Molecular phylogenetics of geographically restricted *Acropora* species: Implications for threatened species conservation

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Abstract

To better understand the underlying causes of rarity and extinction risk in *Acropora* (staghorn coral), we contrast the minimum divergence ages and nucleotide diversity of an array of species with different range sizes and categories of threatened status. Time-calibrated Bayesian analyses based upon concatenated nuclear and mitochondrial sequence data implied contemporary range size and vulnerability are liked to species age. However, contrary to the popular belief based upon morphological features that geographically restricted *Acropora* species evolved in the Plio-Pleistocene, the molecular phylogeny depicts some species restricted to the Indo-Australian Archipelago have greater antiquity, diverging in the Miocene. Species age is not related to range size as a simple positive linear function and interpreting the precise tempo of evolution in this genus is greatly complicated by morphological homoplasy and a sparse fossil record. Our phylogenetic reconstructions provide new examples of how morphology conceals cryptic evolutionary relationships in the genus *Acropora*, and offers limited support for the species groupings currently used in *Acropora* systematics. We hypothesize that in addition to age, other mechanisms (such as a reticulate ancestry) delimit the contemporary range of some *Acropora* species, as evidenced by the complex patterns of allele sharing and paraphyly we uncover. Overall, both new and ancient evolutionary information may be lost if geographically restricted and threatened *Acropora* species are forced to extinction. In order to more adequately protect *Acropora* biodiversity and resolve the evolutionary history of *Acropora*, further analyses based on comprehensive and heterogeneous morphological and molecular data utilizing reticulate models of evolution are needed.

1. Introduction

Confronted with rapid and accelerating losses of biodiversity, it is important to know what traits elevate extinction risk (Cardillo et al., 2008), and how these traits interact to make some species more vulnerable than others (Davidson et al., 2009). Rarity is often associated with elevated extinction risk (Gaston, 1994; Brooks et al., 2006). In an ecological context, rarity refers to a species distribution and/or abundance being constrained in comparison to congeneric species (Gaston, 1994; Rabinowitz 1981). The causal mechanisms of rarity are varied and include ancient and contemporary factors such as: 1. Historical biogeography (Benzie, 1999; Nelson et al., 2000; Barber et al., 2000, 2002; Timm et al., 2008); 2. Phylogeny (Johnson, 1998; Ricklefs and Bermingham, 1999; Webb and Gaston, 2000; Carotenuto, et al., 2010); 3. Biology (e.g. life history, body size, dispersal characteristics and habitat specificity (Kunin and Gaston, 1997; Angel et al., 2006; Gaston, 1994); 4. Interactions (e.g. predator-prey, competitors and transmission of diseases, Gaston, 1994); 5. Genetic diversity (Chung et al., 2012; Frankham et al., 2010); and 6. The magnitude of direct and indirect anthropogenic threats (Gaston, 1994; Halpern et al., 2008).

In some taxa, rare and threatened species are concentrated within certain parts of the phylogenetic tree. For example, in plants, rare and vulnerable species cluster within young and rapidly evolving lineages (Davies et al., 2011). Whilst in mammals, ancient, slowly evolving species face the highest level of threat (Schipper et al., 2008). For hard corals, the nature of associations between age, rarity and extinction risk has not been established. If rare species are concentrated within certain clades and these species are pushed to extinction, whole phylogenetic branches may be lost from the tree of life. Such disproportionately large potential losses of phylogenetic diversity must be avoided (Faith and Richards, 2012). For reef-building hard corals, a substantial amount of phylogenetic diversity may be at risk (Huang 2012) because a high proportion of species can be numerically rare and/or geographically restricted on a local scale (Richards et al 2013), and on a global scale, one third of reef-building coral species face an elevated level of extinction this century (Carpenter et al., 2008).

Theoretically, rare species could have evolved at any point throughout history, but they are often considered to be either young or old. They may be "living fossils" or relicts of an old lineage whose populations have declined and/or fragmented through time as they approach the end of their taxon cycle (Ricklefs and Cox, 1972; Byrne et al., 2001; Ricklefs and Bermingham, 2002). Alternatively, they may be newly evolved and still expanding their distribution range (Blackburn and Gaston, 1997; Knowlton, 1993; Knowlton and Jackson, 1994; Schluter, 2000; Thomas et al.1997; Young and Brown, 1996). Both relict and recently-evolved species have high conservation value because they

either retain ancient genetic signatures, or introduce novel genetic elements that encode for characters or character states that either enhance resilience or may prove advantageous in a changing environment.

A predicted consequence of rarity that is likely to influence extinction risk is genetic erosion. In small populations, genetic diversity is lost through genetic drift, founder effects, directional selection and high levels of inbreeding (Wright, 1931; Kimura and Ohta, 1971; Avise, 1994, Frankham *et al.*, 2010; Willi *et al.*, 2006). Through genetic erosion, species become vulnerable to the fixation of deleterious mutations and this can lead, among other things, to a loss of reproductive fitness and a lower capacity to respond to changing environmental conditions (Kimura and Ohta, 1971; Soule 1986; Simberloff 1988; Frankham, *et al.*, 2010). It is predicted that rare species will have lower genetic diversity than common congeners (Kimura, 1983), however theories concerning the causes and consequences of rarity have largely been developed for terrestrial systems; it is not clear to what extent they apply to marine invertebrates.

Acropora provides a good model to further explore the causes and consequences of rarity because there is exceptionally high diversity within the group (134 recognized species, *sensu* Wallace, 1999, Ditley, 2004; Richards and Wallace, 2004; Claereboudt, 2006; Wallace et al., 2012); and 50% of the group are classified in an elevated level of threat under IUCN categories and criteria (Carpenter et al., 2008, IUCN, 2009). Systematics of this genus is based upon skeletal form and structure, and species are organized into 19 species groups (Veron and Wallace, 1984; Wallace and Wolstenholme, 1998; Wallace, 1999). The system of groupings are "… for convenience of identification only and do not imply any taxonomic affinity" (Veron and Wallace, 1984). To date, the level of molecular support for these groups has not been established.

The genus *Acropora* is at least 66 million years old (based upon Palaeocene fossils collected from Somalia and Austria (Carbone et al., 1993, Baron-Szabo, 2006). Paleontological records suggest nine of the species groups have their origins in the mid Eocene (39 – 49 Ma) and that the genus further diversified in the European region until the mid-Miocene (Wallace and Rosen, 2006; Wallace, 2008). These initial diversifications preceded more recent allopatric speciation events in the central Indo West Pacific Ocean over the last 5 million years and it is during this more recent period that the majority of the Indo-Pacific *Acropora* species are thought to have evolved (Pandolfi, 1992; Wilson and Rosen, 1998, Wallace and Rosen, 2006; Wallace, 1999). So far, molecular phylogenies of *Acropora* have been constructed on small subsets of common species (Odorico and Miller, 1997;

Hatta et al., 1999; Márquez et al., 2002; van Oppen et al., 2001, 2002) and the phylogenetic positions of rare species have not been systematically investigated.

Obtaining a better understanding of the history of speciation and nucleotide diversity of *Acropora* species is important given the group's highly threatened status (Carpenter et al., 2008). In this research, the polymorphic and phylogenetically informative single-copy nuclear *Pax-C* 46/47 intron and the mitochondrial *rns-cnox3* control region are used to examine the relative minimum age and nucleotide diversity of geographically restricted *Acropora* (staghorn) corals in contrast with congeneric species that have a widespread distribution. *Acropora* fossils do not preserve well, and it is especially difficult to find pre-Pleistocene fossils that can be attributed with high confidence to a specific living species or ancestor. Hence, our time-calibrated phylogeny includes two calibration points, one of which is a fossil and the other a biogeographic event.

2. Materials and Methods

2.1 Species examined and tissues collected

Thirty-eight species of *Acropora* (*sensu* Wallace, 1999) and one species of *Isopora* are included in this study (Table 1). New data were obtained for 21 species (16 rare/5 common) from five species groups (*Acropora loripes, elegans, nasuta, aspera* and *horrida* groups). Samples were collected from the Palm Island Group, E. Australia (S 18° 36.77' E 146° 29.426'), Rongelap Atoll, Marshall Islands (N 11° 09.207' E 166° 50.189') and Kimbe Bay, Papua New Guinea (N 5° 25.186' E 150° 05.353') (Figure 1). Tissue and skeletal samples were collected from 102 coral colonies and voucher specimens are deposited in the Museum of Tropical Queensland www.mtq.qm.qld.gov.au. New sequence data were combined with existing Genbank sequence data for 17 common species (see Section 2.4).

2.2 Definitions of rarity and extinction risk

Species were classified as rare if they have a restricted global distribution. To determine which species have a restricted global distribution, the maximum global range of the 39 species included in this study was quantified using the WorldWide *Acropora* Database which has 25,000 records based on over thirty years of collections (Wallace 1999). Longitudinal and latitudinal limits for each species were determined from the database, and the range approximated as elliptical in shape. Species were described as restricted if their range is $1/10^{th}$ or less of the species with the largest global range in the database. For ease of interpretation, rare species are marked with (*) throughout the text. Species are categorized as facing an elevated level of extinction risk if they are classified as

'vulnerable', 'endangered' or 'critically endangered' according to IUCN categories and criteria (http://www.iucnredlist.org).

2.3 DNA Extraction, PCR Conditions and Cloning

DNA was extracted from approx. 20 mg of coral branch according to van Oppen et al., (2001). The purified DNA pellet was suspended in 100 μl 0.1 M Tris (pH 9) and stored at -20°C. Target segments were amplified in a Polymerase Chain Reaction (PCR) using the primer pairs, PCR conditions and profile in Tables S1A and B. Successful products were cleaned using MO BIO UltraClean DNA Purification Kit. *Pax-C* PCR products were cloned using the ligation kit, pGEM T easy (Promega) and the cloning protocol described in van Oppen et al., (2001). DNA was isolated from cultures using the RBC Hyfield Plasmid Mini Kit, and sequencing was conducted by Macrogen.

2.4 Single gene-tree analyses

Nexus files were created in Se-Al 2.0a11 (Rambaut, 2002) from sequences aligned manually in Sequencher 4.5. Mitochondrial and nuclear sequence data were analysed separately and together in concatenated format in PAUP* 4.0b10 (Swofford, 2002) and Mr Bayes 3.1.2 (Huelsenbeck and Ronquist, 2001). The optimal model of sequence evolution was identified using Hierarchical Likelihood Ratio tests in Modeltest 3.7 (Posada, 2005 – for PAUP*) and Mr Modeltest 2.2 (Nylander 2004 - for Mr Bayes 3.1.2). Phylogenetic trees were constructed using Maximum-parsimony (MP) (heuristic search, 1000 bootstrap replicates); Maximum-likelihood (ML) (heuristic search, 100 bootstrap replicates) in PAUP* 4.0B10 (a beta version; Swofford, 2002); and Hierarchical Bayesian methods using Mr Bayes 3.1.2 (Huelsenbeck and Ronquist, 2001). Maximum Likelihood and Bayesian settings are listed in Table S1C.

Uncertainty in the topology of the tree, the branch lengths and the parameters of the substitution model, were integrated by the Markov Chain Monte Carlo method (as per Huelsenbeck and Bollback, 2001) whereby posterior probabilities were assigned after 10 million generations. To further reduce uncertainty the first $10^3 - 10^5$ generations were discarded as "burn-in" (See Table S1C), hence the posterior probabilities reported in this paper are based on points that were sampled from the MCMC chain after it had reached a plateau. Branches with >70% bootstrap support values and >0.95 posterior probability are considered significantly supported. Trees were rooted using sequences of *Isopora cuneata* (van Oppen et al., 2001; Fukami et al., 2000) which is sister to the Genus *Acropora* in the Family Acroporidae (Wallace et al., 2007). The sequence alignment data is available from GenBank {accession numbers - EU918202-918288 (mitochondrial data) and EU918771-918925

(nuclear intron data)}. Insertions in the *Pax-C* sequences were blast searched (www.ncbi.nlm.nih.gov) to confirm their identity.

