Adaptive Evolution of Deep-Sea Amphipods from the Superfamily Lysiassanoidea in the North Atlantic

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Abstract

In this study we reconstruct phylogenies for deep sea amphipods from the North Atlantic in order to test hypotheses about the evolutionary mechanisms driving speciation in the deep sea. We sequenced five genes for specimens representing 21 families. Phylogenetic analyses showed incongruence between the molecular data and morphological taxonomy, with some morphologically distinct taxa showing close molecular similarity. Approximate dating of nodes based on available calibration suggested adaptation to the deep sea around the Cretaceous-Palaeogene boundary, with three identified lineages within the deep-sea radiation dating to the Eocene-Oligocene transition. Two of those lineages contained species currently classified in multiple families. We reconstructed ancestral nodes based on the mouthpart characters that define trophic guilds (also used to establish the current taxonomy), and show a consistent transition at the earliest node defining the deep-sea lineage, together with increasing diversification at more recent nodes within the deep-sea lineage. The data suggest that the divergence of species was adaptive, with successive diversification from a non-scavenging ancestor to ‘opportunistic’, ‘obligate’ and ‘specialised’ scavengers. We propose that the North Atlantic species studied provide a strong case for adaptive evolution promoted by ecological opportunity in the deep sea.

Keywords: deep sea; amphipod; invertebrate; adaptation; phylogenetics

1. Introduction

It has been proposed that effectively continuous marine environments with few obvious geographical barriers should allow broad dispersal and promote panmixia (reviewed in Palumbi 1994), inhibiting reproductive isolation and speciation (known as the 'marine speciation paradox'; Bierne et al. 2003). There are two main hypotheses generally put forward to explain the observed patterns of speciation in the marine environment. One is that species divergence is the result of ecological speciation (Puebla 2009) generating adaptive radiations, when multiple lineages evolve from a single common ancestor at a rapid pace. The other involves differentiation across geographic barriers which may include oceanographic factors such as current systems or thermal fronts (though typically less clearly defined than boundary systems in terrestrial environments). According to the first idea, relaxed ecological constraints (abundant resources and reduced competition) may create ecological opportunity in the colonisation of new habitats resulting in adaptive divergence (Schluter 1996; Schluter 2000; Puebla 2009). For example, speciation in the Pacific rockfish genus (*Sebastes*) is associated with divergence in habitat depth and depth-associated morphology, in the absence of geographic barriers (Ingram 2011).

According to the second idea, tectonically-driven changes to ocean basins or oceanographic factors may generate physical barriers to dispersal in vicariance events resulting in allopatric or parapatric speciation (reviewed in Palumbi 1994). Fully allopatric speciation has been observed across barriers such as the Isthmus of Panama (e.g. Marko 2002) but such clear examples are relatively rare in the marine environment. The same mechanisms that generate vicariance may generate ecological opportunity by releasing habitat that can then be colonised.

66 Adaptive radiations can be difficult to identify, but should be characterised by
67 a correlation between phenotype and environment, novel phenotypes providing a
68 selective advantage (difficult to prove without experimentation), and speciation
69 should be rapid, with the emergence of multiple species from a recent common
70 ancestor (see Schluter 2002). They have been frequently described for terrestrial and
71 freshwater ecosystems, including well-known cases such as the Galapagos finches
72 (e.g Schluter & Grant 1984) and cichlids of the African rift lakes (e.g. Seehausen
73 2006).
74 In aquatic ecosystems, habitat shifts from marine to freshwater have been
75 shown to promote species diversification (e.g. Hou et al. 2011). Adaptive radiations
76 described for marine systems include reef fish (e.g. Taylor & Hellburg 2005; Puebla
77 2007) and Antarctic fish species (Clarke & Johnson 1996). However, habitat shifts
78 from shallow to deep-sea environments have been less well supported in the literature
79 (but see Distel et al. 2000). Historically, deep-sea environments were thought to
80 harbour reduced species diversity due to harsh environmental conditions (see Hessler
81 & Sanders 1967). It was further suggested that rates of evolution were much slower
82 at depth, leading to the idea that the deep sea was a refuge for ancient relics
83 (Zenkevitch & Birstein 1960). More recently however, greater species diversity has
84 been documented in various groups in the deep sea, including bivalves, gastropods,
85 polychaetes and isopod crustaceans (reviewed in Wilson & Hessler 1987; Grassle
86 1989).
87 Here we examine the phylogeny of deep-sea amphipods in order to investigate
88 the evolutionary processes driving their speciation in the deep sea. Amphipods
89 occupy almost all aquatic environments as well as some subterranean and terrestrial
90 habitats (Barnard & Karaman 1991). Despite their widespread distribution, the

relationships among and within the major amphipod taxonomic groups are poorly resolved, possibly due to the effects of convergent evolution (Englisch et al, 2003; Macdonald et al, 2005; Hou et al; 2007; Fiser et al, 2008; Ito et al, 2008; Havermans et al, 2010). We focus our analysis on amphipods collected at our study sites at the mid-Atlantic ridge, which can be classified within the superfamily Lysianassoidea (the taxonomy of which remains controversial, see below). Lysianassoid amphipods can be found from the colder waters of the Polar Regions (De Broyer *et al.*, 2004) to the tropics (Lowry & Stoddart, 2009) and from the intertidal to the deepest ocean trenches (Jamieson et al., 2010). Many members of the Lysianassoidea are known to be epibenthic, and infaunal scavengers and carnivores. They are numerically and taxonomically the most important group of deep-sea scavengers (Wolff, 1970; Hessler *et al.*, 1978; Smith, 1985; Thurston, 1990).

There have been numerous studies of the amphipod scavenging fauna in the deep sea, including biodiversity, distribution, ecology, taxonomy, and respiration and pressure effects (e.g. Hargrave, 1985; De Broyer, 2004; Premke & Graeve, 2009; Thurston 1979; 1990). However, despite the fact that the group contains some of the most primitive amphipods (Bousfield & Shih, 1994), little attention has been paid to studies of the molecular phylogeny of this group, and the Amphipoda in general have a history of taxonomic instability in the higher ranks (Superfamily and higher) to the extent that that they are generally listed alphabetically (e.g. see Martin & Davis, 2001). However, a recent study by Havermans et al (2010), looked at the molecular phylogeny of Antarctic lysianassoids in the families, Lysianassidae and Uristidae, based on nuclear 28S rRNA and mitochondrial cytochrome oxidase subunit I genes, and showed that the molecular and morphological taxonomies of these groups are largely incongruent and did not support the monophyly of several of the currently

proposed genera (including *Abyssorchomene, Orchomenella, Pseudorchomene* and *Falklandia*). In particular, their study indicated the need for a revision of the higher level systematics within the Lysianassoidea due to the apparent polyphyly of the Lysianassidae (Tryphosinae).

The major problems appear to stem from the use of the mouthpart morphology in higher level classification. In scavenging amphipods the mouthparts have evolved to fill an ecological niche associated with necrophagy in a sparse environment (Thurston, 1979, Dahl, 1979, De Broyer et al., 2004). For example, species from at least two groups (Uristidae and Lysiassanidae) have evolved morphology characteristic of 'opportunistic' scavengers with a triturative mandibular molar for grinding food and shorter foregut (see De Broyer et al., 2004). It is probable that this has occurred more than once during the evolution of the Lysianassoidea. Other studies of amphipod phylogenetics have also illustrated that morphological and molecular evolution may become uncoupled during their radiation, giving rise to close genetic relatives with extreme morphological and ecological divergence (Macdonald et al., 2005).

In this study we use a multi-locus approach to generate a consensus tree with strong congruence, and consider the resultant lineage structure in the context of phenotypic characteristics related to foraging. We model the evolution of phenotypic traits along the phylogeny in order to test the hypothesis that phenotype and lineage structure are correlated. We assess diversification rate changes among lineages to test the hypothesis that there was an increased diversification rate in the deep-sea lineage, as expected in association with adaptive radiations. We estimate node dates based on published calibration points and test the hypothesis that the amphipods in the deep-sea environments of the North Atlantic radiated when habitat associated with

141 foraging opportunity was made available by environmental change associated with
142 geologic transitions.
143
144 **2. Materials and Methods**
145 *2.1 Sampling*
146 The majority of specimens (see Table S1) used for this study were collected
147 using baited (with mackerel) traps at ~2500m depth, during several expeditions to the
148 Mid-Atlantic Ridge (MAR; Table S2; see Horton et al, 2013 for full sampling details).
149 This represented an extensive sampling program and involved the screening of 4900
150 ethanol-preserved specimens from which the included species were identified.
151 Further samples came from baited traps at the Crozet Islands at 4192m (Cousins et al,
152 in press) and offshore Angola at 2002m. Additional material was obtained from the
153 Museum für Naturkunde in Berlin for 18 outgroup species representing 14 families
154 (sequencing 1-2 samples per species; Table S1) from a range of habitats including
155 marine pelagic and benthic, subterranean groundwater and freshwater. Fourteen
156 ingroup species represented six families from the superfamily Lysianassoidea and one
157 from the family Alicellidae (sequencing 1-4 specimens per species; Table S1).
158 Although baited traps preferentially collect necrophagous amphipods (see Horton et
159 al. 2013), there was good species representation for the Lysianassoid taxa.
160 The species *Abyssorchomene chevreuxi* and *A. abyssorum*, *Orchomenella*
161 *gerulicorbis*, *Paralicella caperesca* and *Eurythenes gryllus*, are thought to have a
162 cosmopolitan distribution, whereas the remaining eight ingroup species are believed
163 to be confined to the Atlantic Ocean. These species include two recently described as
164 new to science (*Hirondellea namarensis*, Horton & Thurston 2013; *Centromedon zoe*,
165 Horton & Thurston 2011) and a further 5 species as yet undescribed but most

probably also new to science (*Cyclocaris* sp. nov., *Tmetonyx* sp. nov., *Orchomene* aff. *oxystoma*, *Orchomene* aff. *pectinata*, *Paracallisoma* sp. 1; see Horton et al. 2013). We focus on the ingroup of species present in the deep-sea habitat near the mid-Atlantic ridge, and the resolution of higher-level taxonomic groupings is beyond the scope of this study. Outgroups were included to provide calibration points and support for assessing the topology of the ingroup.

2.2 DNA Extraction and Amplification

Total genomic DNA was extracted from pereopods or whole organisms using a phenol-chloroform protocol, and many species were represented by multiple specimens (see Table S1). Amplification of the mitochondrial 16S and COI loci, and the nuclear, 18S and 28S rRNA and Histone 3 loci were carried out using both published primers and primers designed in this study (based on the comparison of published sequences in GenBank; Table S3). The reaction mix (50µl) contained a final concentration of 0.2mM each dNTP, 1.5mM $MgCl_2$, 0.5µM each primer and 1.25 units of Taq DNA Polymerase (Promeaga GoTaq). The PCR conditions were as follows: 2 minutes at 95 °C followed by 35 cycles of 40s at 94 °C, 40s at T_a °C (given in Table S3) and 40s at 72 °C, and a final extension for 10 minutes at 72 °C. Purified products were sequenced in both directions using an ABI DNA sequencer. All loci were sequenced for all samples except for a subset which could not be amplified, and some which were available from the Genbank database (see Table S4 for details and accession numbers).

2.3 Phylogenetic Reconstruction

Sequences were aligned using Clustal X (Thompson et al. 1997) after checking sequence accuracy through the assessment of chromatograms and comparison of forward and reverse sequences (no errors detected). As a screen against the inclusion of pseudogenes in the analyses, coding gene sequences were translated into amino acids (using MEGA; Tamura et al. 2011) and checked for stop codons. Phylogenetic analyses were conducted on separate and combined data sets. Parsimony and maximum likelihood analyses were carried out using PAUP 4.0b10 (Swofford 2002). The best evolutionary model was determined using JModeltest 0.1.1 (Posada 2008). Alignment gaps were treated as missing data. Heuristic searches were carried out with random sequence addition (100 replicates) and using tree bisection reconnection (TBR) branch swapping. Branch support was estimated with bootstrap analysis using 1000 replicates. Partitioned Bremer support was used to evaluate the contribution of individual data partitions in the combined analysis (Baker and DeSalle, 1997). This was done by generating constrained trees in TreeRot V.2 (Sorenson 1999) and analysing them in combination with PAUP 4.0b10.

Bayesian analyses were conducted on the combined dataset using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) with five partitions. The best-fit model for each partition was selected using JModeltest 0.1.1 (Posada 2008). Each Bayesian analysis was run for ten million generations sampling every 100 generations (every 1000 generations was also tested, with no change in outcome). The level of convergence was monitored and the 'burn-in' value set accordingly. The first 25% of trees (25,000) were discarded and the remaining trees were used to reconstruct a consensus tree and estimate Bayesian posterior probabilities (BPP). The strategy is summarised in Table S5

2.4 Molecular Dating Analysis

Fossil records of amphipod crustaceans are rare, however several specimens have been found in Baltic amber, dated late Eocene, c. 35-40 Ma. These specimens most resemble the *Niphargus* species of the subgenus *Phaenogammarus* (Jazdzewski and Kulicka 2000; Coleman & Myers 2000), *Paeleogammarus*, a fossil species of the Family Crangonyctidae (Jazdzewski and Kulicka 2002; Coleman 2004) and *Stygobromus* sp. (Coleman 2006). We can therefore date the appearance of these lineages prior to the late Eocene and can use this date for molecular clock calibration. We used this date as an approximation for the upper boundary of divergence time of this monophyletic group of species. The origins of the genus *Gammarus* is proposed to have been ~61 Ma (Hou et al. 2011), and this provided a further reference point to test against dates determined in this analysis (though based on a molecular clock estimate, and therefore not as robust as the fossil calibrations).

