Molecular Ecology
September 2013, Volume 22 (18), pages 4663–4680
http://dx.doi.org/10.1111/mec.12419

High connectivity across the fragmented chemosynthetic ecosystems of the deep Atlantic Equatorial Belt: efficient dispersal mechanisms or questionable endemism?

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Abstract :

Chemosynthetic ecosystems are distributed worldwide in fragmented habitats harbouring seemingly highly specialized communities. Yet, shared taxa have been reported from highly distant chemosynthetic communities. These habitats are distributed in distinct biogeographical regions, one of these being the so-called Atlantic Equatorial Belt (AEB). Here, we combined genetic data (COI) from several taxa to assess the possible existence of cryptic or synonymous species and to detect the possible occurrence of contemporary gene flow among populations of chemosynthetic species located on both sides of the Atlantic. Several Evolutionary Significant Units (ESUs) of Alvinocarididae shrimp and Vesicomyidae bivalves were found to be shared across seeps of the AEB. Some were also common to hydrothermal vent communities of the Mid-Atlantic Ridge (MAR), encompassing taxa morphologically described as distinct species or even genera. The hypothesis of current or very recent large-scale gene flow among seeps and vents was supported by microsatellite analysis of the shrimp species *Alvinocaris muricola*/*Alvinocaris markensis* across the AEB and MAR. Two nonmutually exclusive hypotheses may explain these findings. The dispersion of larvae or adults following strong deep-sea currents, possibly combined with biochemical cues influencing the duration of larval development and timing of metamorphosis, may result in large-scale effective migration among distant spots scattered on the oceanic seafloor. Alternatively, these results may arise from the prevailing lack of knowledge on the ocean seabed, apart from emblematic ecosystems (chemosynthetic ecosystems, coral reefs or seamounts), where the widespread classification of endemism associated with many chemosynthetic taxa might hide wider distributions in overlooked parts of the deep sea.

Keywords : Atlantic equatorial belt ; chemosynthetic habitats ; deep-sea connectivity ; endemic bivalves ; endemic shrimp ; genetic diversity ; microsatellite markers ; mitochondrial COI gene

Introduction

Hydrothermal vents, cold seeps, and other deep-sea sites of organic enrichment (whale- and wood-falls) have in common the use of reduced chemicals as energy source by chemoautotrophic bacteria that function as primary producers, allowing very high biomass production far from the euphotic zone (Desbruyères et al. 2000). Despite sharing chemoautotrophy for primary production of organic matter, chemosynthetic ecosystems differ in many characteristics. Hydrothermal vents, due to their tectonic and volcanic nature, are usually ephemeral and characterized by high temperatures, high sulphide, high heavy metal concentrations, and are generally not strongly sedimented. In comparison, more stable and sediment-rich cold seeps are often associated to cold temperatures and diffusion of methane-rich fluids, harbouring more stable communities, which include species with extreme life span (Bergquist et al. 2000). Hydrothermal vents are scattered along mid-ocean ridges and back-arc spreading centres while cold seeps are patchily distributed along active or passive margins associated with accumulated sediment. Despite their habitat differences, the chemosynthetic communities inhabiting these two kinds of ecosystems harbour common genera and sister species, suggesting a shared history of colonization during the evolutionary history of deep ocean habitats (Hecker 1985; McLean 1985).

Cold seeps, because of their distribution along continental margins, have been suggested as potential stepping-stone habitats for long-distance dispersal of vent species (Craddock et al. 1995). However, very few shared species have been detected among those ecosystems (Peek et al. 2000; Baco et al. 1999; Andersen et al. 2004; Jollivet et al. 2000; Turnipseed et al. 2003; Tyler & Young 1999; Sibuet & Olu 1998). One exception, supported by genetic analyses, is the vestimentiferan tubeworm *Escarpia spicata* found across different kinds of chemosynthetic habitats, namely cold seeps, whale falls and sedimented hydrothermal vents

off the coast of California (Black et al. 1997). In the Northwestern Pacific, several morphologically determined species have been reported to occur in both seeps and vents; genetic connectivity between both ecosystems has been shown for *Lamellibrachia* tubeworms and *Bathymodiolus* mussels, but not for Vesicomyidae bivalves (Watanabe et al. 2010). Additionally, Vesicomyidae bivalves from whale carcasses in the Eastern Pacific have been shown to be genetically close to both seep (*Phreagena kilmeri*) and vent (*Archivesica gigas*) vesicomyids from the same region (Baco et al. 1999).

Since the discovery of these habitats in the mid 1970´s, one of the most puzzling issues has been the influence of past and present connectivity on the nature of species assemblages and their geographic distribution (Corliss et al. 1979). Several studies to date have addressed the biogeography of vent ecosystems (Tunnicliffe 1997; Van Dover et al. 2002; Bachraty et al. 2009). The most recent assessment included 63 hydrothermal vents distributed worldwide, and revealed the existence of five major biogeographic provinces: Mid-Atlantic Ridge, Indian Ocean, Western Pacific, Northeast Pacific Rise and East Pacific Rise, characterized by high levels of endemism, with 95% of the species not shared between provinces (Moalic et al. 2012). Cold seep ecosystems have been grouped into a few provinces: the Gulf of Mexico, Atlantic, Mediterranean, East Pacific and West Pacific (Tyler et al 2003), with low species richness but high endemism (Turnipseed et al. 2003; Tunnicliffe et al. 1998).

The high levels of endemism currently reported for these ecosystems, indicate that vent fauna have in general low dispersal potential, although taxonomic uncertainty might cause an overestimation of endemism (Vrijenhoek 2009). Because access to great depths is extremely challenging, there is insufficient information about species variation in space and time. This knowledge gap might lead to the description, across sites and provinces, of synonymous species (morphologically distinct yet belonging to a single interbreeding species) or on the

93 contrary, single species descriptions including undetected cryptic ones (morphologically
94 indistinguishable but reproductively isolated species). To date, population genetics analyses
95 have surprisingly shown a generally high capacity for long-distance dispersal and gene flow
96 for organisms associated with chemosynthetic habitats (Teixeira et al. 2011a, 2012; Van der
97 Heijden et al. 2012; Thaler et al. 2011; Vrijenhoek 2010; Kyuno et al. 2009; Peek et al. 2000),
98 except for some cases of genetic differentiation (Plouviez et al. 2009; Johnson et al. 2006;
99 Hurtado et al. 2004; Jollivet et al 1995). Thus, whether the high endemism reported among
100 seeps and vents reflects speciation and lack of connectivity among habitats, or is partly
101 overestimated by descriptions of synonymous species due to morphological plasticity requires
102 further in-depth investigations (Vrijenhoek 2009; Samadi et al. 2006).
103 The Atlantic equatorial belt (AEB) has been identified as one of the areas of choice to study
104 connectivity among deep chemosynthetic ecosystems (Tyler et al. 2003). Seep communities
105 have been described along the American and African margins (e.g. Olu et al. 1996; Cordes et
106 al. 2007; Olu et al. 2009) potentially connected through equatorial currents. Genetically and
107 morphologically similar taxa of Bathymodiolinae mussels, were found at seeps from both
108 sides of the Atlantic, raising questions about the past and/or present day connection along the
109 AEB (Olu-Le Roy et al. 2007). A hypothetical west-east passage has been proposed for
110 chemosynthetic species across the equatorial Atlantic, with a possible role of hydrothermal
111 vents distributed along the transform faults of the Mid-Atlantic Ridge (MAR) as conduits to
112 dispersal (Van Dover et al. 2002; Tyler et al. 2003). The most recent biogeographical analysis
113 of taxa across the AEB showed that communities cluster according to depth rather than
114 geographical distances (Olu et al. 2010). Among 72 taxa, only 9 species appeared to be
115 present on both sides of the Atlantic, and the hypothesis of the MAR hydrothermal vents
116 acting as stepping stones for migration between both sides was not supported. Sister species

of mussels of the genus *Bathymodiolus* are segregated among different types of chemosynthetic ecosystems. This is also the case for shrimp, with *Alvinocaris muricola* occurring at cold seeps on both sides of the Atlantic and its sister taxa *Alvinocaris markensis* occurring at vents along the MAR (Olu et al. 2009). However, the presence of *A. muricola* was once suspected in the Logatchev site of MAR (Shank, pers. comm. in Komai & Segonzac 2005). More recently, the Vesicomyidae bivalves *Calyptogena* sp. and *Vesicomya* sp., now re-named *Abyssogena southwardae* (Audzijonyte et al. 2012), were recorded for both Western Atlantic seeps and vents of the Mid-Atlantic Ridge, based on morphological traits (Krylova et al. 2010), and also genetic similarities (at the mitochondrial Cytochrome Oxydase subunit I, COI; Decker et al. 2012; Van der Heijden et al. 2012).

To test the hypothesis of large scale dispersal between seeps and vents of the Atlantic equatorial belt we used partial sequences of the mitochondrial cytochrome oxidase subunit I (COI) gene from several morphologically described species of Alvinocarididae shrimps and Vesicomyidae bivalves. To obtain estimates of gene flow across the Atlantic equatorial belt, the shrimp taxa (*Alvinocaris* sp.) identified as shared among sites on the basis of COI sequence analysis were then further analysed using the nuclear 18S ribosomal RNA gene (18S rRNA) and 9 microsatellite markers.