2.5 Divergence dating of concatenated species-tree

Dating was performed with BEAST 1.7.4 and associated programs (Drummond *et al.*, 2012) on the concatenated alignment. The concatenated data set was not partitioned into individual genes. We are aware of the benefits of data partitioning when using Bayesian analyses (Nylander et al., 2004); however, reducing the complexity of such analyses increases the number of tree topology changes suggested during the MCMC, thus leading to better exploration of tree topology space. A likelihood ratio test for rate constancy (Felsenstein, 1988) was performed in MEGA5 (Tamura et al., 2011) where the likelihood of our ML tree was compared to the likelihood of the same tree with the constraint of a strict molecular clock under the Kimura2-parameter model (+G+I) (Kimura, 1980). The null hypothesis of equal evolutionary rate throughout the tree was rejected at a 5% significance level (P = 8.36) hence a relaxed clock was applied (Table S5).

The concatenated alignment file was loaded into the utility BEAUTI to prepare an xml input file for BEAST and taxon groups corresponding to *Isopora cuneata* (root) and *A. cervicornis* were defined. COI sequence data indicates *Isopora* diverged from its most recent common ancestor prior to the divergence of *Acropora* (Kitahara et al., 2010). The oldest fossil occurrences of *Acropora* are 66 Ma (Budd and Wallace, 2008). For this analysis, a normal distribution was applied as a prior to the calibration node of 66 Ma (±2 SD) which constrains the minimum date of divergence between the genus *Acropora* and *Isopora*. Given the scarcity of *Acropora* species-level fossil records from the Indo-Pacific (See Table S4) we have used an approximate date for the closure of the Isthmus of Panama (3-3.5 Ma, Keigwin 1982) to constrain the minimum age of divergence of Caribbean and Indo-Pacific species (± 1 SD) (see also Chen et al., 2009).

The general time reversible + gamma + invariant sites model was selected in Mr Modeltest, with four gamma categories and the mean substitution rate fixed to 1.0 (the default). A total of 30 independent exploratory runs of 10-50 million generations each were undertaken (taking approximately 8-20 hours each) and every 1000th generation was sampled to optimize the parameters and priors were adjusted to maximize the effective sample size (ESS) values. A final 100 million generation run was conducted with the uncorrelated exponential clock rate variation model with uniform operators. Tracer 1.4 was used to check for convergence of the model likelihood and parameters between each run until reaching stationarity. The maximum clade credibility tree was selected in TreeAnnotator, with 10% discarded as burnin, displayed in FigTree and the graphics file for editing.

Comparative dating analyses were attempted using the mitochondrial dataset only using the calibration points described above. In addition, analyses were also conducted using a published estimate of mtDNA divergence rate (0.237-0.948% Mya^{-1}, Chen, et al., 2009). This rate was calibrated using the closure of the Isthmus of Panama at 3mya and the closure of the Tethyan Sea at 12mya. Unfortunately, even after 100 million generations, ESS values were too low for key likelihood parameters (below the 100 threshold); hence the results of these analyses are not shown or discussed further. The relationship between mean estimated time since divergence and range size was compared via linear regression with (r^2) as the measure of goodness of fit to test the null hypothesis that a positive linear relationship exists between time since divergence and range size.

2.6 Genetic Diversity from Pairwise Distances

Genetic distances were calculated as Kimura 2-parameter distances (Kimura, 1980) because it allows for unequal substitution rates. The distribution of genetic variation among species was compared using the mean haplotype and allele diversities +/- 95% confidence intervals. The significance of the difference in nucleotide diversity between species classified as Vulnerable, Near Threatened and Least Concern categories were examined using a Kruskal Wallace test. The relationships between nDNA and mtDNA diversity and range size were compared using linear regression to test the null hypothesis that a positive linear relationship exists between nucleotide diversity and range size.

3. Results

3.1 Pax-C Nuclear Intron

New sequence data generated from three individuals of each of 21 species (116 sequences in total; see Table 1) were combined with published data, resulting in an alignment consisting of 156 *Pax-C* intron sequences from 38 species. Individual sequences varied in size from 545 bp to 965 bp. Individuals of *A. rongelapensis*, A. pichoni** and *A. tenella** had the shortest sequences (544 bp). Seven other species (*A.nasuta, A. kimbeensis*, A. caroliniana*, A. austera, A. longicyathus, A. digitifera, A. gemmifera*) had sequences between 13-25 bp shorter than the modal size of 574 bp. The alignment consisted of 1681 positions, and its generation required four large indels corresponding to insertions in sequences from *Isopora cuneata* (position 231-370), *A. millepora* and *A. spathulata** (position 370-760), *A. horrida* (position 871 – 1284) and *A. aspera* (position 1403-1546). NCBI Blast searches showed in some cases, these insertions had corresponding regions in other species that were not included in this analysis; e.g. the *A. horrida* insertion resembled those from *A. latistella* and *A. tenuis*, and the *A. aspera* insertion matched sequences from *A. florida* and *A. sarmentosa*. By contrast, the insertion in the *A. millepora* and *A. spathulata** sequences had no

counterpart in other species; this insertion was present in all *A. millepora* sequences, but only approximately half of the *A. spathulata** sequences.

Results of phylogenetic analyses of *Pax-C* intron data (Figure 2) were broadly consistent with published results (van Oppen *et al.*, 2001; Márquez, 2002), but some details differ due to the selection of taxa. Figure 2 shows the tree generated by Bayesian Inference, but with ML support for the major clades also indicated (only where ML support was >70%). To facilitate comparison with previous analyses, clade numbering from previous studies (van Oppen *et al.*, 2001; Márquez, 2002) is included in Figure 2. Note that, as in previous studies (eg. van Oppen et al., 2001), support for many of the minor nodes is relatively weak, and a number of polytomies occur. This is in part due to hybridisation/introgression phenomena (reviewed in Willis et al., 2006), but presumably also reflects incomplete lineage sorting in the case of some recent divergences.

Despite uncertainties around some groupings and branching orders, six major clades (Figure 2) were supported by all of the phylogenetic methods applied, a seventh (III in the present analysis) being supported only by BI. As in previous analyses (van Oppen et al., 2001), Clade I contains sequences from *A. longicyathus* and *A. austera*. A novel aspect of the present analysis is the inclusion of *A. horrida*; significantly, all of the *A. horrida* sequences also form an early diverging clade (clade II), suggesting that this species may have diverged earlier than was predicted by Wallace (1999) on the basis of morphology.

In clade IV, some alleles from the rare species *A. spathulata** cluster with *A. millepora* alleles with strong support whereas other *A. spathulata** alleles are scattered throughout the tree. A polytomy then gives rise to three strongly supported clades; clade V corresponds to the endemic Atlantic species, *Acropora cervicornis* (subclade IIID in van Oppen et al., 2001), whereas clades VI and VII are heterogeneous, containing sequences from 29 species, with very poor internal resolution. A major difference to previous analyses is the resolution of clade VI, which (which the exception of a single allele from *A. valida*) is composed exclusively of alleles from 7 rare species (*A. loisetteae**, *A. batunai**, *A. derawanensis**, *A. jacquelineae**, *A. spathulata**, *A. papillare**, *A. tortuosa**). While each of these rare species also has alleles in other parts of the tree, *A. jacquelineae** is found only in clade VI. Clade VII also contains alleles from 15 rare species, but also includes alleles from 13 common species *A. valida, A. nasuta, A. cerealis, A. vaughnai, A. loripes, A. granulosa, A. speciosa, A. gemmifera, A. elseyi, A. carduus, A. longicyathus, A. pulchra* and *A. microphthalma*.

With the exception of two alleles from *A. spathulata**, all of the sequences from rare species fell into clades VI and VII. Generally, alleles from the same individual clustered within the same clade, but for five individuals this was not the case. Different alleles of *A. papillare** 148; *A. tortuosa** 53; *A. loisetteae** 100; *A. loisetteae** 118; and *A. valida* 23 occurred in both clades VI and VII. In many cases, identical alleles were shared between species e.g. *A. pichoni** and *A. tenella** (Table S3) and in some situations, between species that fall into different groups sensu Wallace (1999), e.g. *A. microphthalma* (*horrida* group) and *A. valida* (*nasuta* group). Cases were also observed of allele sharing between rare and common species e.g. *A. papillare** and *A. gemmifera*.

3.2 Mitochondrial DNA Phylogeny

The alignment of mtDNA control region data included 92 sequences from 37 species of *Acropora*. The sequence length varied from 1081-1268 bp in length. Fifty-eight new mtDNA sequences were obtained from 21 species (Table 1). In most cases, the same individuals as the nDNA analysis were used for the mtDNA analysis however mtDNA data was not available for *A. palmata* and *A. prolifera*. *Acropora tenuis* sequences were included in the mtDNA analysis but not in the nDNA analysis. For the mtDNA analyses presented here, the new data were combined with 34 mitochondrial sequences from 16 common species (van Oppen et al., 2001; Marquez et al., 2002; Fleury unpublished).

It has previously been shown that a number of repetitive sequence elements are present within the mtDNA control region of *Acropora* species (van Oppen et al., 2002). Whilst conserved sequence blocks were present in all of the sequences examined, the new sequences added to the phylogeny lacked the majority of repeat regions previously described. Hence, whereas previous analyses were based on highly edited sequences, those presented here were conducted on a more extensive alignment. We identify three general size classes – those with sequences up to 400 bp shorter than the longest and most common sequence length (*A. cervicornis**, *A. loisetteae**, *A. millepora, A. longicyathus, A. tenuis, A. cerealis* – from 860-863 bp); those with sequences 141-188 bp shorter than the longest sequence (*A. horrida, A. caroliniana**, *A. austera, A. valida* – from 1070-1127 bp) and those with sequences >1268 bp.

Six major clades were resolved in the mtDNA phylogeny (Figure 3). One striking difference between the nDNA and mtDNA topologies is the position of the Caribbean species which occur in Clade I in mtDNA as opposed to Clade V in nDNA. Clade II contains two sister groups (labelled IA, IB in van Oppen et al., 2001), one containing *A. austera* and *A. longicyathus* while the other contained *A. tenuis* with significant ML and BI support as a monophyletic group. Clade III had 100% bootstrap and 100% posterior probability support for *A. horrida* being a monophyletic group. As in the case of

the nuclear intron dataset, the support for the terminal clades (VI, V, and VI) is weak. Nevertheless there is significant bootstrap support for the divergence of clades IV from V and VI however posterior support above the threshold of 0.95 was not obtained for this node. There is also significant ML and BI support for *A. pichoni** and *A. tortuosa** as monophyletic groups.

Clade IV contained 10 species (both rare and common); possibly belonging to three subclades however this clade and the internal subclades were not significantly supported in either analysis. Branch lengths within Clade IV indicate that some individuals were extremely divergent (e.g. *A. derawanensis* DA, A. vaughnai 201*). With the exception of one individual of *A. lokani**, clade V was composed entirely of common species and the majority of rare species included in this analysis clustered within a variety of subclades in an unresolved terminal clade (VI) which was not significantly supported. The entire terminal clade (and most subclades within) remains largely unresolved.

As observed in the nDNA phylogeny, there are numerous cases of polyphyly. For example, *A. jacquelineae** has one haplotype within clade IV and two in a single cluster within clade VI. *A. longicyathus* has one haplotype in clade II and two in clade V (Figure 3). Four cases of haplotype sharing were observed (Table S3) but only on a single occasion was both nDNA and mtDNA shared between species (i.e. *A. kirstyae** and *A. walindii**). For mtDNA, the *Acropora* species included in this analysis are more divergent from the outgroup than observed in nDNA.

3.3 Alleles and haplotypes shared between species

Twenty-two species share a nuclear allele with at least one other species and eight share mtDNA haplotypes with one other species (Table S3). Identical nuclear alleles were shared not only between species but also between species belonging to different species groups. The globally widespread species *A. valida* (*nasuta* group) shares alleles with three members of the *horrida* group – *A. tortuosa*, A, vaughani and A. microphthalma*. *A. tortuosa** shares alleles with two members of the *loripes* group (*A. loripes, A. granulosa*) (Figure 4)

We found nine cases of nuclear polyphyly (i.e. alleles/haplotypes phylogenetically interspersed with those of other species, Funk and Omland, 2003) and nine cases of mtDNA polyphyly. Some species were polyphyletic with respect to the nuclear *Pax-C* allele but monophyletic in mtDNA (e.g. *A. spathulata* and A. papillare**), while some showed the reverse pattern (e.g. *A. jacquelineae*. A vaughani, A. walindii*, A. granulosa, A. microphthalma* and *A. cerealis*). One species was polyphyletic with respect to both markers (*A. longicyathus*).

