CHAPTER II

Diversification of Fijian halictine bees: insights into a recent island radiation

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Abstract

Although bees form a key pollinator suite for flowering plants, very few studies have examined the evolutionary radiation of non-domesticated bees over human time-scales. This is surprising given the importance of bees for crop pollination and the effect of humans in transforming ecosystems via agriculture. In the Pacific, where the bee fauna appears depauperate, their importance as pollinators is not clear, particularly in Fiji where species diversity is even lower than neighbouring archipelagos. Here we explore the radiation of halictine bees in Fiji using phylogenetic analyses of mtDNA COI sequence data. Our analyses indicate the existence of several 'deep' clades whose divergences are close to the crown node, along with a highly derived 'broom' clade showing very high haplotype diversity, and mostly limited to low-lying agricultural regions. This derived clade is very abundant, whereas the more basal clades were relatively rare. Although nearly all haplotype diversity in Fijian *Homalictus* comprises synonymous substitutions, a small number of amino acid changes are associated with the major clades, including the hyper-diverse clade. Analyses of haplotype lineage accumulation show a steep increase in selectively neutral COI haplotypes corresponding to the emergence of this 'broom' clade. We explore three possible scenarios for this dramatic increase: (i) a key change in adaptedness to the environment, (ii) a large-scale extinction event, or (iii) a dramatic increase in suitable habitats leading to rapid population expansion. Using estimated mutation rates of mitochondrial DNA in other invertebrates, we argue that *Homalictus* first colonised the Fijian archipelago in the middle–late Pleistocene, and the rapid accumulation of haplotypes in the hyper-diverse clade occurred in the Holocene, but prior to recorded human presence in the Fijian region. Our results indicate that bees have not been important pollinators of Fijian ecosystems until very recent times. Post-Pleistocene climate change and anthropogenic effects on Fijian ecosystems are likely to have greatly transformed pollinator suites from the conditions when those ecosystems were first being assembled.

Keywords: Fiji; Halictinae; Extinction; Adaptation; Last glacial maximum; mtDNA; COI

1. Introduction

Bees are profoundly important pollinators of angiosperms in almost all terrestrial ecosystems (Michener, 2007). The rapid diversification of angiosperms in the mid-Cretaceous is believed to have driven subsequent diversification in their corresponding pollinators (Grimaldi, 1999). Bees are almost entirely dependent on angiosperms (Michener, 1979a), and their diversification can often be tied to coevolutionary relationships with flowering plants (Dötterl and Vereecken, 2010). The importance of bees as pollinators in island systems, where there are generally reduced levels of species richness but high levels of endemism (Cronk, 1997), can provide very important nsights into the evolution of ecosystems. Plants in island plant– pollinator mutualistic networks often show increased levels of pollinator specialisation, due to an increased plant:pollinator ratio that involves only a narrow range of visiting pollinator groups, some of which are often atypical (Olesen and Jordano, 2002). The result of this reduced interspecific competition between pollinators appears to encourage broadened pollination niches for endemic species, creating 'super generalists' (Olesen et al., 2002) that can act as keystone species, critical to the functioning of the ecosystem.

1.1. Pacific endemism

The islands of the Pacific in particular exhibit some of the highest levels of endemic richness for vascular plants, terrestrial vertebrates, and particularly avian taxa (Kier et al., 2009). Endemic birds have been shown to comprise between 22% and 44% of the known avian diversity in the Solomon Islands, French Polynesia, Fiji, and Hawaii (Groombridge, 1992). While insects show even higher levels of endemicity in the region (e.g. 67% in Hawaii; Miller and Eldredge, 1996), it is sometimes low (e.g. 11% in the Pitcairn Islands; Groombridge, 1992; Benton, 1995). Raised levels of endemism in the Pacific islands, but reduced pollinator diversity, means the loss of generalist pollinators could constitute a great threat to pollination networks (Memmott et al., 2004). A large proportion of threatened species occur in island systems, due to pressures from habitat degradation and loss (Brooks et al., 2002), natural resource exploitation (Rainbird, 2002), and the introduction of invasive species (Zavaleta et al., 2001; Daehler et al., 2004). Furthermore, island species may compete poorly with introduced continental taxa that have undergone extensive selection over relatively long evolutionary periods (Cox and Elmqvist, 2000), and this may facilitate further introductions (Simberloff and von Holle, 1999). Therefore, for island populations there are not only increased risks of extinction, but indirect threats to the disruption of pollinator interactions critical to their persistence. For island plant–pollinator interactions to remain functional in the face of human-mediated changes to ecosystems, there is a need to understand how pollinator groups originated in these systems, how they have radiated, and how they contribute to ecosystem functioning.

1.2. Dispersal patterns in the Pacific

The evolution of island biota is often strongly influenced by geological history, not only for volcanic oceanic islands that typically have short life spans in archipelagos of varying ages (Whittaker et al., 2008), but also for continental rafts that represent source locations. Thus, understanding how different pollinator groups disperse requires confident geological reconstruction. In regions of complex geological history, such as the volcanic hotspots of the Pacific, this is not always easily achieved. Radiation of bees into island groups has been examined in several regions of the world, but not in the south western Pacific (SWP).

The islands of the Hawaiian archipelago have a relatively recent volcanic origin via a step-wise chain that enabled the expansion and diversification of species as new islands emerged and older islands submerged (Clague and Dalrymple, 1987). Magnacca and Danforth (2006) used molecular phylogenetics to show that the Hawaiian *Hylaeus* bee fauna (family Colletidae) appears to have resulted from a single colonisation event followed by rapid diversification. However, species were not distributed in the same way as many other Hawaiian taxa, where basal species are found on older islands with apical species on younger islands. Magnacca and Danforth (2006) suggested this is the result of the coloniser first adapting to open niches on the island of origin before dispersing to other islands and separating into distinct species. But should the original colonising island now be submerged it is possible that the crown age of the *Hylaeus* clade in Hawaii could predate the oldest extant islands, affecting the time frame and perceived dispersal pattern of subsequent lineages.

It is commonly expected that isolated and species-poor islands tend to harbour a subset of the diversity of islands closer to the source population. In the Kepulauan Seribu island archipelago off the coast of west Java in Indonesia, Spengler et al. (2011) found that although bee and wasp assemblages deviated from a random pattern of species distribution, community nestedness was only weakly developed. Gillespie (2002) showed that *Tetragnatha* spiders from neighbouring islands of the

Society Islands, the Marquesas Islands, and the Hawaiian islands were almost completely unrelated, and instead represented repeated colonisation events from the source.

1.3. Geological and recent history of Fiji

Fiji comprises a group of islands that began forming during the Late Eocene with island-arc volcanics and subsequent deposition of shallow water limestones as a result of westward subduction of the Pacific plate, with the earliest emergence of Viti Levu occurring between 29 and 24 Myr (Taylor et al., 2000). By the Mid Miocene (15.97–11.6 Myr) the most significant land mass had formed but it was not until 8–6.5 Myr, after the breakup of the Vitiaz trench and attachment of Viti Levu to the Pacific plate, that Vanua Levu formed (Rodda, 1994; Neall and Trewick, 2008). The next largest volcanic islands of Kadavu and Taveuni emerged within the last 3.43–3.35 Myr (Taylor et al., 2000) and 3.0–0.7 Myr (Nunn, 1998; Monaghan et al., 2006) respectively, with the latter comprised of large deposits of magma. Volcanic origins of both primary, Viti Levu and Vanua Levu, and secondary, Taveuni and Kadavu, islands mean they share similar profiles. Large, heavily forested peaks of high rainfall create dry windward areas where drier forests or savanna persist, particularly the northwestern side of the primary islands. Given the geological age for the islands, all pre-human settlement taxa are believed to have arrived via post-Eocene dispersal (Pole, 1994). Reconstructions of Fijian vegetational history have focussed on anthropogenic impacts, but sedimentary samples from Lake Tagamaucia at c. 800 m on Taveuni provide pre-human records to 27,000 years ago revealing mist forests dominated by *Ascarina* and gymnosperms giving rise to Myrtaceae, *Balanops* and

Pandanus in the mid Holocene (Hope et al., 2009). Recent history of the Pacific saw the rapid human settlement of Melanesia and Polynesia between 3500 and 2500 years ago, followed by a period of extensive land use change via slash-and-burn processes for cultivation (Nunn, 1990). Anthropogenic burning practices have contributed to large-scale conversion of forest to grassland across the SWP islands; however, there is some evidence that suggests the presence of savanna prior to settlement (Latham, 1983; Hope et al., 2009).

