COMPARATIVE PHYLOGEOGRAPHY OF THREE HOST SEA ANEMONES IN THE INDO-PACIFIC

Running Title: Phylogeography of host sea anemones

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DATA ACCESSIBILITY

The multilocus genotype tables for each species have been included as supporting information as one single excel file.

Aim

The mutualistic relationship between anemones and anemonefishes is one of the most iconic examples of symbiosis. However, while anemonefishes have been extensively studied in terms of genetic connectivity, such information is lacking entirely for host sea anemones. Here, we provide the first information on the broad-scale population structure and phylogeographic patterns of three species of host sea anemone, *Heteractis magnifica, Stichodactyla mertensii,* and *Entacmaea quadricolor*. We evaluate if there is concordance in genetic structure across several distinct biogeographic areas within the Indo-Pacific region and to what extent the observed patterns may concur with those found for anemonefishes.

Location

Indo-Pacific, including the Red Sea.

Taxon

Heteractis magnifica, *Stichodactyla mertensii,* and *Entacmaea quadricolor*

Methods

Microsatellite markers and a combination of statistical methods including Bayesian clustering, Isolation by Distance (IBD), Analysis of Molecular Variance (AMOVA), and Principal Components Analysis (PCA) were used to determine population structure. The congruence among distance matrices method (CADM) was used to assess similarity in spatial genetic patterns among species.

Results

Significant population structure was identified in the three host anemone species. Each species is likely composed of at least two genetic clusters corresponding to two biogeographic regions, the Red Sea and the rest of the Indo-Pacific. Two of the three anemone species seem to be experiencing admixture where the two main clusters overlap (the Maldives). IBD analyses in the Red Sea revealed differences in gene flow among species, suggesting more limited dispersal potential for *E. quadricolor* than for *S. mertensii* and *H. magnifica*. Clonality is documented in *S. mertensii* for the first time.

Main conclusions

This research documents the genetic population structure for three ecologically important host sea anemones across the Indo-Pacific and provides valuable insights regarding their biogeography and evolution. Specifically, we found high levels of genetic divergence between populations across different biogeographic regions, suggesting different evolutionary lineages within species. At the same time, common geographic overlap of population structures suggests similar evolutionary histories among all three species. Interestingly, the observed patterns are congruent to some extent with structure reported for several anemonefish species, reflecting their close ecological association.

Keywords: Actiniaria, biogeography, Cnidaria, connectivity, coral reef, gene flow, Indo-Pacific, microsatellites, phylogeography, population genetics.

INTRODUCTION

The roles of historical, environmental, geological, and geographic barriers to gene flow in shaping a species' genetic diversity and ultimately its distribution, can be determined by comparing population structures between species with similar distributions and ecological niches (Bermingham & Moritz, 2002; Arbogast & Kenagy, 2008). For example, different species displaying similar patterns of spatial population structure suggests the presence of congruent evolutionary phylogeographic processes. Alternatively, differences in these patterns between species can provide evidence for the relative importance of individual life history strategies or variance in evolutionary histories among species that can be the result of different effects of historical events (Dawson, Louie, Barlow, Jacobs, & Swift, 2002; Crandall, Frey, Grosberg, & Barber, 2008; Hui et al., 2016).

The Indo-Pacific is a highly diverse biogeographical region that includes the Coral Triangle biodiversity hotspot, the Red Sea, and tropical waters of the Indian Ocean, as well as the central and western Pacific (Hui et al., 2016). It is a broad region with a complex geological history (Hall, 2002), and it encompasses different bodies of water that together represent a mosaic of environmental conditions, geographic settings, and oceanographic features (Bowen et al., 2016). Genetic surveys show that the population structure of Indo-Pacific species can coincide with known historical geological processes, geographical barriers or environmental gradients. For example, low sea levels during glaciations exposed the Sunda (southeast Asia) and Sahul (Australia-New Guinea) continental shelves (Voris, 2000) creating what is known as the Indo-Pacific barrier or the Sunda Shelf barrier (Randall, 1998; Rocha, Craig, & Bowen, 2007). Several studies in different marine fishes and invertebrates have shown a geographic concordance of population genetic structure with this historical barrier with populations in the Pacific being divergent from those in the Indian Ocean (reviewed in Ludt & Rocha, 2015; Crandall et al., 2019). More precisely, documented genetic breaks occur in the eastern Indian Ocean (Christmas Island, Cocos Keeling Islands, Indonesia), representing the division between Indian Ocean and Pacific populations. This division has been attributed to changes in sea-level during the Plio-Pleistocene which resulted in land bridges throughout Indonesia that effectively split Indo-Pacific groups into allopatric Indian and Pacific populations or species (Rocha et al., 2007; Gaither & Rocha, 2013). Similarly, the narrow, shallow Bab-el-Mandab strait (between the Red Sea and western Indian Ocean), has been considered an historical barrier to gene flow to numerous species that display genetic structure between populations in the Red Sea and in the Indian Ocean (Klausewitz, 1989; DiBattista et al., 2016). Yet, despite being widespread across the Indo-Pacific, there is no available information about the distribution of genetic diversity of sea anemones or the role played by historical/geological barriers.

Geographic and environmental settings have also been documented as barriers to gene flow. For instance, the large geographic distances separating some Pacific islands, such as Moorea (French Polynesia), the Marquesas Islands, and Easter Island, represent geographic barriers to gene flow (Randall 1998; Rocha et al., 2007).

Barriers to gene flow have also been identified in the Red Sea, and these are linked to environmental gradients (Saenz-Agudelo et al. 2015). Several studies have shown correlations between genetic distance and these environmental gradients (Nanninga, Saenz-Agudelo, Manica, & Berumen, 2014; Giles, Saenz-Agudelo, Hussey, Ravasi, & Berumen, 2015; Sawall, Al-Sofyani, Banguera-Hinestroza, & Voolstra, 2014; Reimer et al., 2017). However, again, the role of geographic barriers in shaping the genetic diversity of sea anemones has not been examined.

Many marine species are endemic to the Indo-Pacific region and some of these have evolved symbiotic relationships, such as the iconic association between host sea anemones (Order Actiniaria) and anemonefishes (Genera *Amphiprion* and *Premnas*; Fautin & Allen, 1997; Allen, Drew, & Fenner, 2010). A number of studies report large-scale population genetic structure of anemonefishes (Timm & Kochzius, 2008; Nanninga et al., 2014; Dohna, Timm, Hamid, & Kochzius, 2015; Saenz-Agudelo et al., 2015; Steinberg et al., 2016; Huyghe & Kochzius, 2017; O'Donnell, Beldade, Mills, Williams, & Bernardi, 2017). In general these studies indicate that anemonefish species such as *Amphiprion bicinctus*, *Amphiprion ocellaris,* and *Amphiprion perideraion* display genetic structure that coincides with historical geographic barriers such as the Sunda shelf, the Straight of Bab Al Mandeb, between basins (Pacific Ocean and Indian Ocean) or between the eastern and western sides of the Indian Ocean. In contrast, there is a limited understanding of the dispersal abilities and population connectivity of their host sea anemones, and whether or not there are similarities in their genetic structure with that of anemonefishes. Resolving these knowledge gaps could shed light on the processes that have shaped their close ecological relationship.