3.4 Phylogenetic dating and divergence times

The total concatenated data matrix relates to 137 sequences from 35 species for which both nDNA and mtDNA sequences were available for the same individual. Codon positions included were 1st+2nd+3rd+Noncoding. All positions with less than 95% site coverage were eliminated. The alignment consisted of 2536 positions. 542 characters were parsimony informative (21 %). A partition-homogeneity test with a heuristic search (1000 replicates) excluded 1994 characters (1123 characters for subset nDNA, 871 characters for subset mtDNA). The partition-homogeneity test indicated that incongruence was low between the trees generated from phylogenetically informative characters in different gene fragments {probability > 0.01 (0.037)}, and that sequences were not saturated (transition: transversion ratio was 0.74). The ESS results from the independent runs were variable with the highest ESS obtained when the Yule speciation prior was applied (Gernhard 2008). The Yule model is a simple stochastic model for speciation whereby each extant species is equally likely to give rise to a new species. Summary statistics and ESS values from all parameters are presented in Table S6.

The time-calibrated maximum clade credibility tree (Figure 5) closely resembles the *mtDNA* tree (Figure 3) however additional significant posterior probability support values were recovered at nodes not supported by the mtDNA genetree. The endemic Caribbean species *A. cervicornis* diverged from the Indo-Pacific *Acropora* species at least 6.6Ma, well before the closure of the Isthmus of Panama. *A. horrida* split from its most recent ancestor at least 5.12 Ma. Mean divergence estimates suggest 10 geographically restricted and 6 widespread species split from their MRCA in the Miocene (5.3-23.7Ma) while 7 geographically restricted and 11 widespread species diverged in the Pliocene (1.6-5.3Ma, Table 2). There is evidence that some geographically restricted species diverged from their MRCA earlier than expected {e.g. *A. jacqelineae** at least 11.76Ma (and up to 24.02Ma), see lineage arising at the base of Node 5). Another rare species, *A. rongelapensis** (Node 7) also appears to have arisen in the Miocene, diverging from its MRCA at least 5.69 million years ago (up to 15.6Ma). Species that have achieved widespread range distribution also appear to have diverged from their MCRA in the Miocene (e.g. *A. cerealis*), but moreover, mean divergence time estimates suggest the majority of widespread species included in this study split from their MCRA in the Pliocene.

3.5 The relationships between time since divergence and rarity/extinction risk

From this data, there is a tendency for species with a restricted distribution range to have greater antiquity than other congeneric species that have achieved widespread distribution range (Figure 6a). Hence, estimated mean time since divergence (Ma) and range size are not related as a positive linear

function (r^2 = 0.040, SE = 3.799, df = 32, p = 0.254) (Figure 6b). There is a clear signal that extinction risk is related to estimated time since divergence (Figure 6c), because the majority of Vulnerable species have greater antiquity than the majority of congeneric species classified as Near Threatened or Least Concern.

3.6 The relationships between genetic diversity and rarity/extinction risk

As judged by the Kruskal-Wallis test, mean nucleotide diversities at the two loci did not differ significantly between geographically restricted and widespread species *($x^2(2) = 0.887$, $p = 0.346$)*, although there were major differences in diversity levels between species. *A. kirstyae** has low diversity in both haplotypes and alleles whilst *A. longicyathus* has high diversity in both gene fragments. For some species, levels of haplotype and allelic diversity differed dramatically (Figure 7a). For example, *A. horrida* has high allelic diversity and low haplotype diversity whilst *A. lokani** shows the opposite pattern. Limited or no mtDNA diversity was detected among three rare species (*A. kirstyae**, *A. papillare** and *A. tortuosa**)*;* however, considerable levels of nDNA diversity were detected among these species indicating that these individuals were not clonemates.

The relationships between allelic and haplotype diversity and both range size and level of extinction risk are not fitted by positive linear functions (Table S2). Neither allelic nor haplotype diversity are significantly associated with range size because numerous species with a small range have comparatively high nucleotide diversity and numerous species with a large range have comparatively low nucleotide diversity (Figure 7b). Likewise, there is no significant association between level of extinction risk and allelic or haplotype diversity because some species categorized as vulnerable have high nucleotide diversity and others categorized as least concern have low nucleotide diversity (Figure 7c).

4. Discussion

4.1 Evolutionary history of geographically restricted Acropora species

The 34 species included in in our dated phylogeny belong to 7 major lineages that diverged from the late Eocene to the mid Miocene. The phylogenetic placement of geographically restricted species suggests that 59% diverged in Miocene radiation events (2-20my) and the remaining 41% diverged from their MRCA in later Pliocene events (within the last 5.3my). There is little evidence of greater antiquity among widespread species (based on mean divergence times, Table 2), because only 35% of widespread species appear to have arisen in the Miocene, while a greater proportion (65%) diverged from their MCRA in the Pliocene (Figure 6a).

Our study has uncovered new diversity and relationships within *Acropora* and most notably, novel subclades of species that are geographically restricted to the Indo-Australian Archipelago that appear to have diverged from their MRCA at least 17 mya (e.g. Clade VI in nDNA, Figure 2; Node 2 in Figure 5 which comprise five rare species: *A. kirstyae*, A. batunai*, A. walindii*, A. jacquelineae*, A. derawanensis*).* Similar studies on other marine taxa such as gastropods have also reported surprising antiquity among some lineages that are restricted to the Indo-Australian Archipelago (Meyer, 2003; Williams and Read, 2004). Our calibrated phylogeny also confers the Indo-Pacific *Acropora* lineages appear to have last shared a common ancestor with the Caribbean lineage at least 8.3 million years ago, well before the closure of the Isthmus of Panama.

Other than those species restricted to the Caribbean, morphologically similar species did not cluster in any topology. Moreover, species from single groups were not concentrated within distinct clades. For example in mtDNA, members of the *nasuta* group were dispersed through clades IV, V and VI; whilst in nDNA, members of the *aspera* group occured in clades III, IV, VI and VII. This suggests certain morphologies, or the shared characters and character states may have evolved independently in different lineages (i.e. morphological homoplasy, see also Budd et al., 2010). Homoplasy may be a result of convergent/parallel evolution and/or reticulation (Arnold, 1992), but regardless of the mechanism, it greatly complicates the ability to interpret *Acropora* evolution. Overall, these results imply there is limited molecular support for the 'species groups' as constructed by Veron and Wallace (1984) and Wallace (1999).

In the phylogeny presented by Wallace (1999) that is based on morphological features, the evolution of *Acropora* is depicted as a transformation from heavy, simple thick-branched corals in which the axial corallite forms the major skeletal component of the branch, towards light, complex narrow-branching corals in which the axial corallite is slender but repeated more frequently, giving slender branches with more porous skeletons. This molecular reconstruction suggests porous and fine branching species may have evolved earlier than currently understood. For example, in all analyses there is significant support for two lightly structured species {*A. longicyathus* and *A. horrida* (designated as members of the derived *echinata* and *horrida* groups in Wallace, 1999; Wallace and Rosen, 2006) diverging from their MRCA in the Miocene. Our dated phylogeny opens up the possibility that two other lightly-calcified species (*A. cerealis, A. jacquelineae*) may have diverged as early as the Oligocene.

4.2 Phylogenetic Uncertainty

Phylogenetic inference depends upon the reliability of evolutionary reconstructions (Huelsenbeck and Bollback, 2001). While there is significant bootstrap and posterior probability support for early diverging nodes (i.e. nodes 1-3, Figure 5), there is limited support within the terminal nodes, and this limits the extent of phylogenetic inference. A possible explanation for the lack of resolution in terminal parts of the trees is that the markers examined here may not have been able to resolve derived nodes. However, given there is significant divergence among taxa within the polytomy, we concur with van Oppen et al., (2001) that this clade is likely to be a hard polytomy (Hoelzer and Melnick 1994) driven by rapid speciation events. Considering however that only a subset of the entire *Acropora* diversity has been examined (34 of 134 spp., Wallace et al., 2012); it is expected that the addition of missing species and supplementary genomic information, may resolve the polytomy (see also section 4.3 for a discussion of the influence of ancestral polymorphisms v's introgression on the inferred evolutionary relationships).

Uncertainty surrounding the time-calibrated tree also arises as a result of fossil bias. Fossil dates provide only only a minimum age estimates because the fossil is necessarily younger than the phylogenetic event that led to its existence (Benton and Donoghue 2007). Fossil records of modern *Acropora* species are sparse (see Simpson et al., 2011 for a discussion of bias in the coral fossil record); hence only a single fossil-based calibration point and a single geological event calibration point were used in this analysis. In order to obtain a high level of confidence in the time-calibrated phylogeny, ESS values should be above 100 (Rambaut and Drummond, 2009). Values above 100 were obtained for all parameters of the study and the tree likelihood value was above 400 (Table S6). This indicates the time-calibrated tree presented is robust, and for the purposes of this study, which was not to date absolute divergence times, but to estimate the minimum relative divergence times (mean and 95%HPD). Moreover, the dates and analytical approach presented provides a useful guide for dating studies in the future in the light of additional information.

Overall, nodes 1-3 were significantly supported in all analyses; thus a high degree of confidence can be placed in the positioning of deeper nodes. It is possible that the incongruence in support levels across the tree indicates that mutation rates were not constant during *Acropora* evolution. Former studies suggest "rapid diversification events" occurred in the Indo-Pacific after the Miocene (Wallace 1999: van Oppen et al. 2001). While allopatric speciation and vicarance events have been proposed to explain this trend, the influence of molecular mechanisms such as positive selection are not well understood. Positive selection is the force that increases the prevalence of advantageous traits (Sabeti et al., 2006). The existence of positive selection is difficult to detect, however preliminary comparative EST analysis has revealed some candidate fast-evolving genes in *Acropora* species

(Iguchi et al., 2011). Thus, further exploration of available *Acropora* genomic information (e.g., *Acropora digitifera*; http://marinegenomics.oist.jp/genomes/gallery; *A. millepora*: http://coralbase.org/; *A. hyacinthus*, *A. millepora* and *A. tenuis*: http://www.bio.utexas.edu/research/matz_lab/matzlab/Data.html), is likely to reveal more candidate genes under positive selection and would enable the rate and extent of rapid evolution in this genus to be further explored.

4.3 Allele/haplotype sharing in Acropora

Even after the addition of rare species to the phylogeny, many aspects of *Acropora* evolution remain ambiguous. Most perplexing are the complex patterns of allele/haplotype sharing observed in this dataset. For example, 10 species derived from 5 different 'species groups' share identical nuclear alleles (Figure 4). In the case of species with large population sizes (hence, long coalescence times) such patterns of allele sharing could be explained by the retention of ancestral polymorphisms {i.e. incomplete lineage sorting (van Oppen et al., 2001; Vollmer and Palumbi, 2002; Márquez, 2002; Wolstenholme et al., 2003; Nakajima et al., 2012}. However, among rare species, the alternative explanation of hybridization is more likely to explain the genetic overlap, because rare species are likely to have very short coalescence times (Richards et al., 2008).

It is possible that the complex patterns of allele sharing may indicate syngameons (i.e. groups of intermittently interbreeding species). For hybridization to occur between species occurring in sympatry, overlapping spawning times and semi-permeable reproductive barriers are necessary (Harrison, 1990). The observed lack of resolution in the terminal Indo-Pacific clades supports the assumption that few synchronously spawning Indo-Pacific *Acropora* species are fully reproductively isolated (Willis, 1997; van Oppen et al., 2002). However to confirm this, further data are required detailing the reproductive ecologies of these species at the locations examined here. If complex syngameons exist within *Acropora*, it is possible that certain species (in this case *A. valida*) could effectively act as conduits - transferring genetic material between species via introgressive hybridisation. *A. valida* has a widespread distribution, is phenotypically diverse (Wallace, 1999) and may be polyploid (Kenyon, 1997); these are traits that are either consistent with the idea that it may function as a genetic conduit, or would enable it to function in this way. However, it is important to note that it is not likely all *A. valida* individuals function as genetic conduits in all locations; moreover conduits and syngameons would be expected to vary in time and space (Frank and Mokady, 2002).