The divergence times were obtained by applying a Bayesian method implemented in BEAST 1.6.1. We used the relaxed molecular clock model, GTR+ I+G for the substitution model (for all genes except 16S where HKY+I+G was used as above), and a normal distribution with SD of 1 as priors on the calibration node to accommodate for calibration uncertainty. The Markov chain Monte Carlo was run for 50 million generations and sampled every 1,000 generations. Two independent runs were performed to help assess convergence which was examined using the effective sample sizes of each parameter (>200) in TRACER v1.4 (Rambaut & Drummond 2004). The last 40 million generations were used to construct the maximum clade credibility tree and the associated 95% highest posterior density distributions around the estimated node ages.

2.5 Morphological Analyses

We used our consensus phylogeny to examine trait evolution for seven morphological traits (Table 1). The traits were chosen based on gut and mandible morphology (as discussed in De Broyer et al, 2004) to define trophic guilds according to foraging strategy. In particular, species were distinguished as non-scavenger, obligate scavenger, obligate specialist or opportunistic scavenger. These same traits are often used in support of the classification of Lysianassoidea. We used a Bayesian method implemented in the *BayesTraits* v 1.0 package (available at www.evolution.rdg.ac.uk; Pagel et al. 2004) to reconstruct ancestral morphological character states at selected nodes in the phylogeny. BayesTraits uses a reversible-jump Markov chain Monte Carlo (MCMC) method to derive posterior probabilities on the trait values at ancestral nodes (Pagel et al. 2004). BayesMultiState was selected as the model of evolution, allowing for rapid state changes. We used a hyperprior approach specifying a gamma prior with its mean and variance seeded from uniform distributions on the interval 0 to 10. Thus, acceptance rates in the preferred range of 20–40% were achieved as recommended (Pagel et al. 2004). The average acceptance rate was 32.4%.

2.6 Diversification Rate Shifts

We used the program SymmeTree v1.1 (Chan & Moore 2004) to test the hypothesis that the branches of our amphipod phylogeny have diversified at significantly different rates, with respect to a specified node. The tree analysed included only a single copy of each known or putative species. As this tree showed the same topology as our consensus tree (Figure 1), we undertook the single-tree analysis in SymmeTree. We used the random resolution option for resolving

polytomies (only present in outgroup). We report results using the taxon-size sensitive equal-rates Markov random-branching model (TSS-ERM) for the random resolution of polytomies, as the authors note that this is conservative with respect to the null hypothesis (no significant diversification rate variation). The default number of 100,000 replicates was applied for random resolution and for approximating null distributions. Whole-tree rate variation is estimated in the program based on two rate-shift statistics (M_{Π} and M_{Σ}; Chan & Moore, 2002) and a tree imbalance statistic (B_1; Shao and Sokal, 1990). To locate the position of diversification rate shifts we followed default settings and report the significance levels for the two shift statistics, delta 1 and delta 2 (see Chan & Moore 2004).

3. Results

3.1 Phylogenetic Reconstruction

A final combined dataset of 2,442bp (18S – 1141bp, 28S – 345bp, H3 – 242bp, 16S – 354bp and COI - 346bp) was used in the analyses. Different selection criterion (Akaike Information Criterion and Bayesian Information Criterion) identified the same best-fit substitution model: the general time-reversible substitution model (GTR + I + G) for all partitions except 16S, where it identified the Hasegawa, Kishino and Yano model (HKY + I +G) as the best-fit model. A lack of multiple or ambiguous peaks confirmed that nuclear genes for the individuals included were homozygous and not compromised by multiple sequences from different isoforms of a given locus. Partitioned Bremer analysis provided support for homogeneity amongst genes in the combined dataset, though not all genes were informative for all nodes (Figure 1, Figure S2). Similar topologies were obtained for the separate data sets of all five genes, and conflicting nodes received low support. In the combined analyses,

290 lineages were supported by high bootstrap values (ML analysis) and Bayesian
291 posterior probabilities (Figure 2; Parsimony showed similar support – data not
292 shown). Note that although multiple specimens of a given species are including in the
293 tree shown, trees including only one representative of each showed the same lineage
294 structure (e.g. Figure 1).

295 The phylogeny supports four main lineages, one representing the outgroup,
296 and the other three (labelled A, B & C in Figure 2) the deep-sea species. Within the
297 deep-sea lineage, genera are all shown to be monophyletic however the parsimony,
298 maximum likelihood analyses and Bayesian inference all gave clear evidence for
299 polyphyly for the families Uristidae and Lysianassidae (Figure 2). In all analyses,
300 three main lineages consistently received high bootstrap values and Bayesian
301 posterior probabilities with one of these lineages incorporating species from four
302 named families, and another incorporating two (Figure 2). *Hirondellea namarensis*,
303 recently described as new to science (see Horton & Thurston 2013) and
304 *Paracallisoma* sp. 1 are sister-species to the three well-supported lineages, which is
305 consistent with the understanding that these genera are more 'primitive' scavengers,
306 based on their morphology, without close relationships to other extant lysianassoid
307 groups (Lowry & Stoddart 2010).
308
309 *3.2 Ancestral State Reconstruction & Molecular Dating Analysis*

310 The ancestral state reconstruction (see Table 1; Figure S1) indicates that five
311 shifts have occurred: one transition from non-scavenger to opportunistic scavenger;
312 two independent shifts from opportunistic scavenger to obligate scavenger and one
313 shift from obligate to 'specialised' scavenger in *Stephonyx biscayensis* (Figure 3).
314 The transition from non-scavenger to opportunistic scavenger occurred at the most

315 recent common ancestor (MRCA) to the deep-sea species (node a, Figure 4). Based
316 on the reference points and our data, this node can be dated to ~60 Ma (95%HPD: 45
317 – 90 Ma) overlapping the transition to the Palaeogene. The origins of the genus
318 *Gammarus* (dated at ~61 Ma (95%HPD: 45 – 83Ma) by Hou et al. 2011) is illustrated
319 with a black dot in Figure 3 and is dated to 55 - 105 Ma (95 % HPD) in our study.
320 The shifts from opportunistic to obligate scavenger occur independently twice,
321 firstly in the root of Lineage C (Figure 2) dated at 40 Ma (95%HPD: 30 – 65 Ma)
322 (node d, Figure 4) and then again more recently along the branch to *Abyssorchomene*
323 and *Orchomenella* dated at 20 Ma (95%HPD: 15 - 30 Ma) (node e, Figure 4). The
324 shift from obligate to 'specialised' scavenger in *Stephonyx biscayensis* can be dated to
325 33 Ma (95%HPD: 24 – 50 Ma; node c, Figure 4). The fossil evidence for the
326 *Stygobromus*, *Crangonyx*, and *Niphargus* specimens found in amber dates the origin
327 of their shared lineage to before 35-40 Ma. This provides a calibration node at the
328 base of this lineage (illustrated with a grey dot, Figure 4).
329 In general, over all traits, lineage A retains the state of the ancestral node
330 representing the origin of the deep-sea lineage, whereas multiple shifts occur in the
331 other two lineages and the end node states are mostly derived (see Table 1; Figure
332 S1). The radiations of lineages A, B and C (Figure 2) can be dated to approximately
333 35 Ma (95%HPD: 28 – 55 Ma), 33 Ma (95%HPD: 26 – 50 Ma) and 40 Ma (95%HPD:
334 30 – 65 Ma) respectively (Figure 4).
335
336 *3.4 Diversification Rate Shifts*
337 Among the four tests for significant diversification across the full tree, three
338 were significant after Bonferonni correction (I_c = 0.008; M_{II} = 0.004; M_{Σ} = 0.005) and
339 one was not (B_1 = 0.107). The results of two likelihood-ratio tests to locate shifts in

diversification identified one node, closest to significance at the 0.05 level, indicated in Figure 2 by a star, and reflecting the base of the deep-sea lineage ($p\Delta 1 = 0.066$; $p\Delta 2 = 0.066$).

4. Discussion

4.1 Polyphyly of Scavenging Amphipods

Our phylogeny does not support the current taxonomy within our focal deep sea ingroup. Most genera were monophyletic (apart from paraphyly in *Abyssorchomene*) however, two families, Uristidae and Lysianassidae, are polyphyletic, appearing in multiple well-supported lineages (Figure 2). One of these lineages contains specimens from four different families (Uristidae, Alicellidae, Eurytheneidae, Cyclocaridae) as currently classified (lineage C in Figure 2). Our focus in this study is on the nature of the radiation of this group of species in the deep-sea environment, and most details about the taxonomy will be published elsewhere. However, we focus briefly on the positioning of *Orchomenella gerulicorbis* and *Stephonyx biscayensis* as illustrative.

The molecular data suggests that *Orchomenella gerulicorbis* would be better placed in a clade alongside *Abyssorchomene*, and it could perhaps be argued that since the genus *Abyssorchomene* is likely a derived group of deep-sea scavengers within the Orchomenid group, that *Abyssorchomene* should be placed within the family Lysianassidae rather than placing *Orchomenella* within the Family Uristidae. Havermans et al. (2010) also found a cluster of *Orchomenella* (*Orchomenopsis*) *cavimanus* and the clade of *A. chevreuxi*, *Abyssorchomene* sp. and *A. scotianensis*, and suggested similar changes to the higher level taxonomy of that group.

364 The situation of *Stephonyx biscayensis* is more problematic. It has been
365 classified as Uristidae based, among other characteristics, on the possession of the 7/4
366 crown arrangement of setae on the Maxilla 1 outer plate and the setose tongue
367 mandibular molar. The genus does not have the subchelate (or imperfectly subchelate)
368 gnathopod 1 as defined by Hurley 1963 (see Figure 5), used by Lowry & Stoddart
369 (1992) as a defining characteristic of the Uristidae. Lowry & Stoddart (1997)
370 acknowledge that the assumption that the 7/4 crown arrangement could be used as a
371 synapomorphy for the Uristidae lineage, is tenuous. This is a concern supported by
372 our phylogeny, which shows the Uristidae to be polyphyletic, and therefore suggests
373 homoplasy for this characteristic.
374 It is possible that the chelate gnathopod 1 of *S. biscayensis* (Figure 5) is an
375 adaptation to 'picking' carcasses rather than cutting and slicing flesh as practised by
376 other scavengers, and may indicate a more derived state of this genus from a primitive
377 scavenging ancestor. This and the results of the phylogenetic analysis suggest that
378 *Stephonyx* would be better placed in a new Family. However the nature of this level
379 of classification requires further assessment beyond the scope of this study, in
380 particular given the presence of four named families in the lineage shared by *S.*
381 *biscayensis* in our phylogeny.
382
383 *4.2 Evolution of Trophic Adaptation in the Deep Sea*
384 The current classification of deep-sea scavenging amphipod species is based
385 on traits representing trophic adaptations, especially the morphology of the
386 mouthparts and digestive tract (e.g. Lowry & Stoddart, 1992; 1997; De Broyer et al.,
387 2004; Dahl, 1979). *Centromedon zoe* and *Tmetonyx* sp. 1, (in lineage A, Figure 2)
388 along with *Orchomene* aff. *oxystoma* and *O.* aff. *pectinata* (lineage B) are

389 characteristic of ‘opportunistic’ scavengers, with a triturative mandibular molar for
390 grinding food and shorter foregut (see De Broyer et al., 2004). The results of the
391 Bayestraits analysis show that such traits are likely to have first appeared in the
392 scavenging ancestor (Table 1; Figures 3 & S1). This opportunist ancestor then
393 diverged firstly into a group of genera primarily (but not exclusively) inhabiting deep-
394 sea habitats (*Eurythenes, Paralicella,* and *Cyclocaris,)* and then adapted to obligate
395 necrophagy (lineage C, Figures 2 - 4) with several morphological modifications
396 (Thurston, 1979, Dahl, 1979, De Broyer et al., 2004). This occurred at approximately
397 30 Ma (Figure 4) as discussed below. The split between lineage A and B occurred
398 subsequently, and the ancestral characters are retained in lineage one (*Centromedon*
399 and *Tmetonyx* species) but lineage B is shared by both opportunist (*Orchomene*
400 complex of genera) and obligate (*Abyssorchomene* genus) scavengers (Figure 3, Table
401 1).

402 The morphological adaptations towards necrophagy in scavenging amphipods
403 have been reported elsewhere (Thurston, 1979, Dahl, 1979, De Broyer et al., 2004)
404 and in general the changes include a modification of the mandibular molar (Figure
405 S1) from subcolumnar with a triturative surface (in opportunistic scavengers) capable
406 of tearing and grinding tissue, through to a ridge-shaped mandibular molar with
407 reduced triturative surface in more derived scavengers (e.g *Abyssorchomene*), and
408 ultimately, in those species presumed to be obligate necrophages, to a non-triturative
409 conical flap (e.g. *Hirondellea*, *Eurythenes* and *Paralicella*; De Broyer et al., 2004;
410 Figure S1). These adaptations allow larger fragments of food to be passed directly
411 into the oesophagus, and combined with increased capacity for dilation of the midgut,
412 mean that these species are capable of ingesting 10 times their body weight in food
413 (Thurston, 1979). This suggests that deep-sea scavengers have the potential to survive

for long periods of time without feeding, which is an obvious adaptation to life in an environment where food supply is sparse (Smith & Baldwin, 1982). *S. biscayensis* is probably adapted as a 'specialist' scavenger and is the only species in this study to have adapted the 'pincer'-like chelate gnathopod 1, discussed above (see Figure 5).