Materials and Methods

Sampling and DNA extraction

The Alvinocarididae shrimp and Vesicomyidae bivalve taxa analyzed in this study were collected from cold seeps of the Eastern (Congo margin) and Western (Gulf of Mexico)

Atlantic and from hydrothermal vents (along the Mid Atlantic Ridge – MAR) during several oceanographic cruises. Three cold seep areas along the Congo margin were sampled during the WACS and the Congolobe cruises including the Regab and the Worm Hole pockmarks (Ondréas et al. 2005; Sahling et al. 2008). The terminal lobes of the Congo deep-sea fan, a sedimentary zone where chemosynthetic species are present were also sampled during these cruises (Sibuet & Vangriesheim 2009). Shrimp samples were collected using a slurp-gun from the ROV (Remotely Operated Vehicle) Victor or the manned submersible Nautile, and Vesicomyidae bivalves were mainly collected with nets and sometimes embedded in sediment cores. Prior to each dive, the bowls used for collecting the shrimp were aseptically washed with ethanol (96 %) before being filled with sterile seawater; the nets and cores were similarly cleaned. Once on board, live specimens were either frozen whole or immediately dissected into body parts under sterile conditions and frozen or preserved in 70 % alcohol. DNA extraction was performed using the CTAB (cetyl trimethyl ammonium bromide) method (Doyle & Doyle 1990) on muscle tissue. Sample sizes are described in Table 1.

Taxon sampling of the Vesicomyidae bivalves included five described species of *Abyssogena*: *Abyssogena kaikoi* Okutani & Metivier 1986, Nankai Trough; *A. mariana* Okutani et al. 2013, from the Shinkai seeps; *A. phaseoliformis* Metivier et al. 2006, Kurile Trench; *A. novacula* Krylova et al. 2010, from Peru trench seeps and *A. southwardae* Krylova et al. 2010, Barbados Accretionary Prism, West Florida Escarpment and Logatchev vent field. We also included an undescribed *Abyssogena* specimen, from Ryukyu Trench Kojima et al. 2004.

As Genbank contains data obtained prior to the new taxonomic revision, and/or unnamed/ re-identified sequences, we have renamed these sequences (see Table 1) according to their identification in the most recent taxonomic revision (Audzijonyte et al. 2012). This new nomenclature was used throughout the analysis.

In addition, sequences available in GenBank for morphologically identical or closely related species (Table 1) were integrated in the analysis to define clusters of identical or highly similar groups of sequences or taxa, that would support or challenge morphological taxonomy or identification. All species analysed and their locations are detailed in Figure 1 and Table 1. Based on our sequence results (see results below) the Alvinocarididae shrimp were grouped into two ESU (Evolutionary Significant Unit), and one of these (hereafter referred to as ESU 1) was further investigated with more detailed microsatellite analyses (sample size permitting this analysis).

Polymerase chain reaction, sequencing and genotyping

Part of the mitochondrial COI gene, the 18S gene (ca 1.7 kb) and 9 microsatellite loci were amplified according to the conditions detailed in Table 2. The PCR amplifications were conducted on a Perkin-Elmer Gene Amp System 7200 (Waltham, MA, USA). PCR products obtained for the mitochondrial and the 18S gene were sent to be purified and sequenced commercially at Macrogen, Inc. (Seoul, Korea) and GATC Biotech (Konstanz, Germany), while microsatellite fragments were separated on an ABI 3130 XL automatic sequencer with the internal size standard Rox 350. Alleles were scored using Peak Scanner version 1.0 (Applied Biosystems).

Data analysis

Phylogenetic Reconstruction

184 Alignments of nucleotide sequences were constructed with Clustal X version 1.83 using the
185 default parameters (Thompson & Gibson 1997), and verified by eye in order to maximise
186 positional homology. Only unique haplotypes were included in phylogenetic analyses.
187
188 Three different data sets were analysed: (1) partial sequences of the COI gene of 26
189 Vesicomyidae bivalves (14 from this study) using "undetermined genus" *nautilei* (former
190 *Calyptogena nautilei*) as the outgroup produced an alignment of 502 bp; (2) partial sequences
191 of the COI gene of 39 Alvinocarididae shrimps (35 from this study) using *Stenopus hispidus*
192 as the outgroup produced an alignment of 447 bp, and (3) partial sequences of the nuclear 18S
193 rRNA gene of 20 Alvinocarididae shrimps (17 from this study) using *Eugonatonotus chacei*
194 as the outgroup produced an alignment of 483 bp.
195 Prior to the phylogenetic reconstructions of the Alvinocarididae shrimp, we obtained an initial
196 data set of 198 Alvinocarididae partial COI sequences (28 retrieved from the GenBank
197 representing all available deep-sea chemosynthetic shrimp species), of which we discarded all
198 identical and all highly dissimilar sequences (clustered with ours with a divergence level
199 higher than 4%), this approach resulted in the final phylogenetic dataset of 39 COI sequences
200 analysed.
201 The nuclear 18S rRNA data set included three *Alvinocaris aff. muricola* from West African
202 seeps, four *Alvinocaris muricola* from the Gulf of Mexico seep, six *Alvinocaris markensis*
203 from the Logatchev vent field, and four *Chorocaris chacei* from Lucky Strike vent field. Two
204 sequences retrieved from Genbank: *Alvinocaris muricola* and *Rimicaris hybisae* were also
205 included in the nuclear data set (accession numbers detailed in Figure 4).
206

The Akaike Information Criterion (Akaike 1973) implemented in MODELTEST v.3.7 (Posada & Crandall 1998) was used to determine the evolutionary models that best fit each of the three data sets. PHYML v2.4.4 (Guindon & Gascuel 2003) was used to estimate the maximum likelihood (ML) trees in all data sets, and to test by nonparametric bootstrap proportions (BPs) the robustness of the inferred trees using 1000 pseudoreplicates. The selected model for the COI Alvinocarididae dataset used in ML analysis was the GTR+Γ whereas the JC was the selected model for the nuclear data set. The best-fit evolutionary model for the Vesicomyidae COI data set was the TrN+I. All ML analyses were carried out on the freely available Bioportal (http://www.bioportal.uio.no).

Bayesian inferences (BI) were conducted with MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001) by Metropolis coupled Markov chain Monte Carlo (MCMCMC) analyses were run for four million generations, and sampled every 100 generations. Two independent runs were performed to reduce chances of selecting a local but not a global optimum. The burn-in was set to 2000,000 generations and robustness of the inferred trees was evaluated using Bayesian posterior probabilities (BPPs).

The Alvinocarididae shrimp COI dataset was analysed under the GTR+Γ (Nst=6), the best-fit model for the nuclear data set was JC (Nst=1) and the Vesicomyidae bivalve COI data set was analysed under the TrN+I (Nst=6). All Bayesian analyses were performed on the CCMar Computational Cluster Facility (http://gyra.ualg.pt) at the University of Algarve.

To analyse and illustrate the divergence levels among haplotypes found for the Vesicomyidae bivalves and Alvinocarididae shrimp across sites, median-joining networks were constructed, using the number of mutations as distance, using Network v. 4.1.0.9 (Bandelt et al., 1999) to infer the most parsimonious branch connections between the sampled haplotypes. In this

231 analysis higher sample sizes (all available sequences) were used in relation to the maximum
232 likelihood trees obtained for both organisms (bivalves and shrimp). For the network analysis a
233 total of 44 sequences were used to obtain one haplotype network of *Abyssogena southwardae*
234 (details in Table 1), while for the Alvinocarididae shrimp a total of 199 sequences were used
235 to obtain two haplotype networks (corresponding to ESU 1 and 2; Figure 3), 197 sequences
236 were generated in this study (as explained in the sampling section) and 2 sequences retrieved
237 from GenBank (*Rimicaris hybisae* and *Alvinocaris methanophila*). To obtain the haplotype
238 network corresponding to ESU 1, 101 sequences were used (all generated in this study) and
239 for the network corresponding to ESU 2, 98 sequences were used (two of these were retrieved
240 from GenBank as explained above).

241

242 Atlantic equatorial belt connectivity of *Alvinocaris* shrimp (ESU 1)

243 The clade we named ESU 1 (Figure 3), which includes *Alvinocaris muricola* (from West
244 Africa and Gulf of Mexico seeps) and *A. markensis* (Logatchev vent field) had enough
245 sequences available for further analyses (101 individuals, see results section for details).
246 These were analyzed using ARLEQUIN version 3 (Schneider et al., 2000) to estimate gene
247 diversity and conduct statistical tests on mitochondrial (COI) data.
248 For each sampling location, the following statistics were computed for mitochondrial data:
249 number of private haplotypes (*N*ph), haplotype (*h*) (Nei, 1987) and nucleotide diversities (π_2)
250 (Nei, 1987), and mean number of pairwise differences (π_1) (Tajima, 1983). To assess
251 population differentiation, pairwise F_{ST} values were calculated following the method of
252 Hudson *et al*. (1992) and exact tests of differentiation were conducted following the method
253 of Raymond & Rousset (1995).

For demographic analysis we determined Fu's F_S (Fu, 1996) and Tajima's D (Tajima, 1989), which can detect departures from selective neutrality and changes in population size such as expansions or bottlenecks (Tajima, 1996; Fu, 1997). Both statistics are expected to result in negative values after a population expansion (Ray et al., 2003) or a selective sweep, whereas positive values are expected under balancing selection of recent bottlenecks.

To assess asymmetrical gene flow between the seeps and vent sampled, we used MIGRATE version 3.2.16 (Beerli, 2009). This analysis is based on maximum-likelihood (ML) estimates for both migration rates and effective population sizes using a coalescent approach (Beerli & Felsenstein, 1999). We used an initial random seed number and Θ and M starting parameters calculated from F_{ST}. As searching strategy we used 40 short chains (4000 trees sampled) and 6 long chains (40 000 trees sampled). For each chain the first 300 000 steps were used as a burn-in and adaptive heating was used to ensure an independent, comprehensive search of the parameter space. We performed 4 independent runs and verified their congruence.

For the microsatellite data (9 loci used), the mean number of alleles per locus (allelic diversity), the expected (H_E) and observed (H_O) proportion of heterozygotes, and the inbreeding coefficient (F_{IS}) were estimated using GENETIX 4.05 (Belkhir et al. 1994-2004). Significance levels were estimated using a permutation approach (1000 permutations). The software GENCLONE (Arnaud-Haond & Belkhir 2007) was used to calculate standardized allelic richness (A_{rich}), to compensate for the unequal sample sizes.