Especially among rare species, where the probability of conspecific mating is low, introgression of new alleles into a population may be a critically important source of genetic diversity and adaptive potential (Lewonton and Birch, 1966; Grant, 1973; Dowling and Secor, 1997; Seehausen, 2004; Willis et al., 2006; van Oppen and Gates, 2006). Veron (1995) first suggested that corals with restricted ranges may be morphologically unique hybrids. Subsequently, intra-specific hybridization was confirmed in the case of the two Caribbean *Acropora* species (van Oppen et al., 2000; Vollmer and Palumbi, 2002). However, in the Indo-Pacific, progress towards understanding the role of hybridization has been slow due to the much larger numbers of co-occurring coral species. Hybridization may help to explain the lack of resolution in the terminal clades, however to fully understand the extent and significance of hybridization in Indo-Pacific coral communities it will be important to examine all of the species within specific locations in phylogeographic context in order for parental lineages to be identified.

The results presented here provide further evidence that morphology conceals cryptic evolutionary relationships in the genus *Acropora*. However, the possibility that some species (e.g. *A. walindii** and *A. kirstyae**, which share both nuclear and mitochondrial DNA) are ecomorphs of a single species cannot be ruled out. Although progress is being made, it is clear that resolving evolutionary relationships within this genus is unlikely to be simple and will require large molecular data sets for many more morpho-species. Some of the *Acropora* data are enigmatic; for example, *A. tenella** and *A. chesterfieldensis** share identical nuclear alleles yet are traditionally assigned to distinct species groups (*elegans* and *loripes* groups) and their modern geographic distribution and habitat preferences do not overlap. Most likely these two species are more closely related than currently recognized, but this hypothesis requires validation using larger datasets. Taking into consideration the high likelihood that complex evolutionary processes maintain *Acropora* biodiversity, future phylogenetic studies within the genus *Acropora* should focus on reticulate analytical techniques because traditional phylogenetic assumptions (e.g. strict dichotomous branching amongst lineages) are inappropriate for hybridizing species (Arnold, 1992).

4.4 Nucleotide diversity in rare/common species

In this analysis, some species that are broadly distributed right across the Indo-Pacific (e.g. *A. microphthalma*) are of the same relative age or younger than geographically restricted species hence, age-area relationships are not simple (see also Pigot, et al., 2012). It is possible that genetic erosion may be an alternative cause or consequence of having a restricted distribution range, however surprisingly, the results indicate neither allelic nor haplotype diversity are significantly positively correlated with range size (Figure 7b). Some species with a restricted distribution range have

comparatively high nucleotide diversity (e.g. *A. derawanensis** and *A. batunai**) and numerous species with a widespread distribution range have comparatively low nucleotide diversity (e.g. *A. aspera* and *A. loripes*) (Figure 7a). Likewise, there is no significant association between level of extinction risk and allelic or haplotype diversity because some species categorized as vulnerable have high nucleotide diversity and others categorized as least concern have low nucleotide diversity (Figure 7c).

Furthermore, despite examining relatively small numbers of individuals, high levels of haplotype and/or allelic diversity were detected in a number of rare species. For example, *A. derawanensis** exhibits high haplotype diversity whereas the mean nucleotide diversity in mtDNA for *A. valida*, which has the largest global distribution range of all *Acropora* species, was comparatively low (Figure 7a). This result supports similar findings of non-depletion in some rare species in a companion population-genetic study (Richards and van Oppen 2012) and in plants (Gitzendanner and Soltis, 2000). However, considering genetic diversity is governed by a complex range of factors including genetic drift and gene flow, further robust species-specific population estimates at neutral and functional loci are required to fully understand the genetic causes and consequences of rarity.

4.5 Implications for the conservation of biodiversity in the Indo-Pacific

An important finding of this study is that the majority of species that appear to have diverged in the Miocene are listed as *Vulnerable* by the IUCN (Figure 6c). Thus, if these species that are currently facing an elevated level of threat due to cumulative anthropogenic and climate impacts are pushed to extinction, there is a distinct possibility that ancient evolutionary information will be lost. Of particular conservation concern is our finding of a previously undetected clade comprising five (mtDNA) - seven (nDNA) geographically restricted species. Given that all of these species are listed as Vulnerable on the IUCN red list of threatened species, and one of them *A. jacquelineae** is a sister group to *A. aspera* which is also listed as vulnerable by the IUCN (see Figure 5), there is elevated risk that a deep phylogenetic branch may be lost and this could equate to a disproportionately large loss of phylogenetic diversity (Faith and Richards, 2012).

Another Indo-Pacific species occurring in an early diverging clade that appears to have split from its MRCA in the Mio-Pliocene, *A. horrida* is listed as vulnerable by the IUCN. The loss of this species presents the potential loss of unusual biological and physiological traits. For example *A. horrida* is renowned for having its tentacles extended day and night suggesting it has an increased propensity for heterotrophic feeding (in contrast to other *Acropora* spp. which are primarily autotrophic). Further, a high prevalence of clade ‘A’ *Symbiodinium* strains has been recorded in this species

(Richards unpublished); along with an unusual pattern of fluorescent pigment organization, Salih et al., 1998). Thus, the extinction of *A. horrida* could lead to disproportional loss of evolutionary information from the tree of life.

At the other end of the spectrum, *A. tortuosa* appears to have diverged recently from its MRCA and occurs in a derived position of the terminal lineage (Node 7, Figure 5). This species is primarily found in the Pacific and may be an example of a peripheral speciation event (see Palumbi, 1997; Paulay and Meyer, 2002). In the remote Marshall Island atolls, *A. tortuosa* is prevalent in the lagoons where it has a unique sturdy upright 'tree-like' growth form which is uncommon among other extant *Acropora* species. Considering *A. tortuosa* is listed as vulnerable by the IUCN, the loss of *A. tortuosa* would represent a loss of newly evolved characters and/or character states. In the medium-long term, the loss of newly evolved traits may impede the ability for the Genus *Acropora* to adapt to the rapidly changing coral reef environments.

4.6 Conclusion

The inherent problem of phylogenetic uncertainty plagues phylogenetic studies in diverse groups such as *Acropora*. Nevertheless, considering such a large proportion of this keystone group of corals are geographically restricted and/or vulnerable to extinction, the urgency of implementing threatened species conservation measures precludes the delays inherent in obtaining comprehensive molecular data for all species. The phylogenetic reconstruction presented here suggests that new and ancient evolutionary information is at risk if rare and vulnerable *Acropora* species are not protected. It is important to note that in *Acropora* the age-area relationship is not a simple positive linear correlation and factors additional to age are likely to also play a role in limiting the distribution of rare species. The genetic linkages we observe between morphologically distinct species and species groups reinforces that effecting the conservation of *Acropora* biodiversity is not likely to be straight-forward because the current systematic scheme does not accurately reflect evolutionary relationships. In order to resolve species-level relationships, further analyses based on heterogeneous morphological and molecular data (see Budd et al., 2010) and reticulate models of evolution are needed.

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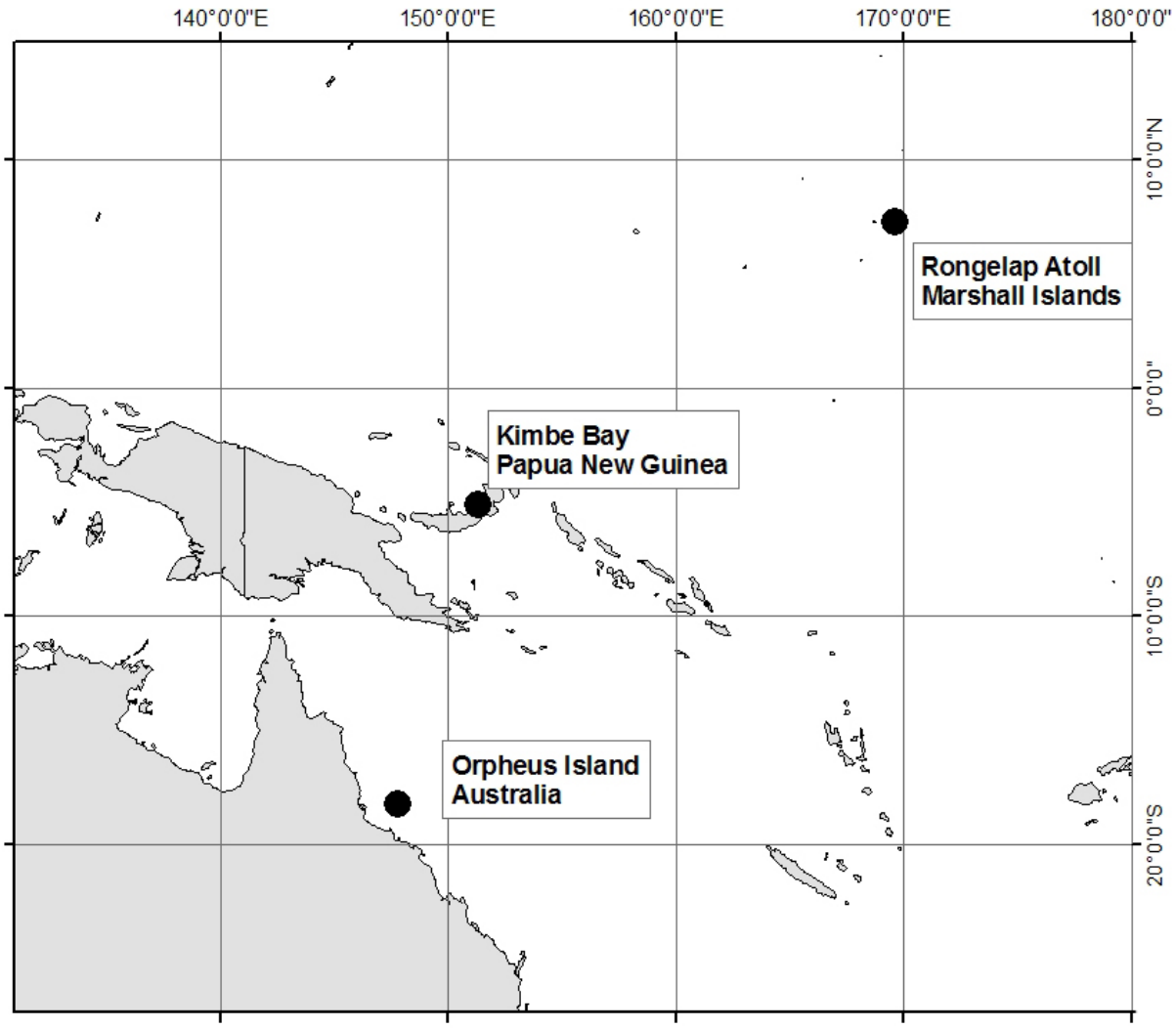

Figure 1. Sampling locations of new sequences obtained in this study.