Our analyses indicate that traits associated with necrophagy have arisen independently multiple times during the radiation of Lysianassoidea in the deep sea, consistent with data presented by Havermans et al. (2010). The fact that multiple end character states have arisen, some independently multiple times, suggests that the deep-sea scavenger species evolved into novel niches as a result of ecological opportunity. Adaptive radiations have been seen in freshwater amphipods elsewhere (Hou et al 2011), and the most extreme example is from Lake Baikal (MacDonald et al. 2005).

We used a method that assesses whole-tree topology to determine if there is a signal for rate differentiation within the tree, indicative of an adaptive radiation. Although not all tests were significant at the 0.05 level, there was evidence in support of rate differentiation, and the suggestion that this occurred in association with the deep-sea lineage. These methods are affected to some extent by taxon sampling, and our ingroup is not meant to be an inclusive representation of the broader group, instead focussing on those species found in the North Atlantic near the mid-Atlantic ridge. Our outgroup reflects available database sequences to some extent. However, the ingroup is if anything under-sampled, which may be expected to make it harder to identify a signal for lineage differentiation.

4.3 Deep-Sea Colonisation and Radiations

Our results indicate that the colonisation of the deep-sea environment by a shallow water ancestor occurred at ~70 Ma at the Cretaceous-Palaeogene boundary and that the three identified lineages among the deep-sea scavenging species date to the Eocene-Oligocene boundary. Accurate dating with such a limited fossil record is difficult, although when interpreted in the context of geological changes, these estimated date ranges, though broad, are realistic and a good fit with a study on the timing of the freshwater diversification of *Gammarus* sp. (Hou et al. 2011; see Figure 4). Further, the available fossils place a minimum date on nodes at the same level in the phylogeny, sometime before the late Eocene.

The Cretaceous-Palaeogene boundary coincides with the timing of the transition of the North Atlantic from narrow, silled basins to the deep marine trenches of the modern Atlantic (Norris et al. 2001). This provided habitat for colonisation in the deep sea, and likely promoted the adaptations towards necrophagy that define this lineage. The Eocene-Oligocene transition was characterised by a climate change from 'hothouse' to 'icehouse' (Lear et al. 2008). During this period atmospheric CO_2 levels decreased, deep-sea waters cooled (Miller et al, 1987; Zachos et al. 2001) and primary productivity increased (Lear et al. 2008; Pearson et. al. 2008). It is suspected that this cooling during the Palaeogene may have caused extinctions in some taxa and this has been well documented for deep-sea Foraminifera and Ostracoda (Benson et al., 1985; Kaiho, 1998). Our results suggest that this is not the case for deep-sea Amphipoda for which the Eocene/Oligocene cooling may instead have been beneficial providing the opportunity for adaptive speciation.

This period is also concurrent with an increased speciation rate in cetaceans, a radiation that is thought to be driven by the development of the Antarctic circumpolar current and increased silicate upwelling which may have spurred the evolution of

463 filter-feeders (Steeman et al. 2009). Increased cetacean diversity and abundance,
464 along with the increased primary productivity during this time, would increase the
465 availability of carcasses on which scavenging amphipods could feed, although of
466 course we have no direct evidence of an association with amphipod diversification.
467 The hypothesis that habitat shifts promote adaptive speciation via ecological
468 opportunity is well studied in terrestrial systems. We propose that the deep-sea
469 Lysianassoidea provide a strong case in support of this hypothesis in the marine
470 environment. The development of the deep-sea habitat, coupled with increased
471 productivity and the availability of novel food resources free from competitive
472 restraints could have provided this opportunity.
473
474
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486
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725

Figure Captions

Figure 1: Strict consensus tree built using PAUP, indicating Bremer Support Indices for each gene (in order: 18S, 28S, COI, H3, 16S) at each node.

Figure 2: Bayesian phylogenetic tree with posterior probabilities based on the combined analysis (18S, 28S, COI, H3, 16S). The three deep-sea clades are labelled A, B & C (*c.f.* Table 1). Families are labelled: Uristidae, ①; Lysiassanidae, ②; Alicellidae, ③; Eurytheneidae, ④; Cyclocaridae group, ⑤; Scopelocheiridae, ⑥; and Hirondelleidae, ⑦). Branch nodes show Bayesian posterior probability support followed by ML bootstrap support (in italics). A shift in rate diversification is suggested by the SymmeTree analysis at the node denoted with a star.

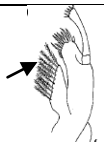
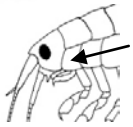
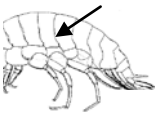
Figure 3: Phylogenetic analysis of scavenger 'type' amongst deep-sea Lysiassanoids. Species were assigned to a trophic guild on the basis of 7 morphological traits. The probability of each trophic type occurring at ancestral nodes is indicated with pie charts at the nodes. Non-scavengers are shown in black (blue online), opportunistic scavengers are shown in dark gray (green online), obligate scavengers are shown in light gray (red online) and specialist in white (purple online).

Figure 4: Maximum clade credibility diagram inferred from a BEAST dating analysis. Nodes a-e marked with open circles (red online) are nodes of interest (see explanation in text), and horizontal bars show 95 % highest posterior density intervals of the posterior distributions. Node 1 (light gray dot, green online) is used for calibration.

The black dot (yellow online) shows the origin of the *Gammarus* genus, dated to ~61Ma (Hou et al. 2011). NG= Neogene; Q = Quaternary.

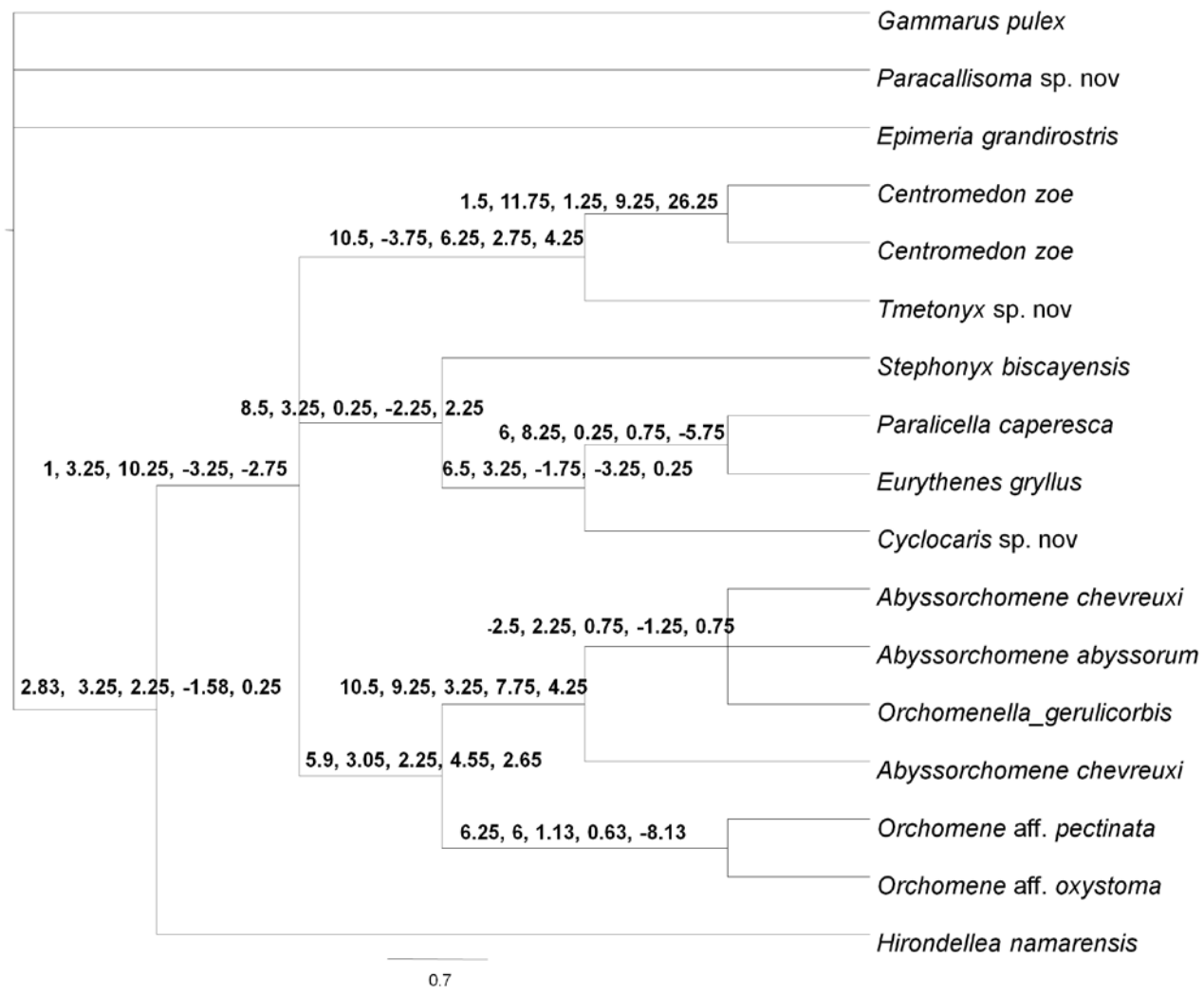
Figure 5: Diagram showing the more specialised chelate gnathopod 1 of *Stephonyx arabiensis* (reproduced from Diffenthal & Horton, 2007), probably used for picking carcasses.

757 Table 1. Maximum probability ancestral character states at nodes (from BayesTraits).
758

Trait	Diagram	Root	Deep-sea ancestral node	Lineage A	Lineage B	Lineage C
Maxilla 1 inner plate setation		**1** (fully setose)	**2** (2 apical setae)	**2**	**2**	**3** (>2 apical setae)
Maxilla 1 outer plate tooth arrangement		**1** (>11 spine teeth)	**2** (7-4 crown)	**2**	**3** (6-5 crown)	**4** (8-3 crown)
Mandibular molar		**1** (columnar)	**2** (coni-colaminate)	**2**	**1&2**	**2**
Gnathopod 1		**1** (sub-chelate)	**1**	**1**	**1**	**2** (para-chelate)
Gnathopod 2		**1** (sub-chelate)	**2** (mitten)	**2**	**2&3** (C: chelate)	**4** (minute)
Coxa 1		**1** (normal)	**2** (tapered)	**2**	**3** (expanded)	**4** (vestigal)
Gut storage		**1** (normal)	**2** (elongated)	**2**	**2**	**3** (midgut)

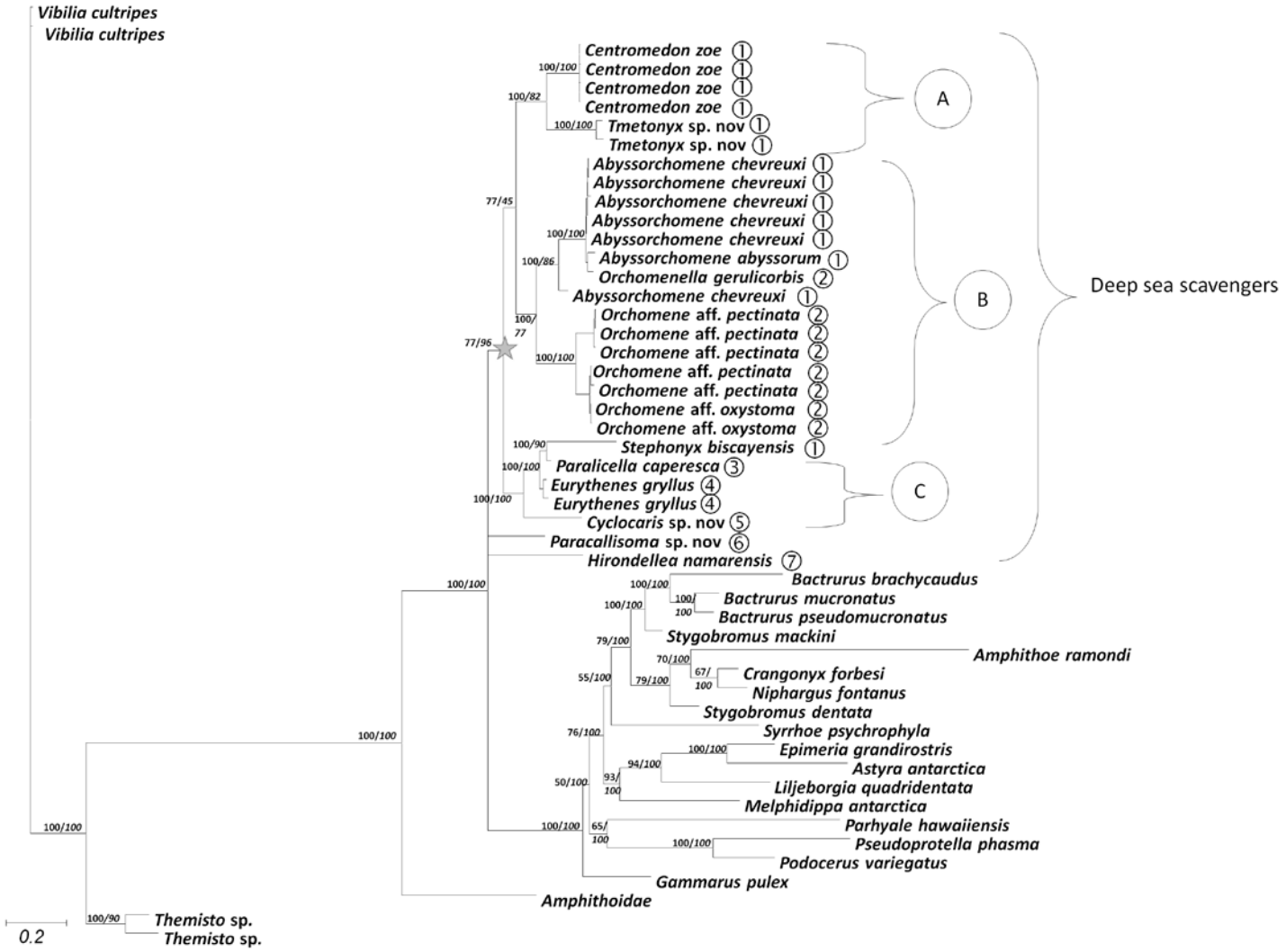
759
760 For each trait, 1-4 represents progressive change (defined parenthetically). The ‘deep-
761 sea ancestral node’ defines lineages A-C (see figures 1&2).
762