The *F* estimator of genetic structure θ (Weir & Cockerham 1984) was calculated for each locus and over all loci. The probability of the *F*-statistics being greater than zero was determined by permutation (10 000 replicates) using GENETIX 4.05 (Belkhir et al. 1994-

277 2004). Correction for multiple testing was performed using the false discovery rate (FDR)
278 approach (Benjamini & Hochberg 1995) in the software QVALUE (Storey 2002).

279 To test for a reduction in effective population size linked to bottleneck or founder events, the
280 Wilcoxon sign-rank test was applied to test for differences between heterozygosities
281 estimated from allele frequencies (H_E) and from the number of alleles and sample size (H_{Eq}).
282 During a bottleneck, allele number decreases faster than heterozygosity, resulting in a
283 transient apparent heterozygosity excess, indicative of a recent bottleneck event (Cornuet &
284 Luikart 1996) whereas the opposite (allele excess) might occur during a population expansion
285 (Maruyama & Fuerst 1984). Tests were implemented by BOTTLENECK 1.2.02 (Cornuet &
286 Luikart 1996) using 1000 iterations. Estimates of H_{Eq} were calculated under the single-step
287 mutation model (SMM) and the two-phase model (TPM), allowing for 10% multi-step
288 mutations.
289

290 ## Results

291 *Vesicomyidae bivalves*

292 Potential Scale Reduction Factors in Bayesian analyses (all data sets) were 1.00, indicating
293 full convergence of the runs (Gelman and Rubin, 1992). Phylogenetic relationships within the
294 genus *Abyssogena* were mostly unresolved and only two well-supported clades were
295 recovered both in the ML and BI analyses (Figure 2). One corresponded to *A. mariana* from
296 the Shinkai Seep Field (Pacific Ocean) and the other clade included the samples of *A.*
297 *southwardae* from the Lobes of the Congo deep-sea fan and West Florida Escarpment.
298 Sequence divergence between the 20 individuals of *A. southwardae* collected at the Western,
299 Eastern and Mid Atlantic Ridge was very low (less than 1.2% maximum divergence). These
300 clustered into three groups that were geographically mixed and poorly statistically supported.

No identical sequences of *A. southwardae* were detected among those available in GenBank for Western Atlantic cold seeps (Florida Escarpment and Barbados Accretionary Prism), Mid Atlantic Ridge (Logatchev vent field) nor the ones generated in this study from Eastern Atlantic cold seeps (Western Africa cold seeps). However the haplotypes from Florida Escarpment and MAR appear in a central position in the network, intermediate between several haplotypes retrieved from Western African cold seeps (Figure 5). While the two Barbados samples of *A. southwardae* (JX196983; AF008279) displayed a large divergence between themselves (5 point mutations); the JX196983 sequence appeared in the haplotype network between the AF008279 sequence and the other *A. southwardae* sequences of the MAR. The JX196983 sequence displayed 1 point mutation divergence from the MAR and a 2 point mutation divergence from the Florida *A. southwardae*. Of the 20 *A. southwardae* sequences analysed of the Atlantic ocean, the AF008279 Barbados sequence was the most divergent (1.2% maximum divergence).

Alvinocarididae shrimp

Potential Scale Reduction Factors in Bayesian analyses (all data sets) were 1.00, indicating full convergence of the runs (Gelman and Rubin, 1992). Both the ML and BI trees based on the COI gene obtained from several Alvinocarididae shrimp species exhibited two well supported and very divergent clades (Figure 3). The first clade, which we call ESU 1 (Evolutionary Significant Unit 1, Figure 3), includes all *Alvinocaris muricola* and *A. markensis* from seeps and vents of the Atlantic. The second clade (ESU 2, Figure 3) includes specimens with sequence divergence bellow 2%. This comprises *Chorocaris chacei* from the MAR vents, the newly described *Rimicaris hybisae* (which shares some identical sequences with *Chorocaris chacei* but still displayed a maximum divergence of 0.8%) from the Cayman Ridge vents and *Alvinocaris methanophila* (1.4% divergence with *Chorocaris chacei*) from

325 Blake Ridge seeps. In accordance with these results, the ML tree obtained using the 18S gene
326 clusters the same taxa as the COI gene for the second clade (ESU 2) with high support (Figure
327 4), while it lacked resolution in the first clade (ESU 1; Figure 4).
328 In ESU 2 (*Chorocaris*/*Rimicaris*/*A. methanophila*) 15 distinct haplotypes were recovered
329 (Figure 5) out of the 98 sequences analysed for COI. Of these, 10 haplotypes were unique
330 (66.7%) and the remaining 5 that were shared, i.e. haplotypes displayed by more than one
331 individual, belonged to *Chorocaris chacei* from the Logatchev and Lucky Strike vent fields
332 (accession numbers KC840928 to KC840940). The *Rimicaris hybisae* sequence had only one
333 point mutation from a *Chorocaris chacei* haplotype and only two point mutations from the
334 most common haplotype. Similarly, the results for the 18S gene also revealed very low
335 divergence between *Rimicaris hybisae* and *Chorocaris chacei* (0 to 0.4% divergence). The
336 *Alvinocaris methanophila* haplotype was more distant from the centre of the haplotype
337 network, with eight point mutations from the most common haplotype (Figure 5). Yet, the
338 ML tree (Figure 3) clusters this specimen in ESU 2 making the genus *Alvinocaris* (ESU 1)
339 non monophyletic, an issue that should be verified with nuclear sequence data.

340 Population analysis

341 For the ESU 1 clade, which had sufficient sampling size for a population analysis, a total of
342 49 haplotypes were recovered out of the 101 individuals analysed, all belonging to the genus
343 *Alvinocaris* (*A.* aff. *muricola* from West African seeps, *A. muricola* from the Gulf of Mexico
344 seep and *A. markensis* from the Logatchev vent field; Figure 5) sampled across the Atlantic
345 Equatorial Belt. A total of 45 (91.8 %) haplotypes were "private" (Figure 5; accession
346 numbers: KC840879 to KC840927). The most common haplotype was present in all
347 populations sampled across the whole study region, and was central to all other haplotypes,

most of these represented by a single individual and divergent by a single or double point mutation, leading to the central haplotype as the core of a star-like topography (Figure 5).

Haplotype diversity (*h*) was high for all populations, ranging from 0.80 (West Africa seeps, n=78) to 0.96 (Logatchev vent, n=11). In contrast with these rather high values of haplotype diversity, nucleotide diversity (π_2) was however low, ranging from 0.0037 to 0.005 (Table 3). Multilocus genotypes at the 9 microsatellite loci analysed for 98 *Alvinocaris* shrimp (ESU1) from the three sites across the Atlantic equatorial belt (*A. muricola* from the Gulf of Mexico and West Africa and *A. markensis* from Logatchev) also revealed high genetic diversity. The mean number of alleles per locus increased with sample size (Table 4), but the standardized allelic richness (A_{rich}) did not show major trends, ranging from 4.67 (Logatchev) to 5.47 (Gulf of Mexico). Unbiased heterozygosity (H_E) varied between 0.59 (West Africa) and 0.65 (Logatchev) and the observed heterozygosity (H_O) varied between 0.52 (Gulf of Mexico) and 0.62 (Logatchev). The tests for Hardy-Weinberg equilibrium revealed heterozygote deficiency, after correction for multiple tests for both seep sites (Table 4) except for the Logatchev vent which showed no significant departure from equilibrium. Significant F_{IS} values were comprised between 0.09 and 0.18 and were homogeneous across loci.

Demographic analyses suggested the occurrence of population expansions, these were significant for all studied sites when analysing mitochondrial data, with significant negative values for Tajima's D and Fu's F_S (Table 3), while with microsatellite data (bottleneck tests) only the West African cold seep population revealed a significant signature of population expansion (for all mutation models tested), as the expected heterozygosity estimated from allele frequencies (H_E) was significantly lower than estimates based on the number of alleles and sample size (H_{Eq}) ($p < 0.02$).

372 Pairwise comparisons between populations from the Gulf of Mexico seeps and the other two
373 regions (Logatchev vent and West Africa seeps) revealed no differentiation (pairwise F_{ST} for
374 COI haplotypes not significantly different from zero; p> 0.05) (Table 5). However they were
375 significant (p<0.05) between the West Africa seeps and the Logatchev vent (Table 5). The
376 pairwise F_{ST} estimates based on microsatellite loci revealed a similar pattern although with
377 low but significant values between the West Africa seeps and the two other sites (Gulf of
378 Mexico and Logatchev vent), after q-value correction for multiple tests (Table 5).
379 Accordingly, the results from the Bayesian analysis performed with MIGRATE (Table 6)
380 supported, consistently across the independent runs, the occurrence of high gene flow from
381 the Logatchev vent to the Gulf of Mexico seeps.

382 For the ESU 2 clade, only *Chorocaris chacei* had sufficient sampling size for a population
383 analysis. The pairwise F_{ST} estimate based on COI, between the two MAR vent locations, was
384 very low and not significant (F_{ST}=0.00017; p> 0.05).

Discussion

The results reported here reveal large scale connectivity across distinct and patchily distributed chemosynthetic ecosystems. This supports the existence of efficient mechanisms facilitating dispersal and localization of suitable habitats. These findings also raise questions regarding the accuracy of endemism estimates, given our limited knowledge of ecosystems and communities distributed on the bottom of the oceans. We provide evidence for the occurrence of synonymous species for three of the taxa analysed in this study, one Vesicomyidae bivalve and two Alvinocarididae shrimp taxa, shared between hydrothermal vents and cold-seeps across the entire Atlantic Equatorial Belt. This effect creates biases in the evaluation of community composition, diversity and connectivity across deep-sea ecosystems. The description of the same taxa or genetic entities under distinct species and genus names also prevents analyses of dispersal levels and directions across the entire distribution of the taxon or meta-population.