1.4. Paucity of Fijian bee fauna

The bee fauna of the south western Pacific (SWP) has been reported as largely depauperate with only 71 species recorded across the four main island groups (Groom and Schwarz, 2011). New Caledonia exhibits a mix of Australian and Indo-Malayan elements, Vanuatu predominantly the latter, and the islands of Fiji and those east of Samoa contain only limited bee diversity (Michener, 1965). However, studies on these island bee faunas have been few and were based on morphology; attempts to determine how well morphological variation reflects species boundaries were limited by restricted sampling areas. The low bee diversity in Fiji suggests that either the extant bee fauna consists of only a small number of supergeneralist species, that the current records do not reflect the true diversity, or that Fijian angiosperms do not rely primarily on bee-pollinated systems. The rarity of bee-focused studies in the region and tendency of islands to harbour super-generalist pollinator species (Kaiser-Bunbury et al., 2010), such as *Xylocopa darwinii* of the Galapagos (McMullen, 1993) or *Bombus canariensis* of the Canary Islands (Olesen, 1985), suggests the former two scenarios are most likely. However, Davies et al.

(2013) show that the Megachilidae of Fiji, which represent the next most abundant group after halictines, are likely to be recent anthropogenic introductions.

1.5. *Lasioglossum* (*Homalictus*)

One bee group, subgenus *Homalictus* in the genus *Lasioglossum*, is particularly abundant throughout Fiji and the SWP. Its highest diversity is in Australia (43 recorded species, Walker, 1986, 1997), and Papua New Guinea (PNG) where 37 species have been recorded (Pauly, 1986). Westwards and northwards from PNG, *Homalictus* is spread thinly, with only a few species through the Indo-Malayan area to Sri Lanka and Vietnam (Michener, 1965). Eastwards *Homalictus* occurs in Micronesia (Krombein, 1950) and through Melanesia into western Polynesia (Perkins and Cheesman, 1928; Cheesman, 1936; Perkins and Cheesman, 1939). However studies outside of the Australian region are limited to those of Michener (1979b Fiji; 1980a PNG; 1980b Philippines) and Pauly (1980 Indonesia; 1984a Borneo; 1984b Solomon Islands; 1986 PNG). Historically, *Homalictus* has been considered a separate genus to *Lasioglossum* based on morphological characteristics (Michener, 1965), however, the studies of Danforth and Ji (2001) and Danforth et al. (2004) were able to show via molecular phylogenetics of the nuclear EF-1α gene region that the group is well nested within *Lasioglossum*. With the earliest arrival of Halictinae in Australia estimated at approximately 20 Myr (Brady et al., 2006), the timing at which *Homalictus* arose and radiated is likely to have been even later. A recent study by Gibbs et al. (2012) used a fossil-calibrated molecular phylogeny to show that *Homalictus* is likely to have a crown age greater than 15 Myr.

There are currently only four described Fijian *Homalictus* species and, based on morphology, Michener (1965) suggested that speciation after a single colonisation event was most plausible. However, species distinctness is highly problematic as noted by Michener (1979b). The Fijian *Homalictus* fauna provide a remarkable opportunity to study how bees are able to disperse and evolve into new habitats across recent time-scales. Here we explore the molecular diversity of *Homalictus* in Fiji to investigate whether it represents a case of very recent radiation and potential ‘speciation-in-action’.

2. Methods

2.1. Collecting localities

Sampling occurred between July 16th and August 20th during the dry season of 2010, and aimed at obtaining a complete or near-complete coverage of *Homalictus* in Fiji by focusing on the main (larger) islands where diversity is expected to be higher. Specimens were collected via sweep netting from inflorescences of both native and introduced plant species across an altitudinal range of 0–906 m above sea level within a 2.6° latitude/longitude square including the four main Fijian islands of Viti Levu, Vanua Levu, Taveuni, and Kadavu (outlined in Fig. 3c). The sampling regime sought to cover both habitat and geographic variability across the islands. Collections were made from a total of 73 sites, however, unique haplotypes represent 27 sites (Table 1). Strict adherence to the key of Michener (1979b) identified the majority of specimens to either *L.* (*H.*) *fijiensis* or *L.* (*H.*) *versifrons*. However, the range in both female and male morphology among specimens was very wide, and some displayed characters of multiple species descriptions. For example, whilst the form of the gonostylus and gonocoxite of male genitalia in some specimens was consistent with the description for *L.* (*H.*) *hadrander* (Michener, 1979b), the majority of characters were more consistent with descriptions of either *L.* (*H.*) *fijiensis* or *L.* (*H.*) *versifrons*. This was also found in some females where key diagnostic characters such as scutal and propodeal sculpturing would each resemble descriptions of *L.* (*H.*) *fijiensis* and *L.* (*H.*) *versifrons*. In such cases identifications were based on a majority rule of described features.

2.2. DNA sequencing

All DNA extraction (from a single leg), PCR, and mtDNA (COI) sequencing was completed by the Canadian Centre for DNA Barcoding (CCDB) at the Biodiversity Institute of Ontario using standard protocols (Ivanova et al., 2006). Bidirectional sequencing was used for the universal primer pair of LepF1/LepR2 (Hebert et al., 2004). From 332 *Homalictus* individuals we retrieved 316 sequences. Subsequent trace files were examined using Geneious Pro v5.6.4 (Drummond et al., 2012) where haplotypes consistent for the presence of at most one ambiguous pair in both forward and reverse directions were removed from analyses. Duplicate sequences were also removed for analyses, leaving 49 consensus sequences of 660 bp (base frequencies – A: 33.5%, C: 13.9%, G: 11.7%, T: 40.9%) with strong AT bias (74.4%) at the 3rd codon position, as is common in Hymenoptera (Crozier and Crozier, 1993; Dowton and Austin, 1997; Schwarz et al., 2004). Sequences were screened via BLAST database search for potential Wolbachia contamination, but are also checked as part of CCDB sequencing quality controls. All specimens are stored in the Schwarz Bee Collection at Flinders University, South Australia. Sequences for outgroup taxa were acquired from GenBank and for halictine bees, specifically *Lasioglossum*, covering both Australian and non-Australian regions.

2.3. Phylogenetic analysis

Our phylogenetic analyses were based on unique COI haplotypes, rather than species, and correspondence between the two are discussed in Sections 3 and 4. The presence of an altitudinal gradient of species diversity was suggested by Michener (1979b), where all species were recorded from two locations between 800 and 1100 m in the Central Highlands of Viti Levu but only *L.* (*H.*) *fijiensis* has been recorded outside of this area. To test for an altitude effect on species composition in our dataset, we separated collection localities from Viti Levu based on an elevation of 600 m as the lower range of Fijian cloud forest most prominent above 800m (Mueller-Dombois and Fosberg, 1998), with the remaining three islands of Vanua Levu, Taveuni, and Kadavu treated as whole entities due to lower topography and smaller sample sizes.

Phylogenetic trees were constructed using maximum parsimony in PAUP* v.4.0b (Swofford, 1999) and Bayesian inference (BI) implemented in MrBayes v.3.1.2 (Huelsenbeck and Ronquist, 2001) and in BEAST v.1.6.2 (Drummond and Rambaut, 2007).

For our BEAST analysis we used a rate-estimated strict molecular clock with a GTR + invariant + γ substitution prior, unlinked model parameters for a dataset partitioned into 1st, 2nd, and 3rd codon positions. Our analysis was run for 50 million iterations, sampling every 1000th iteration, and with a burnin of 15 million generations with default priors. This burnin was based on examination of parameter values using Tracer v.1.5 (Rambaut and Drummond, 2007). Multiple runs in BEAST resulted in highly concordant results. This analysis returned an ultrameric tree where branch lengths can be interpreted as approximate indicators of relative divergence times.

Our MrBayes analysis (Fig. S1) was used to produce a consensus phylogram where branch lengths are approximate indicators of inferred overall genetic change, rather than indicators of divergence times. We used the same three codon position partitions and GTR + invariant + γ substitution prior as for the BEAST analysis. We ran the analysis for a total of 50 million iterations sampling every 1000th tree and, followed by examination of parameter values using Tracer v.1.5, and we used a burnin of 15 million iterations.

2.4. Synonymous and non-synonymous changes in COI

We explored whether or not patterns in our phylogenetic trees were associated with non-synonymous changes in our region of COI because preliminary analyses suggested that some radiations might have been associated with amino acid substitutions. We used three approaches for this: Firstly, DNA sequences were translated into amino acids (aa), and aa substitutions were inferred using parsimony criteria in MacClade v.4.08 (Maddison and Maddison, 2003) and these were mapped onto the phylogram obtained from the BEAST analysis. Secondly, we explored whether the ratio of non-synonymous to synonymous evolutionary rates (dN/dS or ω) varied across the phylogenetic tree using HyPhy v.1.0 (Pond et al., 2005). We applied an MG94xREVxDualRV_GDD model to infer synonymous and non-synonymous changes under both a local and global model where the global model formed the null hypothesis. These two models were then compared using a maximum likelihood ratio test. Thirdly, we produced comparative trees showing synonymous and non-synonymous changes across the topology of the Fijian phylogeny to indicate aa substitutions throughout the tree.

2.5. Reconstruction of Ancestral States in Phylogenies (RASP)

To infer the likely ancestral regions for lineages in our phylogeny, we used an MCMC Bayesian approach implemented in RASP v.2.0b (Yu et al., 2011) based on our BEAST chronogram, pruned to include only Fijian representatives and a single Australian *Homalictus* outgroup. The outgroup was assigned a null distribution so that it did not influence inferred ancestral regions for the Fijian clades. A distribution matrix was compiled using specimen collection regions, as outlined in Section 2.3, as distribution breadth and dispersal possibilities between regions were unrestricted. We ran the MCMC analysis for 100,000 cycles of 10 chains, sampling every 100 iterations using an estimated state frequencies model with Gamma distributed amongst site variation, in order to determine the likely source region for each of the main clades.