Population structure, re-colonization, and replenishment of host sea anemones are influenced by dispersal and reproduction. Of the few reproduction studies that have been conducted, *Entacmaea quadricolor* and *Heteractis crispa* were found to be gonochoric, releasing their gametes in broadcast spawning events in the austral summer and autumn in subtropical Australia (Scott & Harrison, 2005, 2007, 2009). In the Red Sea, male *Stichodactyla mertensii* have been observed spawning on three consecutive days after a boreal spring full moon (Bouwmeester, Gatins, Giles, Sinclair-Taylor, & Berumen, 2016). In the laboratory, most *E. quadricolor* planulae metamorphose within two weeks, although they can remain free-swimming for at least two months (Scott & Harrison, 2007). This suggests that anemone larvae have the potential to travel large distances, potentially facilitating high levels of gene flow, though larval dispersal may be more restricted when asexual reproduction is common. *Heteractis magnifica* and *E. quadricolor* can reproduce asexually using longitudinal fission (Scott, 2017) and the resulting clones can form large assemblages of >100 individuals (Frisch et al., 2019).

In this study, we determine the broad-scale genetic structure and phylogeography of three host anemone species (*H. magnifica, S. mertensii*, and *E. quadricolor*) and test for congruence in genetic structure among species and genetic divergence associated to putative barriers previously reported for other coral reef organisms. Our hypothesis is that given the large geographic distribution of these species, these anemones should display some degree of genetic structure across their distribution range and this structure should be concordant with the well-characterized biogeographic breaks and also with the genetic structure of the anemonefishes that these species host.

MATERIALS AND METHODS

Heteractis magnifica, *S. mertensii*, and *E. quadricolor* are widely distributed across the Indo-Pacific and Red Sea. *H. magnifica* occupies the largest longitudinal range of the three species, from French Polynesia to East Africa and the Red Sea, while *S. mertensii* and *E. quadricolor* range from Micronesia and Melanesia to East Africa and the Red Sea (Fautin & Allen, 1992; Brolund, Tychsen, Nielsen, & Arvedlund, 2004; Gatins, Saenz-Agudelo, Scott, & Berumen, 2018). All three species are present from Australia to the Ryukyu Islands, with *E. quadricolor's* distribution extending further north to Japan (Fautin & Allen, 1992). All three species can be found in extremely shallow waters around 1 m deep (Dunn, 1981) but maximum depths vary. *Heteractis magnifica* and *E. quadricolor* can inhabit mesophotic waters down to around 60 m (Brolund et al., 2004; Bridge, Scott, & Steinberg, 2012), while S. *mertensii* are limited to shallow waters of around 20 m (Dunn, 1981). Tentacle specimens were collected using dissecting scissors and forceps whilst SCUBA diving at 42 sites across the Indo-Pacific and Red Sea (Fig. 1 & Table 1). Specimens were placed in 2 ml vials and stored in 96% ethanol. The GPS coordinates of each anemone were also recorded.

DNA was extracted from 880 specimens using Qiagen's DNeasy Blood and Tissue Kit according to the manufacturer's protocol. A total of 10 unique microsatellite markers were amplified for *H. magnifica,* 11 for *S. mertensii,* and 12 for *E. quadricolor* (Table S1, Supporting information). All forward sequences were labelled with a fluorescent dye (6-FAM, NED, PET, VIC). PCR conditions followed the Qiagen PCR Multiplex kit protocol with modifications as in Gatins et al. (2018); a total of 10 µL was used for each individual reaction mix, including 5 µL of Multiplex PCR MasterMix (Qiagen), 1 µL of primers (2 µM; see Table S1, Supporting information), 3.3 µL of water and 0.7 µL DNA (50-150 ng/µL). The thermocycler conditions for PCR amplifications were: 95 ºC for 15 min, then 25 cycles of 94 ºC for 30 s, annealing at a locus-specific temperature (57/60 ºC, see Table S1, Supporting information) for 90 s, and an extension at 72 °C for 60 s, with a final extension set at 60 °C for 30 min. Further details regarding microsatellite and PCR protocols can be found in Gatins et al. (2018). Final PCR products of 10 µL were diluted with 130 µL MilliQ water before being sent for fragment size analysis using a GeneScan 500-LIZ size standard and an ABI 3730xl genetic analyser (Applied Biosystems, USA) in the Biosciences CORE laboratory at King Abdullah University of Science and Technology, Saudi Arabia. Genotyping was completed using Geneious v. 8.1.6 (Kearse et al., 2012).

The final datasets consisted of 205 *H. magnifica* individuals, 122 *S. mertensii* individuals, and 249 *E. quadricolor* individuals (Table 1; samples with more than three missing loci were excluded). Clonality was investigated and corrected for by comparing multilocus genotypes in GenAlEx v.6.502 (Peakall & Smouse, 2012). Subsequent analyses were conducted using the corrected datasets, leaving only one individual per multi-locus genotype. GenePop v.4.2 (Raymond & Rousset, 1995; Rousset, 2008) was used to check for deviations from Hardy-Weinberg Equilibrium (HWE), and thus the presence of null alleles. Calculations of the inbreeding coefficient F_{IS} (Weir & Cockerham, 1984), deviations from HWE, and linkage disequilibrium in pairwise comparisons of all loci were also tested for using GenePop. Significance values were estimated using Markov chain methods (1000 dememorizations, 100 batches, and 1000 iterations per batch) and were adjusted in R using the false discovery rate (fdr) method (Benjamini & Hochberg, 1995; alpha =

0.05). Summary statistics, including allelic richness, expected and observed heterozygosity, and fixation indices were calculated in GenAlEx. At some locations it was not possible to obtain the minimum requirement of five specimens. We did not estimate summary statistics for these sites to avoid biases associated with small sample sizes.

For each species' dataset, the software Structure v. 2.3.4 (Pritchard, Stephens, & Donnelly, 2000) was used to perform a Bayesian clustering analysis to estimate the most likely number of genetic clusters or putative populations (K) given the genotypic data. Parameters were set to use the admixture model with sampling location as prior and correlated allele frequencies. Analyses were run with a burn-in period of 200,000 iterations, 500,000 MCMC repetitions, K set to the number of sites sampled, and five runs for each value of K for each species. The resulting data were uploaded to Structure Harvester (Earl, 2012) in order to summarize and visualize the change in the mean log likelihood and Evanno's delta K for different population clusters (K). CLUMPAK (Kopelman, Mayzel, Jakobsson, Rosenberg, & Mayrose, 2015) was then used to create a visual representation of the population structure based on several estimates of K, combining the data files (runs) from Structure. We used Principal Component Analysis (PCA) to depict the overall genetic variability among individuals, as an alternative to Structure that has no underlying assumptions to identify genetic structures (Jombart, Devillard, & Balloux, 2010). To do this, we used the dudi.pca function of the ade4 package available in R (Chessel, Dufour, & Thioulouse, 2004). An Analysis of Molecular Variance (AMOVA) was performed in GenAlEx (Peakall & Smouse, 2012) to quantify the magnitude of genetic variation among the groups identified by structure and among locations. Our intention with this analysis was to provide an indication of the magnitude of genetic divergence between clusters identified by structure and compares it among species (Meirmans, 2015). The same program was used to estimate pairwise F_{ST} (Wright, 1965), and F'_{ST} (Meirmans, 2006).