Vesicomyidae bivalve connectivity

The molecular characterization of the vesicomyid *A. southwardae* assigned to *Calyptogena* sp., was first conducted on specimens from the Western Atlantic seeps of Barbados and the Florida Trench (Peek et al. 1997), and later found to be similar to specimens from the MAR Logatchev vents (Peek et al. 2000). Its morphological description (Krylova et al. 2010) also included specimens from the Vema fracture zone and empty valves from the Henry seamount, located close to the Canary Islands at 3500 m depth. Further molecular studies report this species at recently discovered sites in the South MAR hydrothermal vents (Stewart et al. 2008; Van der Heijden et al. 2012). Our study is the first record of this species from the Eastern Atlantic African cold seeps. Although no shared haplotypes were observed between

regions, the few sequences available from GenBank for the Mid and Western Atlantic are evolutionarily close to those reported here from African seeps, indicating that this might be the same species across all the AEB. Only one of the *A. southwardae* from the Barbados seeps was slightly more distant but still exhibited relatively low genetic divergence from the others (less than 1.2%, the maximum divergence level found among the sequences analysed), suggesting that they might all belong to the same taxon. The analysis of more samples, especially from those regions poorly represented in GenBank, would help to elucidate whether there are unsampled shared haplotypes across the AEB, or if, on the contrary, the low divergence levels reflect recent differentiation among those sites.

Even with the extremely low sampling sizes available, it was already possible to reveal a close relationship among all sites, suggesting large-scale connectivity for this species across the Atlantic. Indeed, Vesicomyidae bivalves are distributed worldwide and the genus *Abyssogena* in particular has representatives in at least two Oceans (Atlantic and Pacific; Krylova & Sahling 2010; Figure 2). Other Vesicomyidae bivalve species shared across oceans are also genetically similar, with several species displaying a trans-Pacific or Indo-Pacific distribution (Kojima et al. 2004; Audzijonyte et al. 2012; Decker et al. 2012; Van der Heijden et al. 2012; Figure 2). These results indicate extremely large-scale dispersal capacity. This might have been either followed by persistent connectivity or, if isolated, then the divergence was too recent to be detected on the basis of mitochondrial sequences alone.

Alvinocarididae shrimp connectivity

Our data on the Atlantic Alvinocarididae shrimp revealed two well-supported clades shared across the Atlantic, each composed by samples from seeps and vents. The first clade (ESU 1) comprised *Alvinocaris muricola* from the Western and Eastern Atlantic seeps and *Alvinocaris*

markensis from the MAR vents. We show here that these are synonymous taxa, with shared mitochondrial haplotypes and identical microsatellite polymorphism across the entire AEB including vents and seeps. Perhaps more surprisingly, the second clade (ESU 2) comprised specimens from different genera (*Chorocaris chacei, Rimicaris hybisae and Alvinocaris methanophila*). These showed very low genetic divergence at levels similar to divergence between individuals of the same species. We posit that these taxa belong to the same genus, possibly even the same species.

In the genus *Alvinocaris* of the AEB (ESU 1), the hypothesis of synonymous species with high connectivity between all geographic locations was further supported by the intermingled distribution of haplotypes originating from different regions in a star-like haplotype network, with extremely low and mostly non-significant differentiation for both mtDNA and microsatellite data. The lack of, or very low genetic differentiation found between sites was not due to low genetic diversity, as the 101 *Alvinocaris* individuals from the three localities across the Atlantic had even higher genetic diversity, than the high levels revealed for the shrimp species *Rimicaris exoculata* of the Mid-Atlantic Ridge (Teixeira et al. 2011a; 2012). At most, low sampling sizes for the Western and Central Atlantic could have contributed to the non-rejection of the null hypothesis of panmixia. Yet levels of F_{ST} were so low, that even if increasing sampling size would make them significantly different from zero, they would still be very low levels of differentiation. These results, together with the lack of genetic differentiation also found in shrimp from clade ESU 2 of the MAR vents, support the occurrence of large scale effective migration across the Atlantic Ocean.

Demographic effects

453 For a species to persist as a single genetic entity over a large geographical range in a patchy
454 habitat, gene flow between the separated populations must be high enough to compensate the
455 differentiation originated by random genetic drift within isolated sites. However, a similar
456 signature of low genetic differentiation may also arise when populations have a recent
457 common origin (typical of recently colonized novel habitats; e.g. Neiva et al. 2012) and/or
458 exhibit high effective population sizes limiting the effect of drift and resulting in incomplete
459 lineage sorting despite a lack of connectivity.

460 Both Vesicomyidae bivalves and Alvinocarididae shrimp have been reported to display large
461 population densities (>1000 individuals /m^2; Tyler & Young 1999, Copley et al. 1997). In the
462 absence of temporal fluctuation or significant variance in reproductive success, high
463 population densities might reflect large effective population sizes. However, the star-like
464 topology of our haplotype network of the *Alvinocaris* shrimp together with the neutrality and
465 bottleneck tests, suggest a recent small effective population size followed by expansion. Such
466 events cause instantaneous drift in each independent population which would generate
467 differentiation among populations, unless counteracted by connectivity. The joint observation
468 of large-scale homogeneity and signatures of recent demographic events invalidates the
469 hypothesis of large effective population size hiding ongoing divergence. Instead, it supports
470 the hypothesis that contemporary high connectivity across the Atlantic seeps and vents is the
471 most likely explanation for the results reported here.
472 More taxa should be investigated in the future to further test for the role of MAR as a
473 significant stepping stone between Atlantic seep communities. Our results confirm the role of
474 Mid-Atlantic vents as a stepping stone for at least the two taxa that had sufficient sampling to
475 allow the test of this hypothesis, among the 72 reported thus far from Atlantic seeps (Olu et
476 al. 2010). Additionally, our results highlight that the possible large scale overall connectivity

477 picture might be obscured by the suggested occurrence of synonymous species. In particular,
478 distinct species (and sometimes genus) names discourage genetic studies to address
479 connectivity. Indeed, recently an identical mitochondrial background was revealed among
480 three distinct morphologically described species of tubeworms, *Escarpia* sp., from seeps
481 along the Atlantic and the Eastern Pacific. This finding triggered population genetic studies
482 which revealed large scale dispersal at regional scales (Cowart et al., in press; doi:
483 10.1111/mec.12379).
484
485 *Connectivity*
486 Many factors play a role in effective dispersal, such as fecundity, size of the source
487 population, timing of reproduction, type of larval development, mortality and oceanic currents
488 (Scheltema 1986). Large-scale dispersal has been inferred for many vent organisms regardless
489 of their differences in early life history traits (see review Vrijenhoek 2010) that could
490 influence dispersal ability, suggesting that these are poor predictors of effective dispersal.
491 Indeed *Abyssogena southwardae* and *Alvinocaris* spp. share a wide distribution and patterns
492 of contemporary gene flow across the Atlantic, yet exhibit strikingly distinct larval
493 development.
494 The reproductive biology of Vesicomyidae bivalves is poorly studied and undescribed for
495 *Abyssogena* species. However earlier studies showed that vesicomyid oocytes are usually
496 ~200 μm in diameter, possibly supporting a lecitotrophic development (Lisin et al. 1997;
497 Tyler & Young 1999; Parra et al. 2009). Lecitotrophs are generally assumed to be poor
498 dispersers, but they can have very long pelagic residence times (Shilling & Manahan 1994)
499 and eggs with a greater amount of yolk. Higher reserves may even represent an advantage by

providing nourishment during long-distance dispersal across inhospitable habitats. But to date, no data are available regarding the larval dispersal capacities of Vesicomyidae bivalves.

Alvinocarididae shrimp have been mostly shown to exhibit planktonic larval development with relatively low fecundity (~400 eggs per female). The scarce information available suggests that the development could take place at shallower depths (Pond et al. 2000). For the species *Rimicaris exoculata*, it has been hypothesised that females release their eggs into vent plumes before hatching, as plankton samples at Broken Spur vent field contained eggs, which represented 95% of the biomass (Tyler & Young 1999). Also in the Atlantic, larval stages of *Alvinocaris* sp. and *Chorocaris* sp. have been captured in trawls at mid-water depths and at great distances from known vents (Herring & Dixon 1998). All available data therefore seem to support dispersal potential in the three dimensions of the Atlantic water masses, for at least these Alvinocarididae shrimp species.

Across the Atlantic Equatorial Belt the longitudinal flow of the North Atlantic Deep Water, enhanced by equatorial intermediate jets could theoretically provide a connection pathway along the Equator (Arhan et al. 1998). However, these deep currents have very low velocities, and the time taken to cross the Atlantic may represent a few years. These low velocities may at least in part be compensated by their low temperatures that should slow down larval development, and delay metamorphosis (O'Connor et al. 2007). Besides, warmer and faster surface currents could offer enhanced crossing speed, on the order of few months (Olu et al. 2010). A study on dispersal of deep-sea larvae (Young et al. 2012) among seep communities along the Atlantic American margins indeed showed that shallow dispersal provided greater travelled distances for some of the species analysed. This study further supported that the eastward drift in the North Atlantic is unlikely to carry larvae from North America seeps to Western Africa. The authors suggested that if there is genetic exchange across the Atlantic it

524 is most likely unidirectional, from East to West in the Equatorial current system (Young et al.
525 2012). Accordingly our Bayesian analysis (MIGRATE) of mitochondrial data from *Alvinocaris*
526 seems to consistently support a westward migration from the Mid-Atlantic Ridge to seeps in
527 the Gulf of Mexico. As deep-sea shrimp have been reported as possibly having an ontogenetic
528 vertical migration (Herring & Dixon 1998), they may take advantage of the faster shallow
529 currents across the Equator.
530 A wide range of biological mechanisms and oceanographic pathways therefore exist that may
531 facilitate, speed up or lengthen the duration of dispersal, and contribute to explain the large
532 scale dispersal of larvae across the Atlantic deep sea ecosystems. Yet in a three dimensional
533 ocean, considering the extremely fragmented distribution of vents and seeps, the probability
534 of an individual reaching a suitable chemosynthetic habitat after being diluted in an
535 incommensurable volume of water seems infinitesimal. The existence of dispersal
536 mechanisms such as those delaying metamorphosis (as found for an alvinellid polychaete,
537 Pradillon et al. 2005), or actively guiding larvae (or adults in cases where they are
538 significantly mobile) towards suitable habitat, or through vertical migrations that catch more
539 favourable currents, seem a more parsimonious additional explanation. Indeed, deep-sea
540 larvae can potentially move into water of different temperatures and in some cases different
541 pressures; this may lead to alterations in metabolism, feeding rate, and other vital processes
542 (Young et al. 1996, 1998), extending pelagic larval durations (PLD) (O'Connor et al. 2007). A
543 hypothesis of active directed migration could involve the detection of stimuli such as water
544 chemistry, sound, polarized light, current direction, magnetism and water pressure, as found
545 for other organisms (Kingsford et al. 2002), or a combination of such mechanisms with
546 extremely delayed larval development linked to low temperatures in the deep-sea (O'Connor
547 et al. 2007).