2.6. Patterns of haplotype diversification

We explored temporal patterns of haplotype diversification using log lineage through time (LTT) plots based only on unique Fijian haplotypes produced with Tracer v.1.5 (Rambaut and Drummond, 2007). We produced a single LTT plot based on the maximum credibility tree from the BEAST analysis and then, to explore the effects of phylogenetic uncertainty, we overlaid a further 200 LTT plots based on the last 2000 sampled trees from the BEAST analysis, well after stationarity was reached.

To determine whether any changes in haplotype diversification rates were associated with amino acid (aa) substitutions, we used a Medusa analysis (Alfaro et al., 2009) in the Geiger module (Harmon et al., 2008) for the R statistics environment. For this analysis we collapsed all clades that were associated with aa changes to produce an abbreviated phylogeny, where each clade was defined by at least one aa substitution, along with a ‘richness’ vector, which listed the number of haplotypes contained within each clade. Medusa uses a stepwise Akaike information criterion (AIC) to determine whether changes in birth/death ratios lead to increased model fit, based on a flexible rate shift model by Rabosky et al. (2007).

2.7. Estimating N_e

In order to explore whether a large and recent change in accumulation of haplotypes (see Section 3.3.2) corresponded with an increase in effective population size, we used a Bayesian Skyline Plot (BSP) coalescence analysis in BEAST 1.6.2 (Drummond and Rambaut, 2007). We used an unpartitioned data set and a GTR + I + γ substitution model, after assessing alternative models using ModelTest (Posada and Crandall, 1998). We explored the effects of using a strict clock with default priors, an uncorrelated log-normal relaxed clock with mean (parameter ucld.mean) and standard deviation (parameter ucld.stdev) priors following a gamma distribution (mean = 0.33, offset = 0), and a random local clock with 10 groups and default priors. The rate was fixed to 1.0 mutations/site/generation over 5×10^6 iterations sampling every 1000th tree. Using a fixed rate of 1.0 allows us to explore the effect of varying mutation rates on age estimates. The subsequent log files were viewed in

Tracer v.1.5 (Rambaut and Drummond, 2007), and BSPs produced for each of the three clock models.

2.8. Age of the 'broom' clade

There are very few data to assess variation in de novo mutation rates among taxa (Montooth and Rand, 2008). Two studies have estimated single nucleotide mutation rates for the mitochondrial genome in invertebrates, one for the nematode *Caenorhabditis elegans* (Denver et al., 2000) and one for *Drosophila melanogaster* (Haag-Liautard et al., 2008), with rates of 9.7×10^{-8} and 6.2×10^{-8} per site per generation, respectively. These estimates are within a single order of magnitude of each other, and both species have similar AT bias to our *Homalictus* samples (76% and 82% respectively, compared to 74.4% in *Homalictus*). Consequently, a mutation rate in the region of 10^{-8}–10^{-9} single-nucleotide substitutions per generation might be expected for *Homalictus*. We also note that COI mutation rates for insects have also been estimated by Farrell (2001) and Brower (1994), ranging from 1.0×10^{-7} to 1.0×10^{-9}, and used by Duennes et al. (2012) to place dates on divergences in the bee genus *Bombus*. Whilst these estimates differ across two orders of magnitude, they did not adjust for varying base composition, which can have very strong effects (Montooth and Rand, 2008). Consequently, we believe that the rate of 6.2×10^{-8} per site per generation for *Drosophila* is the most appropriate estimate for our study.

We then used the rate of Haag-Liautard et al. (2008) to provide age estimates for key nodes of our chronogram with the aim of providing a likely time of origin for (i) the

Fijian *Homalictus*, and (ii) the 'broom' clade, and in doing so explore likely causes for the accelerated haplotype accumulation.

We produced maximum credibility trees corresponding to the BEAST analysis used to obtained estimates of effective population size via BSP under the fixed mutation rate of 1.0 mutations/site/ generation as above (see Section 2.7). Fixing the rate to 1.0 allows the resulting branch lengths to be interpreted in units of mean substitutions per site, which can be converted to years given set mutation rates per generation and the number of generations per year. By applying the mutation rate of Haag-Liautard et al. (2008) of 6.2×10^{-8} per site per generation, with a given number of generations per year, we were able to convert estimates for nodes given in mutations per site to calendar years. To account for potential variation to this rate in our taxa, we also calculated age estimates under rates ±50% of that estimated by Haag-Liautard et al. (2008) to explore how the resulting ages may vary.

3. Results

3.1. Phylogenetic relationships

Our Bayesian maximum credibility tree along with posterior probability values for nodes (Fig. 1) from the BEAST analysis indicated monophyly of the Fijian samples with high support (PP=1.0). Within the Fijian tree we identified five clades that have moderate (i.e. 0.75–0.90 PP) to very high (i.e. >0.99 PP) support, which also correspond to patterns in geographical distribution and scutal/propodeal sculpturing. These clades are labelled as A–E in Fig. 1 and we briefly outline their relationships and the importance of their geographical distributions and patterns of diversification will be discussed later. Clade A was recovered as sister to the remaining Fijian samples, and comprises *L.* (*H.*) *versifrons* or *L.* (*H.*) aff. *versifrons,* but its monophyly was only moderately supported (PP = 0.82). Clade B comprised two specimens, identified as *L.* (*H.*) aff. *versifrons,* whose monophyly was also only moderately supported (PP = 0.83). The remaining clades were more consistent in morphological variation, and fit the traits described for *L.* (*H.*) *fijiensis.* Monophyly of clade C was highly supported (PP = 1.00), as was the support of clade D (PP = 1.00). However, support for the clades of D and E as sister to C was not well supported (PP = 0.78). Clade E is important because its monophyly is well supported (PP = 0.98) and it encompasses a disproportionate amount of haplotype diversity in the Fijian samples, despite the relative recency of its crown age.

3.2. Synonymous and non-synonymous changes

All non-Fijian clades were pruned from the maximum credibility tree to explore both synonymous and non-synonymous changes that may have influenced the topology of Fijian haplotypes. Fig. 2 shows a comparison of the two resulting substitution trees, with the number of non-synonymous substitutions (Fig. 2a) and synonymous substitutions (Fig. 2b) indicated for each branch. It is apparent that a limited number of non-synonymous substitutions are associated with each of the major clades of Fijian representatives, including the stem leading to the ‘broom’ clade, with two amino acid changes occurring within the ‘broom’ itself. From this topology only three major clades are supported, incorporating (i) A, (ii) B + C + D, and finally the ‘broom’ of (iii) E. These clades are associated with 18 non-synonymous substitutions. In contrast, the synonymous substitutions (Fig. 2b) display a higher frequency. The topology is largely congruent with our Bayesian phylogeny with Clades A–E. Of particular interest between the two topologies of Fig. 2a and (b) are the contrasting results exhibited in the ‘broom’ clade E, where numerous synonymous substitutions (Fig. 2b) and only two non-synonymous (Fig. 2a) are present.

While Fig. 2 appears to show some amino acid changes associated with diversification in the Fijian clade, our dN/dS analyses do not indicate any changes in rates of adaptive evolution of our sequenced region of COI, and our Medusa analysis does not suggest that diversification rates have changed in response to amino acid substitutions.

3.3. Geographical patterns

3.3.1. Reconstructed ancestral states

Reconstruction of the ancestral source region (Fig. 3b) suggests that the included Fijian haplotypes originated on the main island of Viti Levu from higher elevations (>600 m), which concords with the greater morphotype diversity above 800m (Michener, 1979b). It also provides support for *L.* (*H.*) *fijiensis* being the predominant species of the four described below 600 m as noted by Michener (1979b), with only one occurrence of *L.* (*H.*) aff. *versifrons* collected from 311 m. There is also some evidence of relatively ancient exchanges with Taveuni, which has a similar landscape to Viti Levu with numerous peaks (>1000 m) of cloud forest and high rainfall. The grouping of a number of lineages from the southern island of Kadavu indicate a dispersal event, however, haplotype representatives also occur within the derived broom head clade suggesting a back-dispersal.

3.3.2. Diversification rates

To determine if diversification rate changes corresponded with key features of our chronogram, the log lineage-through-time (LTT) plot (Fig. 3a) is juxtaposed with the chronogram from the BEAST analysis (Fig. 3b). Three key nodes in our chronogram are compared with the LTT plot using grey vertical bars (labelled i–iii).

The shape of our log LTT plot is sigmoidal with a slow increase over time before plateauing after about 2/3rds of the elapsed time period in Fig. 3a. However, after this plateau there is a markedly steep increase in diversification. Nodes (i and ii) fall between the initial period of increase and plateau, however, node (iii) coincides with the commencement of a dramatic increase in diversification rate and a further period of increase arising within the ‘broom’ clade (shaded in grey, Fig. 3). This change in diversification rate is striking and may correspond to a geographical transition from higher elevations into lower coastal areas (Fig. 3b).

