <i>Bathycallisoma</i>
Species	
<i>Abyssorhomene musculosus</i>	Review morphology used to distinguish <i>A. musculosus</i> as a separate species
<i>Eurythenes gryllus</i>	Further revision of species in the <i>Eurythenes</i> following (d'Udekem d'Acoz and Havermans, 2015)
<i>Hirondellea wagneri</i>	Review morphology used to determine <i>H. wagneri</i> as it does not fall within <i>Hirondellea</i>
<i>Paralicella tenuipes/Paralicella caperesca</i>	Review morphology used to determine <i>Paralicella</i> sp. with the inclusion of molecular data to verify species delimitation
<i>Valettietta gracilis</i>	Review morphology used to determine <i>V. gracilis</i> as it does not fall within <i>Valettietta</i>
<i>Uristes</i> sp. nov.	Review morphology used to determine <i>Uristes</i> sp. nov. as it falls within <i>Hirondellea</i>

503
504 Figure 1. Maximum-likelihood tree showing the relationships between 18 key amphipod species based on a fully
505 concatenated dataset. Bayesian posterior probabilities and maximum-likelihood bootstrap support are shown on
506 branch nodes. Values less than 50% were not stated or depicted by an asterisk. Families are denoted by brackets and
507 colours.

508 Figure 2. Maximum-likelihood tree showing the relationships between 24 identified amphipod species based on all
509 16S sequence data, excluding sequences of *Scopelocheirus schellenbergi*. Bayesian posterior probabilities and
510 maximum-likelihood bootstrap support are shown on branch nodes. Values less than 50% were not stated or
511 depicted by an asterisk. Families are denoted by brackets and colours.

512 Figure 3. Maximum-likelihood tree showing the relationships of amphipod species based on a concatenated mtDNA
513 dataset for a) the two putative species *Paralicella tenuipes* and *Paralicella caperesca* and b) the two putative genera
514 *Abyssorchomene* and *Orchomenella*. Trees are rooted using *Hirondellea dubia*. Bayesian posterior probabilities and
515 maximum-likelihood bootstrap support are shown on branch nodes. 3a also shows species groups within *Paralicella*
516 indicated by bPTP analysis.

517 Figure 4. Maximum-likelihood tree showing the relationships between 25 *Eurythenes* species based on 16S sequence
518 data used in a previous *Eurythenes* study (Havermans et al., 2013) augmented by individuals of abyssal and hadal
519 depth from this study (shown with no accession numbers). Bayesian posterior probabilities and maximum-likelihood
520 bootstrap support are shown on branch nodes. Values less than 50% were not stated or depicted by an asterisk if
521 supported by the alternative method. Previously described groups of *Eurythenes* and their sampling locations have
522 been shown by brackets.

523 Supplementary Figure 1. Maximum-likelihood tree showing the relationships between 18 key amphipod species
524 based on 16S sequence data. Bayesian posterior probabilities and maximum-likelihood bootstrap support are shown
525 on branch nodes. Values less than 50% were not stated or depicted by an asterisk.

526 Supplementary Figure 2. Maximum-likelihood tree showing the relationships between 18 key amphipod species
527 based on COI sequence data. Bayesian posterior probabilities and maximum-likelihood bootstrap support are shown
528 on branch nodes. Values less than 50% were not stated or depicted by an asterisk.

529 Supplementary Figure 3. Maximum-likelihood tree showing the relationships between 18 key amphipod species
530 based on 18S sequence data. Bayesian posterior probabilities and maximum-likelihood bootstrap support are shown
531 on branch nodes. Values less than 50% were not stated or depicted by an asterisk.

532 Supplementary Figure 4. Maximum-likelihood tree showing the relationships between 18 key amphipod species
533 based on a mitochondrial concatenated dataset. Bayesian posterior probabilities and maximum-likelihood bootstrap
534 support are shown on branch nodes. Values less than 50% were not stated or depicted by an asterisk.

535 Supplementary Figure 5. Maximum-likelihood tree showing the relationships between 25 known amphipod species
536 based on 16S sequence variation for a single representative individual. Bayesian posterior probabilities and
537 maximum-likelihood bootstrap support are shown on branch nodes. Values less than 50% were not stated or

538 depicted by an asterisk. Superfamilies are denoted by brackets and species belonging to the Scopelocheiridae are
539 denoted by blue.