763 Figure 1:
764

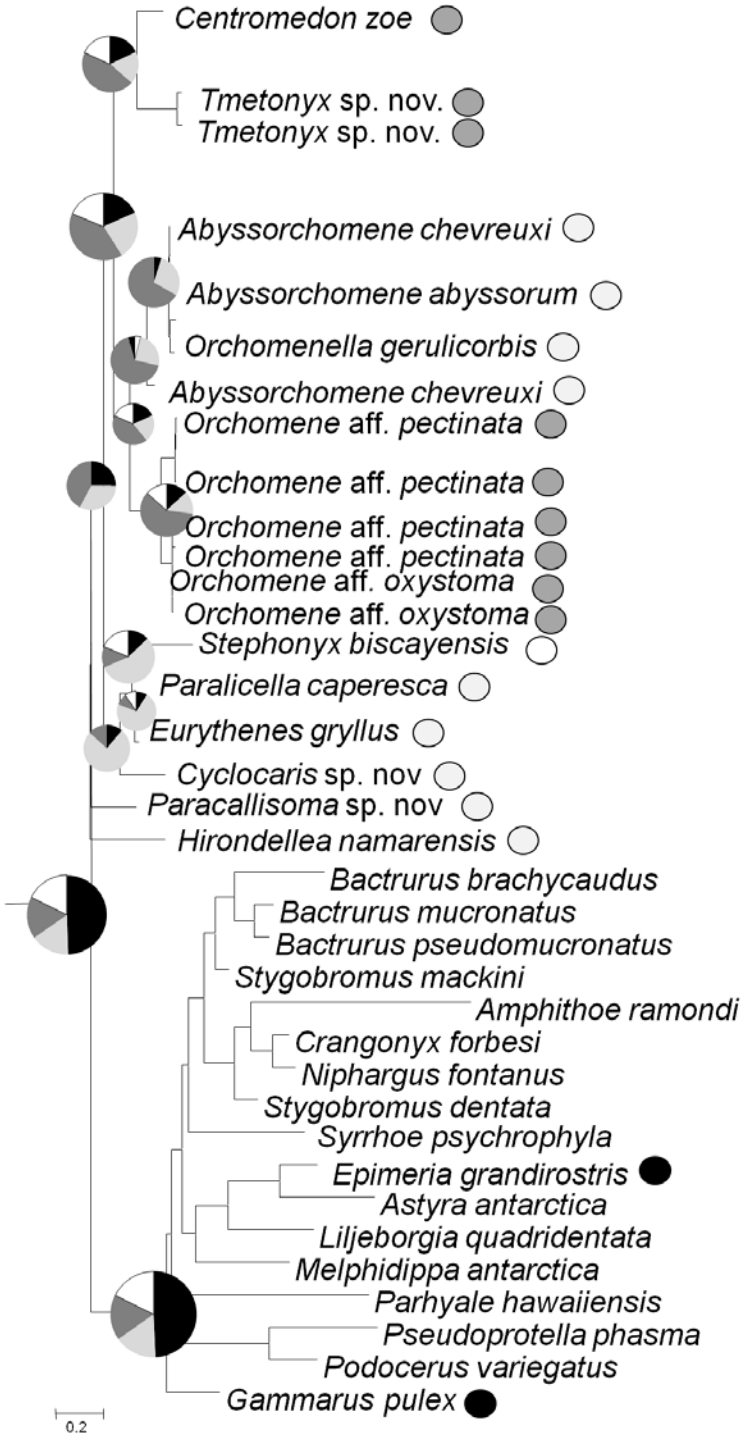


765

Figure 2:


Vibilia cultripes
Vibilia cultripes
Centromedon zoe ①
Centromedon zoe ①
Centromedon zoe ①
Centromedon zoe ①
Tmetonyx sp. nov ①
Tmetonyx sp. nov ①
A
Abyssorchomene chevreuxi ①
Abyssorchomene chevreuxi ①
Abyssorchomene chevreuxi ①
Abyssorchomene chevreuxi ①
Abyssorchomene chevreuxi ①
Abyssorchomene abyssorum ①
Orchomenella gerulicorbis ②
Abyssorchomene chevreuxi ①
Orchomene aff. pectinata ②
Orchomene aff. pectinata ②
Orchomene aff. pectinata ②
Orchomene aff. pectinata ②
Orchomene aff. pectinata ②
Orchomene aff. oxystoma ②
Orchomene aff. oxystoma ②
B
Deep sea scavengers
Stephonyx biscayensis ①
Paralicella caperesca ③
Eurythenes gryllus ④
Eurythenes gryllus ④
C
Cyclocaris sp. nov ⑤
Paracallisoma sp. nov ⑥
Hirondellea namarensis ⑦
Bactrurus brachycaudus
Bactrurus mucronatus
Bactrurus pseudomucronatus
Stygobromus mackini
Amphithoe ramondi
Crangonyx forbesi
Niphargus fontanus
Stygobromus dentata
Syrrhoe psychrophyla
Epimeria grandirostris
Astyra antarctica
Liljeborgia quadridentata
Melphidippa antarctica
Parhyale hawaiiensis
Pseudoprotella phasma
Podocerus variegatus
Gammarus pulex
Amphithoidae
Themisto sp.
Themisto sp.
0.2

769 Figure 3:
770



771

772 Figure 4:
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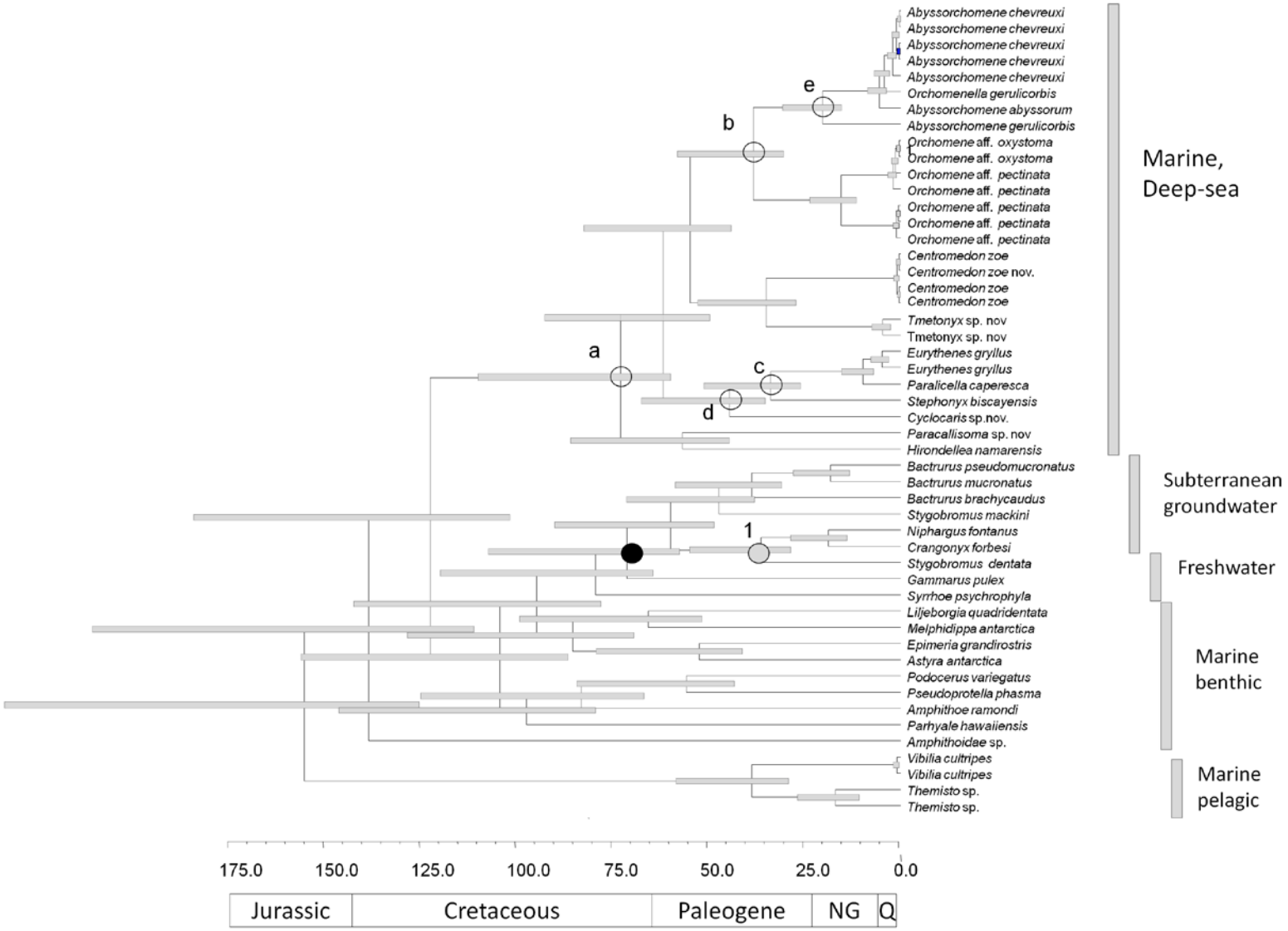
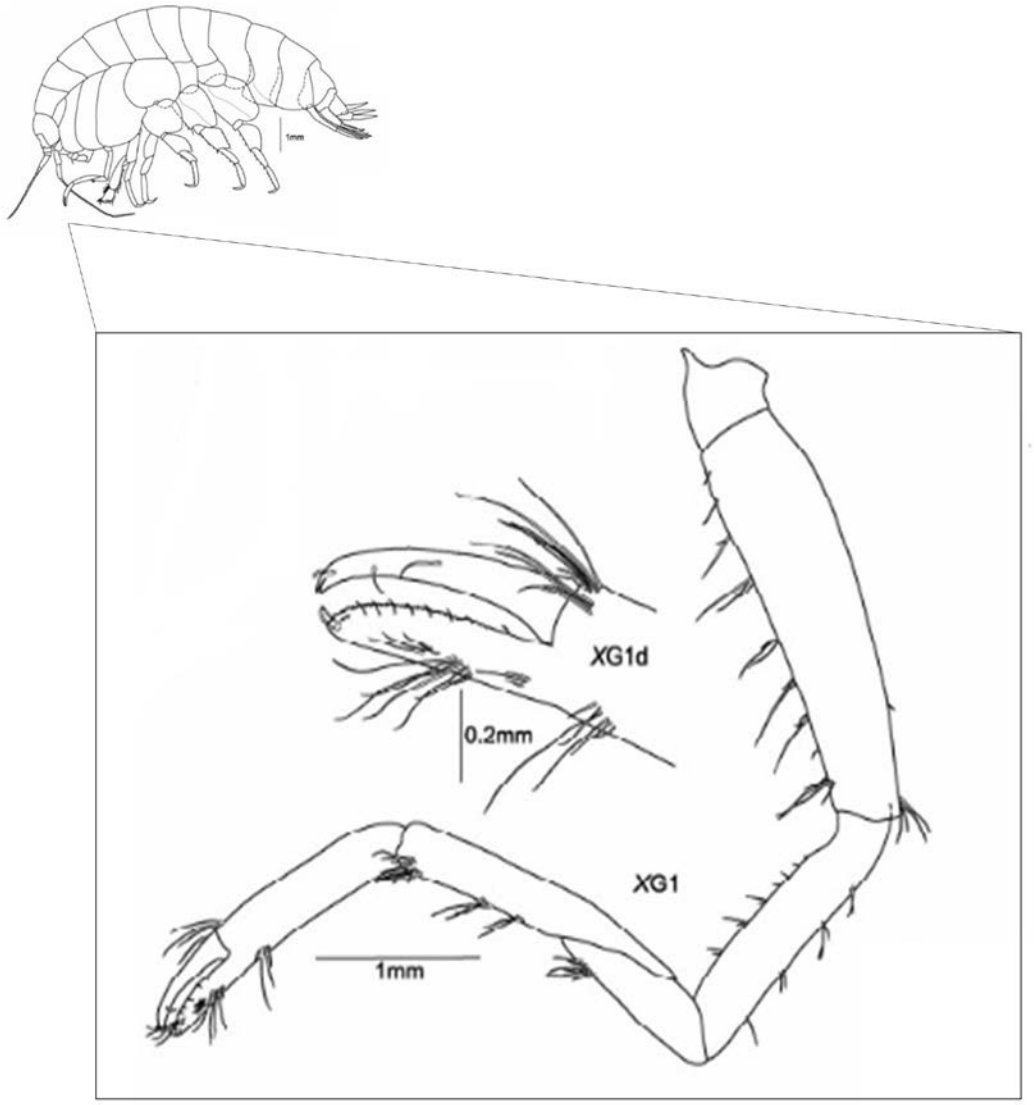


774

775 Figure 5:
776



777

Supplementary material:

Table S1. Data including depth and location of trapped taxa used in the phylogenetic study (including links to taxon pages on the World Register of Marine Species, Appletans et al., 2012). MAR = mid-Atlantic Ridge.