3.3.3. Effective population size estimation

In addition to our Medusa analysis of diversification rate changes, we used Bayesian skyline plots to examine whether the effective population size (N_e) of Fijian *Homalictus* changed over a similar time period. Fig. 4 illustrates the inferred changes in N_e plotted for an enforced strict clock rate of 1.0 mutations/site/generation and indicates a steep increase in N_e as it approaches $t = 0$, following a long period of relative stability. Plots for an enforced rate of 1.0 for a relaxed uncorrelated log normal and random local clock are provided in Figs. S3 and S4 respectively. A maximum credibility tree produced from the trees file of our BSP analysis is provided below the plot (Fig. 4b) to indicate how changes in N_e correspond to topological features. Each plot prior to the increase in N_e, with the exception of the random local clock, shows a drop that coincides with the period prior to the emergence of the ‘broom’ clade.

3.3.4. Estimating crown ages

In order to provide age estimates from the units given in our results we need to know the number of generations of *Homalictus* that are likely to occur within a year. The only studies of *Homalictus* nesting biology are those of Knerer and Schwarz (1976), and those involve a species in a temperate climate of southern Australia with no brood rearing in Autumn and Winter. That study suggests two generations per year, and this is similar to studies of the closely related sympatric subgenus *Chilalictus* (Knerer and Schwarz, 1976, 1978; Kukuk and Sage, 1994; Kukuk, 2002). Rayment (1935) suggested three generations in numerous temperate Australian *Lasioglossum* including *Homalictus*, which Michener (1960) confirms but also notes that tropical Australian species may not cease activity during the winter as in temperate areas. Wcislo et al. (1993) found almost year-round activity in the tropical *L.* (*Dialictus*) *aeneiventre*, which produced three broods per year, whilst the neotropical halictine *Megalopta genalis* has been shown to have an egg to adult period of c. 35 days which would comfortably allow for four generation per year (Wcislo et al., 2004; Wcislo and Gonzalez, 2006; S. Tierney, pers. comm.). The number of generations per year in the tropical habitat of Fiji is therefore likely to comprise three as a minimum, but more likely four generations per year with a reduced wintering period particularly in the north western rain shadow areas.

By applying the number of generations per year to our node age estimates of our maximum credibility tree given in the number of generations (Fig. 4b), we can provide a time frame for the increase seen in N_e (Fig. 4a). The crown age of the Fijian *Homalictus* clade corresponds to 5.13×10^{-2} generations. Given four generations per year in *Homalictus*, this would indicate an age of approximately 200,000 years ago. Using the same procedure, the ‘broom’ clade crown age was estimated at approximately 25,000 years ago. Interestingly, the ‘broom’ crown node corresponds to a change in the median of the skyline plot, where it begins to decrease prior to a steep increase at approximately 13,000 years ago within the ‘broom’ clade. A smaller number of generations per year would push these estimates further back in time, but it seems very unlikely that fewer than three or four generations per year would occur in Fiji given the tropical climate, the complete absence of any reports indicating that *Homalictus* is not active year-round, and the presence of at least three generations per year in southern Australia (Rayment, 1935; Michener, 1960), which has a cool temperate climate. However, based on three generations per year, the estimated crown age still remains relatively recent for the island group at approximately 275,000 years with the 'broom' clade crown at approximately 68,000years. Age estimates under rates +50% (3.1×10^{-8}) and -50% (9.2×10^{-7}) of the rate estimated by Haag-Liautard et al. (2008), produced crown age estimates of 391,421 years and 130,222 years respectively.

The above age estimates are largely congruent with results from applying the same mutation rate to both random (Fig. S3) and relaxed uncorrelated log-normal clock (Fig. S4) models, which produced crown age estimates of approximately 216,000 and 225,000 years respectively based on four generations per year. Confidence limits indicated by the purple bars of Figs. S3 and S4 show that the estimates for the root age have a high degree of uncertainty, although this decreases for nodes closer to the present.

4. Discussion

Previous studies on bee diversity and evolution in the Fijian region, and the South West Pacific more generally, have been hampered by largely taxonomic and geographically piece-meal approaches. Different studies have been related to each other on the basis of often-brief morphological descriptions, and where phylogenetic relationships have not been explicitly explored. Our study represents the first phylogenetic study of Fijian bees, and the first study to explore the likely timing of bee diversification in this region.

Although our molecular phylogenetic analyses are predicated on their use of a relatively small (660 bp) region of COI, the very high level of morphological similarity among our taxa, combined with the very small number of non-synonymous changes and maximum genetic distances of approximately 6% (Fig. S2), suggest that divergences among Fijian lineages are very recent and that adding much slower evolving nuclear genes to our analyses would be unlikely to improve phylogenetic resolution.

An important caveat in our study is that we are unable to firmly outline species boundaries. Previous studies on Fijian *Homalictus* have used minor differences in male genitalia and propodeal and scutal sculpturing, combined with differences in the metallic hues of the integument, to separate species (Perkins and Cheesman, 1928; Krombein, 1951; Michener, 1979b). Whilst our deeper haplotype clades showed more variation in these traits than our broom clade, we found that existing morphological descriptions were frequently difficult to assign specimens to any of the four described species. Ultimately, identification relied on the shape of scutal reticulation from the key of Michener (1979b) to distinguish otherwise largely ambiguous descriptions of *L.* (*H.*) *fijiensis* and *L.* (*H.*) *versifrons.* Given the recency of *Homalictus* in Fiji (see Section 4.2) it is possible that our samples cover a situation(s) of 'speciation-in-action', where species delimitation might require analyses of gene flow via tools such as microsatellites (msat). However, for very recent or even on-going speciation events, even msat analyses may not outline firm species boundaries.

O'Neill et al. (2012) have argued that recent range expansions, following colonisation of new islands, can lead to substantial genetic drift in traits that previously had adaptive significance. That possibility might help explain the wide variation in the morphology of Fijian *Homalictus*, where traits that are usually good indicators of species boundaries (e.g. dorsal surface structure of propodeum, density of scutum puncturing (Walker, 1986)) no longer fall into discrete classes. We now discuss our genetic data indicating a recent colonisation of Fiji.

4.1. Broad phylogenetic patterns of Fijian *Homalictus*

Our analyses suggest several very interesting patterns in the phylogenetic history of *Homalictus* in Fiji, namely: (i) older clades have lower haplotype diversity and are geographically and numerically less abundant; (ii) the recent clade that we refer to as the 'broom' clade is both well supported as a monophyletic group, has a very high haplotype diversity, and appears to be very recent; and (iii) there are some very clear regional patterns in extant and ancestral clades, suggesting that the history of *Homalictus* in Fiji is associated with historical changes in distribution. We now discuss these patterns.

Firstly, the ‘deeper’ Fijian clades in our analyses all have low abundance and were predominantly collected from higher altitudes on Viti Levu (Table 1 and Fig. 3). Some male representatives of these haplotypes have genetalic morphologies that are concordant with Michener’s (1979b) description of *L.* (*H.*) *hadrander*, but are otherwise consistent with the description of *L.* (*H.*) *versifrons.* While others show propodeal sculpturing and colouration not consistent with current species descriptions. Michener (1979b) remarked on the huge morphological variation in *L.* (*H.*) *fijiensis*, but not on variation in the other species. Our much more extensive samples (73 collection localities as opposed to five) suggest that problems of morphological variation extend beyond *L.* (*H.*) *fijiensis* and make species delimitation very problematic.

Secondly, our RASP analysis indicates some clear patterns in historical biogeography. The deeper clades in our tree mostly comprised specimens from higher altitudes on Viti Levu, whilst most specimens from our ‘broom’ clade were from low altitudes, and the intermediate clades largely comprised specimens from Kadavu, a smaller island adjacent to the much larger landmass of Viti Levu. These patterns could be interpreted in multiple ways. They could indicate an early colonisation of high altitude habitats, followed by dispersal to Kadavu and adaptation to lower altitudes, followed by back dispersal to low altitudes in Viti Levu. However, they could also be consistent with an early colonisation of Fiji when the climate was cooler than now, followed by retreat of early lineages to higher altitudes as the climate warmed. Viti Levu, Vanua Levu, and Taveuni were connected during periods of reduced sea level in the Pleistocene, and share similar records of pre-human forest composition, so retreating early lineages may be plausible (Hope et al., 2009). We do not have sufficient data to discriminate between these possibilities, but our results do indicate an important role of either, or both, climate and altitude, as well as dispersal to another island and then back-dispersal, in the genesis of current haplotype diversity. The latter scenario is very interesting because it raises the possibility that founder effects from colonisation of an adjacent island have led to the evolution of traits that allow a back-dispersing clade to more effectively exploit its original habitat.