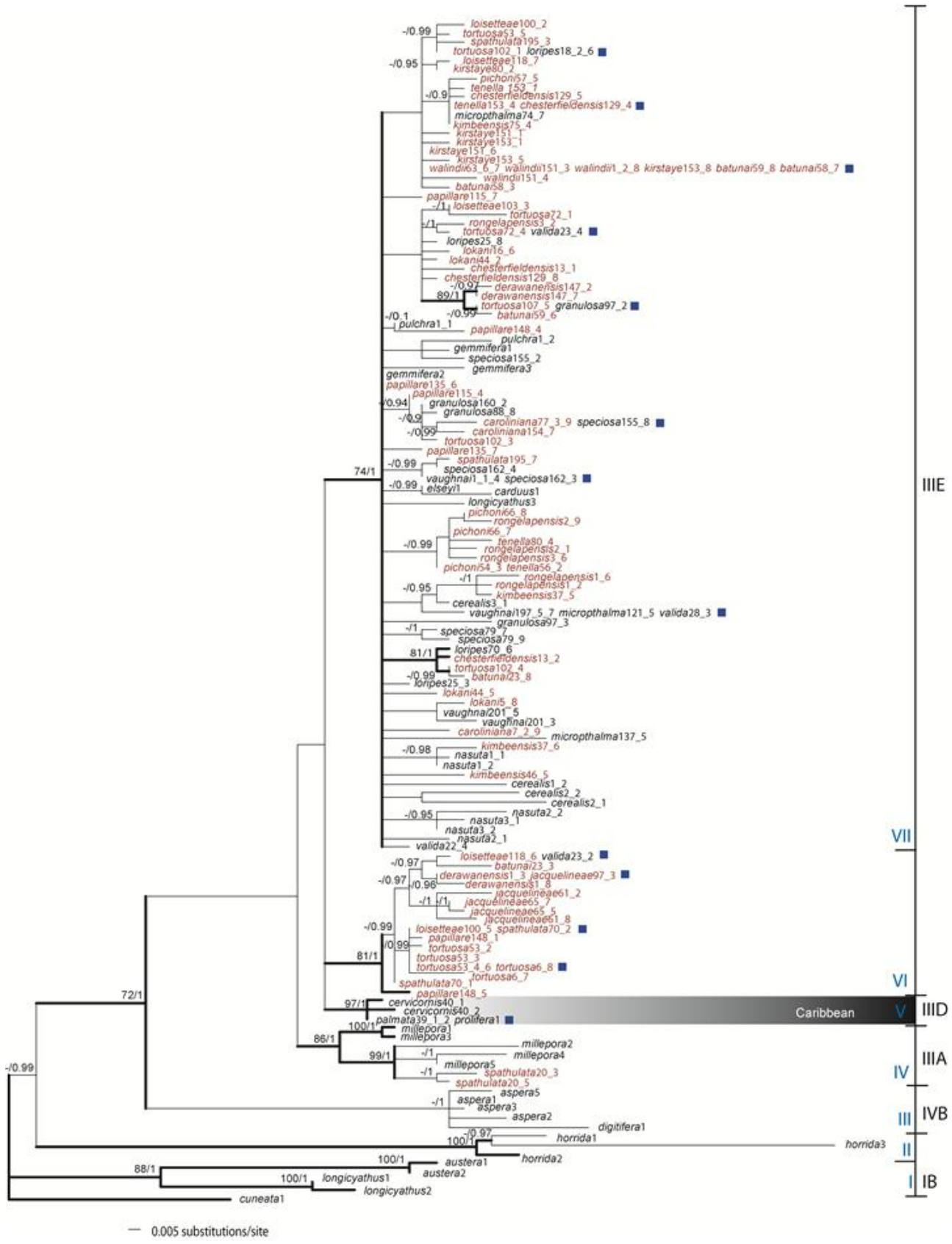

Figure 2. Nuclear Gene-Tree Bayesian topology denoting maximum likelihood values >75% (left) and posterior probabilities >0.95 (right) based upon 156 *Pax-C* intron sequences from 38 species. Geographically restricted species are highlighted in red font, widespread species are in black. Clade nomenclature on left in blue reflects this study; nomenclature on the right reflects van Oppen *et al.*, 2001. For ease of interpretation instances of allele sharing are marked with a blue dot.

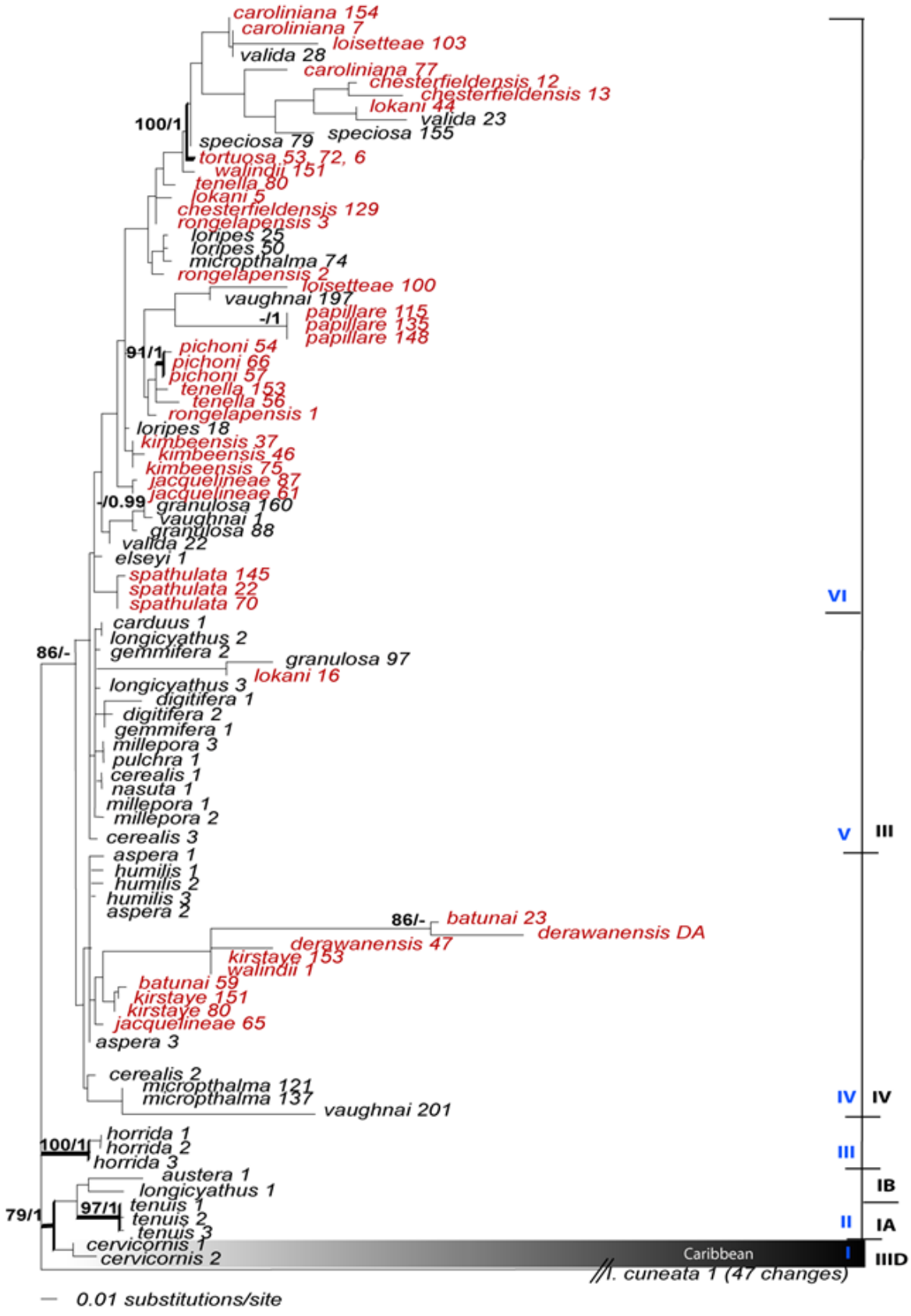

Figure 3. Mitochondrial Gene-Tree Bayesian topology with likelihood bootstrap support values >75% (left) and posterior probabilities >0.95 (right) based upon 92 control region *rns-cox3* sequences from 37 species. Geographically restricted species are denoted by red font, widespread species are in black. Clade nomenclature in blue on the left reflects this study. Clade nomenclature in black on the right reflects van Oppen *et al.*, 2001.

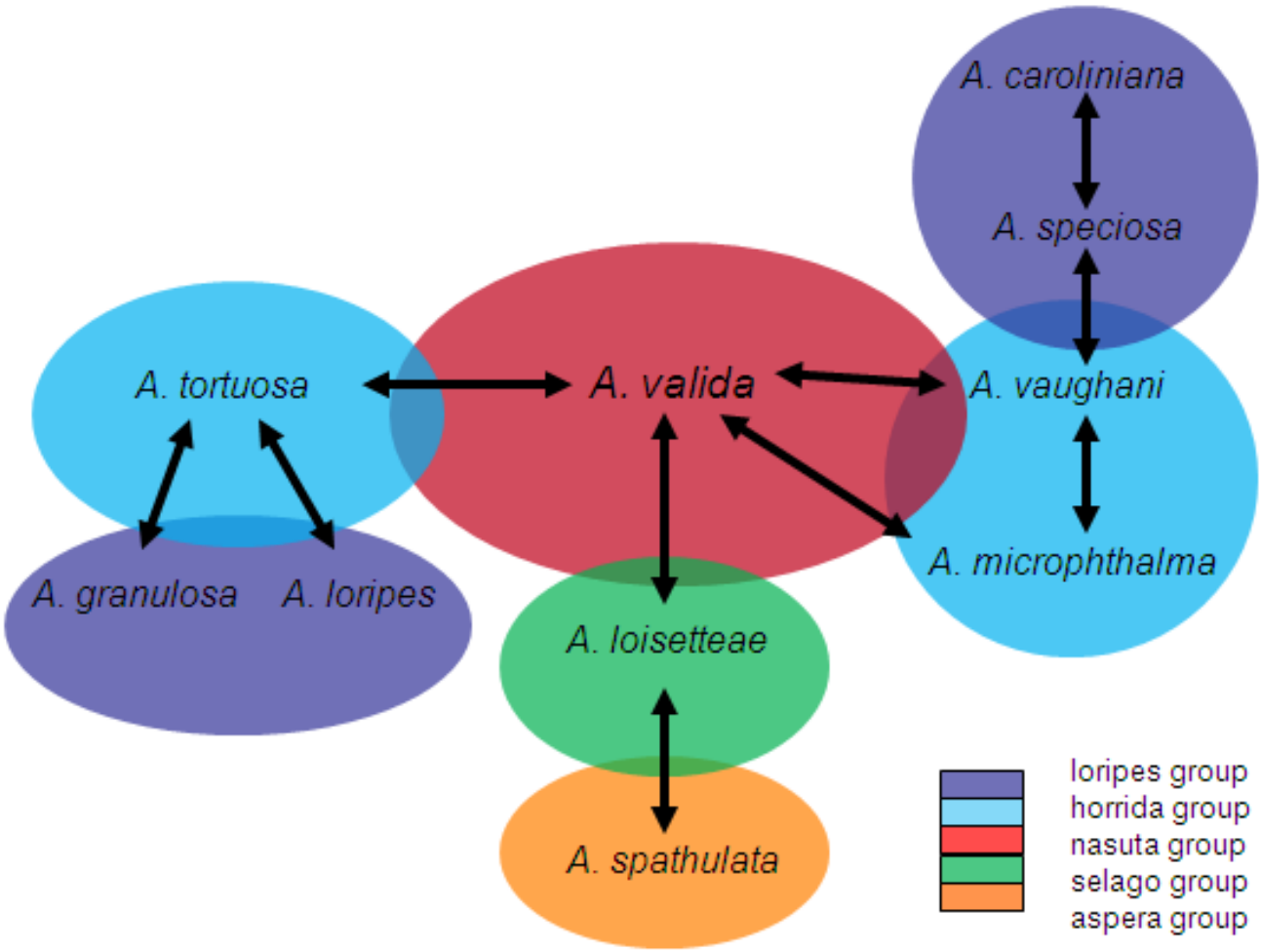

Figure 4. Proposed syngameon showing identical alleles are shared between species currently classified to belong to five different species groups (*sensu* Wallace, 1999).