548 Another non-exclusive hypothesis to explain high connectivity is the possible occurrence of
549 more stepping stone habitats than acknowledged. Additional favourable habitats may exist
550 that might have remained undetected thus far due to an extremely low mapping and
551 exploration effort, though extremely valuable. This might cause an erroneous appraisal of
552 deep-sea species ranges, endemicity and connectivity (Audzijonyte & Vrijenhoek 2010) due
553 to very low, spatially scattered and ecosystem-biased, sampling coverage. Indeed, Samadi et
554 al. (2006) showed that high endemism previously reported for some Pacific seamounts was
555 for a large number of taxa an artefactual observation due to low sampling densities focused on
556 a limited geographical area. By increasing sampling pressure at larger geographical scales,
557 these authors demonstrated that most presumed endemic species were also found in other
558 habitats, leading them to propose that seamounts are biodiversity rather than endemism hot
559 spots.

560 Exploration of deep-sea habitats is very biased toward seamounts, active vents and seeps,
561 because these oases of life can be detected from the surface due to the geological anomalies
562 with which they are associated. The present-day knowledge of the distribution of reducing
563 environments in the whole deep-sea is largely underestimated (Audzyjonite & Vrijenhoek
564 2010). However whale- and wood-falls encountered by chance harboured chemosynthetic
565 communities similar to those observed at seeps and vents. These temporary ecosystems may
566 act as stepping stones (Black et al. 1997) dispersed across the seabed ensuring connectivity
567 among chemosynthetic ecosystems. Indeed, several species of Vesicomyidae bivalves have
568 been for example described from deep-sea expeditions of the early 20th century (e.g. Valdivia,
569 Thiele & Jackel 1931) without description of their biotope (Cosel & Olu 2009) and
570 occasionally these old records are identified as the same species as those from cold seeps (e.g.
571 *Christineconcha regab* from the Bay of Biscay and the Regab pockmark; Krylova & Cosel

572 2011). Incidentally, on an artificial wood deployment about 300 m away from any active site
573 near Logatchev (Mid-Atlantic Ridge), *Alvinocaris* shrimp were observed but unfortunately
574 not collected (SH, pers. obs.). The large scale dispersal observed in previous studies and
575 confirmed here across the whole Atlantic Ocean raises the question of how many suitable
576 habitats not associated to *easily* detectable geological anomalies might remain to be
577 discovered in the depths of the oceans in order to gain a global picture of deep sea
578 biodiversity and biogeography.

579

580 ## Acknowledgements

581 The authors wish to thank Rui Candeias, Rita Castilho and Filipe Alberto for technical help;
582 the captains, chief scientists (K. Olu, C. Rabouille, J. Sarrazin, Y. Fouquet, and C.R. Fisher)
583 and crews of the WACS, Congolobe, Momareto, Serpentine, and Expedition to the Deep
584 Slope cruises; Gareth Pearson for the English revision of the manuscript and Asta
585 Audzijonyte and two anonymous reviewers for their helpful suggestions and comments on
586 earlier versions of this manuscript. Collections from the Gulf of Mexico were supported by
587 the US Bureau of Ocean Energy Management contract M05PC00018, and the National
588 Oceanic and Atmospheric Administration's Office of Ocean Exploration and Research
589 (NOAA OER). S.T. and RLC were supported by a postdoctoral fellowship from FCT,
590 Portuguese Science Foundation and ESF (European Social Fund), C.D. was supported by a
591 postdoctoral fellowship from the national ANR project Congolobe. S.T., and S.A-H. were
592 supported by national ANR projects DeepOases, Congolobe and the FP7 EU project
593 Hermione.

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Author contributions

K.O., C.D., S.H. and S. A. collected the field data. S.T, C.D. and S.F. obtained the genetic data. S.T, C.D. and R.L.C. analysed the data. E.A.S. and S.A. contributed with reagents/ materials/ analysis tools. S.T. and S.A. conceived the ideas. S.T., E.A.S. and S.A. interpreted the data and wrote the article. All authors critically revised the manuscript.

Data Accessability

DNA sequences: GenBank accessions: Abyssogena southwardae COI haplotypes: JX900981 – JX901014; Alvinocarididae shrimp COI haplotypes: KC840879 – KC840940; Alvinocarididae shrimp 18S rRNA haplotypes: KC840876 – KC840878

Aligned sequences are available in the Dryad data repository: doi: 10.5061/dryad.cv910

Microsatellite Genotypic Data: A copy of our microsatellite genotypic data, in Genetix format, are available on Dryad, doi: 10.5061/dryad.cv910

Figure Legends

Figure 1. Location of the specimens and populations sampled across the Atlantic Equatorial Belt (AEB). Legend: Triangles- *Abyssogena southwardae*; circles - species comprised in ESU1 (*Alvinocaris muricola /A. markensis*); squares - species comprised in the ESU2 (*Chorocaris chacei, Alvinocaris methanophila* and *Rimicaris hybisae*). Colour codes: Green- Western Atlantic; Yellow- Mid-Atlantic Ridge; Orange- Eastern Atlantic.

Figure 2. Phylogenetic relationships of the *Abyssogena* bivalves based on the maximum likelihood analysis of partial sequence data of the mitochondrial COI gene using the TrN+I evolutionary model.

Figure 3. Phylogenetic relationships of Alvinocarididae shrimps sampled in the Atlantic seeps and vents based on the maximum likelihood analysis of partial mitochondrial COI sequence data using the GTR+Γ evolutionary model.

Figure 4. Phylogenetic relationships of Alvinocarididae shrimps sampled in the Atlantic seeps and vents based on the maximum likelihood analysis of partial sequences of the 18S ribosomal gene using the JC evolutionary model.

Figure 5. Haplotype networks of the mtDNA haplotypes obtained for *Abyssogena southwardae* bivalves and for both clades (ESU1 and 2) recovered for the Alvinocarididae shrimp of the AEB. Each circle represents a different haplotype, with the size of each circle proportional to the number of individuals displaying that particular haplotype. The colours used represent the locations where the haplotypes were found and within pie charts, the segment size is proportional to the relative frequency of a haplotype in each population where it is present. Mutation steps are represented only when higher than 1.

Tables

Table 1. GenBank accession numbers, specimen collection sites and depth of species used for phylogenetic analyses.

Specimen	Nomenclature according to Audzijonyte et al. 2012	Accession nº	Sample site	Area	Habitat	Depth (m)	Study; sample size
Bivalves							
Abyssogena southwardae		JX900981; JX901014	WormHole pockmark	East Atlantic	Seep	3089	This study; 2
Abyssogena southwardae		JX900982-JX901013	Lobes of the Congo deep-sea fan	East Atlantic	Presumably Seep	4946	This study; 32
Calyptogena kaikoi	*Abyssogena kaikoi*	AB110763	Off Muroto Point, Nankai Trough	Western Pacific	Seep	4800	Kojima *et al.* 2004
Vesicomyidae sp. 'Ryukyu Trench'	*Abyssogena* sp. Ryuku	AB110775	Ryukyu Trench	Western Pacific	Seep	5900	Kojima *et al.* 2004
Calyptogena sp. 6K1234-1/2	*Abyssogena mariana*	AB629938/ 39	Shinkai Seep Field	Western Pacific	Vents	5550	Ohara et al. 2012; Okutani etal. 2013
Calyptogena phaseoliformis	*Abyssogena phaseoliformis*	AB479088	Kurile Trench	Western Pacific	Seep	4819	Okutani *et al.* 2009
Calyptogena sp.	*Abyssogena southwardae*	AF008279; JX196983	Barbados Accretionary Prism	Western Atlantic	Seep	5000	Peek *et al.* 1997; Audzijonyte et al. 2012

Calyptogena n. sp. *West Florida Escarpment*	*Abyssogena southwardae*	AF008280	West Florida Escarpment	Western Atlantic	Seep	3313	Peek *et al.* 1997
Vesicomya sp. MAR	*Abyssogena southwardae*	EU403471	Logatchev	Mid-Atlantic Ridge	Vent	3028	Stewart *et al.* 2008
Abyssogena southwardae		JQ844786; JQ844787	Clueless	Mid-Atlantic Ridge	Vent	2995	Van der Heijden *et al*. 2012
Abyssogena novacula		JX196970	Peru Trench	Pacific	Seep	5528	Van der Heijden *et al*. 2012
Calyptogena nautilei	« Undetermined genus » *nautilei*	AB110759	Zenisu Ridge, Japan	Western Pacific	Seep	3300	Kojima et al. 2004
Arthropods							
Alvinocaris muricola		KC840887-KC840892; KC840894	Gulf of Mexico, GC852 site	Western Atlantic	Seep	1450	This study; 12
Alvinocaris markensis		KC840879-KC840886; KC840893	Logatchev	Mid-Atlantic Ridge	Vent	3028	This study; 11
Alvinocaris markensis		AF125408/409	Snake Pit	Mid-Atlantic Ridge	Vent	3398	Shank *et al.* 1999
Alvinocaris aff. *muricola*		KC840895-KC840927	Regab, West Africa	East Atlantic	Seep	3157	This study; 78
Alvinocaris methanophila		AY163260	Blake Ridge	Western Atlantic	Seep	2155	Van Dover *et al.* 2002
Chorocaris chacei		KC840932-KC840940	Lucky Strike	Mid-Atlantic	Vent	1700	This study; 80

				Ridge			
Chorocaris chacei		KC840928-KC840931	Logatchev	Mid-Atlantic Ridge	Vent	3028	This study; 16
Rimicaris hybisae		JN850607	Mid-Cayman Ridge	Western Atlantic	Vent	4960	Nye *et al.* 2012

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925

926 **Table 2**. Details of primers and PCR conditions used for the different molecular markers
927 amplified.

