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Abstract

Understanding the population structure of tropical anguillids residing in the Pacific is vital for their conservation management. Here, we investigated the population genetic structure of five sympatric freshwater eels across 11 South West Pacific (SWP) islands. We analysed partial nucleotide sequences of the mtDNA control region and the nuclear GTH2b genes of over 300 samples, to elucidate patterns of population differentiation among sampling sites. SWP anguillids are characterised by overall high levels of genetic diversity. Within-species genetic clusters were largely unlinked to sampling locations, despite partly significant differentiation between locations. We confirm the occurrence of hybrids between *Anguilla marmorata* and *A. megastoma*, and found evidence for population growth in all species, as well as population reduction for *A. megastoma*. We suggest that common spawning grounds and larvae mixing by ocean currents could promote the lack of isolation by distance, with significant implications for the future management of anguillids.

Introduction

Sympatric and closely related organisms are excellent models to infer the underpinning mechanisms behind genetic diversity and connectivity in marine ecosystems (Palumbi, 1994; Dawson, 2012). Moreover, multi-species comparative studies examining gene flow have important implications for conservation and management strategies, as they offer a more comprehensive assessment of species' biodiversity and evolution (Weber et al., 2015). Patterns of population structure and phylogeography of economically important species with similar life history characteristics may be concordant or disparate (Mcmillen-Jackson & Bert, 2003; Husemann et al., 2012), providing insights into ecological and anthropogenic factors that might influence them. Interestingly, sympatric species that share large distribution areas are possibly less prone to extinctions due to fishing pressure. However, uncontrolled fisheries and habitat loss due to climate change and fluctuation of sea water levels can have deleterious effects on the habitat of particularly tropical fish, resulting in contracted distributions and reductions in population size (Nicholls & Cazenave, 2010).

The South West Pacific (SWP) hosts six (*Anguilla australis, A. dieffenbachii, A. marmorata, A. megastoma, A. obscura, A. reinhardtii*) out of the 19 global species and subspecies of *Anguilla* eels (Jellyman, 2003, not including *A. bicolor pacifica* and *A. interioris* that occur at the border of the Bismarck Sea). They share catadromous life histories, with growth occurring in freshwater or estuarine areas and spawning in tropical ocean waters (Schmidt, 1923; Tesch, 1977; Aida et al., 2003; Watanabe et al., 2005). All species except *A. bicolor* (Near Threatened) and *A. marmorata* (Least concerned) are classified as Data Deficient or have not been assessed by the

International Union for Conservation of Nature (IUCN) (Jacoby & Gollock, 2014). As the majority of studies on eels have focused on temperate species, large knowledge gaps currently exist on their ecology, population genetic structure and spawning grounds. Previous studies suggested that *A. megastoma* might be separated into eastern and western populations, whereas *A. marmorata*, *A. obscura* and *A. reinhardtii* are comprised of single stocks in the western South Pacific based on vertebral counts (Watanabe et al., 2008, 2011), results that were not always corroborated with molecular data (Ishikawa et al., 2004).

Demographic assessment also requires that species delimitation is taken into account. Natural hybrids have been previously reported between Atlantic eel species (Pujolar et al., 2014; Wielgoss et al., 2014), whereas hybrids between, for example, shortfinned and longfinned eels have been produced under laboratory conditions only (Lokman & Young, 2000; Okamura et al., 2004; Burgerhout et al., 2011). In the Pacific, relatively high levels of hybridisation were recently revealed between the two long-finned eels *A. marmorata* and *A. megastoma* from Gaua Island, Vanuatu, and linked with reproductive sympatry of the species as suggested by satellite tracking (Schabetsberger et al., 2015).

An increasing economic interest in SWP tropical anguillid species has been stimulated by sharp declines in temperate eel species recruitment (Briand et al., 2003; Aoyama et al., 2012; Iglesias & Lobón-Cerviá, 2012; Arai, 2016). East Asian countries are the main consumers of eels and eel products, with more than 90% of *Anguilla* production

based on farming after significant declines of catches of wild stocks due to overfishing (Limburg & Waldman, 2009; Arai, 2014; Shiraishi & Crook, 2015). High demand and exploitation of eels resulted in the classification of European, Japanese and American eels as Critically Endangered or Endangered by the IUCN Red List (Jacoby & Gollock, 2014), with the European eel also listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Until recently, the Philippines and Indonesia have been the main supplier of wild tropical glass eels for aquaculture in East Asia (Crook & Nakamura, 2013). However, recent attempts for the commercial exploitation of wild eel stocks from SWP countries have been reported (Pickering & Sasal, 2017; pers. com. S. Tiitii, Ministry of Agriculture and Fisheries of Western Samoa and D. Kalfatak, Department of Environmental Protection and Conservation, Vanuatu). However, the lack of natural history information and formal stock assessments of eels in this area impedes their management and conservation, and drastic temporal changes in local species compositions attributed to different seasonal spawning dynamics prevent the selective glass eel catch of commercially preferred species (Aoyama et al., 2015). In line with recent discoveries of cryptic species (*A. luzonensis* in the Philippines; Watanabe et al., 2009), there is an urgent need for more information on the distribution of freshwater eels in the SWP to understand their ecology and life history and to facilitate their management (Briand et al., 2003).

The aim of the present study is to document the population structure and demographic history of five sympatric anguillid species across the SWP, using sequence data derived from nuclear and mitochondrial genomes. While a lack of population structure has been

previously documented in a subset of eels in the area based on nuclear and mitochondrial markers (Minegishi et al., 2008, 2011; Shen & Tzeng, 2007a), there are still wide knowledge gaps due to limited spatial sampling and incomplete coverage of uncommon species such as *A. interioris*, *A. reinhardii* and *A. obscura*. To this end, our sampling included new SWP geographic regions to provide novel insights into processes determining gene flow and standing amounts of genetic variation, resulting in important conservation and management implications for five understudied but important species.

Methods

2.1. Study area and PCR amplification

A total of 288 adult or juvenile *Anguilla* specimens were collected during 2015-2016 in freshwater rivers or lakes of South West Pacific Islands, by electrofishing and hand nets in collaboration with local fishermen (Supplementary Table 1). Sampling localities included Bougainville Island (BG: n = 31), Solomon Islands (SI: n = 37), New Caledonia (NC: n = 124), Western Samoa (WS: n = 57) and American Samoa (AS: n = 39; Figure 1, Supplementary Table 1). Samples were treated according to each permit's requirements, as issued by local authorities. Eels were sacrificed or anaesthetised in a freshwater bath containing 40 mg l^{-1} metomidate (Marinil™, Wildlife Labs) or clove oil until motionless. They were subsequently measured, genetically sampled and released (except all individuals from Bougainville). Total length and the distance from lower jaw to the anus and to the dorsal fin were measured to the nearest mm. All individuals were morphologically assigned to a species by assessment of body proportions, coloration,

and dentition of the upper jaw following Ege (1939). Small pieces of tissue and fin clips were stored in 95% ethanol until genomic DNA extraction was conducted using the DNeasy Blood and Tissue kit (Qiagen) as per the manufacturer's instructions, or a standard phenol chloroform procedure (Sambrook et al., 1989).

2.2. Molecular species identification

To genetically assign all individuals to known species, mitochondrial (control region, CR) and nuclear (gonadotropin II-beta subunit, GTH2b) sequence variation was examined using MTF-MTR and GTH2bF-GTH2bR primers in 20 µl reaction volumes (see Schabetsberger et al., 2015 for details). Briefly, Polymerase Chain Reactions (PCRs) were carried out with GoTaq ×5 reaction buffer (1.5 mM $MgCl_2$ in ×1 concentration), 200-250 µM of each deoxyribonucleotide triphosphate (dNTP; Bioline), 0.2-1.0 µM of each primer (MWG-Biotech), 1.0-2.5 U GoTaq polymerase (Promega) and thermal PCR profiles for the CR and GTH2b genes as described in Schabetsberger et al. (2015). PCR products were sequenced commercially (Macrogen, Netherlands).