The congruence among distance matrices (CADM, Legendre & Lapointe, 2004) method was used to quantify the similarity in genetic structure patterns among the three species. This was conducted using the R package 'ape' 3.0 (Paradis, Claude, & Strimmer, 2004). Here, a coefficient of concordance among all matrices (Kendall's W) is generated and ranges from zero to one. Zero indicates no concordance and one indicates complete concordance (Kendall & Smith, 1939). A posteriori tests of pairwise similarity among matrices (r_M) were then conducted. For tests of congruence, distance matrices (F_{ST}) were generated for only the co-sampled sites where at least three samples were collected for each of the three species (n= 4 sites). The Holm (1979) method was used to correct P-values following multiple testing.

Correlations between pairwise genetic differentiation and geographic distances were estimated for each species (Isolation by Distance, IBD) in the Red Sea. We did this to evaluate whether patterns of genetic structure differed among species at a smaller scale. We ran this analysis in the Red Sea because this is the only region where sample coverage was sufficient to compare IBD patterns among species. For this analysis, we estimated pairwise genetic and geographic distance matrices for each species for all sites that had at least five specimens (after clone correction). We used F'_{ST} (Meirmans, 2006) for genetic distances, a standardized estimate of genetic differentiation that accounts for genetic variation within populations and enables comparisons among different datasets. We used shortest overwater distances in kilometres for geographic distances. We explored IBD for each species using Mantel

tests with 10,000 permutations to test for significant correlations between distance matrices.

RESULTS

Clonality and allelic richness

We found evidence of clonality across all species despite no prior record of clonality in *S. mertensii. Heteractis magnifica* had the highest proportion of clones, with the highest rates in Moorea (French Polynesia) (13 out of 26; 50%) and at Red Sea sites (58 out of 120; 48%); clones were also present at Djibouti (2 out of 5; 40%). Two *S. mertensii* clone pairs were identified. One in Fi'ran (Red Sea) and a second pair in Djibouti. *Entacmaea quadricolor* clones were mostly found in the Indian Ocean (3 out of 7; 43%) and eastern/southeastern Australia (13 out of 77; 17%), as well as and one in Fsar (Red Sea) (Table 1). In general, *H. magnifica* clones were more common at the periphery of its distribution while *E. quadricolor* clones were found in the Indian Ocean and across the eastern Indo-Pacific.

Overall, after clone removal, there were no consistent deviations from HWE for any given locus in any of the three species. We found no consistent evidence for linkage between pairs of loci across multiple sites for any of the three species and thus all loci were kept for further analyses. Summary statistics across loci per sampling site are provided in Table S2 (Supporting information). Briefly, highest mean allelic richness across loci for *H. magnifica*, *S. mertensii*, and *E. quadricolor* was found at Kimbe Island, Lizard Island, and Jazirat Burcan, respectively, while the lowest was found in Obhur and Dumsuq for *H. magnifica*, Abu Dauqa, Abu Madafi, and Dhi Dahaya for *S. mertensii*, and Abrolhos Island for *E. quadricolor*. Highest observed heterozygocities were in Moorea, Kimbe Island, and North Solitary Island, respectively, while lowest observed heterozygosities were in Fsar, Djibouti, and Kimbe Island.

Broad-scale genetic structure

Heteractis magnifica, S. mertensii, and *E. quadricolor* all formed at least two main genetic groups across the Indo-Pacific, and these groups were arranged geographically (Fig. 2). When K was set to 2, for all species, the Red Sea sites and Djibouti (when sampled) clustered together in one group (hereafter referred to as the Red Sea cluster). For all species the main genetic break coincided at the Maldives. Interestingly, *H. magnifica* and *S. mertensii* showed evidence of admixture at the Maldives between the two genetic groups while *E. quadricolor* did not (one sample clustered with specimens from the Indo-Pacific while the other specimens clustered together with the Red Sea). Further genetic structure was revealed when K was increased (Fig. 2). For *H. magnifica,* specimens from Moorea (French Polynesia) clustered as a separate group when K was set to 3 and 4, and the Maldives clustered as a separate (admixed) group when K was set to 4 (Fig. 2A). For *S. mertensii*, setting K to 3 suggested weak structure within the Red Sea (Gulf of Aqaba appeared different from other locations) and setting K to 4 resulted in clustered specimens from the Maldives and the Bismarck Sea with varying degrees of admixture (Fig. 2B). For *E. quadricolor*, setting K to 3 clustered together the specimens from Abrolhos Island (eastern Indian Ocean), the Bismarck Sea, and Lizard Island (north Great Barrier

Reef) in one group and specimens from Lord Howe Island (southeastern Australia) in another group. Specimens from the Keppel, Northwest, and Heron Islands (southern Great Barrier Reef) and North Solitary Island (eastern Australia) appear to have admixed genotypes from Lizard and Lord Howe Islands (with a major proportion being from the Lord Howe cluster). Interestingly, a few individuals from Keppel and North Solitary Islands also showed potential admixture with the Red Sea cluster. Setting K to 4 showed a clear isolation by distance pattern within Red Sea sites (Fig. 2C). Mean log likelihood and Evanno's Delta K plots for K = 1 to K = 25 from Structure runs can be found in Supporting information (Fig. S1). Structure barplots for K = 5 to K = 8 for all species can be found in Supporting Information (Fig. S2, Fig. S3 and Fig. S4). According to Evanno´s Delta K the most likely number of clusters for all three species is 2.

PCA provided similar results to Structure. For all species, Red Sea specimens clustered together with Djibouti and separately from the rest of the specimens. Denser clusters indicate less genetic variation among individuals compared to other locations. For *H. magnifica*, specimens from Moorea (French Polynesia) formed a distinct cluster from all other Pacific specimens, and specimens from the Maldives fell between the Bismarck Sea specimens and the Red Sea specimens (Fig. 3A). For *S. mertensii*, there is greater genetic variation between individuals outside the Red Sea with separation between specimens from the Bismarck Sea and the Pacific but with some degree of overlap. Specimens from the Indian Ocean fell in between the Red Sea cluster and the rest of the specimens but closer to the Pacific specimens (Fig. 3B). Finally, for *E. quadricolor*, there was separation between specimens from the Pacific and Indian Oceans, and the specimens from the Bismarck Sea fell in between these two groups with some specimens overlapping with each of these two groups (Fig. 3C). All *E. quadricolor* regions within the Indo-Pacific appeared to cluster almost equidistant to the Red Sea, with a few specimens from the Indian Ocean and the Pacific clustered within it, as expected from the Structure results.