Family	Genus	Species	Number of specimens sequenced	Depth, m	Locality
Ingroup taxa:					
Uristidae Hurley, 1963	*Tmetonyx*	sp. nov.	2	2500	MAR
Uristidae Hurley, 1963	*Abyssorchomene*	*chevreuxi* (Stebbing, 1906)	3	2564	MAR
Uristidae Hurley, 1963	*Abyssorchomene*	*abyssorum* (Stebbing,1888)	1	2500	MAR
Uristidae Hurley, 1963	*Centromedon*	*zoe* (Horton & Thurston 2011)	4	2453-2564	MAR
Uristidae Hurley, 1963	*Stephonyx*	*biscayensis* (Chevreux, 1908)	2	2564	MAR
Lysianassidae Dana, 1849	*Orchomenella*	*gerulicorbis* (Shulenberger & Barnard, 1976)	1	4192	CROZET
Lysianassidae Dana, 1849	*Orchomene*	aff. *pectinata*	4	2500	MAR
Lysianassidae Dana, 1849	*Orchomene*	aff. *oxystoma*	3	2500	MAR
Alicellidae Lowry & De Broyer, 2008	*Paralicella*	*caperesca* (Schulenberger & Barnard, 1976)	1	4192	CROZET
Eurytheneidae Stoddart & Lowry, 2004	*Eurythenes*	*gryllus* (Lichtenstein, 1822)	2	2453	MAR
Cyclocaridae Lowry & Stoddart, 2011	*Cyclocaris*	sp. nov.	1	1975	ANGOLA
Scopelocheiridae Lowry & Stoddart, 1997	*Paracallisoma*	sp. nov.	1	2500	MAR
Hirondelleidae Lowry & Stoddart, 201	*Hirondellea*	*namarensis* (Horton & Thurston, 2012)	1	2500	MAR
Outgroup taxa:					
Vibiliidae Dana, 1852	*Vibilia*	*cultripes* (Vosseler, 1901)	2		MAR
Hyperiidae Dana, 1852	*Themisto*	*sp.*	2		MAR
Crangonyctidae Bousfield, 1973	*Bactrurus*	*brachycaudus* (Hubricht & Mackin, 1940)	1		
Crangonyctidae Bousfield, 1973	*Crangonyx*	*forbesi* (Hubricht & Mackin, 1940)	1		
Crangonyctidae Bousfield, 1973	*Stygobromus*	*dentata* (Hubricht, 1943)	1		
Crangonyctidae Bousfield, 1973	*Stygobromus*	*mackini* Hubricht, 1943	1		
Crangonyctidae Bousfield, 1973	*Bactrurus*	*mucronatus* (Forbes, 1876)	1		
Crangonyctidae Bousfield, 1973	*Bactrurus*	*pseudomucronatus* (Koenemann & Holsinger, 2000)	1		
Hyalidae Bulycheva, 1957	*Parhyale*	*hawaiiensis*(Dana, 1853)	1		

Ampithoidae Stebbing, 1899	*Amphithoe*	*ramondi* (Audouin, 1826)	1		
Gammaridae Leach, 1814	*Gammarus*	*pulex* (Linnaeus, 1758)	1		
Epimeriidae Boeck, 1871	*Epimeria*	*grandirostris*(Chevreux,1912)	1		
Pariambidae Laubitz, 1993	*Pseudoprotella*	*phasma* (Montagu, 1804)	1		
Niphargidae Bousfield, 1977	*Niphargus*	*fontanus* (Bate, 1859)	1		
Stilipedidae Holmes, 1908	*Astyra*	*antarctica* (Andres, 1997)	1		
Synopiidae Dana, 1853	*Syrrhoe*	*psychrophyla* (Monod, 1926)	1		
Melphidippidae Stebbing, 1899	*Melphidippa*	*antarctica* (Schellenberg, 1926)	1		
Liljeborgiidae Stebbing, 1899	*Liljeborgia*	*quadridentata* (Schellenberg, 1931)	1		
Podoceridae Leach, 1814	*Podocerus*	*variegatus* (Leach, 1814)	1		

Table S2: Sample site location and sampling protocol. Time given is GMT. Duration = deployment time.

Site	Station #	Latitude	Longitude	Depth	Deployed	Time	Surfaced	Time	Duration	Trap Type
MAR NE	JC011/098	54°04.08'N	34°09.43'W	2500	09Aug2007	1313	11Aug2007	1215	46: 58	VET/DEMAR
MAR NE	JC011/114	54°02.31'N	34°09.60'W	2453	12Aug2007	1725	13Aug2007	1540	22: 15	VET/DEMAR
MAR NW	JC011/079	53°56.44'N	36°11.56'W	2564	05Aug2007	1951	07Aug2007	1400	42:09	VET/DEMAR
MAR NW	JC037/060	53°58.46'N	36°06.12'W	2340	27Aug2009	2143	30Aug2009	1115	61:32	VET/CORE
MAR SE	JC011/013	49°01.16'N	27°42.29'W	2627	19Jul2007	2322	20Jul2007	1230	13:08	VET/DEMAR
MAR SE	JC037/013	49°02.00'N	27°43.44'W	2501	08Aug2009	2235	10Aug2009	1620	41:45	VET/DEMAR
MAR SE	JC037/018	49°01.20'N	27°42.03'W	2500	10Aug2009	1920	17Aug2009	2108	169:48	VET/DEMAR
MAR SE	JC037/025	49°02.23'N	27°53.66'W	1830	17Aug2009	2311	18Aug2009	1520	16:09	VET/DEMAR
ANGOLA	56755#2	6.30342°S	10.68768°W	1975	26Oct2005	-	-	-	-	ROBIO
CROZET	15775#24	48°59'S	51°13'E	4192	03Jan2006	0631	04Jan2006	09:25	24:45	FRESP

Table S3 Primer sequences T_a

Locus	Primer	Primer sequence 5' - 3'	T_a (°C)	Reference
COI	COI2f	TTYGAYCCIDYIGGRGGAGGAGATCC	45	Otto & Wilson 2001
	COIuR	TAAACTTCAGGGTGACCAAAAAATCA		
16S	16Sbr	CCGGTTTGAACTCAGATCATG	49	France & Kocher 1996
	16Sar	CGCCTGTTTATCAAAAACAT		
18S	18S1f	CGATAAGATACCGCCCTA	55	This study
	18S1r	GTCTCGTTCGTTATCGGA		
H3	HisH3f	AAATAGCYCGTACYAAGCAGAC	45	This study
	HisH3r	ATTGAATRTCYTTGGGCATGAT		
28S	28Sftw	AGGCGGAATGTTGCGT	50	This study
	28Srtw	CTGAGCGGTTTCACGGTC		

Table S4: Sequence data summary; accession numbers for previously published sequences shown in italics. 'No amp' means that the PCR reaction did not produce usable product.

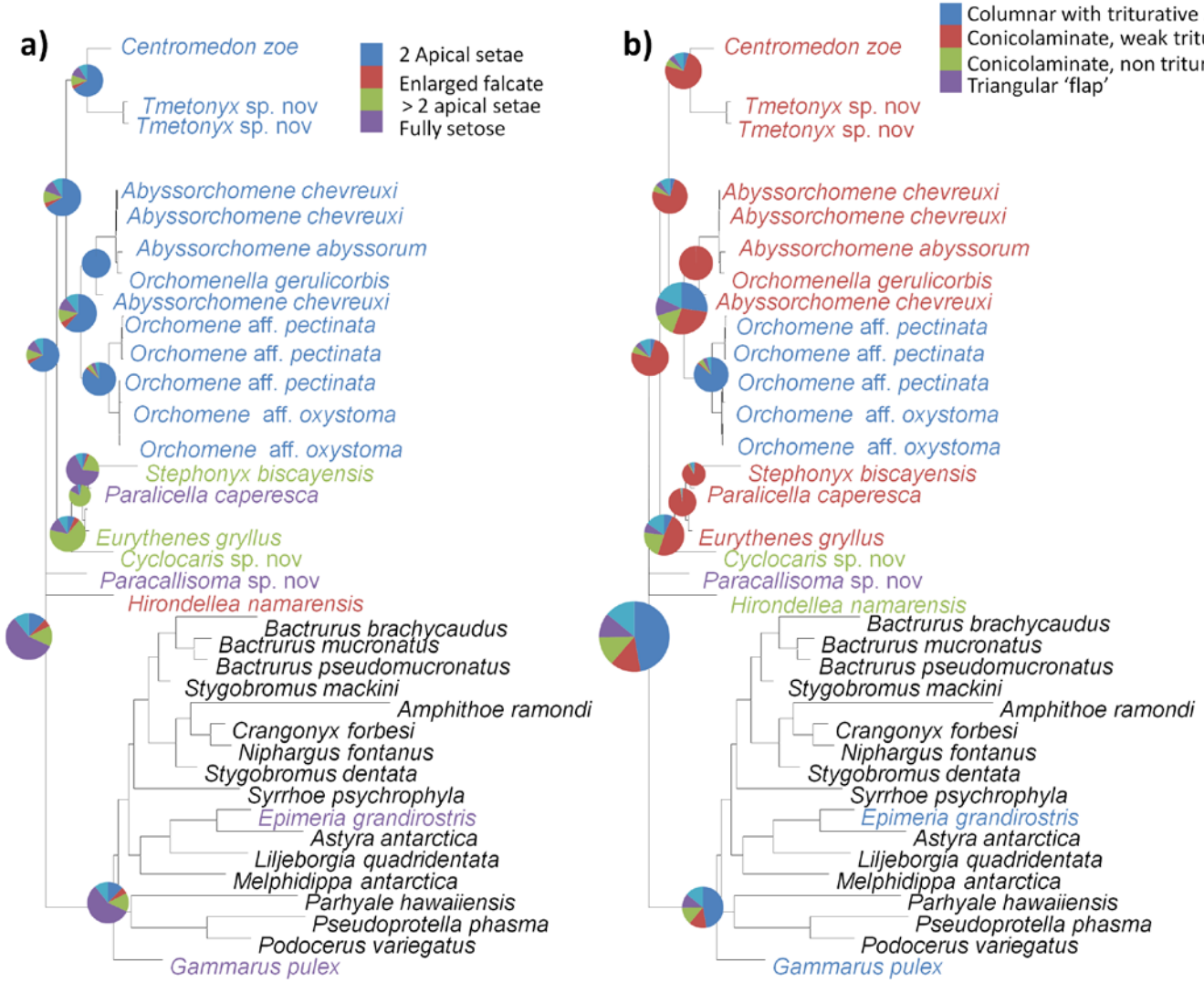
Genus	Species	16S	COI	18S	28S	Histone 3
Tmetonyx	sp. nov.	KF430274	KF430247	KF430232	KF430304	KF484703
Abyssorchomene	*chevreuxi*	KF430265	KF430238	KF430223	KF430295	KF484694
Abyssorchomene	*abyssorum*	KF430266	KF430239	KF430224	KF430296	KF484695
Centromedon	*zoe*	KF430263	KF430236	KF430221	KF430293	KF484692
Stephonyx	*biscayensis*	KF430264	KF430237	KF430222	KF430294	KF484693
Orchomenella	*gerulicorbis*	KF430267	KF430240	KF430225	KF430297	KF484696
Orchomene	aff. *pectinata*	KF430268	KF430241	KF430226	KF430298	KF484697
Orchomene	aff. *oxystoma*	KF430269	KF430242	KF430227	KF430299	KF484698
Paralicella	*caperesca*	KF430270	KF430243	KF430228	KF430300	KF484699
Eurythenes	*gryllus*	KF430273	KF430246	KF430231	KF430303	KF484702
Cyclocaris	sp. nov.	KF430272	KF430245	KF430230	KF430302	KF484701
Paracallisoma	sp. nov.	KF430271	KF430244	KF430229	KF430301	KF484700
Hirondellea	*namarensis*	KF430275	KF430248	KF430233	KF430305	KF484704
Vibilia	*cultripes*	KF430277	No amp	KF430235	KF430307	KF484706
Themisto	*sp.*	KF430276	KF430249	KF430234	KF430306	KF484705
Bactrurus	*brachycaudus*	KF430278	No amp	*AF202984*	KF430308	KF484707
Crangonyx	*forbesi*	KF430285	KF430256	*AF202980*	No amp	KF484714
Stygobromus	*dentata*	KF430281	No amp	*AF419233*	KF430311	KF484710
Stygobromus	*mackini*	KF430287	KF430257	*DQ377995*	KF430316	KF484716
Bactrurus	*mucronatus*	KF430291	KF430261	*AF202978*	KF430322	KF484722
Bactrurus	*pseudomucronatus*	KF430292	KF430262	*AF202985*	KF430323	KF484723
Parhyale	*hawaiiensis*	KF430279	KF430250	*AY826957*	KF430309	KF484708
Amphithoe	*ramondi*	KF430280	KF430251	*DQ378024*	KF430310	KF484709
Gammarus	*pulex*	KF430282	KF430253	*AF202982*	KF430312	KF484711
Epimeria	*grandirostris*	KF430283	KF430254	*DQ378007*	KF430313	KF484712