Thirdly, we have a very large and recent ‘broom’ shaped clade collected from a wide geographical range, but all of these were in lower coastal areas. This ‘broom’ clade also exhibited a high diversity of haplotypes, and the LTT plots in Fig. 3 indicate a dramatic increase in haplotype diversity within this clade. The nature of this ‘broom’ is particularly intriguing, and we propose three broad hypotheses for its existence:

(i) The sudden appearance of haplotype diversity in the ‘broom’ clade might be due to a massive population expansion, perhaps due to environmental changes or a key adaptation(s), opening up a previously unavailable niche.

(ii) A massive extinction event followed by radiation of one or a few surviving lineages.

(iii) A genetic sweep resulting from a *Wolbachia*-like invasion of the species represented by the ‘broom’ clade, where the ‘sweeping’ mitochondrial genome accumulates mutations as it spreads;

Distinguishing between these alternatives is not clear-cut, and we begin by considering the last possibility. Under this *Wolbachia*-like scenario, the increase in haplotype accumulation in the ‘broom’ clade essentially represents the gradual replacement of all genomes by a single one, hitchhiking with the contagion, but which also accumulates synonymous changes as it spreads over time. However, this scenario would not explain the accelerated accumulation rate in the ‘broom’ clade, compared to other clades, since substitution rates will be a function of bee N_e rather than spread of a contagion. For a *Wolbachia*-like scenario, the bee N_e of the ‘broom’ clade would have to be unusually large to begin with, suggesting it had already undergone a massive expansion relative to the other clades.

We now consider the possibility of a massive extinction event. Crisp and Cook (2009) explored the contrasting effects of a massive extinction and a change in diversification rates on LTT plots using phylogenetic simulations. They found that the differing shape of LTT plots under these two scenarios was sufficient to discriminate between them. Specifically, a massive extinction rate resulted in a sharper change in log LTT plots, compared to an increase in diversification rate.

Interestingly, in both scenarios, the LTT plots showed an approximately linear or slowly decreasing slope after the modelled extinction/rate-shift events. This would be expected if surviving lineages radiate out into niches in the same fashion (in terms of birth/death ratios) as prior to the extinction event. In contrast, our LTT plots show an accelerating curve after the root node for the 'broom' clade, suggesting that haplotype accumulation was accelerating closer to the root.

The remaining possibility for the accelerated haplotype accumulation in the 'broom' clade involves environmental or genetic changes that opened up a new niche space allowing the population to expand and accumulate mutations as it did so. We believe that this possibility best fits our results, and it is concordant with the remarkable abundance of the 'broom' clade compared to other, deeper, clades. The pattern of N_e change shown in our BSPs lends further support to this hypothesis, where a largely stable population appears to have been followed by a period of adaptation that has then resulted in proliferation of one clade. This appears to have occurred within the 'broom' clade, which contains all of the included representatives from the lower coastal areas of Fiji. If the 'broom' clade is the result of a key adaptive or environmental change, or both, identifying such changes is important. Intriguingly, our BSP plots (Figs. 4a, S3a and S4a) suggest that N_e may have dropped before the accelerated accumulation of haplotypes in the 'broom', raising the possibility that some factor(s) were leading to a decline in *Homalictus* populations prior to an event that resulted in massive population expansion.

4.2. Timing of the ‘broom’ clade expansion

Our estimated time of origin for the ‘broom’ clade places it within a very recent time frame and potentially influenced by climatic changes. The end of the last glacial maximum (LGM), approximately 19,000 years ago, saw a steady increase in atmospheric CO_2 levels, as well as in sea levels and surface temperatures of the tropical Pacific (Clark et al., 2012). In the south western Pacific, this climate change coincided with a shift in vegetation communities from drier, colder adapted montane taxa to a higher abundance of lowland rainforests (Pickett et al., 2004) before present-day forests became established by 10,000 years ago (Hope, 1996). Human occupation from approximately 3000 years ago has significantly impacted the landscape of Fiji (Burley et al., 2012), with widespread burning practices that have converted large tracts of the lowland forests into savanna grasslands. Some studies have argued that this savanna existed pre-settlement and arose during a period of aridification some 15,000 years ago (Southern et al., 1986). Our results are concordant with a scenario where this shift in lower elevation vegetation communities and warmer climates in Fiji is related to an increase in haplotype diversity within *Homalictus* within the last 20,000 years. Furthermore, our confidence intervals based on applying a molecular clock and the exploring ranges in divergence ages in post-burnin trees and using BSP (Figs. S3 and S4) all show that the upper confidence age estimates precede human occupation. We cannot rule out that vegetation changes following human occupation also had some effects over the last 2000–3000 years, but they cannot explain the very rapid cladogenesis event in the ‘broom’ clade.

Lastly, our estimates for the crown age of *Homalictus* in Fiji depend on whether the crown age of our haplotype lineages is significantly younger than what would be obtained with more complete sampling of Fijian *Homalictus*. We think this is unlikely. Of the four described species, our samples cover characters of all described species except *L.* (*H.*) *achrostus*. That species exhibits male genitalic traits that are clearly apomorphic within the Fijian clade (Michener, 1979b), and although it likely to represent a basal lineage, it is unlikely to significantly affect the estimated crown age of the group.

5. Conclusion

The findings of our study have major implications for understanding the history of Fijian terrestrial biodiversity. They suggest that the major bee group in Fiji, *Homalictus*, has a relatively recent Pleistocene origin, and that the current abundance of these bees is an even more recent feature. This suggests that the pollination ecology of Fiji has probably changed dramatically from its earlier Miocene and Pliocene periods. We may be witnessing a major disruption to those earlier, pre-bee, plant–pollinator systems. Exploring such possible effects may be important for conserving ecosystem elements that were in place long before the rise of bees in Fiji. Our data also indicate the likely occurrence of 'speciation-in-action' following the recent colonisation of Fiji by *Homalictus* followed by some clear biogeographic and climate shifts. These events provide an excellent opportunity to examine how speciation follows colonisation of new island habitats and how this might impact on plant–pollinator dynamics.

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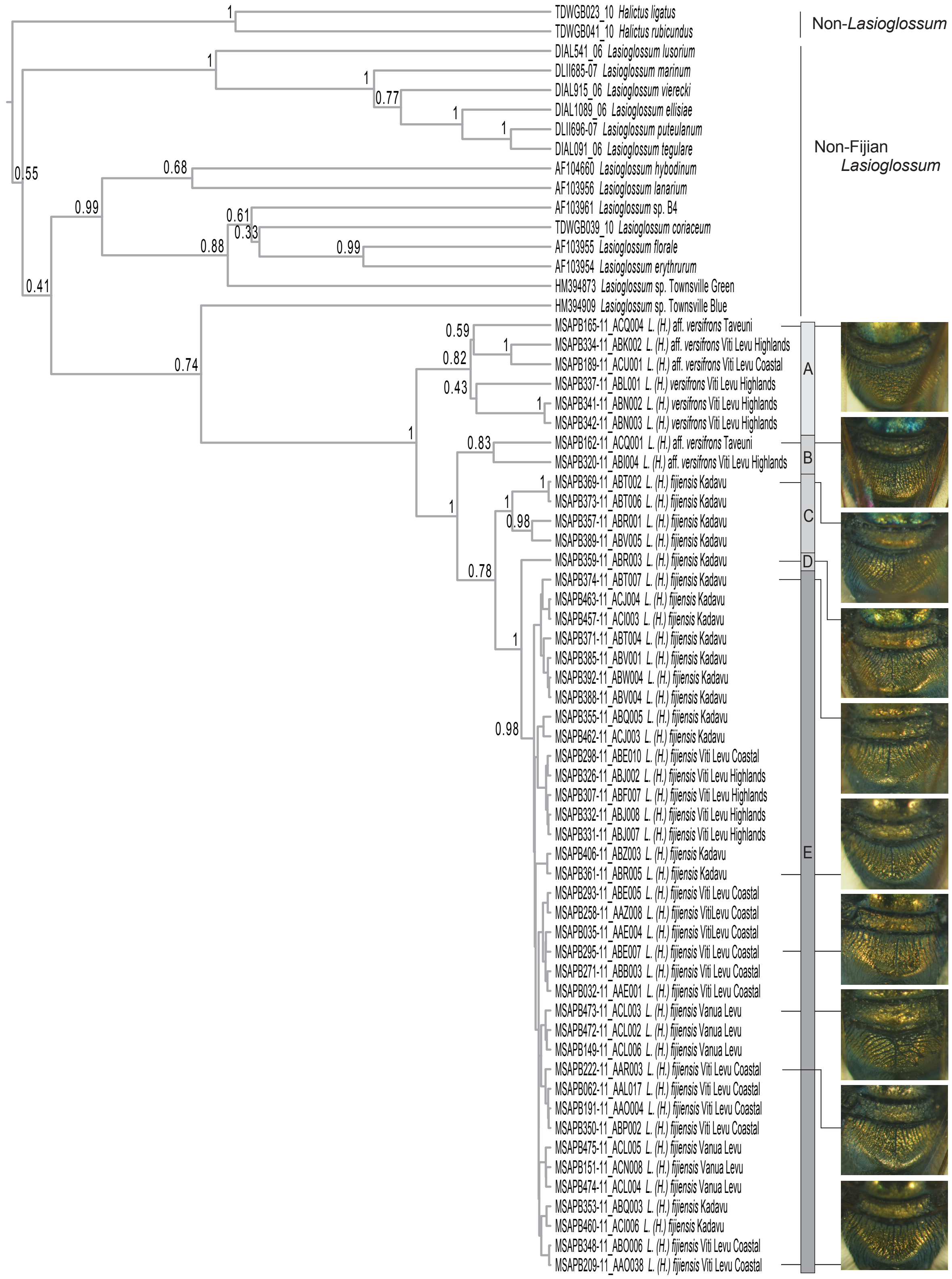
Glossary

Propodeum (adj., propodeal): Last dorsal segment of the thorax. The ventral surface of the propodeum frequently provides taxonomically diagnostic features in halictine bees.