Partitioning of genetic variation

Results from hierarchical AMOVAs that included two regions (the Red Sea and the rest of the Indo-Pacific) indicated that genetic variation (other than that within individuals; >43%) among regions differed considerably among species. The lowest variation among groups was for *H. magnifica* (14%; F_{RT} = 0.143), followed by *E. quadricolor* (22%; F_{RT} = 0.220) and finally *S. mertensii* (42%: F_{RT} = 0.408). Interestingly, the amount of genetic variation explained by differences among populations within groups did not display the same patterns as variation among regions. *Stichodactyla mertensii* displayed the lowest variation at this level (1.1%, F_{RS} = 0.019), followed by *E. quadricolor* (5%; F_{SR} = 0.064) and then *H. magnifica* (9.7%; F_{RS} = 0.113). Global F_{ST} values were lower for *H. magnifica* (F_{ST} = 0.240) and *E. quadricolor* (F_{ST}= 0.270) compared to *S. mertensii* (F_{ST} = 0.419) (Table 2).

In all three species, all pairwise F_{ST} comparisons that involved one site from the Red Sea and one from elsewhere were statistically greater than zero after fdr corrections (Table S3, Supporting information). Highest pairwise F_{ST} and F'_{ST} values were found between Obhur (Red Sea) and Moorea (French Polynesia) for *H. magnifica* (F_{ST} = 0.384 and F'_{ST} = 0.749, respectively), between Obhur and Lizard Island (northern Great Barrier Reef) and Abu Madafi (Red Sea) and Lizard Island for *S. mertensii* (F_{ST} = 0.427 and F'_{ST} = 0.823), and between Dhi Dahaya (Red Sea) and

392 Lord Howe Island (southeastern Australia) for *E. quadricolor* (F_{ST} = 0.347 and F'_{ST} =
393 0.736). Lowest F_{ST} and F'_{ST} values were consistently found between sites within the
394 Red Sea for all three species (Table S3, Supporting information).

395

396 *Congruence among genetic distances and Isolation By Distance in the Red Sea*

397 Concordance based on genetic distance (F_{ST}) was high and significant among
398 the three species (W= 0.82, X^2= 12.33, adjusted p = 0.011). Pairwise similarity among
399 matrices tested a posteriori was also high, ranging from 0.657-0.771, but was only
400 significant for the comparison of *E. quadricolor* and *S. mertensii*. Given this, we can
401 only reject the null hypothesis of no concordance for *E. quadricolor* and *S. mertensii*
402 that, from this test, appear to display very similar patterns of genetic structure based
403 on F_{ST}. Comparing pairwise F'_{ST} values among species and as a function of
404 geographic distance in the Red Sea revealed differences among species (Fig. 4). Only
405 *E. quadricolor* displayed a positive correlation between genetic and geographic
406 distance within the Red Sea cluster (Fig. 4 & Table S4, Supporting information).

407

408 DISCUSSION

409 This study represents the first broad-scale study of the population genetics of
410 sea anemones that provide essential habitat for anemonefishes. Significant population
411 structure was identified in *H. magnifica, S. mertensii,* and *E. quadricolor* across the
412 Indo-Pacific. Our data indicate the existence of at least two geographically segregated
413 genetic groups for all species, namely the Red Sea cluster and the rest of the Indo-
414 Pacific cluster. Interestingly, the major genetic break that separates these two clusters
415 coincides at the Maldives. Overall, our results indicate widespread connectivity of *H.*
416 *magnifica*, *S. mertensii*, and *E. quadricolor* within the Red Sea cluster and to a lesser
417 extent within the rest of the Indo-Pacific. Below we compare and discuss the
418 differences in genetic structure among the three anemone species studied and discuss
419 how our findings complement our current understanding of the phylogeography and
420 population connectivity in the Indo-Pacific and Red Sea regions.

421 The abundance and location of clones varied among the three host anemone
422 species. *Heteractis magnifica* clones were restricted to the periphery of their ranges,
423 which agrees with Dunn (1981). These sites likely experience limited gene flow,
424 which may decrease the chances of successful larval recruitment and increase the
425 importance of asexual reproduction (Hoffmann, 1986; Eckert, 2001; Billingham,
426 Reusch, Alberto, & Serrão, 2003; Johannesson & André, 2006). In contrast, *E.*
427 *quadricolor* clones were found across their range including at central sites in the
428 Indian Ocean, which may be explained by Dunn's (1981) additional observation that
429 *E. quadricolor* clonality varies with depth rather than geographical location. As
430 reflected by our findings, a sea anemone's ability to undergo asexual reproduction is
431 species-specific and dependant on the ecological conditions found at each habitat
432 throughout a particular species' range (Sebens, 1980). In general, clusters of *H.*
433 *magnifica* and *E. quadricolor* appear to be common (Harriott, & Harrison, 1997;
434 Brolund et al., 2004; Richardson, Scott & Baird, 2015), and this seems to be
435 consistent with our results in terms of proportions of clones found. Neither asexual
436 reproduction nor clusters of several individuals have been documented before in *S.*
437 *mertensii*. Together with the low levels of clonality in *S. mertensii* reported in this
438 study it seems that this mode of reproduction is rare in this species.

We found that there is a significant congruence of patterns of genetic differentiation among species and that the Maldives (western/central Indian Ocean) represents a location of overlap or possible hybridization among distinct anemone populations or lineages (Sheppard et al., 2013). Previous studies have reported genetic discontinuity between the eastern and western Indian Ocean in different taxa including coral reef fishes (Bay, Choat, van Herwerden, & Robertson, 2004; Leray et al., 2010; Gaither et al., 2011; Huyghe & Kochzius, 2017) and invertebrates such as echinoderms (Vogler et al., 2012; Otwoma & Kochzius, 2016) and giant clams (Hui et al., 2016). Only one of these included samples from the Maldives (Vogler et al., 2012) and did not report overlap of different clades at these islands. Since none of the other studies sampled the Maldives it is not possible to confirm whether genetic discontinuity and the co-occurrence of different lineages at the Maldives is expected for other taxa. Other studies describe the presence of genetic discontinuities towards the eastern Indian Ocean (Christmas Island, Cocos Keeling Islands, Indonesia) for other coral reef species such as fishes, echinoderms, molluscs, and arthropods (reviewed in Ludt & Rocha, 2015; Crandall et al., 2019). However, most of the studies that document this break did not include specimens from the western Indian Ocean. Taken together, differences among studies are most likely due to the lack of consistency in sampling efforts among taxa and studies in this region, as pointed out by Crandall et al. (2019). This is a main limitation to draw general conclusions when comparing patterns of genetic structure that can only be resolved through better coordination among research groups. Our results are in agreement with previous studies and suggest that genetic exchange among eastern and western Indian Ocean might be limited by the presence of only a few islands that could facilitate connectivity via island hopping (Sheppard et al., 2013; Otwoma & Kochzius, 2016). The Maldives seems to be one of these important crossroads where different lineages meet. Yet, this hypothesis remains to be tested further as limited or ancient dispersal events are not the only possible processes that could have produced the observed genetic structure and our sampling size at several locations is small.