	Cytochrome Oxydase subunit I (Folmer et al., 1994)	**18S rRNA** (López-García et al., 2003)	**Microsatellites** (Teixeira et al., 2011b ; Zelnio et al. 2010)
Primer names	LCOI1490 ; HCOI2198	18S–82F ; 18S–1498R	Rim 11 ; 12 ; 26 ; 30 ; 32 ; 42 ; CHO 83 ; 91
DNA (ng)	50	50	10
$MgCl_2$ (mM)	2.5	3	3
dNTP (mM)	0.8	0.8	0.8
Taq (U/µl)	0.4	0.4	0.5

Final volume (µl)	50	20	10
Annealing temperature (ºC)	52	56	(Teixeira et al., 2011b ; Zelnio et al. 2010)

928

929

930 **Table 3.** Genetic diversity indices based on COI partial sequences (517 bp) of the deep-sea
931 shrimp *Alvinocaris* sampled across the Atlantic equatorial belt calculated for each sampled
932 site. (n) sample size; (*k*) number of polymorphic sites; (*N*h) number of haplotypes; (*N*ph)
933 number of private haplotypes, i.e. the number of haplotypes exclusive of a population; (*h*)
934 haplotype diversity; (π_1) mean number of pairwise differences; (π_2) nucleotide diversity.
935 Neutrality and population expansion tests: *D*= Tajima's D-test; F_S= Fu's F_S test. All values
936 obtained for the neutrality tests were significant at the 5 % level.

Site	*n*	*k*	*N*h	*N*ph	*h*	π_1	π_2	*D*	F_S
Gulf of Mexico	12	14	9	5	0.91±0.08	2.47	0.0047	-1.99	-4.83
Logatchev	11	11	9	7	0.96±0.05	2.87	0.0050	-1.01	-4.83
West Africa	78	43	37	33	0.80±0.05	1.95	0.0037	-2.51	-27.45

937

938 **Table 4**. Descriptive statistics based on 9 microsatellite loci for *Alvinocaris* shrimp from
939 ESU1 from all sampled locations. Number of individuals sampled (n), mean number of alleles
940 across loci (*A*), A_{rich} standardized allelic richness for a minimum of 12 individuals, observed

941 (H_O) and expected (H_E) heterozygosities and heterozygote deficiency (F_{IS}). Bold numbers
942 indicate significant values ($^{***}p<0.001$) after q-value correction.

Site	n	*A*	A_{rich}	H_E	H_O	F_{IS}
Gulf of Mexico	14	5.8	5.47	0.64	0.52	**0.18*****
Logatchev	17	5.4	4.67	0.65	0.62	0.04
West Africa	67	8.8	4.89	0.59	0.53	**0.09*****

943

944 **Table 5**. Pairwise F_{ST} values based on haplotype (COI) and allele frequencies (9
945 microsatellite loci) for the ESU1 *Alvinocaris* species sampled across the Atlantic equatorial
946 belt. Significant levels are indicated ($^{*}p<0.05$; $^{***}p<0.001$).

	COI marker		Microsatellite markers	
Sites	Gulf of Mexico	Logatchev	Gulf of Mexico	Logatchev
Gulf of Mexico				
Logatchev	-0.005		0.022	
West Africa	0.047*	-0.007	0.041***	0.078***

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948

949 **Table 6.** Estimation of *M* and Θ generated in MIGRATE analysis of mtDNA sequences (COI)
950 of the ESU1 *Alvinocaris* species sampled across the AEB. Values in parentheses denote the
951 95% profile likelihoods for each estimate; all values were obtained in one independent run.

Sites	Gulf of Mexico Θ=0.09	Logatchev Θ= 0.0154	West Africa Θ= 0.034
Gulf of Mexico	-	$4.1x10^{-6}$ ($3.1x10^{-6}$ – 0.01)	$1.4x10^{-5}$ ($1.1x10^{-5}$ – 0.04)
Logatchev	$2x10^{3}$ ($1.1x10^{3}$-$3.5x10^{3}$)	-	$3.1x10^{3}$ ($2.4x10^{3}$-$4.8x10^{3}$)
West Africa	$3.7x10^{-5}$ ($2.8x10^{-5}$ – 0.09)	$4.1x10^{-6}$ ($3.1x10^{-6}$ – 0.01)	-

952 Donor populations represent the lines, recipient populations represent the columns

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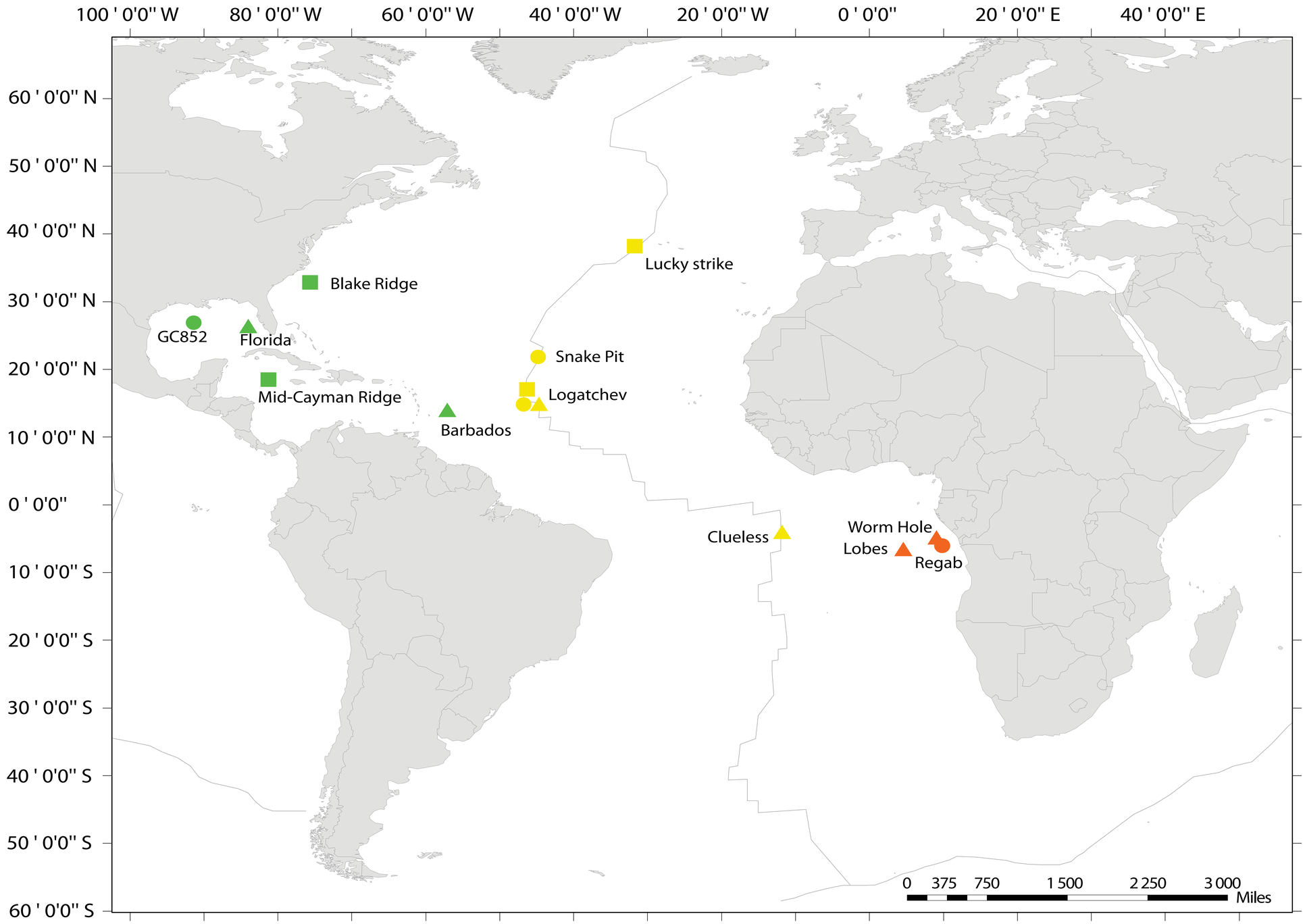
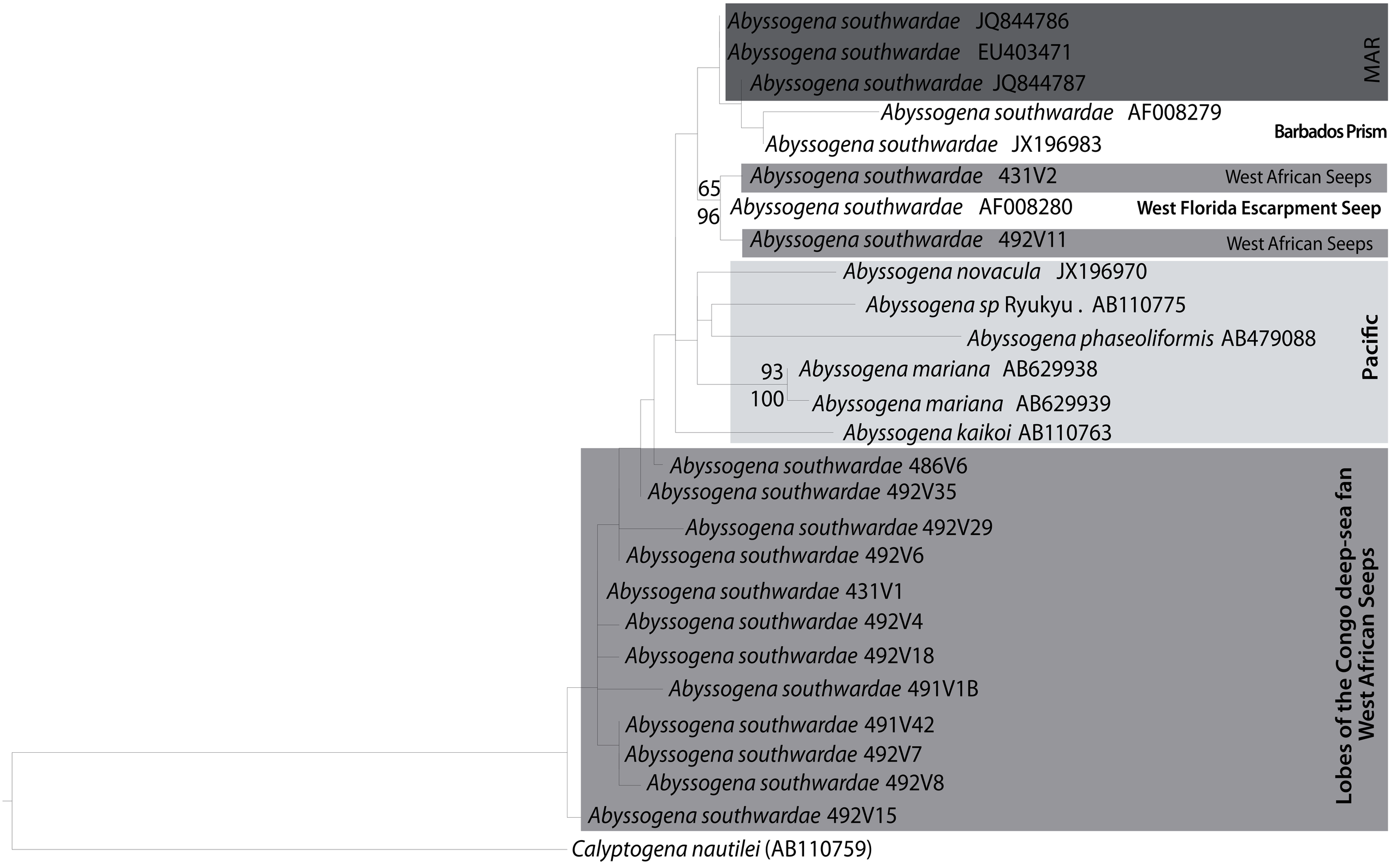