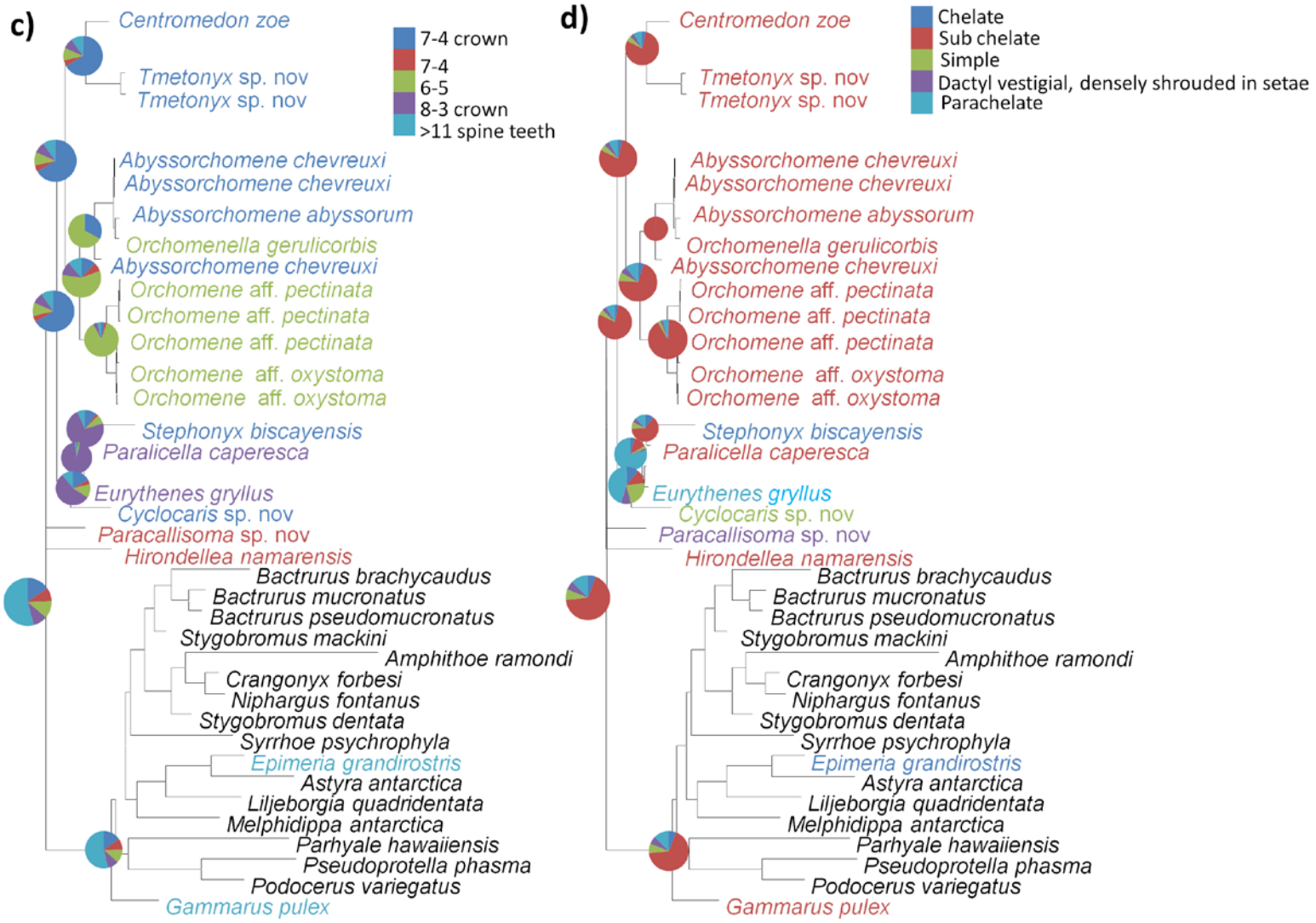
Pseudoprotella	*phasma*	KF430284	KF430255	*DQ378041*	KF430314	KF484713
Niphargus	*fontanus*	KF430286	*DQ064702*	*AF202981*	KF430315	KF484715
Astyra	*antarctica*	KF430288	KF430258	*DQ377999*	KF430317	KF484717
Syrrhoe	*psychrophyla*	No amp	KF430259	*DQ378030*	KF430318	KF484718
Melphidippa	*antarctica*	KF430289	No amp	*DQ377998*	KF430319	KF484719
Liljeborgia	*quadridentata*	KF430290	KF430260	*DQ378013*	KF430320	KF484720
Podocerus	*variegatus*	No amp	No amp	*DQ378022*	KF430321	KF484721

Table S5: Substitution models and model parameter prior for each gene in the MrBayes runs.

Gene Partition	Model	Rate Variation	Substitution Rates	Nucleotide frequencies	Shape parameter	Proportion of Invariable sites	Topology	Branch lengths
16S	HKY	Invgamma	Dirichlet (1,1,1,1)	Dirichlet (1,1,1,1)	Uniform (0,200)	Uniform (0,1)	Uniform	Unconstrained: Exp(10.0)
COI	GTR	Invgamma	Dirichlet (1,1,1,1)	Dirichlet (1,1,1,1)	Uniform (0,200)	Uniform (0,1)	Uniform	Unconstrained: Exp(10.0)
18S	GTR	Invgamma	Dirichlet (1,1,1,1)	Dirichlet (1,1,1,1)	Uniform (0,200)	Uniform (0,1)	Uniform	Unconstrained: Exp(10.0)
28S	GTR	Invgamma	Dirichlet (1,1,1,1)	Dirichlet (1,1,1,1)	Uniform (0,200)	Uniform (0,1)	Uniform	Unconstrained: Exp(10.0)
H3	GTR	Invgamma	Dirichlet (1,1,1,1)	Dirichlet (1,1,1,1)	Uniform (0,200)	Uniform (0,1)	Uniform	Unconstrained: Exp(10.0)

Figure S1: Bayesian trees and Bayestrait analyses of a) maxilla 1 inner plate setation; b) mandibular molar; c) maxilla 1 outer plate tooth arrangement; d) gnathopod 1; e) gnathopod 2; f) coxa 1; g) gut storage. Pie charts illustrate relative trait probabilities at a given node.




c)
Centromedon zoe
Tmetonyx sp. nov
Tmetonyx sp. nov
7-4 crown
7-4
6-5
8-3 crown
>11 spine teeth
Abyssorchomene chevreuxi
Abyssorchomene chevreuxi
Abyssorchomene abyssorum
Orchomenella gerulicorbis
Abyssorchomene chevreuxi
Orchomene aff. pectinata
Orchomene aff. pectinata
Orchomene aff. pectinata
Orchomene aff. oxystoma
Orchomene aff. oxystoma
Stephonyx biscayensis
Paralicella caperesca
Eurythenes gryllus
Cyclocaris sp. nov
Paracallisoma sp. nov
Hirondellea namarensis
Bactrurus brachycaudus
Bactrurus mucronatus
Bactrurus pseudomucronatus
Stygobromus mackini
Amphithoe ramondi
Crangonyx forbesi
Niphargus fontanus
Stygobromus dentata
Syrrhoe psychrophyla
Epimeria grandirostris
Astyra antarctica
Liljeborgia quadridentata
Melphidippa antarctica
Parhyale hawaiiensis
Pseudoprotella phasma
Podocerus variegatus
Gammarus pulex
d)
Centromedon zoe
Tmetonyx sp. nov
Tmetonyx sp. nov
Chelate
Sub chelate
Simple
Dactyl vestigial, densely shrouded in setae
Parachelate
Abyssorchomene chevreuxi
Abyssorchomene chevreuxi
Abyssorchomene abyssorum
Orchomenella gerulicorbis
Abyssorchomene chevreuxi
Orchomene aff. pectinata
Orchomene aff. pectinata
Orchomene aff. pectinata
Orchomene aff. oxystoma
Orchomene aff. oxystoma
Stephonyx biscayensis
Paralicella caperesca
Eurythenes gryllus
Cyclocaris sp. nov
Paracallisoma sp. nov
Hirondellea namarensis
Bactrurus brachycaudus
Bactrurus mucronatus
Bactrurus pseudomucronatus
Stygobromus mackini
Amphithoe ramondi
Crangonyx forbesi
Niphargus fontanus
Stygobromus dentata
Syrrhoe psychrophyla
Epimeria grandirostris
Astyra antarctica
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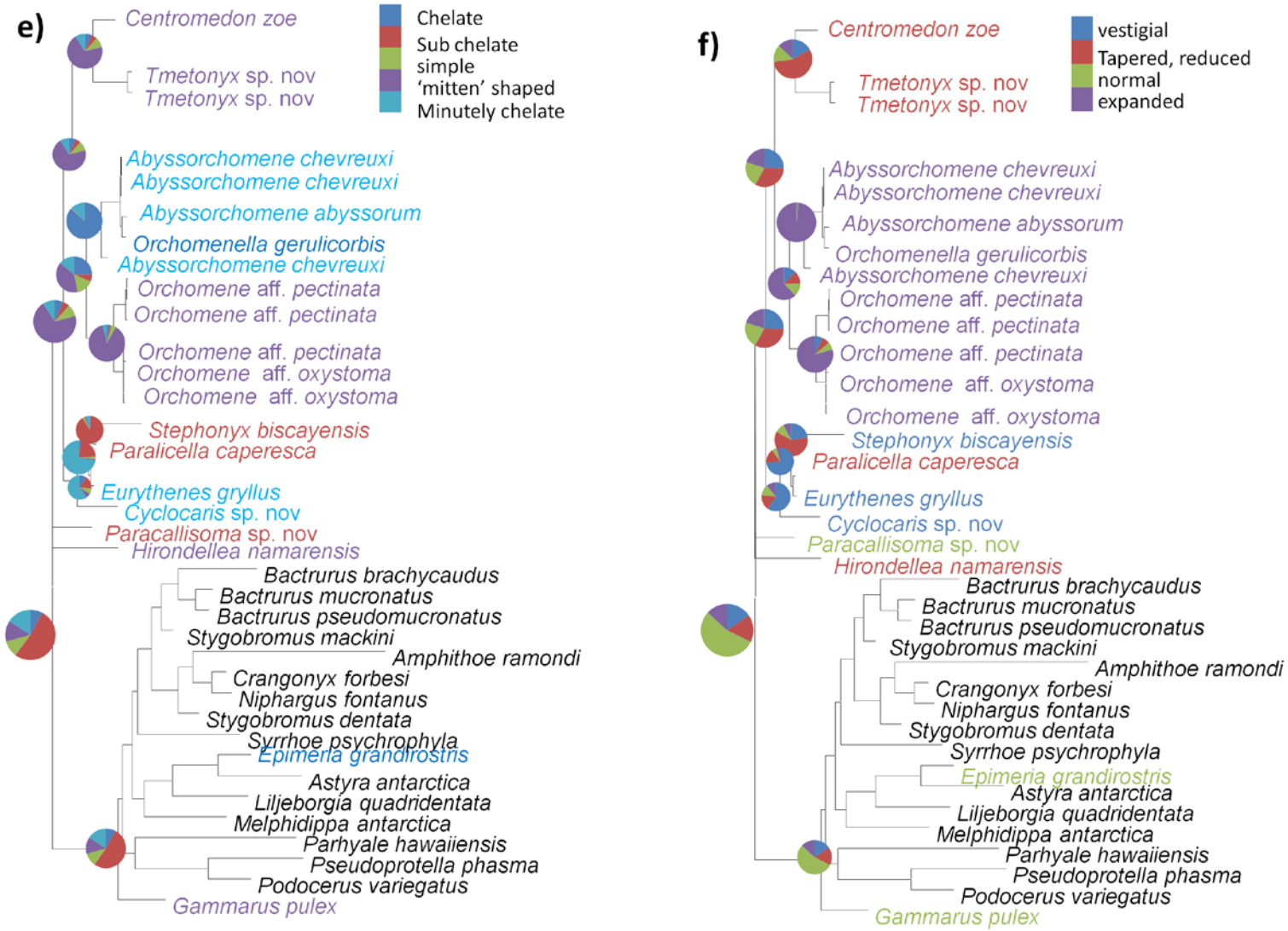
Melphidippa antarctica
Parhyale hawaiiensis
Pseudoprotella phasma
Podocerus variegatus
Gammarus pulex


e)
Chelate
Sub chelate
simple
'mitten' shaped
Minutely chelate
Centromedon zoe
Tmetonyx sp. nov
Tmetonyx sp. nov
Abyssorchomene chevreuxi
Abyssorchomene chevreuxi
Abyssorchomene abyssorum
Orchomenella gerulicorbis
Abyssorchomene chevreuxi
Orchomene aff. pectinata
Orchomene aff. pectinata
Orchomene aff. pectinata
Orchomene aff. oxystoma
Orchomene aff. oxystoma
Stephonyx biscayensis
Paralicella caperesca
Eurythenes gryllus
Cyclocaris sp. nov
Paracallisoma sp. nov
Hirondellea namarensis
Bactrurus brachycaudus
Bactrurus mucronatus
Bactrurus pseudomucronatus
Stygobromus mackini
Amphithoe ramondi
Crangonyx forbesi
Niphargus fontanus
Stygobromus dentata
Syrrhoe psychrophyla
Epimeria grandirostris
Astyra antarctica
Liljeborgia quadridentata
Melphidippa antarctica
Parhyale hawaiiensis
Pseudoprotella phasma
Podocerus variegatus
Gammarus pulex
f)
vestigial
Tapered, reduced
normal
expanded
Centromedon zoe
Tmetonyx sp. nov
Tmetonyx sp. nov
Abyssorchomene chevreuxi
Abyssorchomene chevreuxi
Abyssorchomene abyssorum
Orchomenella gerulicorbis
Abyssorchomene chevreuxi
Orchomene aff. pectinata
Orchomene aff. pectinata
Orchomene aff. pectinata
Orchomene aff. oxystoma
Orchomene aff. oxystoma
Stephonyx biscayensis
Paralicella caperesca
Eurythenes gryllus
Cyclocaris sp. nov
Paracallisoma sp. nov
Hirondellea namarensis
Bactrurus brachycaudus
Bactrurus mucronatus
Bactrurus pseudomucronatus
Stygobromus mackini
Amphithoe ramondi
Crangonyx forbesi
Niphargus fontanus
Stygobromus dentata
Syrrhoe psychrophyla
Epimeria grandirostris
Astyra antarctica
Liljeborgia quadridentata
Melphidippa antarctica
Parhyale hawaiiensis
Pseudoprotella phasma
Podocerus variegatus
Gammarus pulex