540 Supplementary Figure 6. Haplotype network of *Paralicella* spp. based on a mitochondrial concatenated dataset.

541 Circle size is proportional to the number of samples within a given haplotype and lines between haplotypes

542 represent mutational steps within alleles. Colours denote which species individuals have been assigned to –

543 *Paralicella tenuipes* in purple and *Paralicella caperesca* in orange.

544 Supplementary Figure 7. Haplotype network of *Eurythenes* spp. based on a mitochondrial concatenated dataset.

545 Circle size is proportional to the number of samples within a given haplotype and lines between haplotypes

546 represent mutational steps within alleles.

547 **References**

548 Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J.
549 Mol. Biol 215, 403–410.

550 Barnard, J., Karaman, G.S., 1991. The Families and Genera of Marine Gammaridean Amphipoda (Except
551 Marine Gammaroids) Part 1. Records of the Australian Museum.

552 Barnard, J., Shulenberger, E., 1976. Clarification of the abyssal amphipod, *Paralicella tenuipes* Chevreux.
553 Crustaceana. 31, 267-274

554 Barnard, J.L., 1964. Some bathyal Pacific Amphipoda collected by the USS Albatross. Pac. Sci. 18, 315–
555 335.

556 Beliaev, G.M., 1989. Deep sea ocean trenches and their fauna. Scripps Inst. Oceanogr.

557 Bell, J., 2008. A simple way to treat PCR products prior to sequencing using ExoSAP-IT. Biotechniques 44,
558 834.

559 Blankenship, L.E., Levin, L.A., 2007. Extreme food webs : Foraging strategies and diets of scavenging
560 amphipods from the ocean's deepest 5 kilometers. Limnol. Oceanogr. 52, 1685–1697.

561 Blankenship, L.E., Yayanos, A., Cadien, D.B., Levin, L.A., 2006. Vertical zonation patterns of scavenging
562 amphipods from the Hadal zone of the Tonga and Kermadec Trenches. Deep Sea Res. Part I Oceanogr.
563 Res. Pap. 53, 48–61. doi:10.1016/j.dsr.2005.09.006

564 Blankenship-Williams, L.E., Levin, L.A., 2009. Living Deep: a synopsis of hadal trench ecology. Mar.
565 Technol. Soc. J. 43, 137–143. doi:10.4031/MTSJ.43.5.23

566 Bousfield, E., Shih, C., 1994. The phyletic classification of amphipod crustaceans: problems in resolution.
567 Amphipacifica. 1, 76-134

- 568 Corrigan, L.J., Horton, T., Fotherby, H., White, T. a., Hoelzel, a. R., 2013. Adaptive Evolution of Deep-Sea
569 Amphipods from the Superfamily Lysiassoidea in the North Atlantic. *Evol. Biol.*
570 doi:10.1007/s11692-013-9255-2
- 571 d'Udekem d'Acoz, C., Havermans, C., 2012. Two new Pseudorchomene species from the Southern Ocean,
572 with phylogenetic remarks on the genus and related species (Crustacea: Amphipoda: Zootaxa 3310, 1–
573 50.
- 574 d'Udekem d'Acoz, C., Havermans, C., 2015. Contribution to the systematics of the genus Eurythenes SI
575 Smith in Scudder, 1882 (Crustacea: Amphipoda: Lysianassoidea: Eurytheneidae). *Zootaxa* 3971, 1–80.
576 doi:10.11646/zootaxa.3971.1.1
- 577 Dahl, E., 1957. From depths exceeding 6000 meters. *Galathea Rep.* 211.
- 578 Dahl, E., 1979. Deep-sea carrion feeding amphipods: evolutionary patterns in niche adaptation. *Oikos* 167–
579 175.
- 580 Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and
581 parallel computing. *Nat. Methods* 9, 772–772.
- 582 Dawson, M.N., Hamner, W.M., 2008. A biophysical perspective on dispersal and the geography of
583 evolution in marine and terrestrial systems. *J. R. Soc. Interface* 5, 135–50. doi:10.1098/rsif.2007.1089
- 584 De Broyer, C., 1983. Recherches sur la systématique et l'évolution des crustacés amphipodes gammarides
585 antarctiques et subantarctiques. *Dissert. Univ. Cath. Louvain.*
- 586 De Broyer, C., 1984. Evolution du complexe Orchomene Boeck (Amphipoda, Lysianassidae). *Annls. Soc. r.*
587 *zool. Belg.* 114, 197–198.
- 588 De Broyer, C., 1985a. Description de Falklandia gen. n. de l'Océan Austral et définition des Lysianassoidea
589 uristidiens (Crustacea, Amphipoda). *Zool. Scr.* 14, 303–312.
- 590 De Broyer, C., 1985b. Amphipodes lysianassoïdes nécrophages des îles Kerguelen (Crustacea). 1:
591 Orchomenella guillei n. sp. *Bull. du Muséum Natl. d'histoire Nat. Sect. A, Zool. Biol. écologie Anim.*
592 7, 205–217.
- 593 De Broyer, C., Lowry, J., Jazdzewski, K., Robert, H., 2007. Catalog of the Gammaridean and Corophiidean
594 Amphipoda (Crustacea) of the Southern Ocean with distribution and ecological data. *Census Antarct.*
595 *Mar. Life Synopsis Amphipoda South. Ocean.*
- 596 Donoghue, M., Sanderson, M., 1992. The suitability of molecular and morphological evidence in
597 reconstructing plant phylogeny. *Mol. Syst. plants* 340–368.
- 598 Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the
599 BEAST 1.7. *Mol. Biol. Evol.* 29, 1969–1973.
- 600 Englisch, U., Coleman, C.O., Wägelee, J.W., 2003. First observations on the phylogeny of the families
601 Gammaridae, Crangonyctidae, Melitidae, Niphargidae, Megalurotidae and Oedicerotidae
602 (Amphipoda). *J. Nat. Hist.* 37, 2461–2486.
- 603 Eustace, R.M., Kilgallen, N.M., Lacey, N.C., Jamieson, A.J., 2013. Population structure of the hadal
604 amphipod *Hirondellea gigas* (Amphipoda: Lysianassoidea) from the Izu-Bonin Trench. *J. Crustac. Biol.*
605 33, 793–801. doi:10.1163/1937240X-00002193