Scutum (pl., scuta; adj., scutal): Second and largest dorsal segment of the thorax. In halictine bees this often provides diagnostic character in terms of surface sculpturing, especially coloration, punctations and striations.


1
TDWGB023_10 Halictus ligatus
TDWGB041_10 Halictus rubicundus
Non-Lasioglossum
1
DIAL541_06 Lasioglossum lusorium
1
DLII685-07 Lasioglossum marinum
0.77
DIAL915_06 Lasioglossum vierecki
1
DIAL1089_06 Lasioglossum ellisiae
1
DLII696-07 Lasioglossum puteulanum
DIAL091_06 Lasioglossum tegulare
Non-Fijian Lasioglossum
0.55
0.68
AF104660 Lasioglossum hybodinum
AF103956 Lasioglossum lanarium
0.99
0.61
AF103961 Lasioglossum sp. B4
0.33
TDWGB039_10 Lasioglossum coriaceum
0.88
0.99
AF103955 Lasioglossum florale
AF103954 Lasioglossum erythrurum
0.41
HM394873 Lasioglossum sp. Townsville Green
HM394909 Lasioglossum sp. Townsville Blue
0.59
MSAPB165-11_ACQ004 L. (H.) aff. versifrons Taveuni
1
MSAPB334-11_ABK002 L. (H.) aff. versifrons Viti Levu Highlands
0.82
MSAPB189-11_ACU001 L. (H.) aff. versifrons Viti Levu Coastal
0.74
0.43
MSAPB337-11_ABL001 L. (H.) versifrons Viti Levu Highlands
1
MSAPB341-11_ABN002 L. (H.) versifrons Viti Levu Highlands
MSAPB342-11_ABN003 L. (H.) versifrons Viti Levu Highlands
1
0.83
MSAPB162-11_ACQ001 L. (H.) aff. versifrons Taveuni
MSAPB320-11_ABI004 L. (H.) aff. versifrons Viti Levu Highlands
1
MSAPB369-11_ABT002 L. (H.) fijiensis Kadavu
1
MSAPB373-11_ABT006 L. (H.) fijiensis Kadavu
1
0.98
MSAPB357-11_ABR001 L. (H.) fijiensis Kadavu
MSAPB389-11_ABV005 L. (H.) fijiensis Kadavu
0.78
MSAPB359-11_ABR003 L. (H.) fijiensis Kadavu
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MSAPB457-11_ACI003 L. (H.) fijiensis Kadavu
1
MSAPB371-11_ABT004 L. (H.) fijiensis Kadavu
MSAPB385-11_ABV001 L. (H.) fijiensis Kadavu
MSAPB392-11_ABW004 L. (H.) fijiensis Kadavu
MSAPB388-11_ABV004 L. (H.) fijiensis Kadavu
MSAPB355-11_ABQ005 L. (H.) fijiensis Kadavu
0.98
MSAPB462-11_ACJ003 L. (H.) fijiensis Kadavu
MSAPB298-11_ABE010 L. (H.) fijiensis Viti Levu Coastal
MSAPB326-11_ABJ002 L. (H.) fijiensis Viti Levu Highlands
MSAPB307-11_ABF007 L. (H.) fijiensis Viti Levu Highlands
MSAPB332-11_ABJ008 L. (H.) fijiensis Viti Levu Highlands
MSAPB331-11_ABJ007 L. (H.) fijiensis Viti Levu Highlands
MSAPB406-11_ABZ003 L. (H.) fijiensis Kadavu
MSAPB361-11_ABR005 L. (H.) fijiensis Kadavu
MSAPB293-11_ABE005 L. (H.) fijiensis Viti Levu Coastal
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MSAPB473-11_ACL003 L. (H.) fijiensis Vanua Levu
MSAPB472-11_ACL002 L. (H.) fijiensis Vanua Levu
MSAPB149-11_ACL006 L. (H.) fijiensis Vanua Levu
MSAPB222-11_AAR003 L. (H.) fijiensis Viti Levu Coastal
MSAPB062-11_AAL017 L. (H.) fijiensis Viti Levu Coastal
MSAPB191-11_AAO004 L. (H.) fijiensis Viti Levu Coastal
MSAPB350-11_ABP002 L. (H.) fijiensis Viti Levu Coastal
MSAPB475-11_ACL005 L. (H.) fijiensis Vanua Levu
MSAPB151-11_ACN008 L. (H.) fijiensis Vanua Levu
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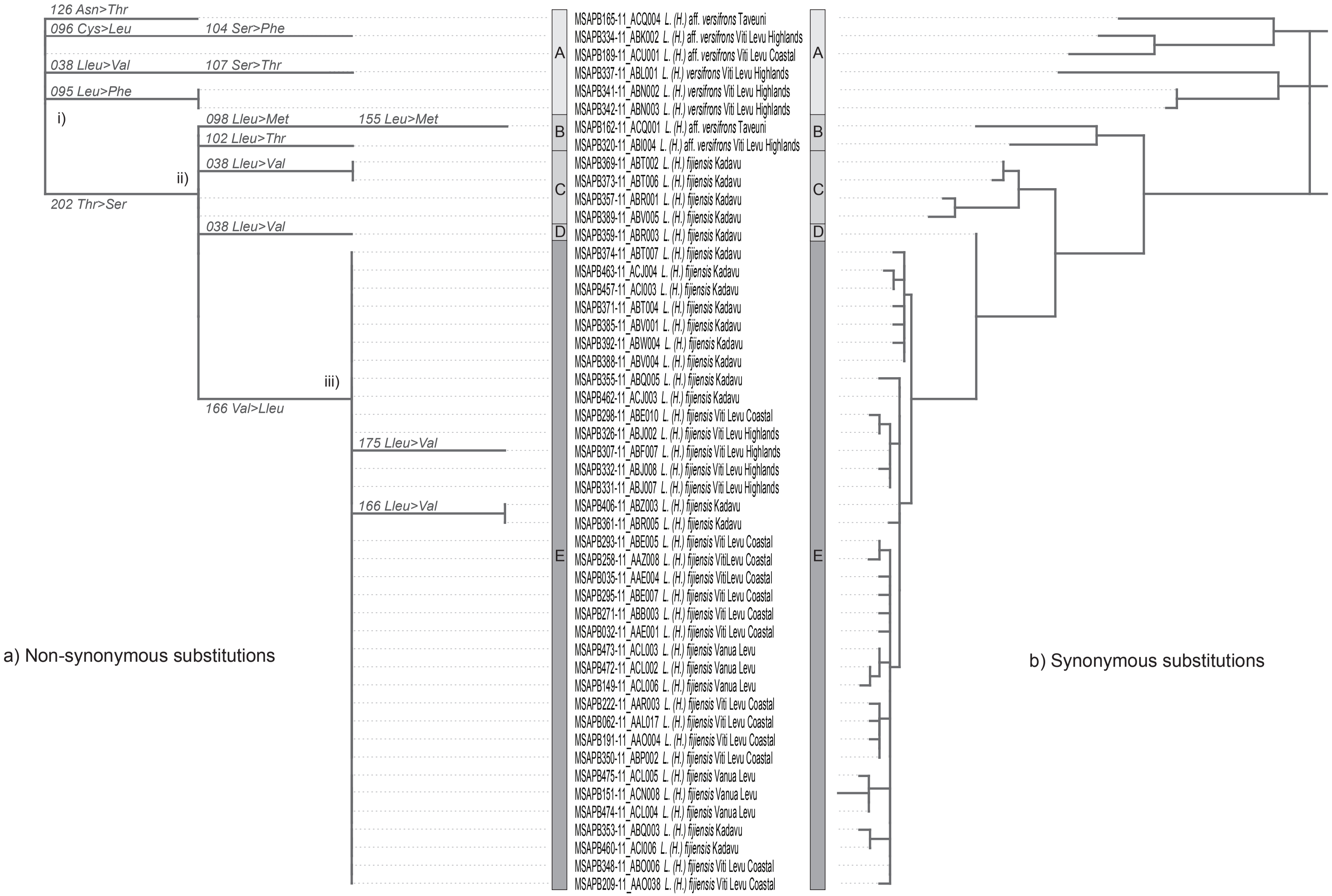
MSAPB353-11_ABQ003 L. (H.) fijiensis Kadavu
MSAPB460-11_ACI006 L. (H.) fijiensis Kadavu
MSAPB348-11_ABO006 L. (H.) fijiensis Viti Levu Coastal
MSAPB209-11_AAO038 L. (H.) fijiensis Viti Levu Coastal
A
B
C
D
E

Fig. 1. Bayesian phylogeny based on mtDNA (COI) dataset. Phylogeny estimated from a rate-estimated strict molecular clock with GTR + I + Γ substitution prior, with model parameters unlinked for codon positions. Bars labeled A-E indicate haplotype clades referred to in the text. Posterior probability values >0.40 are given above all nodes outside of the 'broom' of Clade E. Propodeal images indicate sculpture variation across the tree topology.



a) Non-synonymous substitutions

b) Synonymous substitutions

Fig. 2. Comparative trees for dN/dS changes in Fijian *Homalictus* haplotypes. Comparison of inferred non-synonymous (dN) and synonymous (dS) substitutions using HyPhy and based on a Bayesian phylogeny pruned to only Fijian representatives. DNA-converted amino acid changes inferred using a parsimony criterion corresponds to non-synonymous substitutions as indicated above branches, with key changes marked i) – iii). Bars labeled A-E indicate haplotype clades referred to in the text.