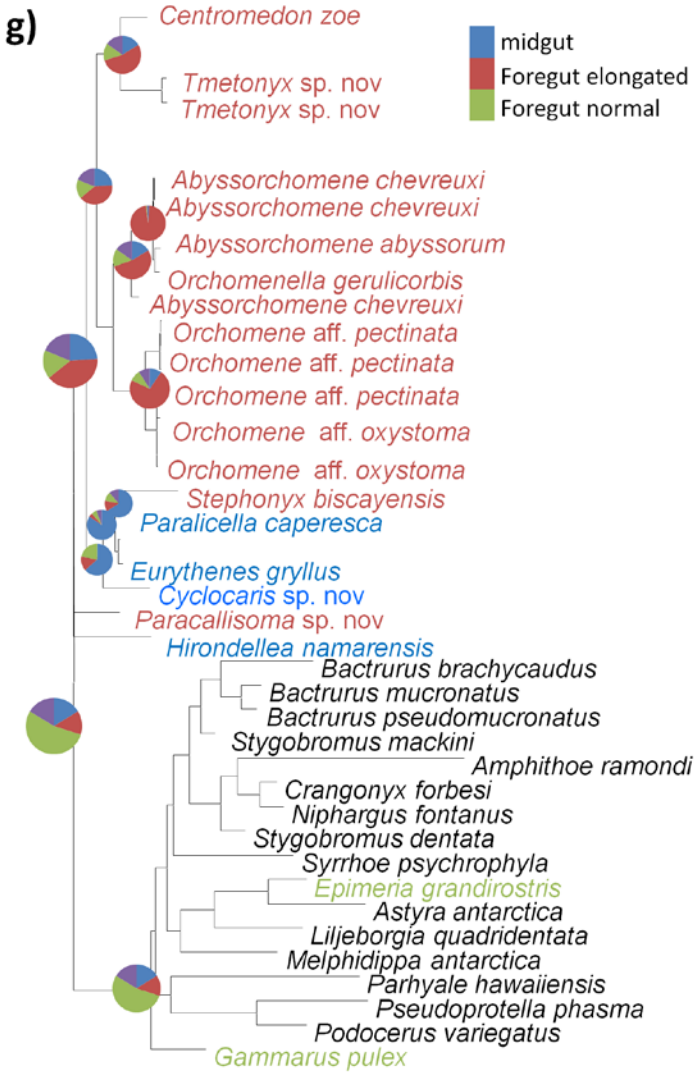
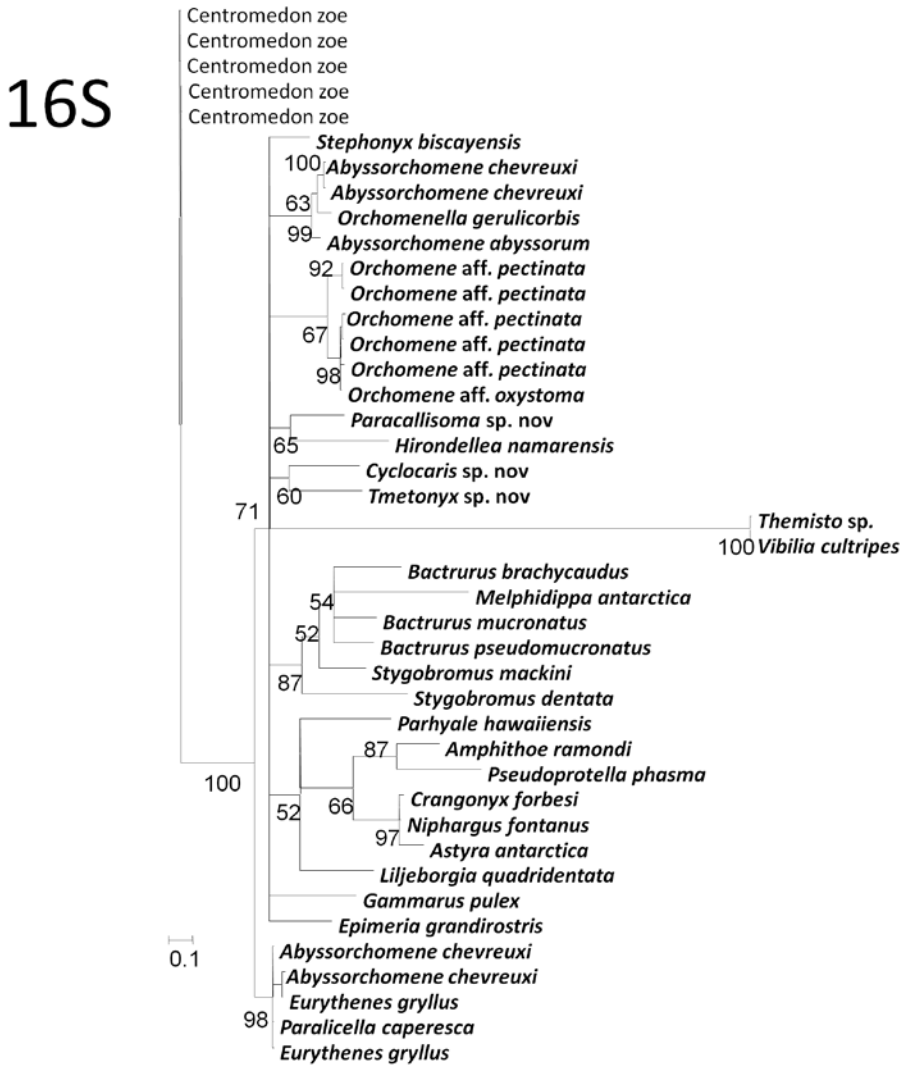
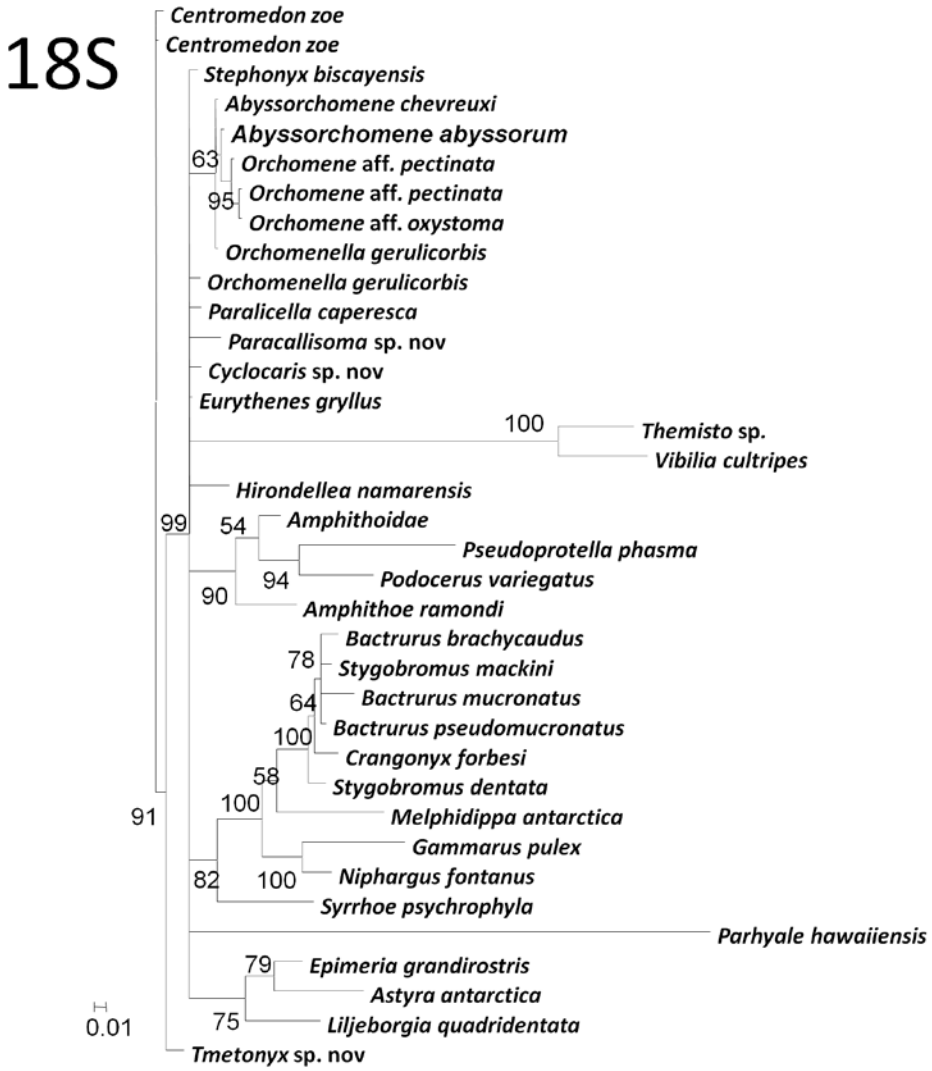
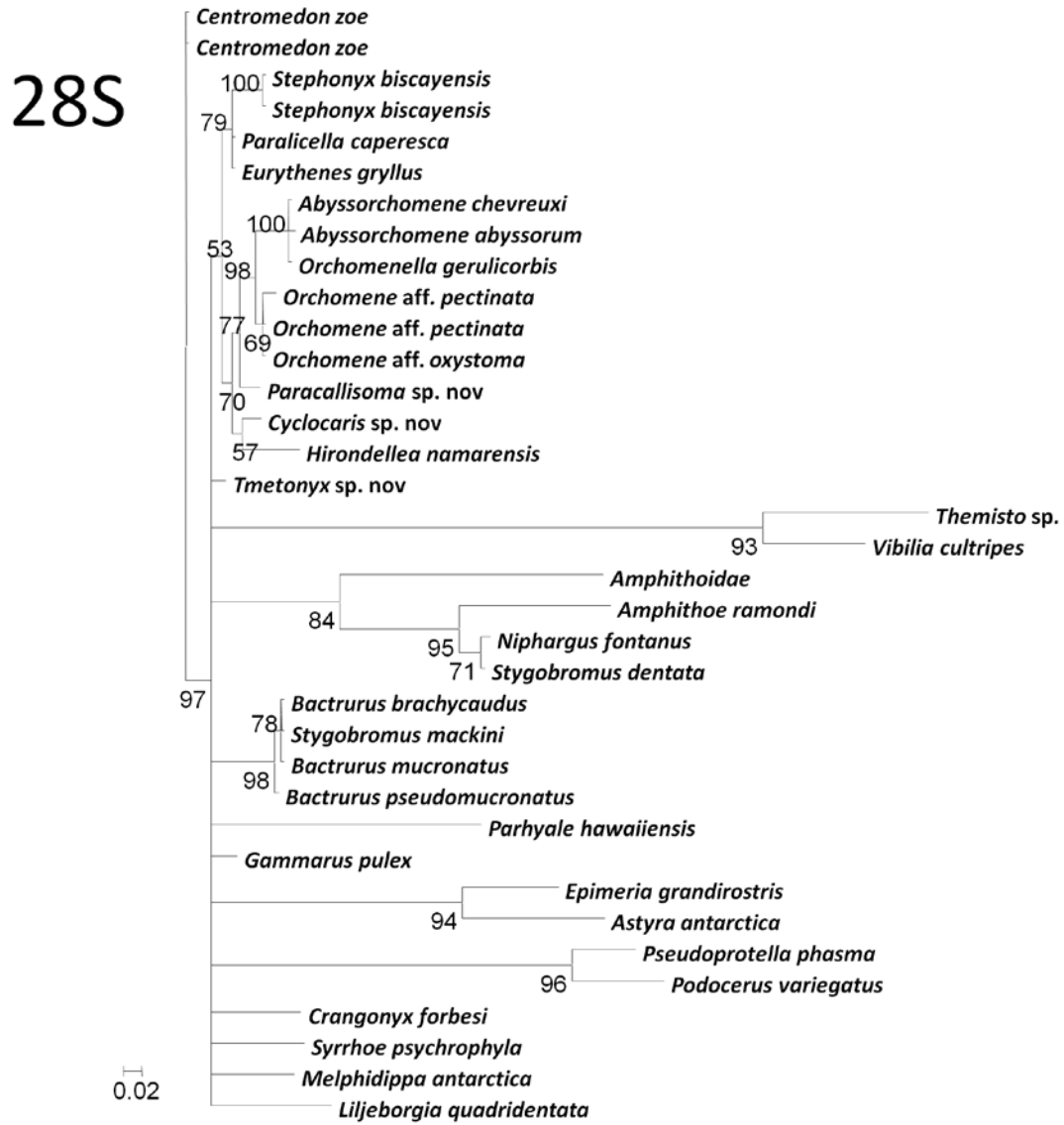
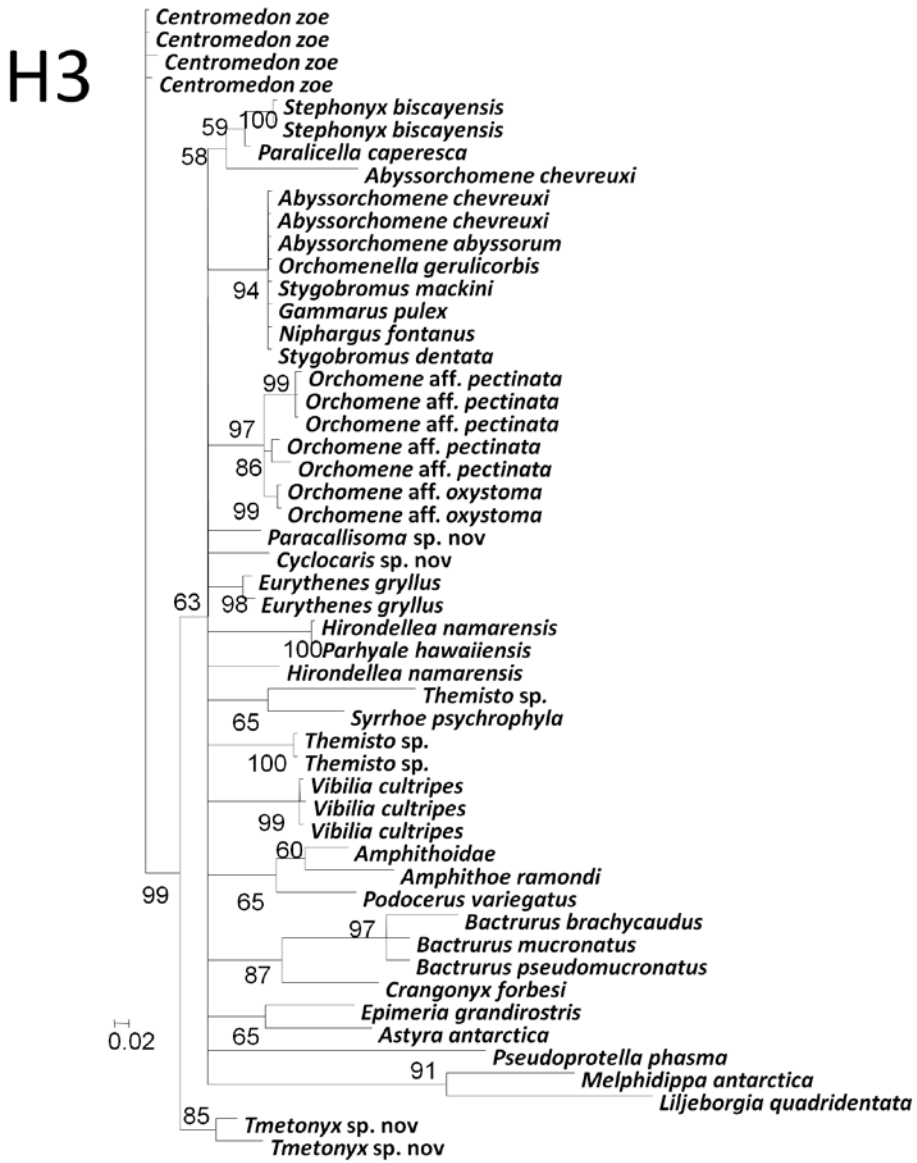