We can only speculate as to why there was no genetic admixture in *E. quadricolor* at the Maldives. Our data are limited (only four specimens), but suggest that both lineages coexist in the same place. It is possible that for *E. quadricolor*, these two clusters are the result of lineages that may have achieved reproductive isolation while ongoing gene flow is still possible for *H. magnifica* and *S. mertensii*. In this sense, IBD results indicate that *E. quadricolor* has more limited dispersal than *H. magnifica* and *S. mertensii* within the Red Sea. However, given our uneven sampling design among species, these results should be interpreted with caution. These differences could be explained either by variation in pelagic larval duration among species or variation in relative species abundance. *Entacmaea quadricolor* larvae can settle 48 hours after spawning; yet, they can remain in the plankton for up to 57 days (Scott & Harrison, 2007). However, little is known about larval dispersal of the other two species and no information is available regarding relative abundances of these anemones in the regions we sampled. Assuming that population connectivity is mostly driven by larval exchange, and that differences in abundance of these three species are negligible it appears that *H. magnifica* and *S. mertensii* larvae may have higher dispersal potential than *E. quadricolor* larvae. This higher restriction in gene flow might have facilitated the appearance of reproductive isolation among *E. quadricolor* clades. However, these hypotheses require further investigation.

The genetic structure and evolutionary history of host anemones and anemonefishes should be inextricably linked due to the obligate nature of the

symbiosis for the anemonefishes. We found at least three cases where there are similarities in the genetic structure of anemonefishes and that of their host anemones that warrant discussion. First, a previous study also employing microsatellite markers has reported isolation by distance and environment within the Red Sea for *Amphiprion bicinctus* (Nanninga et al., 2014), which inhabits *H. magnifica*, *E. quadricolor*, *S. mertensii*, and to a lesser extent *Heteractis crispa* and *Heteractis aurora.* IBD has been reported for other anemonefishes (Pinsky, Montes, Jr., & Palumbi, 2010; Pinsky et al., 2017) and has been attributed to the relatively short pelagic larval duration of these species (aprox. 12d). Here, only *E. quadricolor* displayed IBD in the Red Sea suggesting a shorter PLD compared to the other anemones and perhaps similar to the PLD of *A. bicinctus*. Second, deep genetic differences between the eastern and western Indian Ocean have been reported for *Amphiprion akallopisos* (Huyghe & Kochzius, 2017), which inhabits both *S. mertensii* and *H. magnifica*. This coincides with our results, however the lack of samples of *A. akallopisos* from the Maldives leaves the question open as to whether these two lineages of *A. akallopisos* meet at the Maldives as seems to be the case for the three anemone species in this study. Third, it has been shown that populations of the only anemonefish species present in Moorea (French Polynesia), *Amphiprion chrysopterus*, appear to be clearly different from populations elsewhere (Litsios, Pearman, Lanterbecq, Tolou, & Salamin, 2014). This coincides with our finding that the population of *H. magnifica* from Moorea is highly divergent from other *H. magnifica* populations. Similarly, moderate genetic structure in *E. quadricolor* was found between Lord Howe Island (located in southeastern Australia and to which *Amphiprion mccullochi* is endemic), and locations along the Great Barrier Reef (where its closest relative *Amphiprion akindynos* is commonly found) (van der Meer, Jones, Hobbs, & van Herwerden, 2012). As pointed out previously, there is limited geographic overlap between our study and previous studies of broad scale genetic structure in anemonefishes that prevent us from making thorough comparisons, but our results do suggest that there are some similarities in terms of genetic structure among anemonefishes and their host sea anemones.

Studies of population genetics in the Indo-Pacific region have demonstrated variation in patterns of genetic differentiation across a broad range of species, with evidence of several genetic breaks including between the Indian and Pacific Oceans, between the Red Sea and the Indian Ocean, and between sub-regions of the Indian Ocean (reviewed in Crandall et al., 2019). Here, we studied the broad-scale population genetics of host anemone species for the first time and identified distinct genetic groups with deep divergences at least between the Red Sea and the rest of the Indo-Pacific region and possibly also in Moorea. These deep divergences suggest possible species complexes within these species, which has also been suggested at least for *E. quadricolor* in a recent phylogenetic reconstruction of the clownfish hosting sea anemones (Titus et al., 2019). Overall, the patterns of population structure documented here are similar across *H. magnifica*, *S. mertensii,* and *E. quadricolor*, suggesting shared evolutionary processes. These divergences coincide with the Western Indian / Western Indo-Pacific barrier and the Central Indo-Pacific / Eastern Indo-Pacific biogeographic barriers and are most likely the result of complex changes involving larval connectivity and population sizes associated with Pleistocene sea-level fluctuations (Ludt & Rocha, 2015). The incongruence of our findings compared to other coral reef associated taxa that display genetic discontinuities elsewhere (such as the Sunda self) is most likely associated to differences among species linked to genetic drift (Crandall et al., 2019). Within the identified groups, connectivity is

relatively high for all species, but seems to be more restricted in *E. quadricolor* than in the other two species, at least in the Red Sea. However, our results need to be interpreted with caution because our sampling scheme was limited in terms of the number of samples per location and the congruence of sampling sites among different anemone species. Clearly further studies are needed to elucidate the role of evolutionary forces and demographic history in shaping the genetic structure of populations of these three sea anemones. We hope that our results serve as a road map to further develop these questions regarding the drivers of evolution and population structure of host sea anemones.

549 TABLES

550 Table 1. Summary of specimens for *Heteractis magnifica, Stichodactyla mertensii,*
551 and *Entacmaea quadricolor* across the Indo-Pacific, including number of specimens
552 successfully genotyped (N) and number of unique genotypes following clone
553 correction (N_{cc}). Latitude (Lat) and longitude (Lon) are expressed in decimal degrees.
554