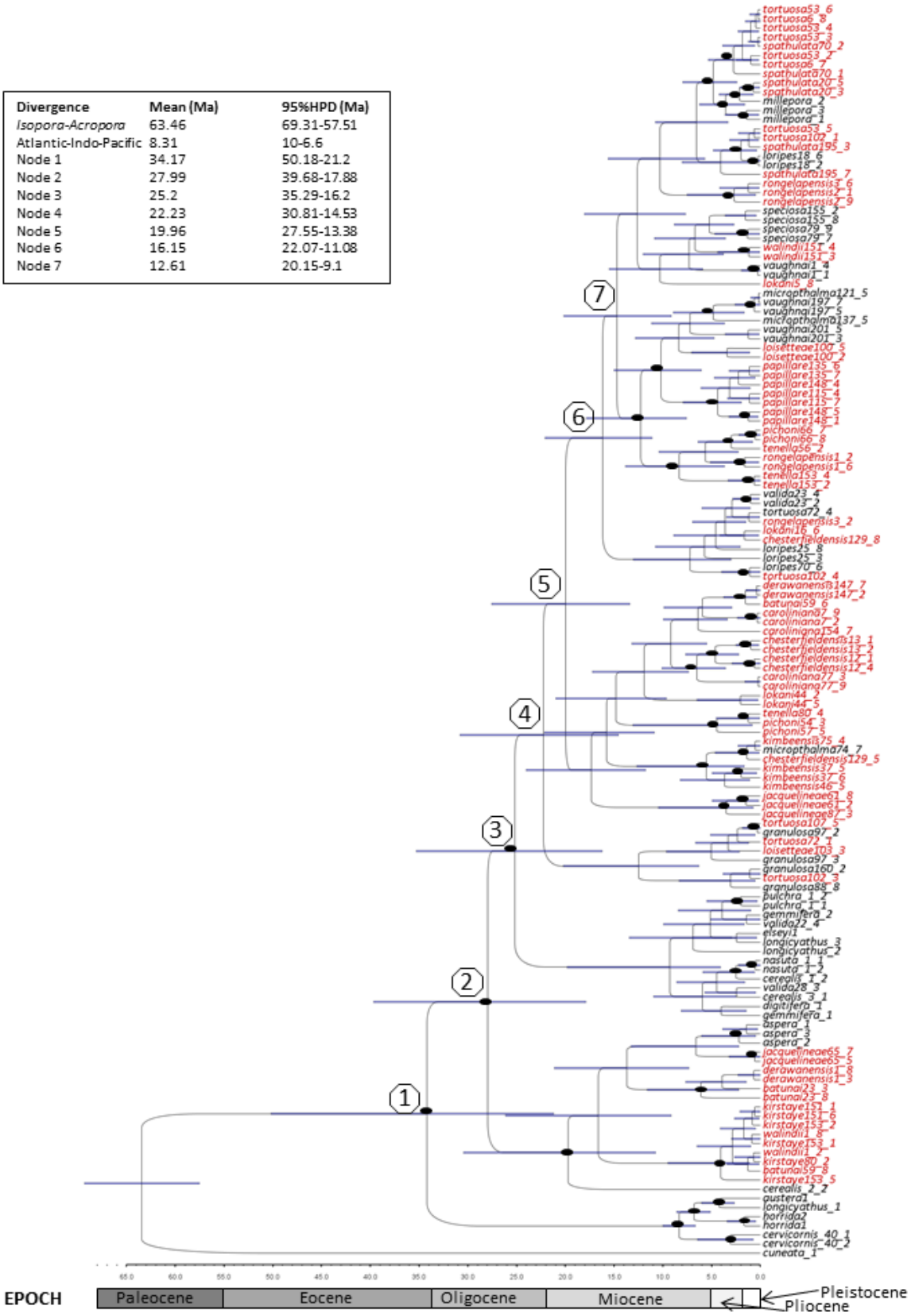

Figure 5. Divergence time estimates from the Bayesian analysis of the concatenated dataset. The time-calibrated maximum clade credibility tree is based upon nDNA and mtDNA pertaining to 35 species and the outgroup. Geographically restricted species are denoted by red font. Major nodes are labeled 1-7. Node bars depict the 95%HPD (Ma) estimates of divergence times. Nodes with significant posterior probability support (over 0.95) are denoted by a black oval. The geological epochs are reported according to the 1999 Geologic Time Scale of the Geological Society of America.

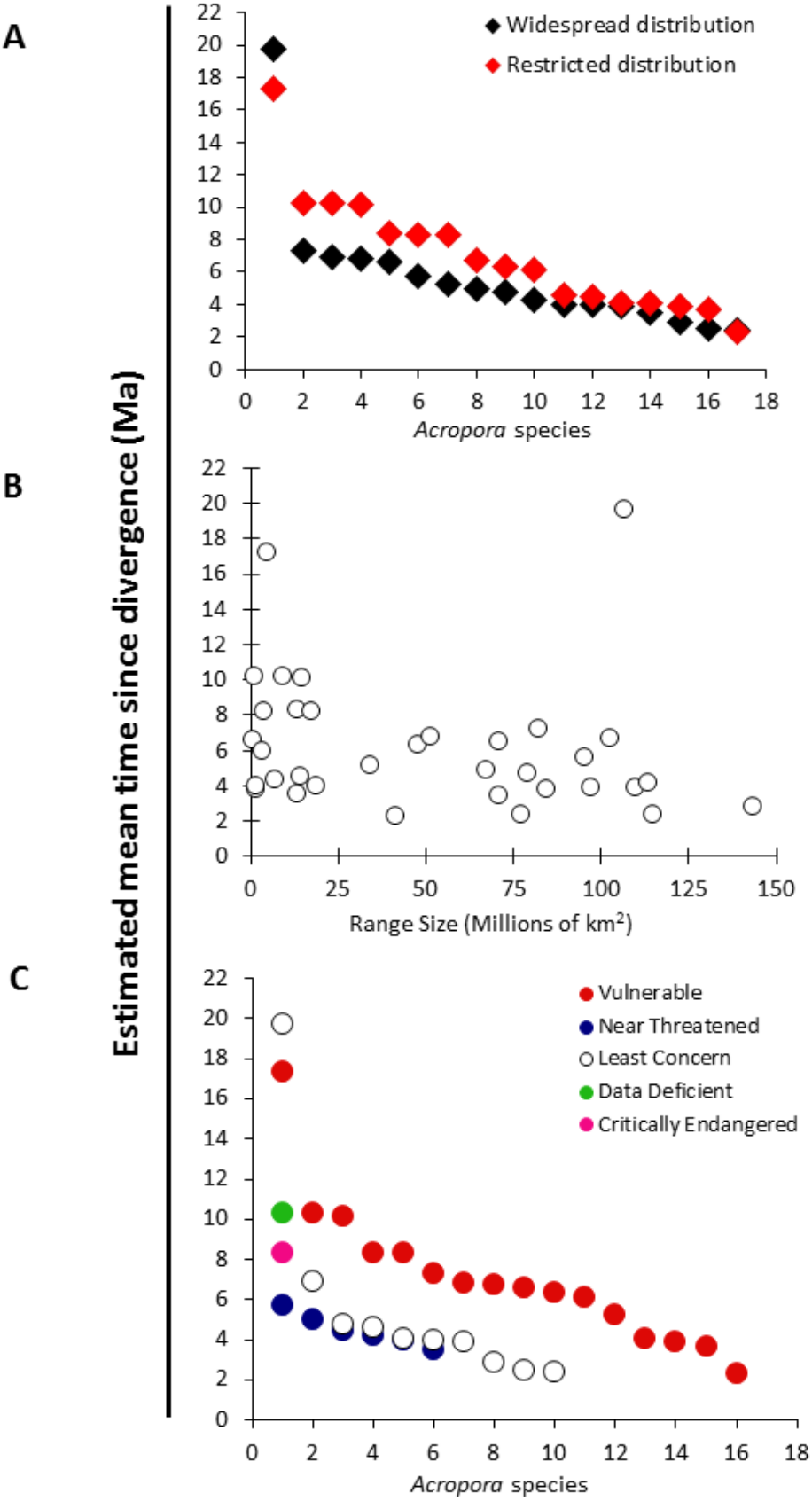

Figure 6. Relationship between mean estimated time since divergence (Ma) and A). Distribution pattern; B). Range size as determined by linear regression; C). Global extinction risk (according to IUCN categories and criteria (see Carpenter et al., 2008).

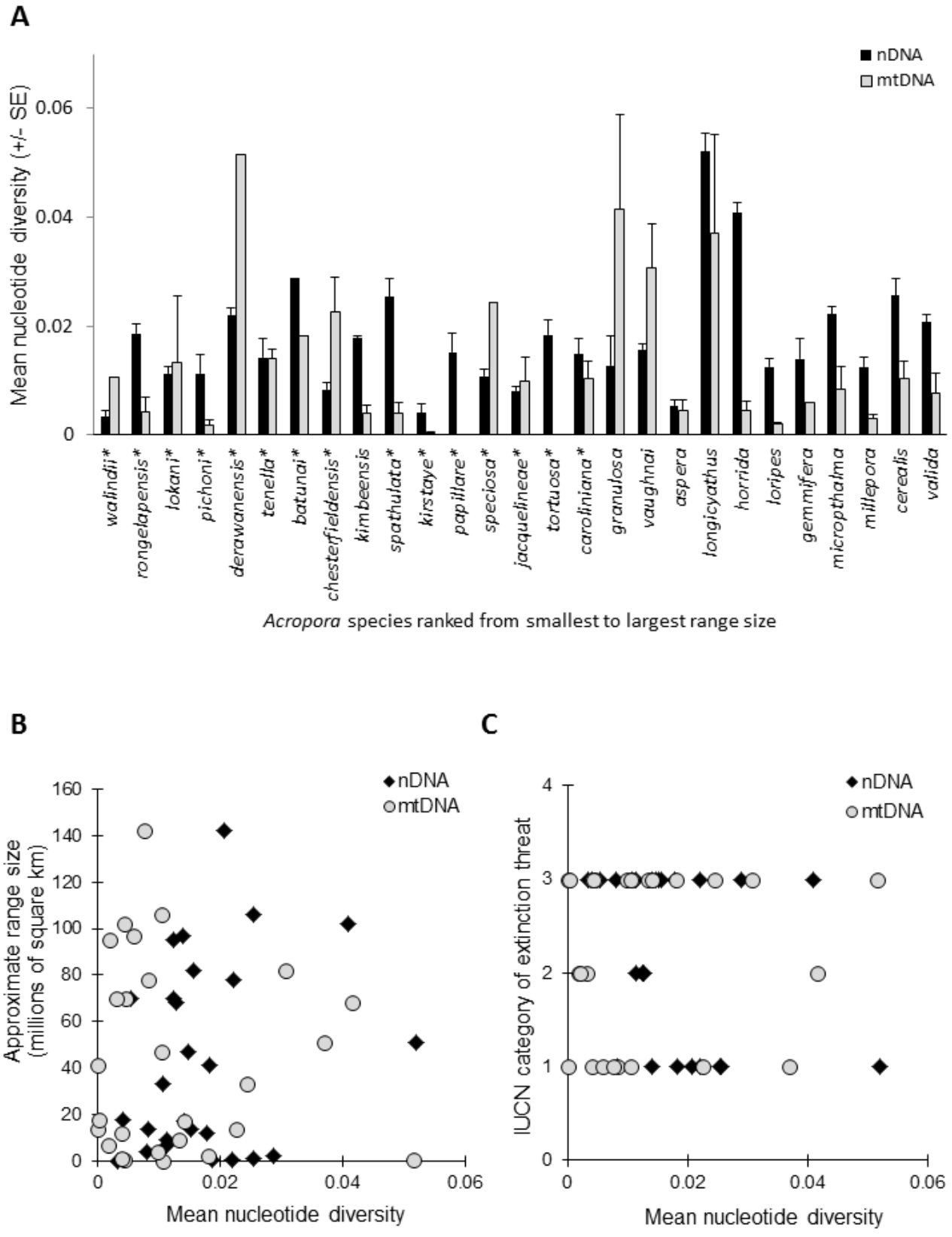

Figure 7. A). Mean pairwise K2P distances (± SE) for nDNA and mtDNA calculated in PAUP* 4.0B10 (a beta version; Swofford 2002). * indicates rare species; B). The relationship range size and nucleotide diversity as determined by linear regression; C). The relationship between global extinction risk and nucleotide diversity where 1= Least Concern, 2 = Near Threatened, 3 = Vulnerable (according to IUCN categories and criteria, see Carpenter et al., 2008).