Alvinocaris muricola and *A. markensis*

Chorocaris chacei, Alvinocaris methanophila, Rimicaris hybisae

Abyssogena southwardae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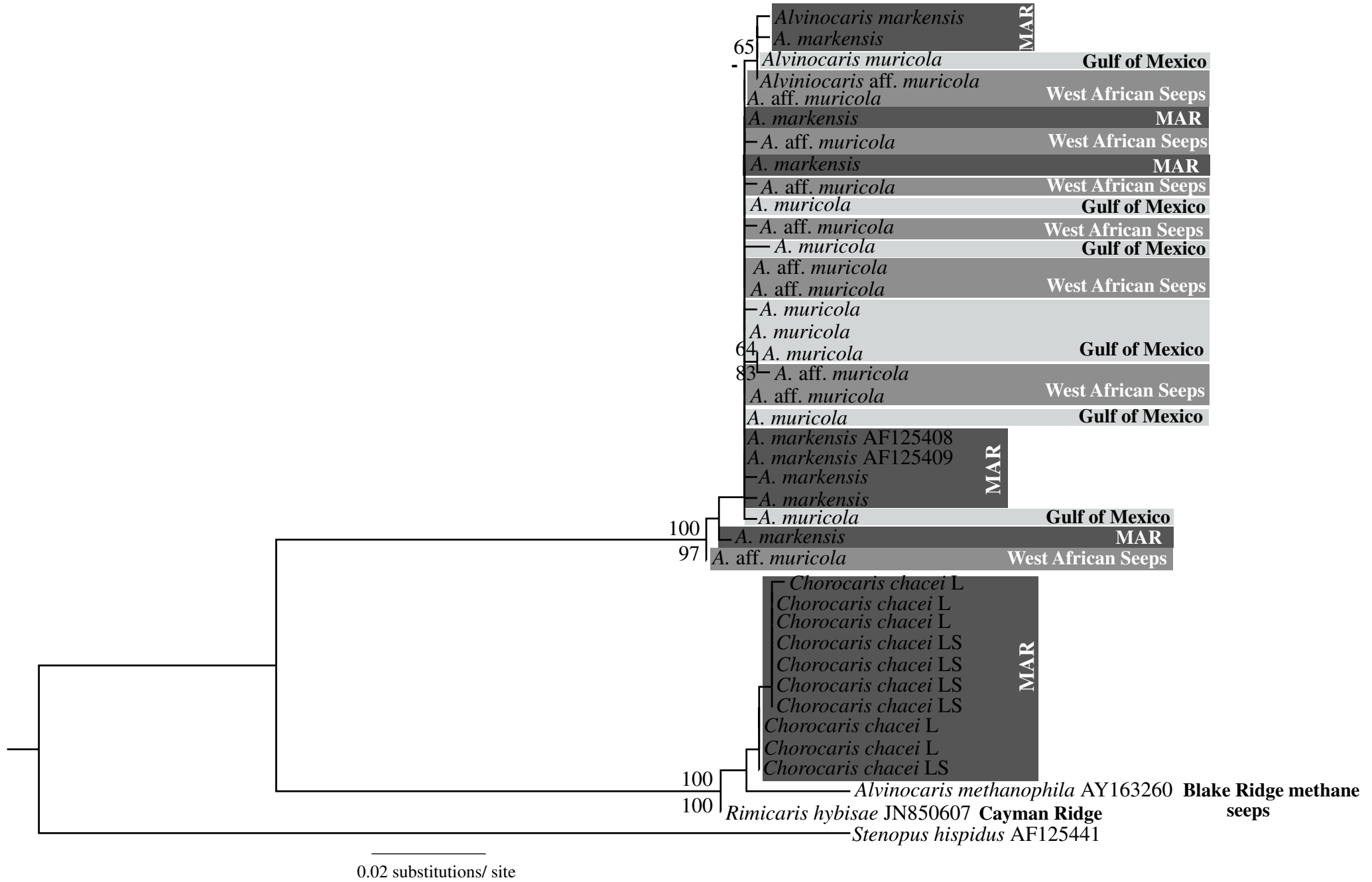
Abyssogena southwardae 431V2
Abyssogena southwardae AF008280
Abyssogena southwardae 492V11
Abyssogena novacula JX196970
Abyssogena sp Ryukyu . AB110775
Abyssogena phaseoliformis AB479088
Abyssogena mariana AB629938
Abyssogena mariana AB629939
Abyssogena kaikoi AB110763
Abyssogena southwardae 486V6
Abyssogena southwardae 492V35
Abyssogena southwardae 492V29
Abyssogena southwardae 492V6
Abyssogena southwardae 431V1
Abyssogena southwardae 492V4
Abyssogena southwardae 492V18
Abyssogena southwardae 491V1B
Abyssogena southwardae 491V42
Abyssogena southwardae 492V7
Abyssogena southwardae 492V8
Abyssogena southwardae 492V15
Calyptogena nautilei (AB110759)
65
96
93
100
MAR
Barbados Prism
West African Seeps
West Florida Escarpment Seep
West African Seeps
Pacific
Lobes of the Congo deep-sea fan
West African Seeps
0.02 substitutions/site