- 606 Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*. 39,
607 783–791.
- 608 Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of
609 mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol.*
610 *Biotechnol* 3, 294–299.
- 611 France, S.C., 1993. Geographic variation among three isolated populations of the hadal amphipod
612 *Hirondellea gigas* (Crustacea : Amphipoda : Lysianassoidea) 92, 277–287.
- 613 France, S.C., Kocher, T.D., 1996. Geographic and bathymetric patterns of mitochondrial 16S rRNA
614 sequence divergence among deep-sea amphipods, *Eurythenes gryllus*. *Mar. Biol.* 126, 633–643.
- 615 Fujii, T., Kilgallen, N., Rowden, A., Jamieson, A., 2013. Deep-sea amphipod community structure across
616 abyssal to hadal depths in the Peru-Chile and Kermadec trenches. *Mar. Ecol. Prog. Ser.* 492, 125–138.
617 doi:10.3354/meps10489
- 618 Havermans, C., Nagy, Z.T., Sonet, G., De Broyer, C., Martin, P., 2010. Incongruence between molecular
619 phylogeny and morphological classification in amphipod crustaceans: a case study of Antarctic
620 lysianassoids. *Mol. Phylogenet. Evol.* 55, 202–9. doi:10.1016/j.ympev.2009.10.025
- 621 Havermans, C., Sonet, G., d'Udekem d'Acoz, C., Nagy, Z.T., Martin, P., Brix, S., Riehl, T., Agrawal, S.,
622 Held, C., 2013. Genetic and Morphological Divergences in the Cosmopolitan Deep-Sea Amphipod
623 *Eurythenes gryllus* Reveal a Diverse Abyss and a Bipolar Species. *PLoS One* 8, e74218.
624 doi:10.1371/journal.pone.0074218
- 625 Hessler, R.R., Ingram, C.L., Yayanos, A.A., Burnett, B.R., 1978. Scavenging amphipods from the floor of
626 the Philippine Trench. *Deep. Res.* 25, 1029–1047.
- 627 J. K. Lowry, C.D.B., n.d. Alicellidae and Valettiopsidae, two new callynophorate families (Crustacea:
628 Amphipoda).
- 629 Jamieson, A., 2015. *The Hadal Zone Life in the Deepest Oceans*, Cambridge University Press. Cambridge
630 University Press.
- 631 Jamieson, A., Fujii, T., 2011. Trench Connection. *Biol. Lett.* 7, 641–643.
- 632 Jamieson, A.J., Fujii, T., Mayor, D.J., Solan, M., Priede, I.G., 2010. Hadal trenches: the ecology of the
633 deepest places on Earth. *Trends Ecol. Evol.* 25, 190–7. doi:10.1016/j.tree.2009.09.009
- 634 Jamieson, A.J., Fujii, T., Solan, M., Matsumoto, A.K., Bagley, P.M., Priede, I.G., 2009. Liparid and
635 macrourid fishes of the hadal zone: in situ observations of activity and feeding behaviour. *Proc. Biol.*
636 *Sci.* 276, 1037–45. doi:10.1098/rspb.2008.1670
- 637 Jamieson, A.J., Kilgallen, N.M., Rowden, A.A., Fujii, T., Horton, T., Lörz, A., Kitazawa, K., Priede, I.G.,
638 2011. Bait-attending fauna of the Kermadec Trench, SW Pacific Ocean: Evidence for an ecotone across
639 the abyssal–hadal transition zone. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 58, 49–62.
640 doi:10.1016/j.dsr.2010.11.003
- 641 Jamieson, A.J., Lacey, N.C., Lörz, A.-N., Rowden, A.A., Piertney, S.B., 2013. The supergiant amphipod
642 *Alicella gigantea* (Crustacea: Alicellidae) from hadal depths in the Kermadec Trench, SW Pacific
643 Ocean. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 92, 107–113. doi:10.1016/j.dsr2.2012.12.002