All sequences were checked with Proseq 3.2 (Filatov, 2009), and all mtDNA (CR) sequences (from 498bp – *A. australis*, to 526bp – *A. marmorata*) were compared with those available in GenBank using the Standard Nucleotide BLAST (blastn) against the Nucleotide collection (nr/nt) database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The 268bp nuclear sequences validated the morphological species identification at two species-specific Single Nucleotide Polymorphisms (SNPs) for some of the studied species (C/T at base position 86, and T/C at position 169; *A. megastoma* and *A. obscura* show genotype C and T, whereas *A. marmorata* has the genotype T and C, respectively,

see Schabetsberger et al. (2015) for details; their hybrids possess heterozygote genotypes at the two species-specific nuclear SNPs and were excluded from further species-specifc analyses presented herein). The newly acquired samples were further combined with 83 sequences already available from Gaua Island (GA, Schabetsberger et al., 2015), as well as sequences provided in Minegishi et al. (2008; Fiji, FJ: 20 individuals; Tahiti, TH: 19 individuals; New Caledonia, NC: 9 individuals; Papua New Guinea, PNG: 15 individuals).

2.3. Population genetic analyses

Measures of genetic diversity, such as number of haplotypes (*H*), polymorphic sites (s), haplotype (*h*) and nucleotide (π) diversities (Nei, 1987) were calculated at the level of each species per marker and sampling site were calculated with Arlequin 3.5.2.2 (Excoffier et al., 2005). The nuclear phased haplotypes were estimated using Bayesian methods in Phase 2.1 (Stephens & Donnelly, 2003). Gametic phases with posterior probabilities equal to or higher than 0.7 were considered resolved (Harrigan et al., 2008). Past recombination for all datasets was investigated using a PHI Test (Bruen et al., 2006) in SplitsTree 4.14.4 (Huson & Bryant, 2006). No significant signals of recombination were detected, and the full non-recombining sequence was used. Average intraspecific genetic p-distances for CR within and between sampling locations were calculated in MEGA 7.0.21 (Kumar et al., 2015) with pairwise deletion for the gaps/missing data treatment. Additionally, the degree of genetic differentiation among localities was estimated using genetic distance-based Φ_{ST}, with 10,000 permutations in Arlequin. Genetic variation across temporally adjacent years in New Caledonia were assessed for *A. marmorata*, the species with the highest sample size and widest

sampling area, by calculating pairwise Φ_{ST} values between the newly acquired samples and the data presented in Minegishi et al. (2008) in Arlequin. Sequential Bonferroni corrections to the significance level of 0.05 were applied (Rice, 1989). A hierarchical analysis of molecular variance (AMOVA) was performed in Arlequin, to test for significant differences between groups of *A. marmorata* samples. Data were grouped according to geographical location, with the following hierarchy: North of SWP (Papua New Guinea, Bougainville, Solomon Islands, Vanuatu) and Caledonia (New Caledonia and New Caledonia from Minegishi et al., 2008), Fiji, Samoa Islands (Western Samoa and American Samoa) and Tahiti. Additionally, a Mantel test was used to test for significant correlation between genetic distances and geographical distances in GenAlEx 6.5 (Peakall & Smouse, 2012).

Haplotype networks for each marker were constructed in PopART 1.7 (Leigh and Bryant, 2015) using statistical parsimony (Clement et al., 2002) to visualize the geneological relationships between haplotypes across the region. The overall genetic structure of the four eel species with sufficient sample size (*A. marmorata*, *A. megastoma*, *A. obscura* and *A. reinhardtii*) was assessed separately using the Bayesian clustering implemented in BAPS 6.0 (Corander et al., 2008) for the mtDNA dataset. We firstly applied a non-spatial genetic mixture analysis to both individuals and groups (sampling localities) using the "linked loci" option. BAPS was run with the number of groups (*K*) set to range between 1 and 12, and each run was replicated five times. We then performed an admixture analysis based on the produced mixture clustering.

Phylogenetic Maximum-Likelihood (ML) analysis of the CR sequences were performed on RAxML 8.0 (Stamatakis et al., 2008). ML analyses were restricted to *A. australis* to determine whether the small number of New Caledonian individuals (three specimens) group with those caught in Australia or New Zealand (Accession Numbers: AB278891-AB278942). We used the GTRGAMMA model and 1,000 replications for the final tree calculations. Sequences of *A. obscura* were used as outgroup.

2.4 Demographic analysis

Recent demographic changes, gene flow-drift or selection and fluctuations in population sizes were assessed using Tajima's *D* (Tajima, 1989) and Fu's F_S (Fu, 1997) for both markers in Arlequin for the species with sufficient sample size. Furthermore, for the mitochondrial dataset, a Bayesian Skyline Plot (BSP) model was generated in BEAUti and Beast 1.8.0 (Drummond et al., 2005) and plotted using the 95% highest posterior density. Program default parameters were used, and different mutation models per species were selected chosen by jModeltest 2.1.9 (Guindon & Gasquel, 2003; Darriba et al., 2012) using the Bayesian Information Criterion (BIC). BSP analyses were conducted using a strict molecular clock with a substitution rate for the control region of 5% between lineages per one million years (Minegishi et al., 2011). The Markov Chain Monte Carlo (MCMC) sampling schemes were set to between 50 and 300 million iterations, depending on the length required for convergence. All runs were sampled every 1,000 iterations, after discarding the first 10% as burn-in. The BSP outputs were analysed using Tracer 1.5 with 10% burn-in (Rambaut et al., 2013).

Results

Overall, 275 and 281 partial mitochondrial (CR) and nuclear (GTH2b) sequences were aligned, respectively (Accession numbers: XXXXX-YYYYY and XXXXX-YYYYY, Supplementary Table 1). The CR sequences comprised 37 individuals from the Solomon Islands, 31 from Bougainville Island, 118 from New Caledonia, 56 from Western Samoa and 33 from American Samoa (Supplementary Table 1). The GTH2b sequences originated from 35 samples from the Solomon Islands, 29 samples from Bougainville Island, 121 samples from New Caledonia, 57 samples from Western Samoa and 39 samples from American Samoa (Supplementary Table 1). After combining morphological evidence with sequence data (mtDNA and nuclear markers), we assigned 175 individuals to *A. marmorata*, 10 individuals to *A. megastoma*, 26 individuals to *A. obscura*, 69 individuals to *A. reinhardtii*, three individuals to *A. australis* and one individual to *A. interioris*. Two individuals exhibited morphological and mtDNA disagreement combined with nuclear heterozygosity. Therefore, they were considered as admixed and removed from the further population genetic analyses presented in this study. The *A. interioris* sample as identified by its mtDNA was recorded in Bougainville Island, representing a range expansion of the species. However, it exhibited ambiguous bases in both positions (86 and 169) of the nuclear GTH2b sequence, which we assumed to be uninformative with respect to species status due to the absence of reference sequences.

For the mtDNA CR dataset, interspecies haplotype diversity ranged from 0.986 (*A. megastoma*) to 1.000 (*A. obscura* and *A. australis*), and corresponding nucleotide diversity values ranged from 0.056 (*A. megastoma*) to 0.072 (*A. australis*; Table 1). The number of haplotypes and variable sites ranged from 3 and 50 in *A. australis* to 218 and

371 in *A. marmorata*, respectively (Table 1), with intraspecific diversity values exhibiting similar ranges (Supplementary Table 2a-c). Three out of the five Variable Number of Tandem Repeat (VNTR) regions firstly reported by Ishikawa *et al*. (2004) were observed only in *A. marmorata*, resulting in the highest ranges of pairwise nucleotide differences (from 0% to 30.9%). Following the terminology of Minegishi *et al*. (2008), VNTR types 1 and 4 were found in all locations; one individual from Tahiti had Type 3, whereas Types 2 and 5 were absent. The lowest mean intraspecific difference in mtDNA CR was detected in *A. megastoma* (5.6%; Supplementary Table 3a), whereas *A. marmorata* exhibited the highest intraspecific variability (from 0% to 30.9%; Figure 2; Supplementary Table 3a). Interspecific variation exhibited values that ranged from 31.6% (between *A. reinhardtii* and *A. australis*; Supplementary Table 3b) to 51.3% (between *A. marmorata* and *A. obscura*; Supplementary Table 3b). The corresponding summary statistic values for GTH2b were overall lower than for CR (Table 1).