Table 1. Locality, biogeography and sequence information for samples used in this study.

Species Group	Species	#nDNA sequences (clones)	#mtDNA sequences	Collection Location and Source	Mean depth (meters)	Range (million km^2)	Distribution Category
rudis	*A. austera*	2 (2)	1	*Palm Islands, GBR (van Oppen et al., 2001)*	2.3 (+/-2.1)	113.004	Widespread
aspera	*A. aspera*	4 (4)	3	*Palm Islands, GBR (van Oppen et al., 2001)*	3 (+/-2.1)	70.088	Widespread
	A. millepora	5 (5)	3	*Palm Islands, GBR (van Oppen et al., 2001)*	4 (+/-3.4)	70.233	Widespread
	A. pulchra	1 (1)	1	*Heron Island, GBR (van Oppen et al., 2001)*	2.6 (+/-2.3)	83.850	Widespread
	A. papillare	3 (7)	3	*Palm Islands, GBR (This study)*	1.8 (+/-1.8)	14.093	Restricted
	A. spathulata	3 (6)	3	*Palm Islands, GBR (This study)*	3.5 (+/-2)	0.986	Widespread
echinata	*A. carduus*	1 (1)	1	*Davies Reef, GBR (van Oppen et al., 2001)*	10.4 (+/-5)	48.631	Widespread
	A. elseyi	1 (1)	1	*Davies Reef, GBR (van Oppen et al., 2001)*	6.5 (+/-4.9)	76.464	Widespread
	A. longicyathus	3 (3)	3	*Palm Islands, GBR (van Oppen et al., 2001)*	8.4 (+/-5.7)	50.754	Widespread
	A. batunai	3 (6)	2	*Kimbe Bay, PNG (This study)*	15.3 (+/-11)	2.522	Restricted
elegans	*A. pichoni*	3 (4)	3	*Kimbe Bay, PNG (This study)*	27.4 (+/-8.3)	6.504	Restricted
	A. tenella	3 (4)	3	*Kimbe Bay, PNG (This study)*	28.6 (+/-11.8)	16.845	Restricted
	A. walindii	3 (6)	2	*Kimbe Bay, PNG (This study)*	21.5 (+/-11.4)	0.000	Restricted
horrida	*A. horrida*	3 (3)	3	*Big Broadhurst Rf, GBR (Fleury unpubl.)*	10.1 (+/-6.1)	101.858	Widespread
	A. microphthalma	3 (3)	3	*Palm Islands, GBR (This study)*	6.7 (+/-4.4)	78.444	Widespread
	A. vaughani	3 (6)	3	*Palm Islands, GBR (This study)*	8.9 (+/-5.9)	81.753	Widespread
	A. tortuosa	5 (12)	3	*Rongelap Atoll, RMI (This study)*	14.9 (+/-9.6)	40.590	Restricted
	A. derawanensis	2 (4)	2	*Kimbe Bay, PNG (This study)*	19.6 (+/-6.6)	0.631	Restricted
	A. kirstyae	3 (6)	3	*Palm Islands, GBR (This study)*	14.6 (+/-5.6)	18.093	Restricted
humilis	*A. digitifera*	1 (1)	2	*Magnetic Is. GBR (van Oppen et al., 2001)*	3 (+/-3)	109.297	Widespread
	A. gemmifera	3 (3)	2	*Trunk Reef, GBR (van Oppen et al., 2001)*	5 (+/-3.8)	96.815	Widespread
	A. humilis	-	3	*Trunk Reef, GBR (van Oppen et al., 2001)*	5 (+/-4.1)	113.474	Widespread
loripes	*A. granulosa*	3 (4)	3	*Rongelap Atoll, RMI (This study)*	18 (+/-8.9)	66.784	Widespread
	A. loripes	3 (5)	3	*Rongelap Atoll, RMI (This study)*	10.8 (+/-6.3)	94.956	Widespread
	A. speciosa	3 (6)	2	*Rongelap Atoll, RMI (This study)*	19.6 (+/-9.3)	33.299	Restricted
	A. caroliniana	3 (5)	3	*Palm Islands, GBR (This study)*	13.9 (+/-7)	46.918	Restricted
	A .chesterfieldensis	3 (6)	3	*Rongelap Atoll, RMI (This study)*	19.5 (+/-6)	13.661	Restricted
	A. jacquelineae	3 (5)	3	*Kimbe Bay PNG (This study)*	18 (+/-7.6)	4.131	Restricted
	A .lokani	3 (4)	3	*Kimbe Bay PNG (This study)*	11 (+/-3.3)	8.532	Restricted
	A. rongelapensis	3 (4)	3	*Rongelap Atoll, RMI (This study)*	23.8 (+/-4.8)	0.446	Restricted
nasuta	*A. cerealis*	3 (4)	2	*Trunk Reef, GBR (van Oppen et al., 2001)*	9 (+/-6.6)	105.971	Widespread
	A. nasuta	3 (6)	1	*Trunk Reef, GBR (van Oppen et al., 2001)*	4.8 (+/-4.4)	114.162	Widespread
	A. valida	3 (4)	3	*Magnetic Is. GBR (van Oppen et al., 2001)*	7.1 (+/-6.3)	142.762	Widespread
	A. kimbeensis	3 (4)	3	*Kimbe Bay PNG (This study)*	10 (+/-3.4)	12.413	Restricted
selago	*A. loisetteae*	3 (4)	2	*Rongelap Atoll, RMI (This study)*	17 (+/-3.2)	12.623	Restricted
	A. tenuis	-	3	*Trunk Reef, GBR (van Oppen et al., 2001)*	4 (+/-3.4)	79.816	Widespread
cervicornis	*A. cervicornis*	1 (2)	2	*SBI, Atlantic Ocean (van Oppen, 2000)*	6 (+/-6.7)	3.062	Restricted
	A. palmata	1 (1)	-	*SBI, Atlantic Ocean (van Oppen, 2000)*	2 (+/-0.6)	3.012	Restricted

Table 2. Estimates of mean divergence times, and respective 95% CL intervals given by Bayesian analyses of concatenated dataset based upon *nDNA* and *mtDNA*. Estimates of divergence times expressed in million years. For species where multiple individuals were examined, the oldest mean date of appearance is shown. The geological epochs corresponding to divergence times followed the 1999 Geologic Time Scale of the Geological Society of America. Oli.: Oligocene; Mio.: Miocene; Pli.: Pliocene. Definitions of Distribution and Threatened Status are described in Section 2.2.

Divergence from MRCA	Mean	95% CL	Geological Epoch	Distribution	Threatened status
A. cerealis	19.72	30.42-10.73	Late Olig-Mid Mio.	Widespread	Least Concern
A. jacquelineae	17.32	24.02-11.76	Late Olig-Mid Mio.	Restricted	Vulnerable
A. lokani	10.3	15.5-5.91	Mid Mio.-Late Plio.	Restricted	Vulnerable
A. rongelapensis	10.28	15.6-5.69	Mid Mio.-Late Mio.	Restricted	Data Deficient
A. papillare	10.17	15.02-6.02	Mid Mio.-Late Mio.	Restricted	Vulnerable
A. loisetteae	8.36	12.82-4.75	LateMio.-Pleistocene	Restricted	Vulnerable
A. tenella	8.31	13.82-3.68	Mid Mio.-Early Plio.	Restricted	Vulnerable
A. cervicornis	8.31	10-6.66	Late Miocene	Restricted	Critically Endangered
A. vaughnai	7.28	12.02-3.77	Mid Mio.-EarlyPlio.	Widespread	Vulnerable
A. longicyathus	6.88	13.45-2.94	Mid Mio.-Late Plio.	Widespread	Least Concern
A. horrida	6.81	8.61-5.12	Late Mio.-Early Plio.	Widespread	Vulnerable
A. walindii	6.72	10.87-3.59	Late Mio.- Early Plio.	Restricted	Vulnerable
A. aspera	6.59	13.26-2.2	Mid Mio.-Late Plio.	Widespread	Vulnerable
A. caroliniana	6.38	9.96-3.36	LateMio.-Early Plio.	Restricted	Vulnerable
A. batunai	6.1	11.59-2.21	Mid Mio.-Late Plio.	Restricted	Vulnerable
A. loripes	5.74	10.77-2.05	Late Mio.-Late Plio.	Widespread	Near Threatened
A. speciosa	5.25	8.82-2.69	Late Mio.-Late Plio.	Widespread	Vulnerable
A. granulosa	5.01	9.62-2.14	Late Mio.-Late Plio.	Widespread	Near Threatened
A. microphthalma	4.81	8.92-1.65	Mid Mio.-Late Plio.	Widespread	Least Concern
A. chesterfieldensis	4.62	7.67-2.22	Late Mio.-Late Plio.	Restricted	Least Concern
A. pichoni	4.46	13.06-0.82	Mid Mio.-Pleistocene	Restricted	Near Threatened
A. austera	4.23	6.02-2.66	Late Mio.-Late Plio.	Widespread	Near Threatened
A. kirstyae	4.05	9.47-1.11	Late Mio.-Pleistocene	Restricted	Vulnerable
A. spathulata	4.04	8.04-1.16	Mid Mio.-Pleistocene	Restricted	Least Concern
A. digitifera	3.99	8.11-1.45	Late Mio.-Late Plio.	Widespread	Near Threatened
A. gemmifera	3.99	8.11-1.45	Mid Mio.-Pleistocene	Widespread	Least Concern
A. pulchra	3.92	8.42-0.95	Late Mio.-Pleistocene	Widespread	Least Concern
A. derawanensis	3.89	7.64-1.44	Late Mio.-Pleistocene	Restricted	Vulnerable
A. kimbeensis	3.66	8.22-1.08	Late Mio.-Pleistocene	Restricted	Vulnerable
A. millepora	3.54	6.28-1.59	Late Mio.-Pleistocene	Widespread	Near Threatened
A. valida	2.91	5.99-1.03	Late Mio.-Pleistocene	Widespread	Least Concern
A. nasuta	2.5	8.59-1.58	Late Mio.-Pleistocene	Widespread	Least Concern
A. elseyi	2.44	6.03-0.37	Late Mio.-Pleistocene	Widespread	Least Concern
A. tortuosa	2.36	5.11-0.54	Early Plio.-Pleistocene	Restricted	Vulnerable

Supplementary Material

Table S1. A). Target Primer Pairs; B). PCR Conditions and Profiles; C). Likelihood and Bayesian Settings.

A). Target Primer Pairs

Primer Name	*Primer Sequence (5' – 3')*
PaxC_Intron_FP1	TCCAGAGCAGTTAGAGATGCTGG
PaxC_Intron_RP1	GGCGATTTGAGAACCAAACCTGTA
Rns_FP1	GGTTTCTAATACCTCCGAGG
Cox3_RP1	TACATAACACTGCCCACAGT
CR_FP1	TCTGATGAGACCCTTGTC
CR_RP1	AATTCTTAGGCAACCCCC

B). PCR Conditions and Profiles

PaxC - The forward primer was located at 112-90 bp upstream of the intron. The reverse primer annealed to the 3' end of the intron. Conditions for the PCR reaction included using 150-200 ng of DNA template and 0.13μl*Taq* polymerase in a 25 μl reaction in the presence of 10x reaction buffer (Fisher Biotech), 2 μl MgCl2 (25 mM), 1.5 μl dNTPs (2 mM), 2 μl forward and reverse primers and 14.87 μlpcr grade H2O. The PCR profile consisted of an initial denaturation step of 95° for 3 mins followed by 35 cycles of 30 sec at 94°C, 30 sec at 55°C and 1 min at 72°C.

***Rns*-Cox3** - In some cases, DNA had become unstable and the mtDNA segments proved difficult to amplify. A number of optimization trials were conducted on these samples whereby the PCR conditions and master mix composition were varied. An initial denaturation step of 30 sec at 94°C, followed by 35 cycles of 10 sec at 94°C, 60 sec at 54°C and 60 sec at 68°C and 5 min at 68°C proved optimal in some cases. An additional 10 cycles were required in some cases to amplify the large fragments. For other samples, increasing the amount of Taq polymerase assisted amplification. The most degraded samples required the use of a Qiagen Core PCR Kit (CAT 201223) or Platinum Taq (Invitrogen) for amplification.