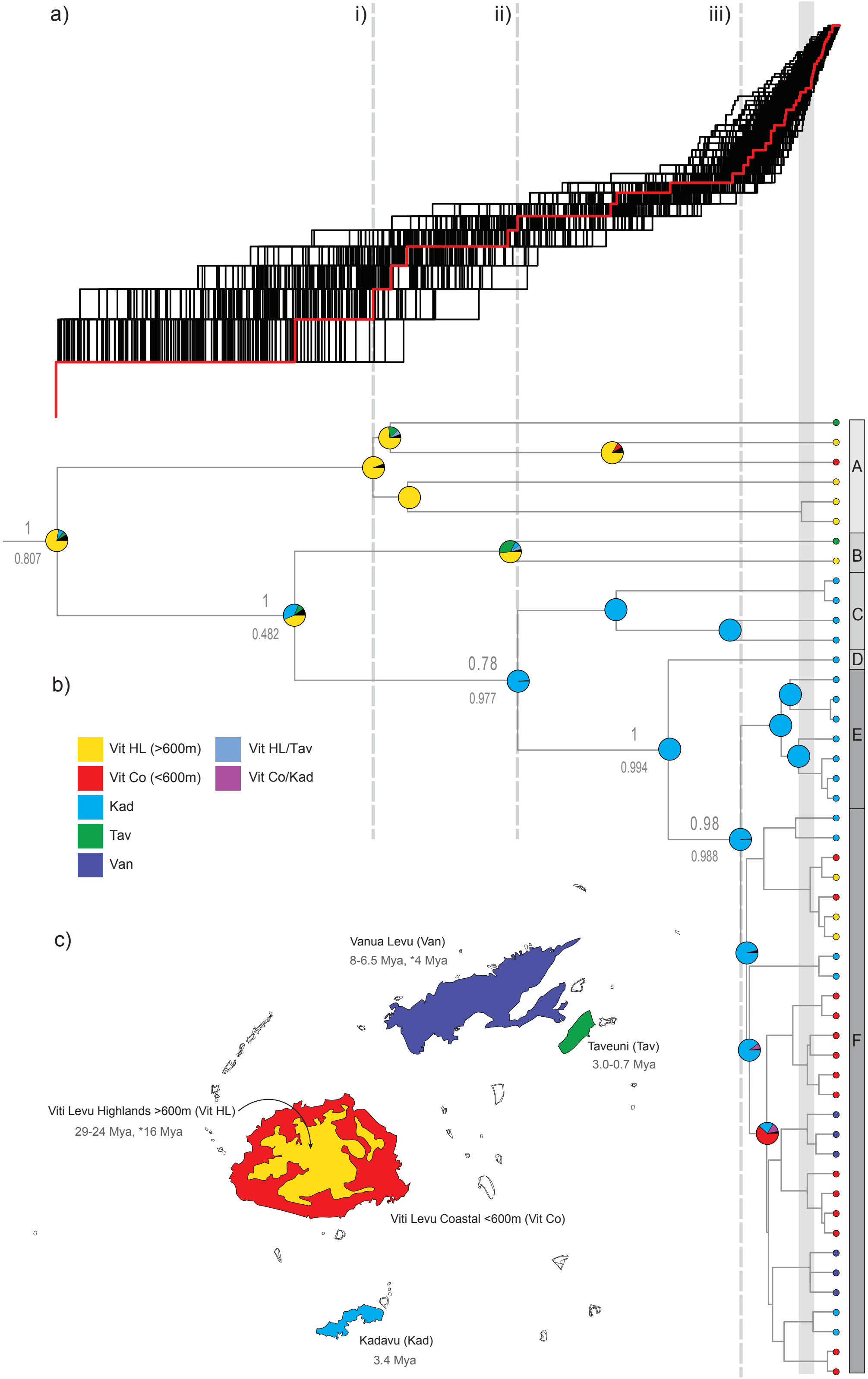

a)
i)
ii)
iii)
b)
1
0.807
1
0.482
0.78
0.977
1
0.994
0.98
0.988
A
B
C
D
E
F
Vit HL (>600m)
Vit Co (<600m)
Kad
Tav
Van
Vit HL/Tav
Vit Co/Kad
c)
Vanua Levu (Van)
8-6.5 Mya, *4 Mya
Taveuni (Tav)
3.0-0.7 Mya
Viti Levu Highlands >600m (Vit HL)
29-24 Mya, *16 Mya
Viti Levu Coastal <600m (Vit Co)
Kadavu (Kad)
3.4 Mya

Fig. 3. Lineage accumulation and inferred ancestral region of Fijian *Homalictus* topology. (a) Lineage-through-time (LTT) plots of Fijian *Homalictus* haplotypes (above) and the corresponding ultrameric tree (below) from a BEAST analysis. The LTT plot comprises 200 randomly selected post-burnin trees, in black, and the red line represents the maximum credibility (MC) tree. Vertical lines (i-iii) flag key changes in the LTT plot, while a shaded grey area marks a period of increasing diversification. Coloured circles at branch tips indicate specimen collection region, and internal circles are pie charts where slices represent the likelihoods of alternative ancestral distributions inferred from a RASP analysis. PP node support values are given above key nodes, and probability values for primary inferred ancestral distributions are given below nodes. (b) Colour codes for inferred ancestral regions are grouped into two sections: those where only single ancestral regions are permitted (left colour codes), and those where joint distributions are allowed (right colour codes). Black slices in the pie charts represent inferred ancestral distributions where probabilities were individually less than 5%. Abbreviations are as follows: Viti Levu High Lands (Vit HL), Viti Levu Coastal (Vit Co), Kadavu (Kad), Vanua Levu (Van), and Taveuni (Tav). (c) Map of key Fijian regions covered by our specimens, with age ranges provided for emergence of earliest landmasses. Asterisks indicate the minimum age of the two main islands of Viti Levu and Vanua Levu.