The CR pairwise comparisons for the temporal sampling in New Caledonia (new data presented herein compared to data presented in Minegishi et al., 2008) revealed a lack of differentiation (Φ_{ST}=0.0739, non-significant after Bonferroni corrections), and all samples were pooled for subsequent analyses (Table 2a). For *A. marmorata,* pairwise genetic distances between islands ranged from -0.0506 to 0.1627, and from -0.0038 to 0.1287 for CR and GTH2b, respectively (Table 2a). In total, 25 out of 45 pairwise comparisons showed significant differences in Φ_{ST} after Bonferroni correction. However, we neither detected a a significant association between genetic and geographic distances across sample collections (R^2=0.0398, p=0.080), nor sharp

geographic clines within the SWP (Table 2a). Additionally, the hierarchical AMOVA neither showed significant variation among geographically pre-defined groups (F_{CT}=0.0505, p=0.0574, 5.05%), nor significant variation among populations within the main regions of *A. marmorata* occurences (F_{SC}=0.0482, p<0.0001, 4.58%), although within population genetic variation was highest for this species (F_{ST}=0.0963, p<0.0001, 90.37%). Bougainville Island was the only locality with significant pairwise differentiation for GTH2b. No significant genetic structure among sites was found for both CR and GTH2b for the other two species with sufficient sample sizes (*A. megastoma* and *A. obscura*, Table 2b-c).

For CR, haplotypes did not assort by sample location for any of the studied species (Supplementary Figure 1I). While similar lack of geographical structure was evident in the GTH2b networks (Supplementary Figure 1II), a number of haplotypes were unique to Bougainville Island for *A. marmorata*, supporting the distinctiveness of this region (Supplementary Figure 1IIa). To assess the overall within-species genetic structure, BAPS identified clusters which consisted of patchily distributed groups without pronounced geographic partitioning for all species (Figure 3). *Anguilla reinhardtii* consisted of two clusters, including one admixed individual (p<0.01). The population structure for both *A. obscura* and *A. megastoma* was best explained by five clusters. Three genetically differentiated groups (K=3) were determined for *A. marmorata*, with 24 specimens showing significant admixture (p<0.01, found across all sampling localities except Papua New Guinea, Fiji and Tahiti).

The ML topology based on the CR revealed three lineages for *A. australis*, with very low support values for individuals from both Australia and New Zealand

(Supplementary Figure 2). All New Caledonian samples grouped with the second lineage.

Demographic history

Demographic analyses revealed contrasting findings. *Anguilla marmorata* was the only species with significantly negative Tajima's *D* and Fu's F_S values for both markers, conforming to a model of population expansion (Table 1), while the indices were not significant for *A. megastoma* for both markers. For the mtDNA datasets of *A. obscura* and *A. reinhardtii,* values of Tajima's *D* was not significant despite significantly negative Fu's F_S values, providing evidence of population expansion; however, both indices were not significant for GTH2b. The BSP analysis based on mtDNA data indicated increasing population sizes for *A. reinhardtii*, *A. obscura* and *A. marmorata* in the past 750,000 years, whereas *A. megastoma* showed a decline in the last 100,000 years (Figure 4).

Discussion

This study represents the most comprehensive analysis of genetic variation in sympatric freshwater eels of the South West Pacific to date. A main finding was that each species was characterised by low levels of population structure in the SWP basin, without notable genetic differentiation between geographic localities. Previous studies revealed the clear existence of distinct genetic groups separated by the Indian and Pacific Oceans in *A. marmorata* (Ishikawa et al., 2004; Minegishi et al., 2008, 2011; Watanabe et al., 2008; Gagnaire et al., 2011). Within the SWP, fast-evolving nuclear markers such as microsatellites and AFLPs revealed either a lack (*A. reinhardtii*, Shen & Tzeng, 2007a;

A. marmorata, Gagnaire et al., 2011) or the presence (*A. australis*, Shen & Tzeng, 2007b) of population structure, whereas no structure was detected with mtDNA markers for *A. australis* (Watanabe, 2001). In the present study, we used both mtDNA and nuclear sequences and provided no evidence for geographic clines in five species anywhere in the SWP. In comparison to previous findings on spatial structure based of the number of vertebrae, our findings were in accordance with Watanabe et al. (2008, 2011; for *A. obscura*, *A. marmorata* and *A. reinhardtii*), but differed from Watanabe et al. (2006, 2011; for *A. australis* and *A. megastoma*).

Analyses of eel population structure can be confounded by temporal genetic variation (previously evidenced in *A. anguilla*; Dannewitz et al., 2005; Pujolar et al., 2006). In this study, we found no temporal genetic differentiation for *A. marmorata* in New Caledonia, whereas temporal genetic heterogeneity was for example detected in the Indian Ocean by Gagnaire et al. (2009). In general, our findings corroborate that freshwater eels exhibit low levels of genetic structure, which is credited to the migration of adults from different archipelagos to shared spawning grounds. Additionally, larval mixing in ocean gyres and/or transport of larvae by the complex system of ocean currents in the SWP (Schabetsberger et al., 2016) could further reinforce genetic homogeneity. BAPS identified more than one cluster for all studied species, however largely without spatial partitioning (Figure 3), suggesting that geographic barriers for sympatric eels of the SWP are absent. The mixing of individuals can also be attributed to the prolonged spawning and recruitment of tropical anguillids, which has been suggested to last nearly the entire year, facilitating spawning groups that originate from different generations (Shen & Tzeng, 2007a; Kuroki et al., 2009; Arai & Abdul Kadir,

2017). The spawning ecology of tropical eels differs significantly from those found in temperate waters, enabling interbreeding between silver eels from different areas and cohorts (Kotake et al., 2007; Acou et al., 2008; Verreault et al., 2012).

Arai & Abdul Kadir (2017) suggested that a year-round spawning ability may help to maintain high population sizes in the Indian Ocean. However, the continuous and targeted fisheries, the unregulated removal of recruits for eel farming and the lack of population assessment may nevertheless lead to stock depletion in the area. Therefore, the consequences of overexploitation of SWP tropical eels could be worse than previously documented, as their spawning ecology and life history are still not yet well documented. Schabetsberger et al. (2015) recently proposed that *A. marmorata* and *A. megastoma* from Vanuatu perform their oceanic migration towards the border between the westward South Equatorial Current (SEC) and the eastward South Equatorial Counter Current (SECC). Both current systems are characterised by intra- and interannual variability and could potentially transport leptocephali to both directions, leading to genetic homogeneity in the SWP (Schabetsberger et al., 2016). It remains unknown where *A. obscura*, *A. reinhardtii* and *A. australis* are spawning in the western South Pacific (but see Jellyman & Bowen, 2009). The lack of pronounced spatial genetic structure demonstrated in the present study could be attributed to the mixing of fish from spawners originating from distant areas across the species' distribution (New Caledonia, Australia and New Zealand).

Using the same set of genetic markers as applied in the present study, high levels of hybridisation between *A. marmorata* and *A. megastoma* have previously been

recorded from Vanuatu, with evidence for different levels of admixture (about 15% of individuals; Schabetsberger et al., 2015). In the present study, hybrids between the same species were also detected in American Samoa (one individual, 2.56%) and in Bougainville (one individual, 3.13%). A more detailed analysis of patterns of hybridisation is currently ongoing using a large panel of SNP markers (Gubili et al. in preparation), and might explain why hybridisation was higher in Vanuatu compared to other sampling localities. Comparable levels of hybridisation between *A. rostrata* and *A. anguilla* have also been detected in Iceland, with proportions of admixed individuals ranging from 10.7% to 15.5% of examined samples attributed to difference between years and/or sampling locations (Albert et al., 2006; Pujolar et al., 2014). Moreover, the high occurrence of hybrids in Iceland could be related to intermediate duration and development time of larval phase and/or larvae transport from one branch of the North Atlantic Current to Iceland (Pujoral et al., 2014). Similarly, the increased survival of potential hybrids in Vanuatu could be linked to the geographic proximity to the hypothetical spawning area northwest of Fiji (Schabetsberger et al., 2015). Interestingly, an individual from Bougainville that was morphologically classified as *A. megastoma* was identified as *A. interioris* by mtDNA. Considering the small number of *A. interioris* caught in the wild and a morphological distinction based on the number of vertebrae (*A. interioris* typically has between 100-106 vertebrae and *A. megastoma* between 108-116; Froese & Pauly, 2011), an initial misclassification is not overly surprising.