- 644 Kilgallen, N.M., Lowry, J.K., 2015. A review of the scopelocheirid amphipods (Crustacea, Amphipoda,
645 Lysianassoidea), with the description of new taxa from Australian waters. *Zoosystematics Evol.* 91, 1–
646 43. doi:10.3897/zse.91.8440
- 647 Leigh, J., Bryant, D., 2015. popart: full-feature software for haplotype network construction. *Methods Ecol.*
648 *Evol.* doi:10.1111/2041-210X.12410
- 649 Lowry, J., Broyer, C. De, 2008. Alicellidae and Valettiopsidae, two new callynophorate families (Crustacea:
650 Amphipoda). *Zootaxa* 1843, 57–66.
- 651 Lowry, J.K., Stoddart, H.E., 2011. The tryphosine genera *Photosella* gen. nov. and *Tryphosella* Bonnier,
652 1893 (Crustacea: Amphipoda: Lysianassoidea: Lysianassidae: Tryphosinae) in Australian waters.
653 *Zootaxa* 2956, 1–76.
- 654 Löytynoja, A., Goldman, N., 2010. webPRANK: a phylogeny-aware multiple sequence aligner with
655 interactive alignment browser. *BMC Bioinformatics* 11, 579.
- 656 Macdonald III, K.S., Yampolsky, L., Duffy, J., 2005. Molecular and morphological evolution of the
657 amphipod radiation of Lake Baikal. *Mol. Phylogenet. Evol.* 35, 323–343.
- 658 Palumbi, S., Martin, A., Romano, S., 1991. The Simple Fool's Guide to PCR, Version 2.0. Priv. Publ. Doc.
659 Compil. by S. Palumbi. Dept. Zool. Univ. Hawaii.
- 660 Rambaut, A., 2012. FigTree 1.4. Inst. Evol. Biol. Univ. Edinburgh.
- 661 Rambaut, A., Suchard, M., Xie, D., Drummond, A., 2014. Tracer v1. 6. Comput. Progr. Doc. Distrib. by the
662 author, website <http://beast.bio.ed.ac.uk/Tracer> [accessed 21 June 2015].
- 663 Ricklefs, R.E., 2004. A comprehensive framework for global patterns in biodiversity. *Ecol. Lett.* 7, 1–15.
664 doi:10.1046/j.1461-0248.2003.00554.x
- 665 Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models.
666 *Bioinformatics* 19, 1572–1574.
- 667 Swofford, D., 2002. PAUP* - phylogenetic analysis using parsimony (*and other methods). Version 4.
668 Sunderland. Sinauer Assoc.
- 669 Tamura, K., Stecher, G., Peterson, D., Filipowski, A., Kumar, S., 2013. MEGA6: Molecular Evolutionary
670 Genetics Analysis Version 6.0. *Mol. Biol. Evol.* 30, 2725–2729.
- 671 Wolff, T., 1960. The hadal community, an introduction. *Deep Sea Res.* 6, 95–124. doi:10.1016/0146-
672 6313(59)90063-2
- 673 Wolff, T., 1970. The concept of the hadal or ultra-abysal fauna. *Deep Sea Res. Oceanogr. Abstr.* 17, 983–
674 1003. doi:10.1016/0011-7471(70)90049-5
- 675