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

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Highlights:

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

Abstract

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

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

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

1. Introduction

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

This level of geographic isolation, coupled with potent selection pressures that promote local adaptation, has meant that hadal trenches have traditionally been considered centres of high 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

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

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

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

2. Materials and Methods

2.1. Sample Collection

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

2.2. DNA Extraction and PCR Amplification

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

contained 0.2mM each dNTPs, 2.5mM $MgCl_2$, 0.5µM each primer, 0.5U of *Taq* DNA polymerase (Bioline), 10-40ng DNA template in 1x NH4 buffer (Bioline) in a total reaction volume of 20µl. PCR conditions for 16S amplicons were: initial denaturation at 94°C for 1 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing 50°C for 30 s, extension at 72°C for 30 s before a final elongation step at 72°C for 1 min. The PCR conditions for COI amplicons were: initial denaturation at 94°C for 1 min, followed by 30 cycles of denaturation at 94°C for 45 s, annealing 45°C for 45 s, extension at 72°C for 45 s before a final elongation step at 72°C for 1 min. PCR conditions for 18S amplicons were: initial denaturation at 94°C for 1 min, followed by 30 cycles of denaturation at 94°C 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 1 min.

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

2.3. Phylogenetic analyses

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

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

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

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

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

3. Results

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

The Alicellidae family have been described as comprising of the genera *Apotectonia*, *Diatectonia*, *Transtectonia*, *Alicella*, *Paralicella* and *Tectovalopsis* (Lowry and De Broyer, 2008). Here we present molecular data on *Alicella*, *Paralicella* and *Tectovalopsis* which shows these three genera are reciprocally monophyletic, but do not group as a monophyletic family given that *Valettietta* is sister to *Paralicella*, and *Cyclocaris* is sister to *Tectovalopsis* in the concatenated dataset, and *Paralicella* is sister to *Hirondellea* in the 16S dataset. Taxonomic revision should aim to correctly delimit genera in the Alicellidae. This will require more molecular data since the most robust phylogeny can be difficult to ascertain given the instability of internal topologies and poor node support. Discordance 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 differentiation 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 *Abyssorchormene* 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

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

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

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

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

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

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

5. Conclusions

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

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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								
Alicella gigantea	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	---	---
Paralicella caperesca	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	---	---
Paralicella tenuipes	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	---	---
Tectovalopsis wegeneri	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								
Cyclocaris 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								
Cyphocaris sp.	Peru-Chile	1037m	25°58'S	70°52'W	KP456133	KP713952	---	---
Eurytheneidae								
Eurythenes gryllus	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								
Hirondellea brevicaudata	New Hebrides	6948m	20°38'S	168°36'E	KP456082	KP713900	---	---
Hirondellea dubia	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	---	---
Hirondellea gigas	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	---	---
Hirondellea sonne	Peru-Chile	7050m	17°25'S	73°37'W	KP456073	---	---	---
	Peru-Chile	7050m	17°25'S	73°37'W	KP456074	---	---	---
Hirondellea	Peru-Chile	7050m	17°25'S	73°37'W	KP456076	---	---	---

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

496

497

498 Table 2. Output of bPTP species delimitation analysis.

Taxon	No. Seqs	(H)	bPTP Parameters	Delimitation Probabilities T(Max-Likelihood/Heuristic Search)
Eurythenes 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.
Paralicella 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 *Hirondellea*
Scopelocheiridae	Reinstate *Bathycallisoma* as a genus Review *Bathycallisoma* as a member of the Scopelocheiridae
Uristidae	Review the polyphyly of the Uristidae with *Abyssorchomene* and *Orchomenella*
Genera	
Abyssorchomene	Further revise the polyphyly of *Abyssorchomene* with *Orchomenella*
Bathycallisoma	Revise the collapse of *Scopelocheirus* into *Bathycallisoma*
Species	
Abyssorchomene musculosus	Review morphology used to distinguish *A. musculosus* as a separate species
Eurythenes gryllus	Further revision of species in the *Eurythenes* following (d'Udekem d'Acoz and Havermans, 2015)
Hirondellea wagneri	Review morphology used to determine *H. wagneri* as it does not fall within *Hirondellea*
Paralicella tenuipes/Paralicella caperesca	Review morphology used to determine *Paralicell*a sp. with the inclusion of molecular data to verify species delimitation
Valettietta gracilis	Review morphology used to determine *V. gracilis* as it does not fall within *Valettietta*
Uristes sp. nov.	Review morphology used to determine *Uristes* sp. nov. as it falls within *Hirondellea*

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.

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

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

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

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

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

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

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

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

Supplementary Figure 6. Haplotype network of *Paralicella* spp. based on a mitochondrial concatenated dataset. Circle size is proportional to the number of samples within a given haplotype and lines between haplotypes represent mutational steps within alleles. Colours denote which species individuals have been assigned to – *Paralicella tenuipes* in purple and *Paralicella caperesca* in orange.

Supplementary Figure 7. Haplotype network of *Eurythenes* spp. based on a mitochondrial concatenated dataset. Circle size is proportional to the number of samples within a given haplotype and lines between haplotypes represent mutational steps within alleles.

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