Site code	Site Name	Region	Lat, Lon	*Heteractis magnifica*		*Stichodactyla mertensii*		*Entacmaea quadricolor*	
				N	N_{cc}	N	N_{cc}	N	N_{cc}
1	Gulf of Aqaba	North Red Sea	28.185, 34.638					5	5
2	Jazirat Burcan	North Red Sea	27.910, 35.065					24	24
3	An Numan	North Red Sea	27.139, 35.751	3	3			14	14
4	Nuwayshziyah	North Red Sea	26.624, 36.095					20	20
5	Mashabi	North Red Sea	25.582, 36.549	1	1			11	11
6	Abu Matari	North Red Sea	24.723, 37.151					1	1
7	Yanbu	North Red Sea	24.150, 37.675	3	1	13	13	5	5
8	Qita' Al-Girsh	Central Red Sea	22.436, 39.001	2		11	11	28	28
9	Shib Nazar	Central Red Sea	22.331, 38.863	2	1	7	7		
10	Fsar	Central Red Sea	22.227, 39.030	20	14			11	10
11	Abu Madafi	Central Red Sea	22.074, 38.778			6	6	1	1
12	Obhur	Central Red Sea	21.671, 38.844	8	5	25	25	3	3
13	South Reef (Al-Lith)	Central Red Sea	19.876, 40.114	7	3				
14	Lagoon2	South Red Sea	19.559, 40.055	1	1				
15	Abu Dauqa	South Red Sea	19.209, 40.109	1	1	5	5		
16	Dorish	South Red Sea	18.506, 40.670	3	3	4	4		
17	Sumayr	South Red Sea	17.787, 41.442	1	1	4	4		
18	Mamali Kabir	South Red Sea	17.605, 41.671			2	2		
19	Joey's Bluff	South Red Sea	17.476, 41.786	4	3	1	1		
20	Fi'ran	South Red Sea	17.177, 42.205			2	1		
21	Ghurab	South Red Sea	17.109, 42.069	2	1	2	2		
22	Baghlah	South Red Sea	16.980, 41.385	7	1	3	3		
23	Dhi Dahaya	South Red Sea	16.875, 41.440	7	4	5	5	7	7
24	Duraka	South Red Sea	16.860, 42.322	25	5				
25	Zahrat Durakah	South Red Sea	16.840, 42.305			1	1	3	3
26	Mazagnef	South Red Sea	16.592, 42.335	3	2				
27	Hindiya	South Red Sea	16.577, 42.240	4	3				
28	Dumsuq	South Red Sea	16.518, 42.041	11	5	1	1		
29	Djibouti	Gulf of Aden	12.221, 43.439	5	3	8	7		
30	Maldives	Indian Ocean	3.090, 72.976	6	6	3	3	4	4
31	Christmas Island	Indian Ocean	-10.460, 105.629			3	3		
32	Abrolhos Island	Indian Ocean	-28.790, 113.863					7	7
33	Tuare	Bismarck Sea	-5.087, 150.190	1	1	4	4	21	20
34	Kapeppa	Bismarck Sea	-5.091, 150.202					2	2
35	Kimbe Island	Bismarck Sea	-5.203, 150.374	51	51	5	5	5	5
36	Lizard Island	Coral Sea	-14.673, 145.451	1	1	7	7	3	3
37	Keppel Islands	Coral Sea	-23.155, 150.956					9	9
38	Northwest Island	Coral Sea	-23.280, 151.748					13	13
39	Heron Island	Coral Sea	-23.461, 151.934					6	6
40	North Solitary Island	Coral Sea	-29.925, 153.390					7	5
41	Lord Howe Island	Tasman Sea	-31.530, 159.077					39	28
42	Moorea	Pacific Ocean	-17.539, -149.830	26	13				
			Total individuals	**205**	**133**	**122**	**120**	**249**	**234**

555 Table 2. Analysis of molecular variance results for *Heteractis magnifica,*
556 *Stichodactyla mertensii,* and *Entacmaea quadricolor* sampled from across the Indo-
557 Pacific. Sites with less than five specimens were not included in this analysis. Sites
558 were also assigned to one of two regions prior to analysis, the Red Sea (here including
559 Djibouti), and the rest of the Indo-Pacific.

Source of Variation	d.f.	SS	Variance	% of	F-statistic
Heteractis magnifica					F_{RT} = 0.143
Among regions	2	75.8	0.519	14.3%	F_{SR} = 0.113
Among sites within regions	4	40.1	0.352	9.7%	F_{ST} = 0.240
Among individuals within sites	92	268.6	0.162	4.5%	F_{IS} = 0.059
Within individuals	99	257.0	2.596	71.6%	F_{IT} = 0.285
Total	197	641.6	3.629	100%	
Stichodactyla mertensii					F_{RT} = 0.408
Among regions	1	72.4	1.651	41.8%	F_{SR} = 0.019
Among sites within regions	8	30.2	0.046	1.1%	F_{ST} = 0.419
Among individuals within sites	81	239.1	0.600	14.8%	F_{IS} = 0.255
Within individuals	91	159.5	1.753	43.3%	F_{IT} = 0.567
Total	181	501.3	4.050	100%	
Entacmaea quadricolor					F_{RT} = 0.220
Among regions	2	249.7	1.029	22.1%	F_{SR} = 0.064
Among sites within regions	14	134.9	0.235	5.0%	F_{ST} = 0.270
Among individuals within sites	200	762.8	0.414	8.9%	F_{IS} = 0.121
Within individuals	217	648.0	2.986	64.0%	F_{IT} = 0.359
Total	433	1795.5	4.664	100%	

560

561

562 FIGURE LEGENDS

563 Figure 1. Numbered sampling sites (42) for *Heteractis magnifica, Stichodactyla*
564 *mertensii,* and *Entacmaea quadricolor* across the Indo-Pacific. Individual anemones
565 were sampled opportunistically, so not every species was sampled at each site. Size of
566 points is proportional to sample sizes. Specific information regarding the number of
567 specimens collected per site and other geographic information such as longitude and
568 latitude can be found in Table 1. Colours of points represent regions: Red Sea (blue),
569 Gulf of Aden (red), Indian Ocean (green), Bismarck Sea (purple), and Pacific Ocean
570 (orange). Photos of the three anemones are included, but it should be noted that
571 multiple morphotypes of *Entacmaea quadricolor* exist. Map created using an
572 equatorial projection centered on a prime Meridian with equally spaced straight
573 meridians and equal-area.

574

575 Figure 2. Structure output for *Heteractis magnifica, Stichodactyla mertensii,* and
576 *Entacmaea quadricolor* sampled from across the Indo-Pacific, for K = 2 to 4. Sites
577 are arranged from northwest to southeast. Numeric codes for each site correspond to
578 the codes used in Table 1. Major oceanographic regions are also indicated in a
579 coloured bar at the bottom of each graph for reference: Red Sea (blue), Gulf of Aden
580 (red), Indian Ocean (green), Bismarck Sea (purple), and Pacific Ocean (orange).

581

582 Figure 3. Results of Principal Components Analyses performed on the genetic data
583 from (a) *Heteractis magnifica,* (b) *Stichodactyla mertensii,* and (c) *Entacmaea*
584 *quadricolor* sampled from across the Indo-Pacific. Points are coloured according to
585 the oceanographic region of their origin. Major oceanographic regions are indicated in
586 the legend at the top: Red Sea (RS), Gulf of Aden (GA), Indian Ocean (IO), Bismarck
587 Sea (BS), and Pacific Ocean (PO). The values indicated along each axis correspond to
588 the percentage of inertia explained by the corresponding axis.

589

590 Figure 4. Scatterplots from the Isolation by Distance analysis comparing the pairwise
591 matrices of overwater geographic distance (in hundreds of km) and standardized
592 genetic distance (F'_{ST}) between sites with five or more *Heteractis magnifica* (yellow),
593 *Stichodactyla mertensii* (light blue), and *Entacmaea quadricolor* (grey) specimens,
594 within the Red Sea.