g)
midgut
Foregut elongated
Foregut normal
Centromedon zoe
Tmetonyx sp. nov
Tmetonyx sp. nov
Abyssorchomene chevreuxi
Abyssorchomene chevreuxi
Abyssorchomene abyssorum
Orchomenella gerulicorbis
Abyssorchomene chevreuxi
Orchomene aff. pectinata
Orchomene aff. pectinata
Orchomene aff. pectinata
Orchomene aff. oxystoma
Orchomene aff. oxystoma
Stephonyx biscayensis
Paralicella caperesca
Eurythenes gryllus
Cyclocaris sp. nov
Paracallisoma sp. nov
Hirondellea namarensis
Bactrurus brachycaudus
Bactrurus mucronatus
Bactrurus pseudomucronatus
Stygobromus mackini
Amphithoe ramondi
Crangonyx forbesi
Niphargus fontanus
Stygobromus dentata
Syrrhoe psychrophyla
Epimeria grandirostris
Astyra antarctica
Liljeborgia quadridentata
Melphidippa antarctica
Parhyale hawaiiensis
Pseudoprotella phasma
Podocerus variegatus
Gammarus pulex

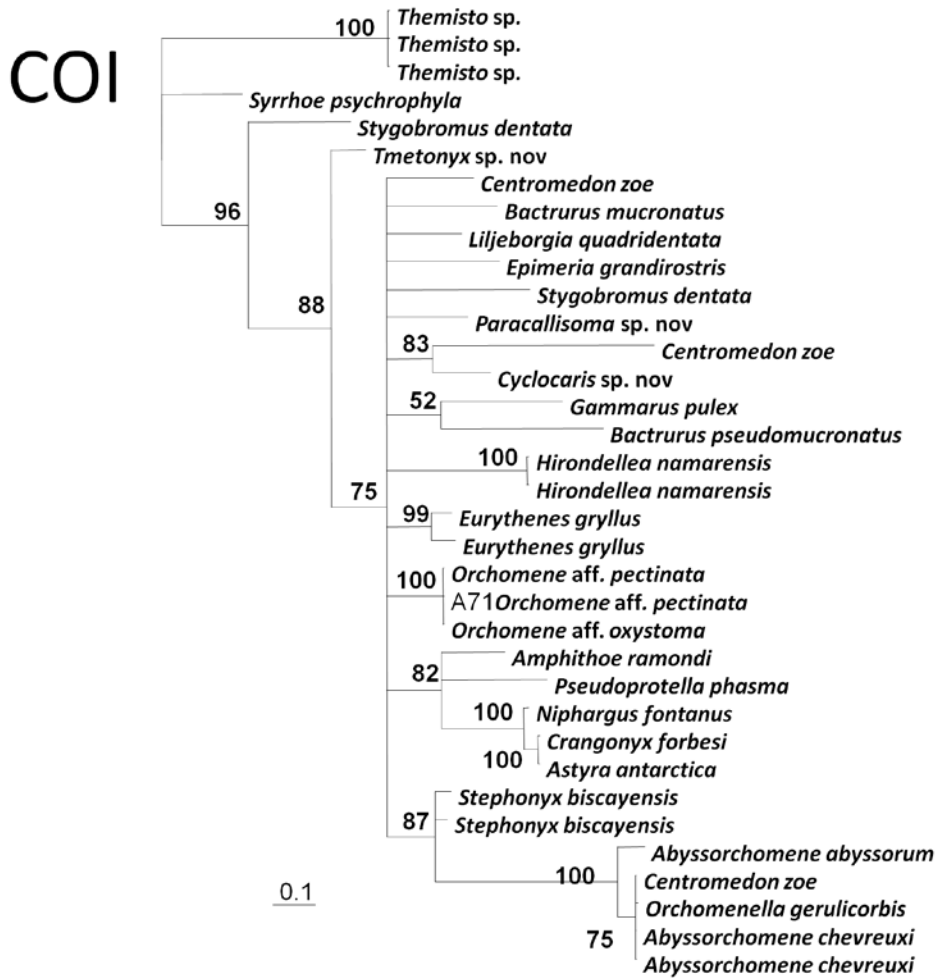
Figure S2: MrBayes phylogenies based on single genes showing node support (Bayesian Posterior Probabilities with 10,000 trees sampled).




18S
Centromedon zoe
Centromedon zoe
Stephonyx biscayensis
Abyssorchomene chevreuxi
Abyssorchomene abyssorum
Orchomene aff. pectinata
Orchomene aff. pectinata
Orchomene aff. oxystoma
Orchomenella gerulicorbis
Orchomenella gerulicorbis
Paralicella caperesca
Paracallisoma sp. nov
Cyclocaris sp. nov
Eurythenes gryllus
Themisto sp.
Vibilia cultripes
Hirondellea namarensis
Amphithoidae
Pseudoprotella phasma
Podocerus variegatus
Amphithoe ramondi
Bactrurus brachycaudus
Stygobromus mackini
Bactrurus mucronatus
Bactrurus pseudomucronatus
Crangonyx forbesi
Stygobromus dentata
Melphidippa antarctica
Gammarus pulex
Niphargus fontanus
Syrrhoe psychrophyla
Parhyale hawaiiensis
Epimeria grandirostris
Astyra antarctica
Liljeborgia quadridentata
Tmetonyx sp. nov
63
95
100
99
54
94
90
78
64
100
58
100
91
82
100
79
75
0.01


28S
Centromedon zoe
Centromedon zoe
100
Stephonyx biscayensis
Stephonyx biscayensis
79
Paralicella caperesca
Eurythenes gryllus
Abyssorchomene chevreuxi
100
Abyssorchomene abyssorum
53
Orchomenella gerulicorbis
98
Orchomene aff. pectinata
77
Orchomene aff. pectinata
69
Orchomene aff. oxystoma
Paracallisoma sp. nov
70
Cyclocaris sp. nov
57
Hirondellea namarensis
Tmetonyx sp. nov
Themisto sp.
93
Vibilia cultripes
Amphithoidae
Amphithoe ramondi
84
Niphargus fontanus
95
71
Stygobromus dentata
97
Bactrurus brachycaudus
78
Stygobromus mackini
Bactrurus mucronatus
98
Bactrurus pseudomucronatus
Parhyale hawaiiensis
Gammarus pulex
Epimeria grandirostris
94
Astyra antarctica
Pseudoprotella phasma
96
Podocerus variegatus
Crangonyx forbesi
Syrrhoe psychrophyla
Melphidippa antarctica
0.02
Liljeborgia quadridentata


H3
Centromedon zoe
Centromedon zoe
Centromedon zoe
Centromedon zoe
Stephonyx biscayensis
Stephonyx biscayensis
Paralicella caperesca
Abyssorchomene chevreuxi
Abyssorchomene chevreuxi
Abyssorchomene chevreuxi
Abyssorchomene abyssorum
Orchomenella gerulicorbis
Stygobromus mackini
Gammarus pulex
Niphargus fontanus
Stygobromus dentata
Orchomene aff. pectinata
Orchomene aff. pectinata
Orchomene aff. pectinata
Orchomene aff. pectinata
Orchomene aff. pectinata
Orchomene aff. oxystoma
Orchomene aff. oxystoma
Paracallisoma sp. nov
Cyclocaris sp. nov
Eurythenes gryllus
Eurythenes gryllus
Hirondellea namarensis
Parhyale hawaiiensis
Hirondellea namarensis
Themisto sp.
Syrrhoe psychrophyla
Themisto sp.
Themisto sp.
Vibilia cultripes
Vibilia cultripes
Vibilia cultripes
Amphithoidae
Amphithoe ramondi
Podocerus variegatus
Bactrurus brachycaudus
Bactrurus mucronatus
Bactrurus pseudomucronatus
Crangonyx forbesi
Epimeria grandirostris
Astyra antarctica
Pseudoprotella phasma
Melphidippa antarctica
Liljeborgia quadridentata
Tmetonyx sp. nov
Tmetonyx sp. nov
0.02


COI
100
Themisto sp.
Themisto sp.
Themisto sp.
Syrrhoe psychrophyla
Stygobromus dentata
Tmetonyx sp. nov
96
Centromedon zoe
Bactrurus mucronatus
Liljeborgia quadridentata
Epimeria grandirostris
Stygobromus dentata
88
Paracallisoma sp. nov
83
Centromedon zoe
Cyclocaris sp. nov
52
Gammarus pulex
Bactrurus pseudomucronatus
100
Hirondellea namarensis
Hirondellea namarensis
75
99
Eurythenes gryllus
Eurythenes gryllus
100
Orchomene aff. pectinata
A71Orchomene aff. pectinata
Orchomene aff. oxystoma
Amphithoe ramondi
82
Pseudoprotella phasma
100
Niphargus fontanus
Crangonyx forbesi
100
Astyra antarctica
Stephonyx biscayensis
87
Stephonyx biscayensis
Abyssorchomene abyssorum
100
Centromedon zoe
Orchomenella gerulicorbis
75
Abyssorchomene chevreuxi
Abyssorchomene chevreuxi
0.1

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