The SWP hosts the largest number of sympatric anguillid species (Jellyman, 2003). All four species studied in the present paper showed patterns of population expansion

around 750,000 years before present. However, *A. megastoma* also appeared to decline around 100,000 years ago. Clearly, our time estimates of these demographic events critically depends on assumed mutation rates, and thus should be treated cautiously. Nonetheless, this period coincide with the glacial and interglacial cycles of the Pleistocene (1,600,000 to 10,000 years before present; Imbrie et al., 1992), which are known to have had profound effects on the genetic architecture of many other marine and freshwater species (Grant & Waples, 2000). The exact timing of population expansion events depends largely on assumed mutation rates, and most likely occurred during the Pleistocene period. Accordingly, the remarkably simultaneous range expansion of SWP anguillids might have been triggered by climate oscillations that became more pronounced after the mid-Pleistocene (< 780,000 years ago; Lowe & Walker, 2014). High levels of haplotype diversity combined with relatively low nucleotide diversity, are indicative of drastic reduction in population size followed by sudden expansion (Grant & Bowen, 1998). The *A. megastoma* population decline in the last 100,000 years could be linked to the species residing in rivers of higher altitude, compared to other freshwater eels in the study region. Hence, habitat alterations such as, erosion, deposition, and sea level changes could have negatively impacted the species' demographic history.

The assessment and management of eel stocks in the SWP are currently hampered by lack of information on migrations and population structure. The main finding of this study is that each species largely consists of a single population without pronounced geographic structure, revealing high levels of connectivity among locations. Moreover, hybridisation between long-finned eels has been documented across the SWP due to

sympatric spawning, with the occurrence of hybrids being more restricted outside Vanuatu. Finally, tropical eel populations exhibit different demographic histories that appear to have initially undergone expansion due to climatic oscillations in mid-Pleistocene. These findings have important implications for sustainable management required to ensure future stock viability, particularly as trade shifts towards tropical species to satisfy current and future demand.

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Table 1: Summary statistics for the total mtDNA control region and nuclear GTH2b partial gene sequences of six *Anguilla* species (putative hybrid individuals were excluded from this analysis). *n*: number of individuals; *h*: number of different haplotypes; *h*: haplotype diversity; π: nucleotide diversity; *s*: number of polymorphic sites. *D*: Tajima's *D*; *F*: Fu's F_S; Standard deviations are in brackets. * p <0.05; ** p < 0.01; *** p < 0.001. Two sequences per individual eel for GTH2b were included for the analysis.

Table 2: Pairwise measures of population structure. Values in lower diagonal relate to the Control Region and upper diagonal values are from GTH2b. Italic and bold Φ_{ST}

values are significant before and after sequential Bonferroni correction respectively, *p* values included in brackets. a) *Anguilla marmorata*, b) *A. megastoma* and c) *A. obscura*.

Figure 1: Sample collection locations, with numbers of individuals analysed (mitochondrial/nuclear markers). Sampling localities included Bougainville Island (BG), Solomon Islands (SI), New Caledonia (NC), Western Samoa (WS) and American Samoa (AS) and are represented with a cross (+). Sampling sites from previous studies included Papua New Guinea (PNG), Vanuatu (EA), Fiji (FJ) and Tahiti (TH) and are depicted with a dot (.).

Figure 2: Intraspecific pairwise sequence differences in the mtDNA control region between individuals of four *Anguilla* species (*A. marmorata*, *A. megastoma*, *A. obscura* and *A. reinhardtii*).

Figure 3: Genetic clustering in tropical anguillids of the South West Pacific as inferred by Bayesian Analysis of Population Structure (BAPS). a) *A. marmorata*; b) *A. megastoma*; c) *A. obscura*; d) *A. reinhardtii*.

Figure 4: Bayesian skyline plots for each species collection showing the maternal effective population size (mean and 95% confidence interval) back in time (years) since present day. a) *A. marmorata*, b) *A. megastoma*, c) *A. obscura*, and d) *A. reinhardtii*.

Supplementary Table 2: Summary statistics for the total mtDNA control region and nuclear GTH2b partial gene sequences of a) *Anguilla* marmorata, b) *A. megastoma* and c) *A. obscura* (putative hybrid individuals were excluded from this analysis). *n*: number of individuals; *h*: number of different haplotypes (2 haplotypes per individual eel for GTH2b); *h*: haplotype diversity; π: nucleotide diversity; *s*: number of polymorphic sites. *D*: Tajima's *D*; F_S: Fu's F_S; Standard deviations are in brackets. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; † includes samples from Minegishi et al. (2008).

a)	Control region							GTH2b						
A. marmorata	*n*	*H*	*h*	π	*s*	*D*	F_S	*n*	*H*	*h*	π	*s*	*D*	F_S
New Caledonia†	55	51	0.997 (±0.005)	0.070 (±0.034)	262	-1.364	-16.045**	94	5	0.181 (±0.053)	0.001 (±0.001)	4	-1.473*	-4.134**
Bougainville	24	24	1.000 (±0.012)	0.069 (±0.035)	170	-0.852	-6.325**	46	13	0.672 (±0.076)	0.008 (±0.005)	16	-1.221	-4.439*
Solomon Islands	36	34	0.997 (±0.008)	0.037 (±0.019)	160	-1.669*	-12.832**	68	6	0.222 (±0.067)	0.001 (±0.001)	5	-1.468*	-3.994**
Vanuatu	29	26	0.993 (±0.011)	0.034 (±0.017)	104	-1.069	-6.638*	48	6	0.200 (±0.077)	0.001 (±0.001)	3	-1.328	-6.044***
American Samoa	31	31	1.000 (±0.008)	0.097 (±0.048)	235	-0.663	-7.642**	72	6	0.160 (±0.059)	0.001 (±0.001)	6	-1.797**	-4.728**
Western Samoa	30	30	1.000 (±0.009)	0.098 (±0.048)	238	-0.580	-7.207**	62	2	0.064 (±0.042)	<0.001 (±<0.001)	1	-0.894	-1.070

b)	Control region							GTH2b						
A. megastoma	*n*	*H*	*h*	π	*s*	*D*	F_S	*n*	*H*	*h*	π	*s*	*D*	F_S
Bougainville	6	6	1.000 (±0.096)	0.059 (±0.035)	67	0.196	0.521	8	4	0.643 (±0.184)	0.006 (±0.005)	5	-0.503	-0.155
Solomon Islands	1	1	NA	NA	0	NA	NA	2	1	NA	NA	0	NA	NA
Vanuatu	32	22	0.976 (±0.013)	0.050 (±0.025)	105	-0.089	-0.294	56	5	0.367 (±0.075)	0.004 (±0.003)	5	-0.216	-0.042
American Samoa	2	2	1.000 (±0.500)	0.103 (±0.104)	52	0.000	3.951	4	4	1.000 (±0.177)	0.007 (±0.006)	2	1.090	-2.017*
Western Samoa	1	1	NA	NA	0	NA	NA	2	1	NA	NA	0	NA	NA

c)	Control region							GTH2b						
A. obscura	*n*	*H*	*h*	π	*s*	*D*	F_S	*n*	*H*	*h*	π	*s*	*D*	F_S
New Caledonia	1	1	NA	NA	0	NA	NA	2	1	NA	NA	0	NA	NA
Vanuatu	7	7	1.000 (±0.076)	0.030 (±0.018)	32	0.694	-0.923	14	2	0.440 (±0.112)	0.005 (±0.004)	3	1.218	3.293

Western Samoa	25	25	1.000 (±0.011)	0.079 (±0.040)	153	-0.292	-6.609**	50	9	0.627 (±0.067)	0.006 (±0.004)	8	-0.337	-2.126