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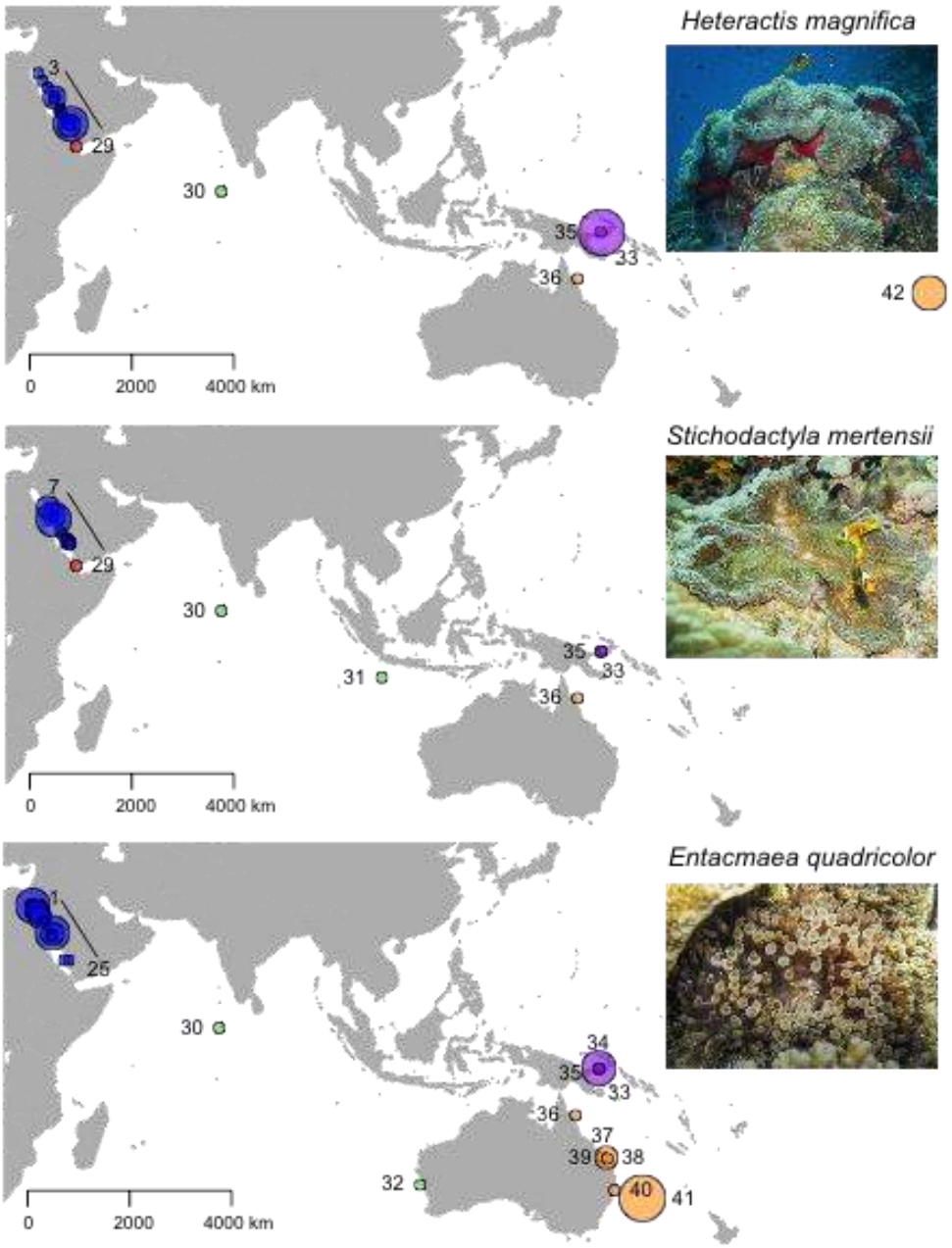
840 BIOSKETCH

841 Madeleine A. Emms is interested in studying population structure,
842 connectivity, and demography in coral reef systems. All other co-authors are broadly
843 interested in the ecology, evolution and conservation of coral reefs. PSA and MLB
844 conceived and designed the study. RAG and PSA carried out the laboratory work.
845 MAE, ECG, and PSA analysed the data. MAE and PSA led the writing of the

manuscript with assistance from ECG and MLB. All authors contributed tissue specimens and contributed to the manuscript.

Figure 1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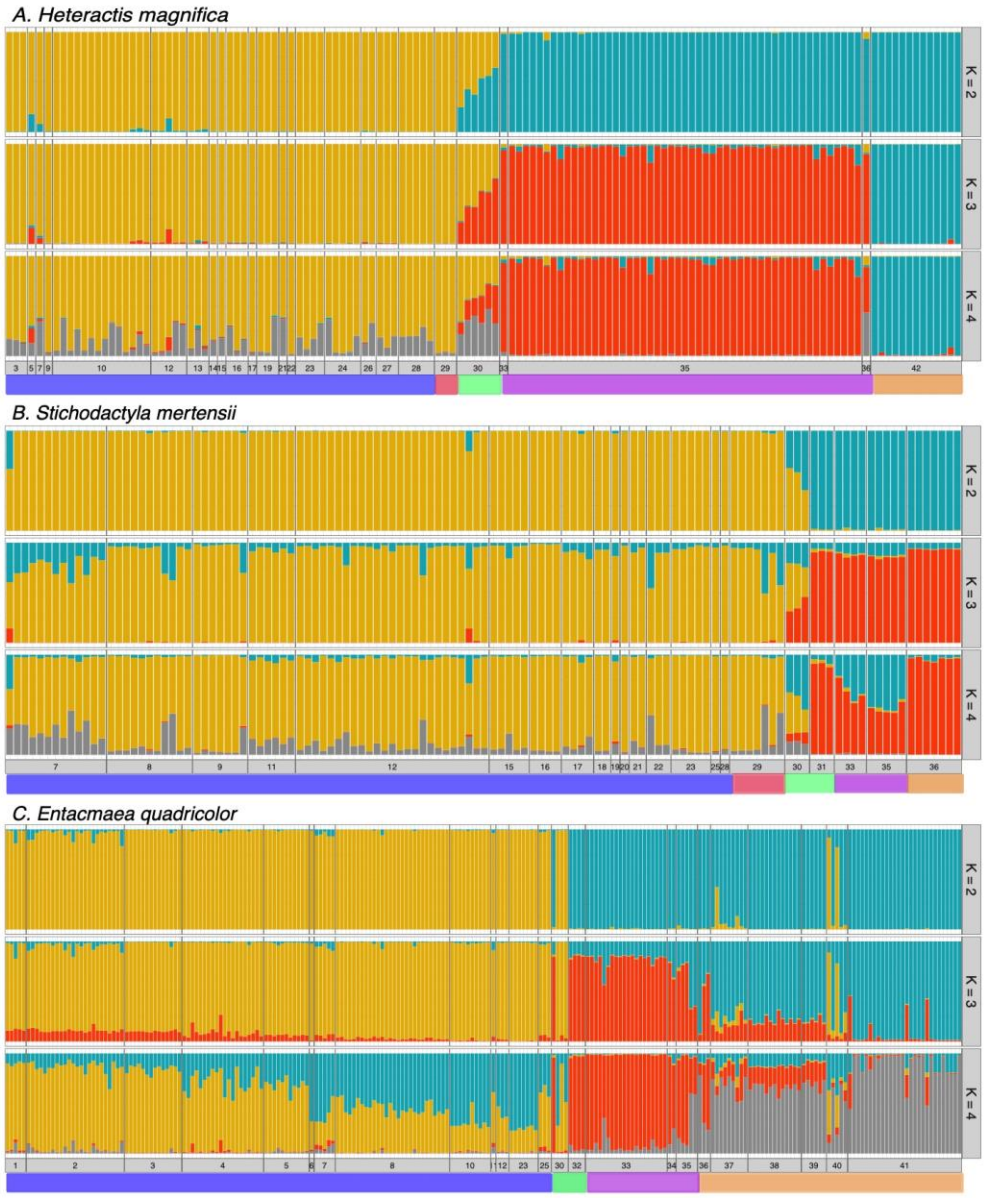
865 Figure 2