Alvinocaris markensis
A. markensis
MAR
65
Alvinocaris muricola
Gulf of Mexico
Alviniocaris aff. muricola
A. aff. muricola
West African Seeps
A. markensis
MAR
A. aff. muricola
West African Seeps
A. markensis
MAR
A. aff. muricola
West African Seeps
A. muricola
Gulf of Mexico
A. aff. muricola
West African Seeps
A. muricola
Gulf of Mexico
A. aff. muricola
A. aff. muricola
West African Seeps
A. muricola
A. muricola
64
A. muricola
Gulf of Mexico
83
A. aff. muricola
A. aff. muricola
West African Seeps
A. muricola
Gulf of Mexico
A. markensis AF125408
A. markensis AF125409
A. markensis
A. markensis
MAR
A. muricola
Gulf of Mexico
100
A. markensis
MAR
97
A. aff. muricola
West African Seeps
Chorocaris chacei L
Chorocaris chacei L
Chorocaris chacei L
Chorocaris chacei LS
Chorocaris chacei LS
Chorocaris chacei LS
Chorocaris chacei LS
MAR
Chorocaris chacei L
Chorocaris chacei L
Chorocaris chacei LS
100
Alvinocaris methanophila AY163260
Blake Ridge methane seeps
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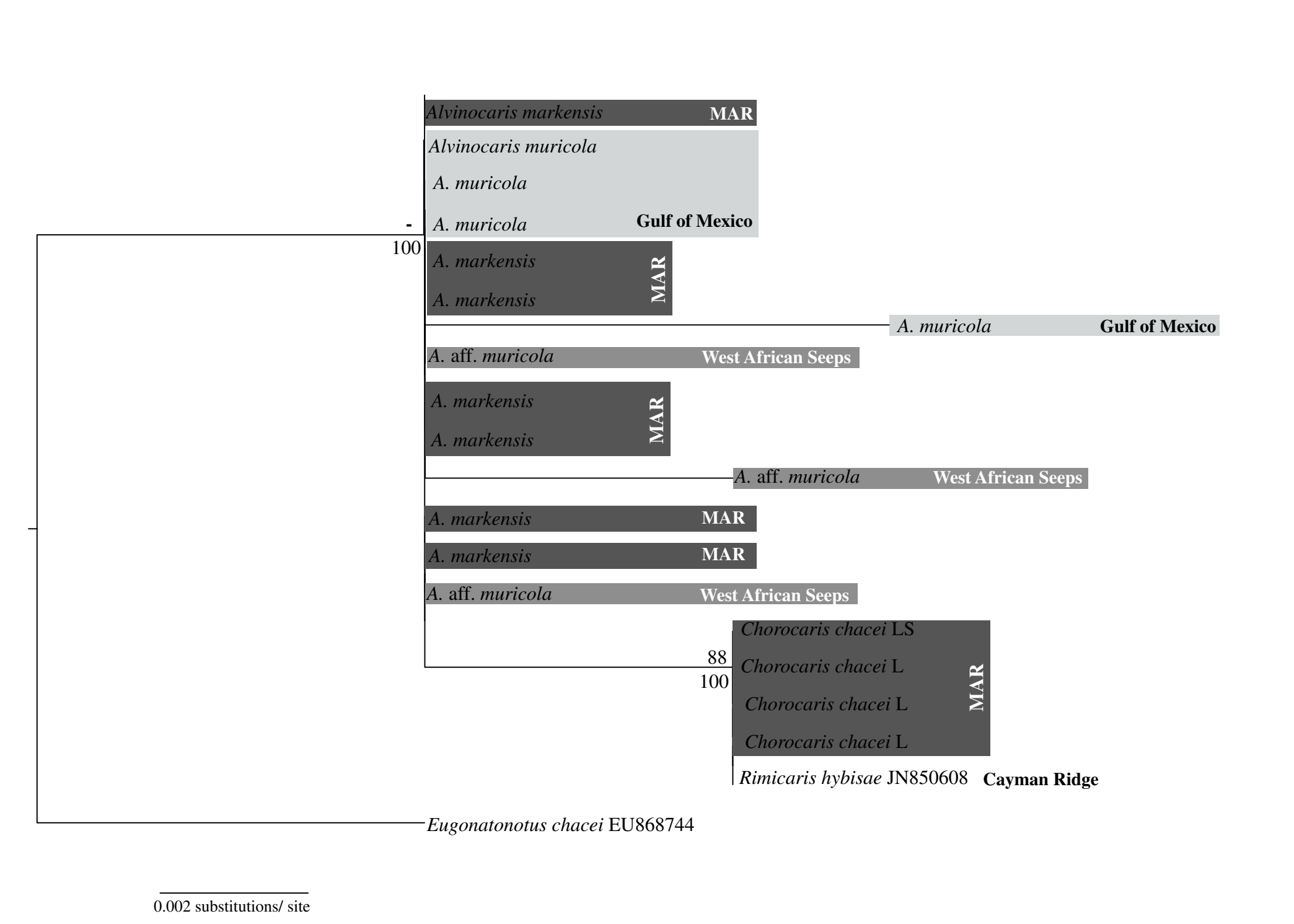
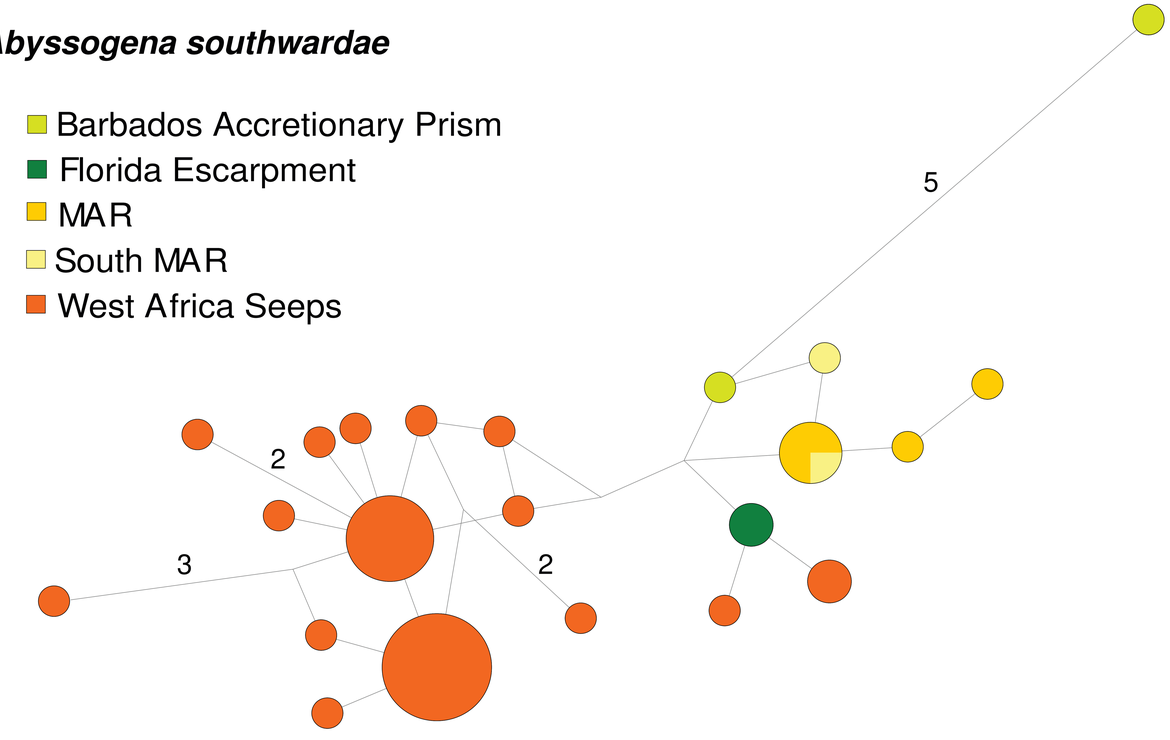
Rimicaris hybisae JN850607
Cayman Ridge
Stenopus hispidus AF125441
0.02 substitutions/ site


Alvinocaris markensis MAR
Alvinocaris muricola
A. muricola
A. muricola Gulf of Mexico
-
100
A. markensis
A. markensis
MAR
A. muricola Gulf of Mexico
A. aff. muricola West African Seeps
A. markensis
A. markensis
MAR
A. aff. muricola West African Seeps
A. markensis MAR
A. markensis MAR
A. aff. muricola West African Seeps
Chorocaris chacei LS
88
Chorocaris chacei L
100
Chorocaris chacei L
Chorocaris chacei L
MAR
Rimicaris hybisae JN850608 Cayman Ridge
Eugonatonotus chacei EU868744
0.002 substitutions/ site

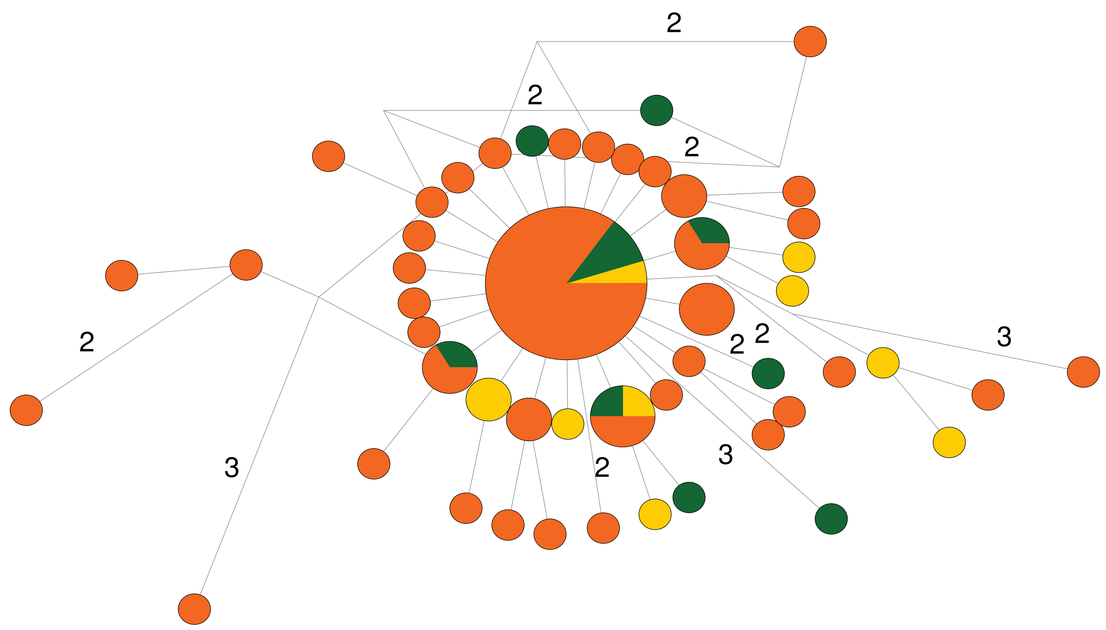
Abyssogena southwardae

- Barbados Accretionary Prism
- Florida Escarpment
- MAR
- South MAR
- West Africa Seeps



Alvinocarididae ESU1

- *Alvinocaris markensis* MAR (Logatchev)
- *Alvinocaris muricola* Gulf of Mexico
- *Alvinocaris aff. muricola* West Africa



Alvinocarididae ESU2

- *Chorocaris chacei* MAR (Logatchev)
- *Chorocaris chacei* MAR (Lucky Strike)
- *Alvinocaris methanophila* Blake Ridge seep
- *Rimicaris hybisae* Cayman Ridge