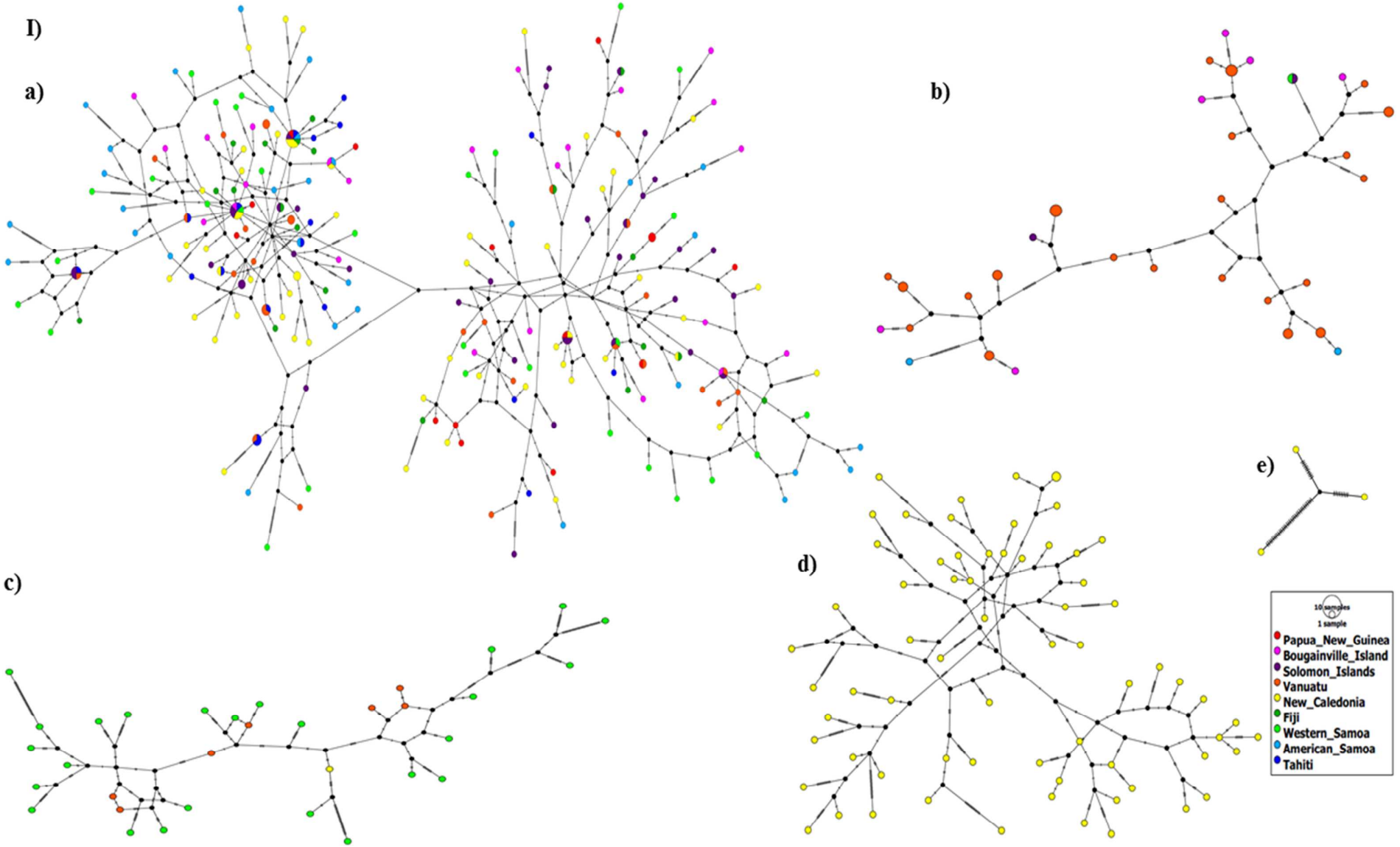
Supplementary Table 3: a) Mean and ranges of intraspecies sequence differences among mtDNA Control Region (CR) of *Anguilla* species, *n*: number of individuals; b) Interspecies variation values of the mtDNA CR between *Anguilla* species.

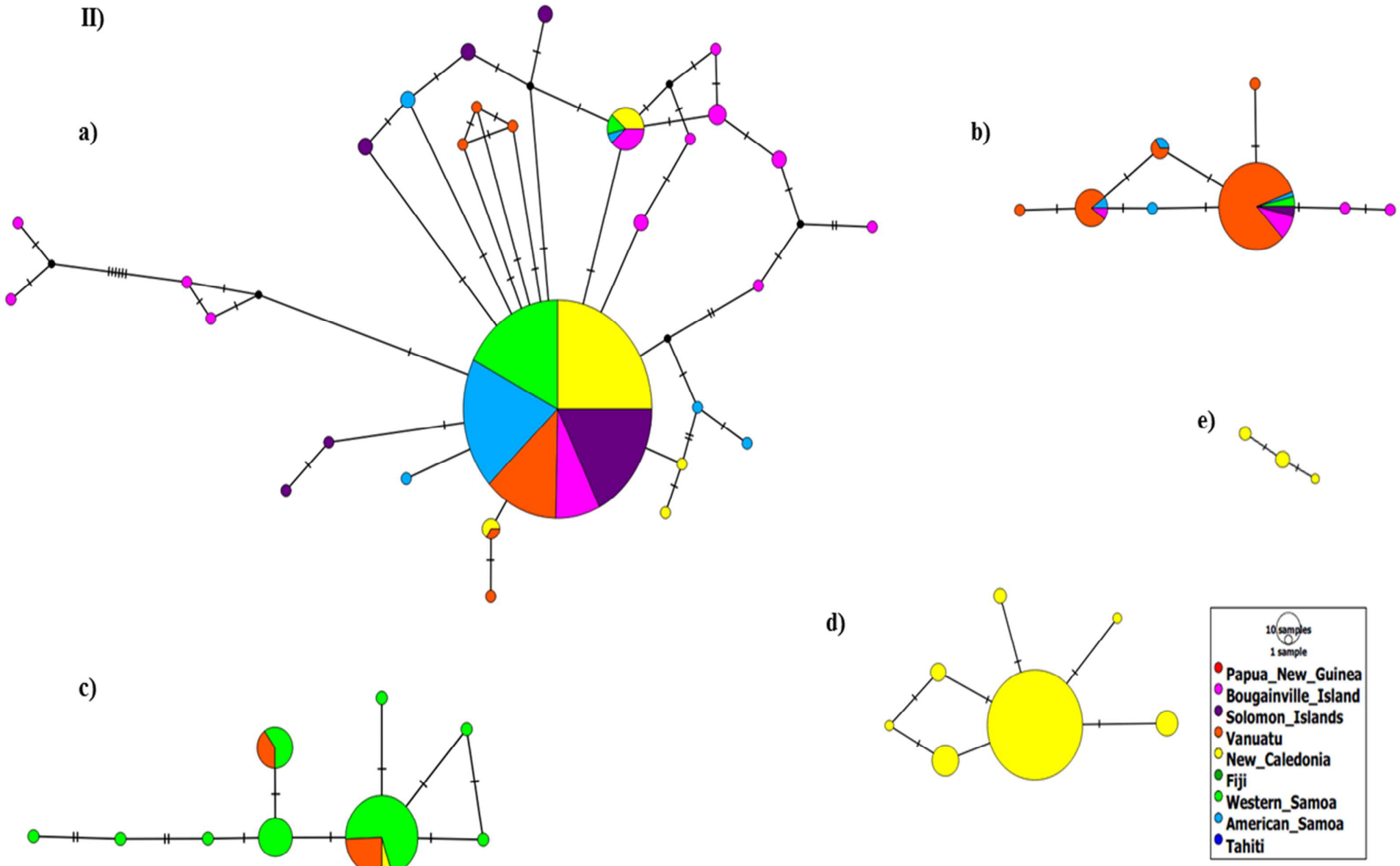
a)	**Control region**			**b)**					
Species	*n*	*Mean*	*Intraspecific*		*A. marmorata*	*A. megastoma*	*A. reinhardtii*	*A. obscura*	*A. australis*
A. marmorata	196	0.071	0.000-0.309	*A. marmorata*	-				
A. megastoma	42	0.056	0.000-0.141	*A. megastoma*	0.505	-			
A. reinhardtii	68	0.067	0.006-0.158	*A. reinhardtii*	0.410	0.327	-		
A. obscura	33	0.071	0.002-0.177	*A. obscura*	0.508	0.474	0.415	-	
A. australis	3	0.072	0.037-0.090	*A. australis*	0.513	0.338	0.316	0.476	-
A. interioris	1	NA	NA						

Supplementary Table 4: Temporal pairwise measures of population structure for *Anguilla marmorata* for the Control Region. Italic and bold F_{ST} values are significant before and after sequential Bonferroni correction respectively, *p* values included in brackets.