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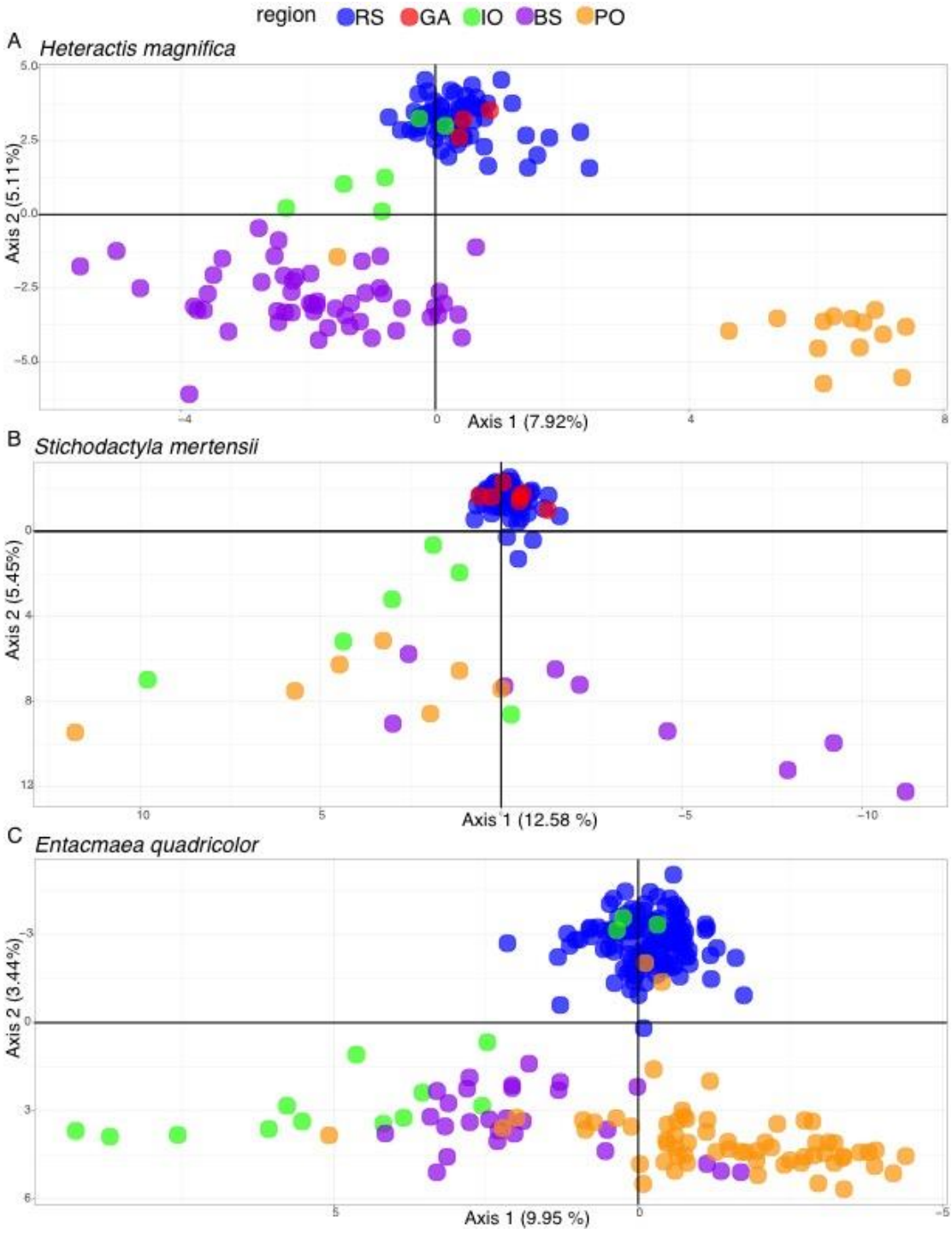
870 Figure 3



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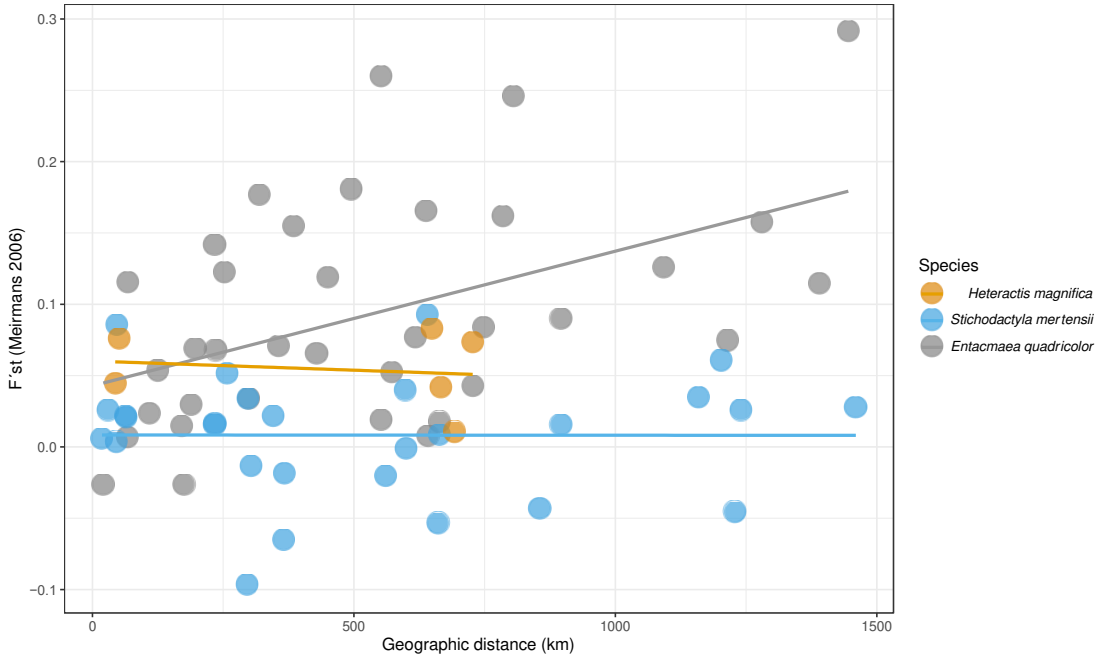
873 Figure 4



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