C). Likelihood and Bayesian Settings

nDNA - ML settings from best fit model HKY+G selected via hLRT in Modeltest 3.7: Lset Base=(0.2902 0.2214 0.2108) Nst=2 TRatio=1.4024 Rates=gamma Shape=1.6262 Pinvar=0. BI analyses used likelihood settings from best-fit model HKY+G selected by hLRT in MrModeltest 2.0: Prset statefreqpr =dirichlet (1,1,1,1); Lset nst=2 rates=gamma; burnin=30,000.

mtDNA - ML likelihood settings from best-fit model HKY+I+G selected by hLRT in Modeltest 3.7: Lset Base=(0.2418 0.1958 0.2604) Nst=2 TRatio=0.7400 Rates=gamma Shape=0.3209 Pinvar=0.1591. BI analyses used likelihood settings from best-fit model HKY+I+G selected by hLRT in MrModeltest 2.0: Prset statefreqpr =dirichlet (1,1,1,1); Lset nst=2 rates = invgamma; burnin= 50, 000.

Concatenated tree - Best-fit model (GTR+I+G) was selected by a hierarchical likelihood ratio test (hLRT) in Modeltest 3.7 with the following parameters: {Lset Base= (0.2726 0.1893 0.2444) Nst=6 Rmat= (1.5528 1.7561 0.9752 1.2138 2.4355) Rates=gamma Shape=0.4766 Pinvar=0.2396; burnin= 50, 000.

1

Table S2. The relationship between mean allelic/mean haplotype diversity and range size/extinction risk examined as a linear function.

Relationship	R^2	Adj R^2	df	F	P value	Sig (0.05)
nDNA -Range Size	0.056	0.018	26	1.475	0.236	ns
nDNA – Extinction risk	0.098	0.060	25	2.606	0.120	ns
mtDNA – Range Size	0.006	-0.033	26	0.163	0.690	ns
mtDNA – Extinction risk	0.005	-0.036	25	0.120	0.731	ns

Table S3. Summary of cases where nuclear alleles (nDNA) and mitochondrial haplotypes (mtDNA) were shared between species.

Species	*nDNA*	*ntDNA*
*A. walindii**	*A. kirstyae*, A. batunai**	*A. kirstyae**
*A, pichoni**	*A. tenella**	
*A. derawanensis**	*A. jacquelineae**	
*A. tenella**	*A. chesterfieldensis*, A. pichoni**	
*A. chesterfieldensis**	*A. tenella**	
*A. prolifera**	*A. palmata**	
*A. spathulata**	*A. loisetteae**	
*A. palmata**	*A. prolifera**	
*A. kirstyae**	*A. walindii*, A. batunai**	*A. walindii**
*A. batunai**	*A. walindii*, A. kirstyae**	
*A. papillare**	*A. gemmifera*	
*A. jacquelineae**	*A. derawanensis**	
*A. tortuosa**	*A. loripes, A. gramulosa, A. valida*	
*A. loisetteae**	*A. spathulata*, A. valida*	
*A. caroliniana**	*A. speciosa*	
A. gramulosa	*A. tortuosa**	
A. speciosa	*A caroliniana*, A. vaughnai*	
A. carduus		*A. longicyathus*
A. vaughani	*A. micropthalma, A. valida, A. speciosa*	
A. pulchra		*A. millepora*

2

A. longicyathus		*A. carduus*
A. loripes	*A. tortousa**	
A. gemmifera	*A. papillare**	
A. microphthalma	*A. vaughani, A. valida*	
A. millepora		*A. pulchra*
A. cerealis		*A. nasuta*
A. nasuta		*A. cerealis*
A. valida	*A. vaughani, A. microphthalma, A. loisetteae*, A. tortuosa**	

Table S4: Fossil data of species included in this analysis

Species	Oldest Fossil Record for species	Fossil record for species group lineage	Source
*A. walindii**	None	*elegans* group - none	
*A. rongelapensis**	None	*loripes* group - none	
*A. loisetteae**	None	*selago* group – Holocene: 10, 000 years to present	Wallace 1999
*A. pichoni**	None	*elegans* group - none	
*A .lokani**	None	*loripes* group - none	
*A. derawanensis**	None	*horrida* group - none	
*A. tenella**	None	*elegans* group - none	
*A. batunai**	None	*echinata* group - none	
*A. chesterfieldensis**	None	*loripes* group - none	
*A. kimbeensis**	None	*nasuta* group - Pliocene	Wallace 1999
*A. prolifera**	None	*cervicornis* group– Lutenian (49-41.3my) – Bartonian (41.4 – 37my)	Budd et al 1999; Wallace 2008
*A. spathulata**	None	*aspera* group - Bartonian 41.4-37.5 my	Wallace 2008
*A. palmata**	Pleistocene - 1.81 –10, 000 years Holocene – 10,000 years to present	*cervicornis* group – Lutenian (49-41.3my) – Bartonian (41.4 – 37.5 my)	Budd et al 1999; Wallace, 1999 Wallace 2008
*A. kirstyae**	None	*horrida* group - none	
*A. cervicornis**	Pleistocene - 1.81 – 10, 000 years	*cervicornis* group – Eocene: Lutenian (49-41.3my– Bartonian 41.4 – 37.5my); A. cervicornis Mio-Pliocene	Budd et al 1999; Wallace, 1999; Wallace 2008 Budd and Wallace 2008
*A. papillare**	None	*aspera* group– Eocene: Bartonian 41.4-37.5 my	Wallace 2008
*A. speciosa**	None	*loripes* group - none	
*A. jacquelineae**	None	*loripes* group - none	
*A. tortuosa**	None	*horrida* group - none	
*A. caroliniana**	None	*loripes* group - none	
A. granulosa	none	*loripes* group - none	
I. cuneata	Pliocene - 5.32 – 1.81my Pleistocene - 1.81my – 10,000 years	Genus *Isopora*–Mio-Pliocene	Budd and Wallace 2008
A. vaughani	Pliocene - 5.32 – 1.81my Pleistocene - 1.81my – 10,000 years	*horrida* group - Pliocene - 5.32 – 1.81Ma to Pleistocene - 1.81my – 10,000 years	Wallace, 1999
A. pulchra	Pliocene - 5.32 – 1.81my Pleistocene - 1.81my – 10,000 years	*aspera* group – Eocene: Bartonian (41.4 – 37.5Ma)	Wallace, 1999 Yabe and Sugiyama, 1935
A. aspera	Pliocene - 5.32 – 1.81my Pleistocene - 1.81my – 10,000 years	*aspera* group– Eocene: Bartonian (41.4 – 37.5Ma)	Wallace, 1999 Pickett et al, 1985
A. longicyathus	Pleistocene - 1.81 – 10, 000 years	*echinata* group - Pleistocene - 1.81 – 10, 000 years	Pickett et al, 1985
A. loripes	None	*loripes* group - none	
A. gemmifera	Pleistocene - 1.81 – 10, 000 years	*humilis* group II – Eocene; Priabonian (36-34.2Ma)	Pandolfi, 1996; Wallace 2008
A. microphthalma	Miocene - 25 – 5.32 my; Pleistocene - 1.81 – 10, 000 years	*horrida* group - none	Wells, 1964 Pickett et al, 1985
A. millepora	Pliocene - 5.32 – 1.81my Pleistocene - 1.81my – 10,000 years	*aspera* group lineage – Eocene: Bartonian (41.4-37.5Ma)	Wallace 1999 Pickett et al, 1985
A. digitifera	Holocene – 10,000 years to present	*humilis* group I - Eocene	Camoin et al.,1997; Wallace 2008
A. humilis	Miocene - 25 – 5.32 my; Pleistocene - 1.81 my – 10, 000 years; Holocene – 10,000 years to present	*humilis* group II – Eocene – Priabonian (36-34.2Ma)	Wallace 1999; Wells 1964; Wallace 2008
A. austera	Pleistocene - 1.81 my – 10, 000 years	*rudis* group - none	Wallace 1999
A. cerealis	Pliocene - 5.32 – 1.81my	*nasuta* group - Pliocene	Wallace 1999
A. nasuta	none	*nasuta* group - Pliocene	Wallace 1999
A. valida	Pleistocene - 1.81 my – 10, 000 years	*nasuta* group - Pliocene	Pickett et al, 1985; Wallace, 1999

Table S5. Results from a test of molecular clocks using the Maximum Likelihood method.

	ln*L*	Parameters	(+*G*))	(+*I*)
With Clock	-8691.728	139	0.377	0.53
Without Clock	-8168.575	274	0.31	0.44

Table S6. Summary statistics for the results the time-calibrated tree performed with a relaxed molecular clock in BEAST 1.7.4 and associated programs (Drummond *et al.*, 2012) based on the concatenated alignment containing *nDNA* and *mtDNA* sequences.

Summary Statistic	mean	stderr of mean	median	geometric mean	95% HPD lower	95% HPD upper	auto-correlation time (ACT)	effective sample size (ESS)
posterior	-15181.932	1.4108	-15182.0127	n/a	-15225.672	-15135.891	1.70E+05	264.2865
prior	-1094.0128	1.2862	-1094.0227	n/a	-1129.9959	-1055.7887	2.04E+05	220.73
likelihood	-14087.919	0.5982	-14087.5043	n/a	-14112.908	-14064.476	1.05E+05	426.9948
treeModel.rootHeight	63.4599	3.20E-02	63.46	63.3876	57.51	69.3065	5025.5943	8954.3639
tmrca(caribbean)	8.3233	2.45E-02	8.3123	8.2791	6.6646	10.0028	37121.024	1212.278
tmrca(root)	63.4528	3.55E-02	63.4555	63.3807	57.5577	69.3331	6207.9151	7248.9715
yule.birthRate	0.1652	1.65E-03	0.163	0.163	0.1139	0.2184	1.66E+05	271.5427
ac	0.601	1.26E-03	0.5978	0.598	0.4883	0.7204	19778.145	2275.2893
ag	0.717	2.03E-03	0.7125	0.7131	0.5776	0.8675	32714.156	1375.5819
at	0.385	8.90E-04	0.3835	0.3831	0.3069	0.4565	24170.475	1861.817
cg	0.4537	1.01E-03	0.451	0.4508	0.3516	0.5517	17227.566	2612.1507
gt	0.3782	9.09E-04	0.3751	0.3761	0.2985	0.453	23781.5	1892.2692
frequencies1	0.2748	2.12E-04	0.2748	0.2747	0.2587	0.291	29400.499	1530.6203
frequencies2	0.1877	1.86E-04	0.1876	0.1876	0.1747	0.2031	30678.788	1466.8441
frequencies3	0.2449	1.85E-04	0.2446	0.2448	0.2291	0.2599	24572.656	1831.3446
frequencies4	0.2926	2.22E-04	0.2925	0.2925	0.2746	0.3082	30881.601	1457.2107
alpha	0.4966	2.15E-03	0.4905	0.4916	0.3637	0.6322	41187.443	1092.5903
pInv	0.2027	1.48E-03	0.2038	0.196	0.1068	0.2955	41130.422	1094.105
uced.mean	2.21E-03	2.69E-05	2.17E-03	2.17E-03	1.52E-03	2.96E-03	2.28E+05	197.1317
meanRate	2.16E-03	2.19E-05	2.13E-03	2.13E-03	1.54E-03	2.80E-03	1.99E+05	225.602
coefficientOfVariation	1.0635	3.77E-03	1.0624	1.0623	0.9692	1.1613	2.66E+05	169.4755
covariance	0.1688	2.98E-03	0.1679	n/a	4.68E-02	0.2932	1.00E+05	448.568
treeLikelihood	-14087.919	0.5982	-14087.504:	n/a	-14112.908	-14064.476	1.05E+05	426.9948
speciation	-383.6194	1.3179	-383.7566	n/a	-418.7029	-345.6715	2.21E+05	203.7558

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