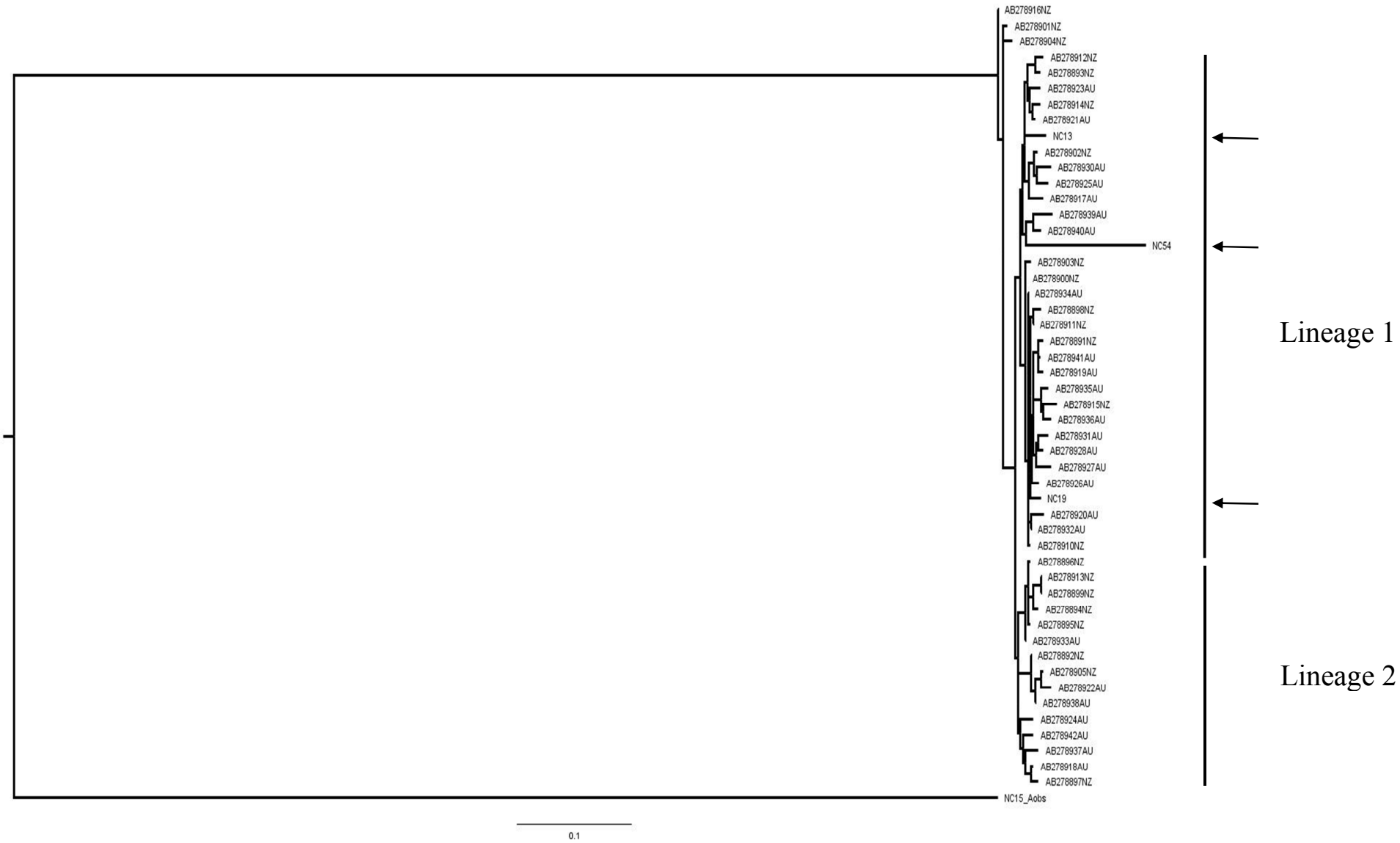
	Bougainville	**Papua New Guinea**	**New Caledonia**	**New Caledonia Minegishi**
Bougainville	-			
Papua New Guinea	**0.1202** (0.0000)	-		
New Caledonia	**0.0328** (0.0072)	**0.1016** (0.0000)	-	
New Caledonia Minegishi	**0.0930** (0.0075)	-0.0506 (0.9662)	**0.0739** (0.0103)	-

Supplementary Figure 1: Parsimony networks for the I) mtDNA control region and II) nuclear GTH2b of a) *A. marmorata*; b) *A. megastoma*; c) *A. obscura*; d) *A. reinhardtii*; e) *A. australis*.




II)
a)
b)
c)
d)
e)
10 samples
1 sample
Papua_New_Guinea
Bougainville_Island
Solomon_Islands
Vanuatu
New_Caledonia
Fiji
Western_Samoa
American_Samoa
Tahiti

Supplementary Figure 2: A Maximum-likelihood (ML) topology inferred from the control region of mtDNA of *Anguilla australis*. The tree is rooted with *Anguilla obscura*. Samples from New Caledonia are highlighted with a black arrow. AU: Australia; NZ: New Zealand; NC: New Caledonia.



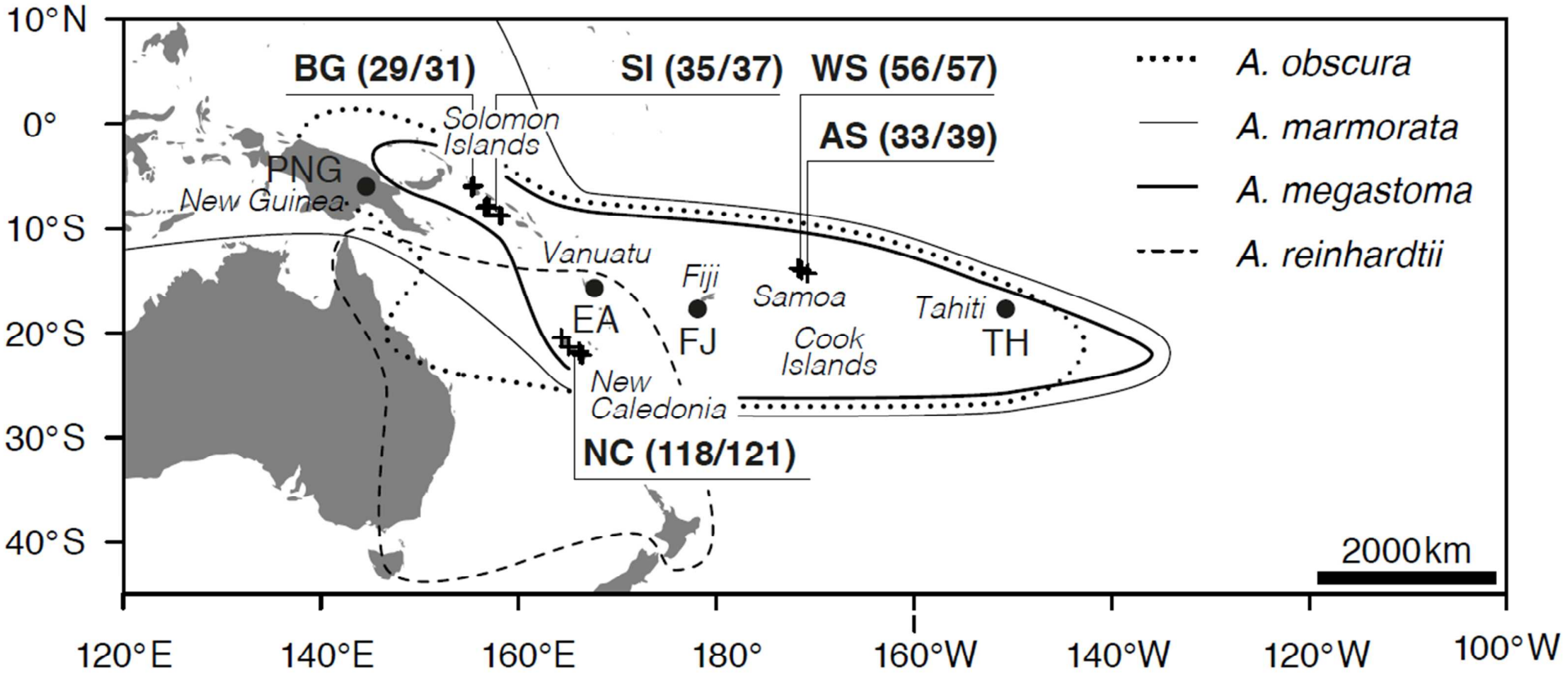
	Control region							GTH2b						
Species	*n*	*H*	*h*	*π*	*s*	*D*	*F*	*n*	*H*	*h*	*π*	*s*	*D*	*F*
A. marmorata	259	218	0.998 (±0.001)	0.065 (±0.032)	371	-1.492*	-23.631*	390	29	0.239 (±0.029)	0.002 (±0.002)	25	-2.261***	-29.456***
A. megastoma	42	32	0.986 (±0.008)	0.056 (±0.028)	149	-0.735	-3.595	72	8	0.421 (±0.068)	0.004 (±0.003)	7	-0.584	-2.081
A. reinhardtii	68	67	0.999 (±0.003)	0.065 (±0.032)	218	-1.135	-24.098***	136	7	0.292 (±0.050)	0.002 (±0.002)	6	-1.088	-3.161
A. obscura	33	33	1.000 (±0.008)	0.069 (±0.034)	157	-0.538	-12.283***	66	9	0.580 (±0.062)	0.006 (±0.004)	8	-0.313	-1.906
A. australis	3	3	1.000 (±0.272)	0.072 (±0.054)	50	0.000	2.398	6	3	0.733 (±0.155)	0.003 (±0.003)	2	-0.050	-0.427
A. interioris	1	1	NA	NA	NA	NA	NA	1	1	NA	NA	NA	NA	NA

a)	Papua New Guinea	Bougainville	Solomon Islands	Vanuatu	New Caledonia	New Caledonia Min	Fiji	Western Samoa	American Samoa	Tahiti	
Papua New Guinea	-										
Bougainville	**0.1202** (0.0001)	-									
Solomon Islands	*0.0553* (0.0126)	**0.0930** (0.0000)	-								
Vanuatu	*0.0862* (0.0065)	**0.0994** (0.0001)	-0.0026 (0.4827)	-							
New Caledonia	**0.1016** (0.0000)	*0.0328* (0.0067)	**0.0671** (0.0000)	**0.0650** (0.0000)	-						
New Caledonia Min	-0.0506 (0.9682)	*0.0930* (0.0055)	0.0440 (0.0666)	0.0613 (0.0552)	*0.0739* (0.0101)	-					
Fiji	0.0363 (0.0890)	**0.1238** (0.0001)	*0.0630* (0.0066)	*0.0512* (0.0228)	**0.0805** (0.0002)	0.0158 (0.2265)	-				
Western Samoa	**0.1527** (0.0000)	0.0107 (0.1205)	**0.1477** (0.0000)	**0.1433** (0.0000)	**0.0482** (0.0003)	**0.1223** (0.0004)	**0.1533** (0.0000)	-			
American Samoa	**0.1523** (0.0000)	*0.0354* (0.0070)	**0.1369** (0.0000)	**0.1267** (0.0000)	**0.0376** (0.0007)	**0.1141** (0.0009)	**0.1297** (0.0000)	0.0108 (0.0936)	-		
Tahiti	**0.1296** (0.0015)	**0.1560** (0.0000)	**0.1157** (0.0001)	*0.0821* (0.0040)	**0.0933** (0.0002)	**0.0911** (0.0230)	0.0180 (0.1408)	**0.1627** (0.0000)	**0.1283** (0.0000)	-	

b)	Bougainville	Solomon Islands	Vanuatu	Western Samoa	American Samoa
Bougainville	-	-0.1707 (0.9999)	0.0373 (0.2560)	-0.1707 (0.9999)	0.0895 (0.1582)
Solomon Islands	0.0125 (0.9999)	-	-0.1795 (0.9999)	0.0000 (0.9999)	0.2859 (0.3312)
Vanuatu	0.0510 (0.1195)	-0.0610 (0.9999)	-	-0.1795 (0.9999)	*0.2672* (0.0373)
Western Samoa	-0.1236 (0.9999)	1.0000 (0.9999)	0.1426 (0.9999)	-	0.2859 (0.3338)
American Samoa	0. 1706 (0.2119)	-0.2938 (0.9999)	0.2013 (0.0507)	-0.2061 (0.9999)	-

c)	Vanuatu	New Caledonia	Western Samoa
Vanuatu	-	-0.0938 (0.9999)	0.0012 (0.3400)
New Caledonia	-0.1095 (0.8712)	-	-0.1312 (0.7624)

Western Samoa	-0.3262 (0.9618)	0.0479 (0.0859)	-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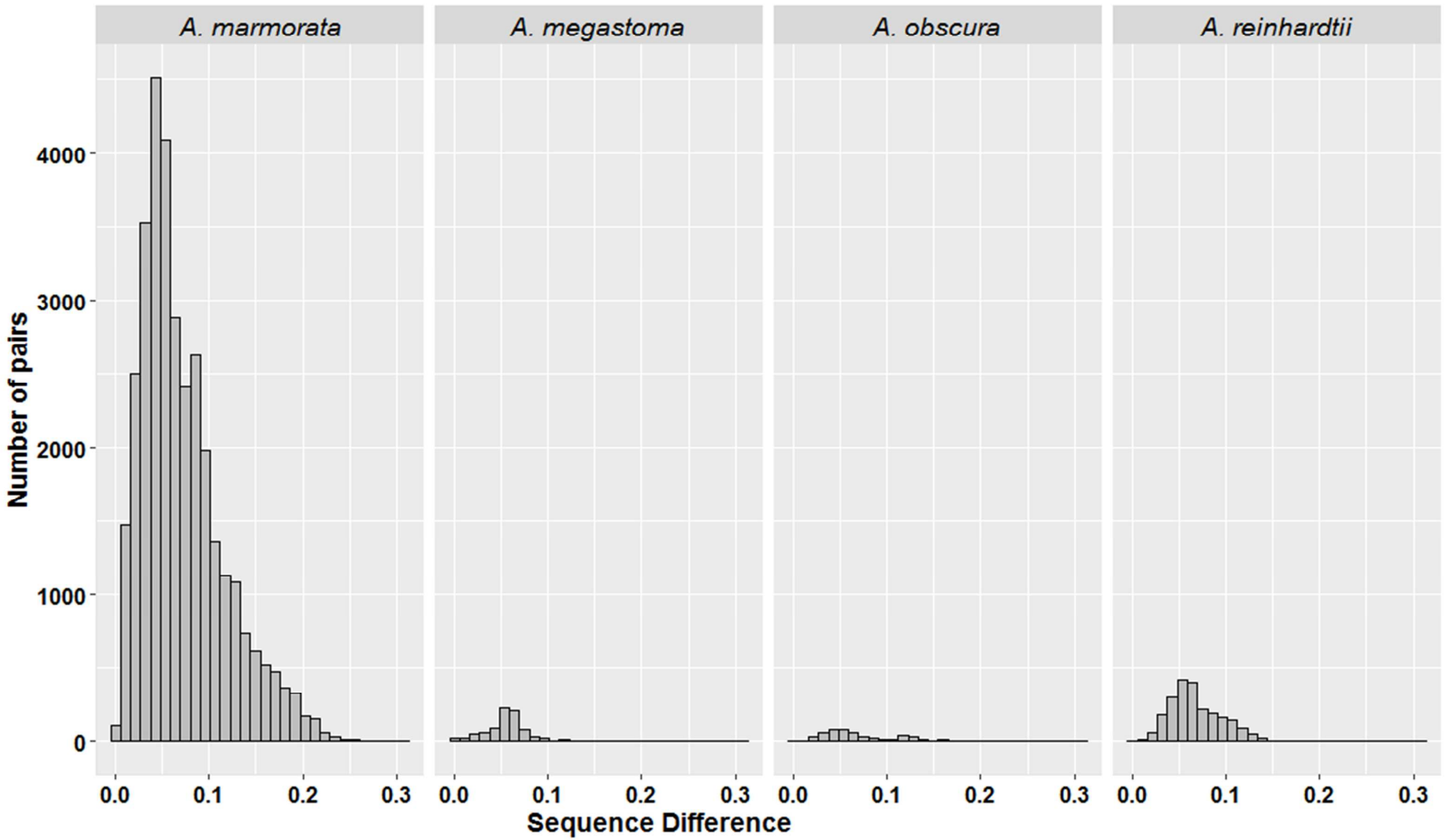
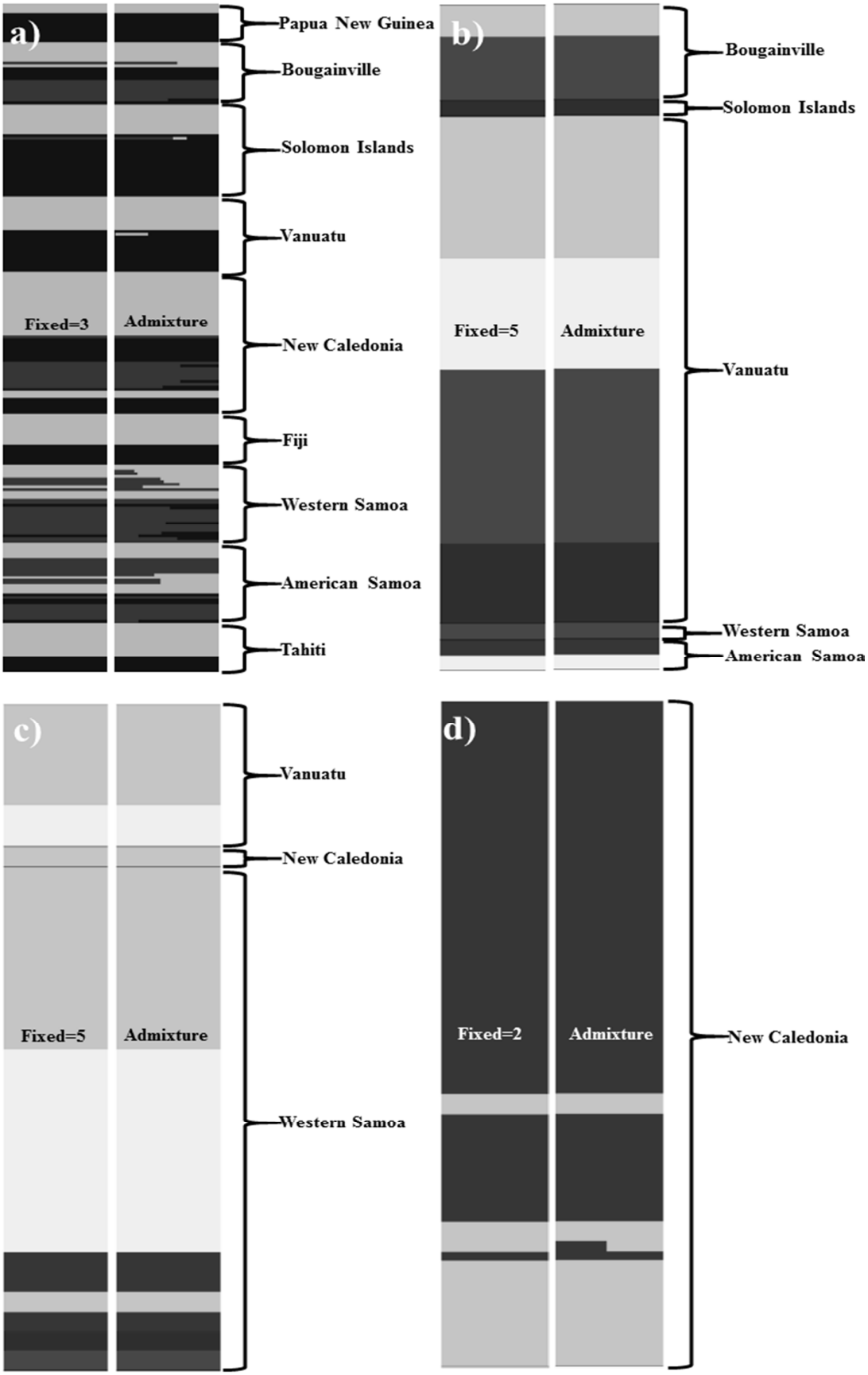
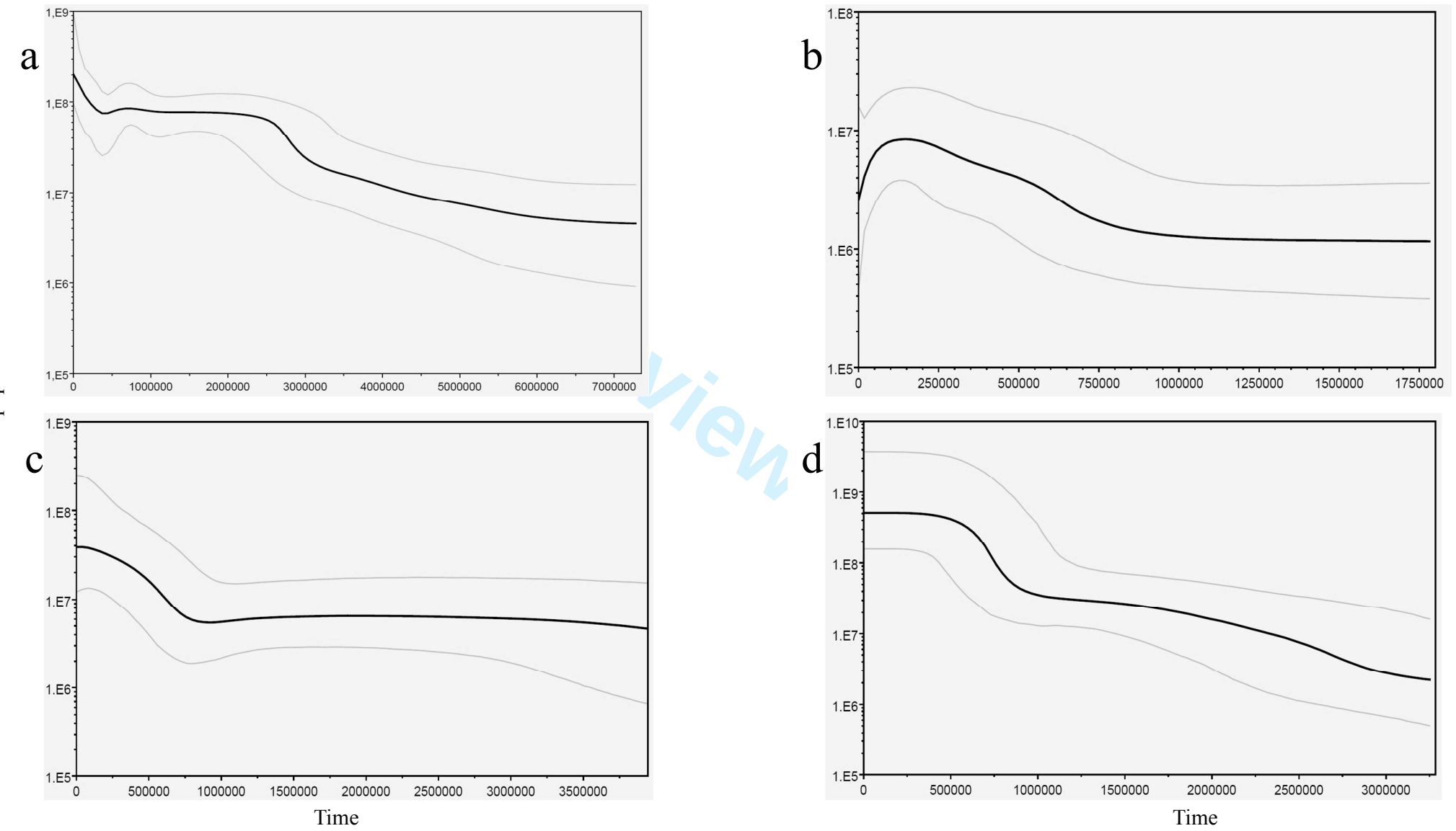
0°
10°S
20°S
30°S
40°S
120°E
140°E
160°E
180°
160°W
140°W
120°W
100°W
BG (29/31)
SI (35/37)
WS (56/57)
AS (33/39)
Solomon Islands
PNG
New Guinea
Vanuatu
Fiji
Samoa
Tahiti
EA
FJ
TH
Cook Islands
New Caledonia
NC (118/121)
2000km
A. obscura
A. marmorata
A. megastoma
A. reinhardtii


A. marmorata
A. megastoma
A. obscura
A. reinhardtii
Number of pairs
4000
3000
2000
1000
0
0.0
0.1
0.2
0.3
Sequence Difference


a)
Papua New Guinea
Bougainville
Solomon Islands
Vanuatu
Fixed=3
Admixture
New Caledonia
Fiji
Western Samoa
American Samoa
Tahiti
b)
Bougainville
Solomon Islands
Fixed=5
Admixture
Vanuatu
Western Samoa
American Samoa
c)
Vanuatu
New Caledonia
Fixed=5
Admixture
Western Samoa
d)
Fixed=2
Admixture
New Caledonia


a
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5000000
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7000000
b
1.E8
1.E7
1.E6
1.E5
0
250000
500000
750000
1000000
1250000
1500000
1750000
c
1.E9
1.E8
1.E7
1.E6
1.E5
0
500000
1000000
1500000
2000000
2500000
3000000
3500000
Time
d
1.E10
1.E9
1.E8
1.E7
1.E6
1.E5
0
500000
1000000
1500000
2000000
2500000
3000000
Time
Effective population size