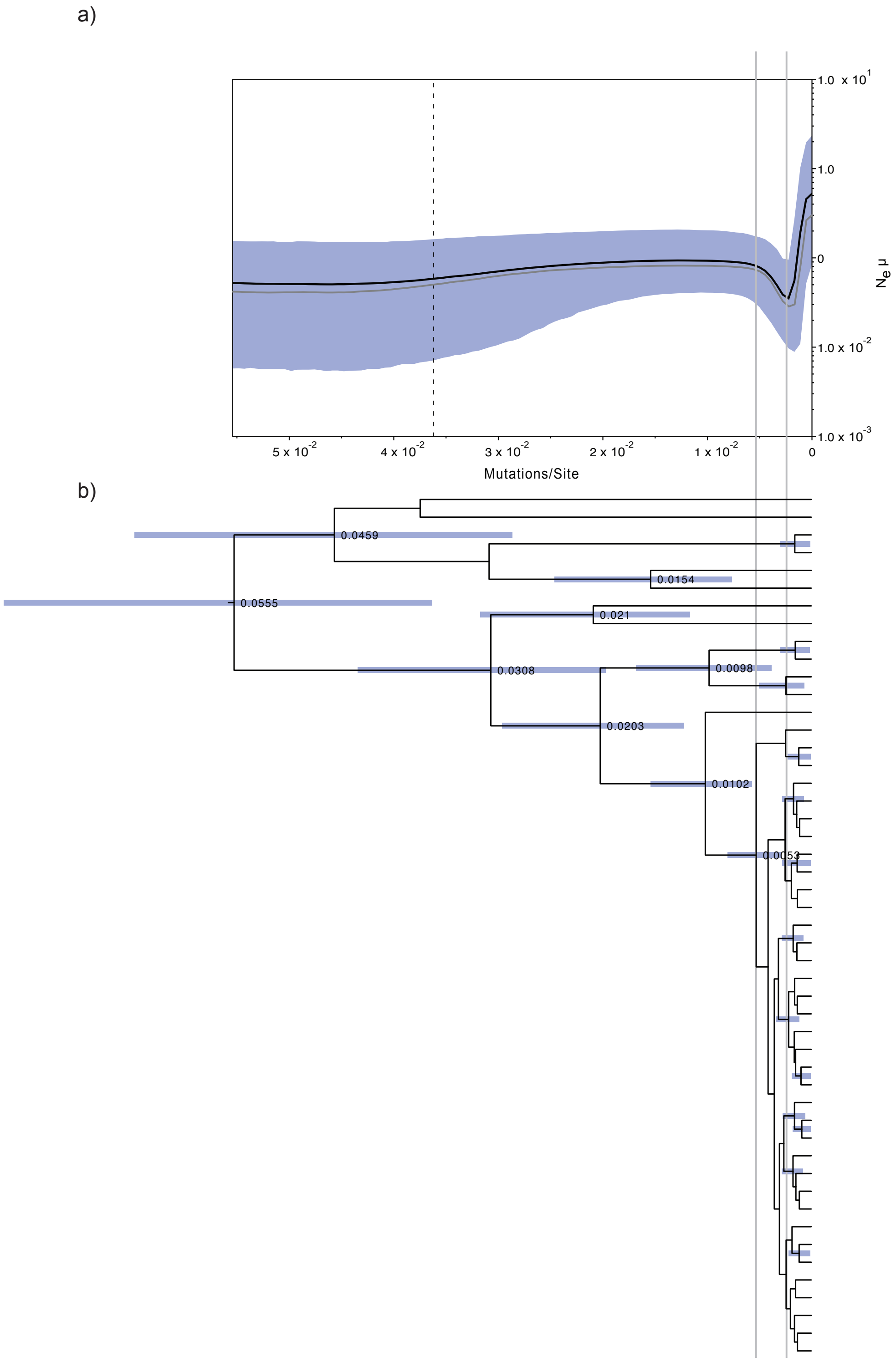
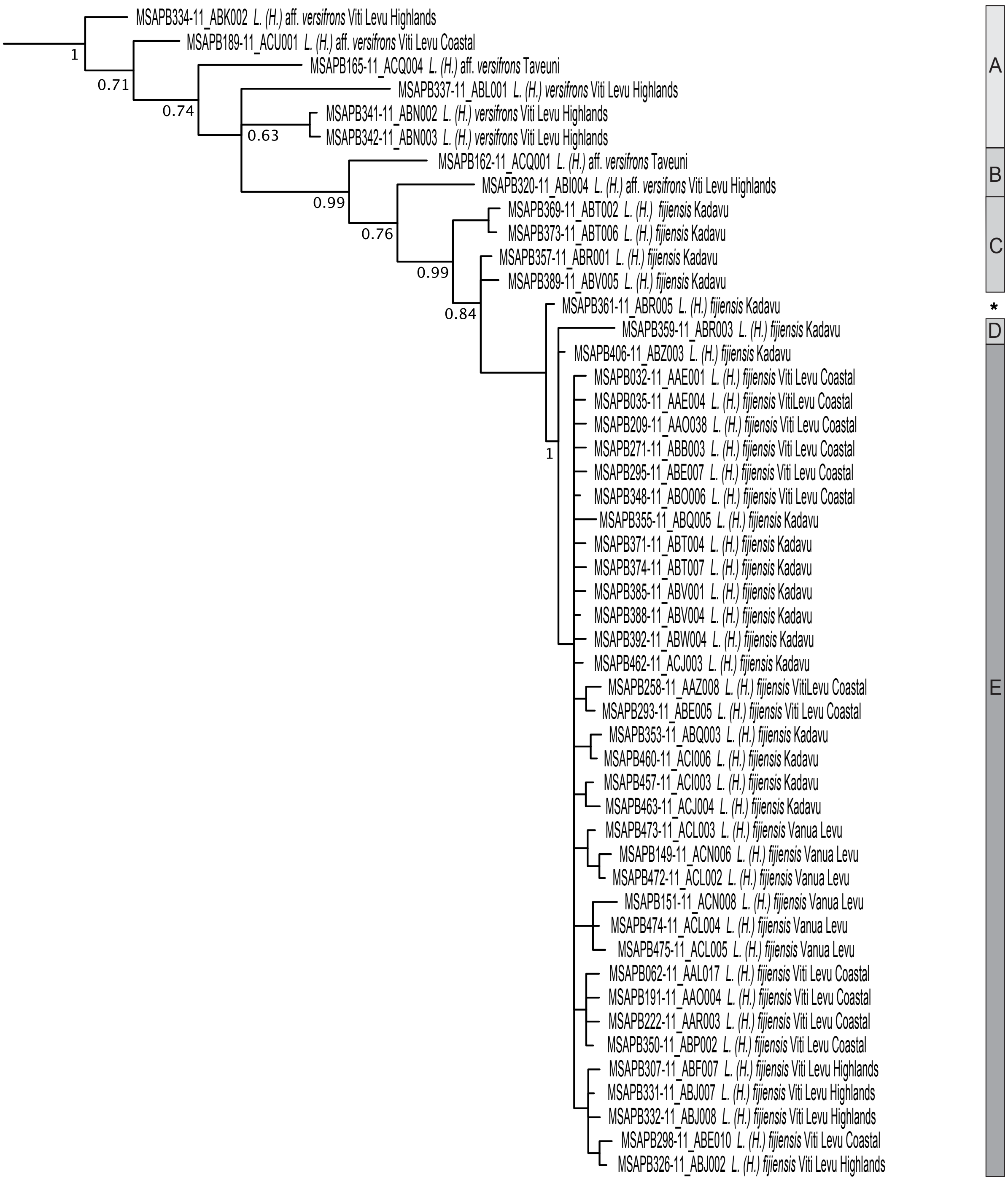

a)
1.0 x 10^{1}
1.0
0
1.0 x 10^{-2}
1.0 x 10^{-3}
N$_e$ μ
5 x 10^{-2}
4 x 10^{-2}
3 x 10^{-2}
2 x 10^{-2}
1 x 10^{-2}
0
Mutations/Site
b)
0.0459
0.0154
0.0555
0.021
0.0308
0.0098
0.0203
0.0102
0.0053

Fig. 4. Effective population size age estimates for Fijian *Homalictus* haplotypes. (a) Bayesian Skyline Plot (BSP) from a strict clock analysis implemented in BEAST. The heavy black line indicates the mean plot values, thin central blue line represents median values, and the shaded area represents upper and lower confidence intervals for mean estimates. The y-axis can be interpreted as N_e x mutation rate/site, and if mutations rates are assumed to be constant, this can be interpreted as a measure of relative N_e over time. The x-axis is in units of mutations per site. (b) Maximum credibility tree from the BSP analysis. Vertical grey bars indicate key changes in the BSP that correspond to features of the phylogenetic tree.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MSAPB189-11_ACU001 L. (H.) aff. versifrons Viti Levu Coastal
MSAPB165-11_ACQ004 L. (H.) aff. versifrons Taveuni
MSAPB337-11_ABL001 L. (H.) versifrons Viti Levu Highlands
MSAPB341-11_ABN002 L. (H.) versifrons Viti Levu Highlands
MSAPB342-11_ABN003 L. (H.) versifrons Viti Levu Highlands
MSAPB162-11_ACQ001 L. (H.) aff. versifrons Taveuni
MSAPB320-11_ABI004 L. (H.) aff. versifrons Viti Levu Highlands
MSAPB369-11_ABT002 L. (H.) fijiensis Kadavu
MSAPB373-11_ABT006 L. (H.) fijiensis Kadavu
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MSAPB361-11_ABR005 L. (H.) fijiensis Kadavu
MSAPB359-11_ABR003 L. (H.) fijiensis Kadavu
MSAPB406-11_ABZ003 L. (H.) fijiensis Kadavu
MSAPB032-11_AAE001 L. (H.) fijiensis Viti Levu Coastal
MSAPB035-11_AAE004 L. (H.) fijiensis VitiLevu Coastal
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MSAPB295-11_ABE007 L. (H.) fijiensis Viti Levu Coastal
MSAPB348-11_ABO006 L. (H.) fijiensis Viti Levu Coastal
MSAPB355-11_ABQ005 L. (H.) fijiensis Kadavu
MSAPB371-11_ABT004 L. (H.) fijiensis Kadavu
MSAPB374-11_ABT007 L. (H.) fijiensis Kadavu
MSAPB385-11_ABV001 L. (H.) fijiensis Kadavu
MSAPB388-11_ABV004 L. (H.) fijiensis Kadavu
MSAPB392-11_ABW004 L. (H.) fijiensis Kadavu
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MSAPB151-11_ACN008 L. (H.) fijiensis Vanua Levu
MSAPB474-11_ACL004 L. (H.) fijiensis Vanua Levu
MSAPB475-11_ACL005 L. (H.) fijiensis Vanua Levu
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MSAPB350-11_ABP002 L. (H.) fijiensis Viti Levu Coastal
MSAPB307-11_ABF007 L. (H.) fijiensis Viti Levu Highlands
MSAPB331-11_ABJ007 L. (H.) fijiensis Viti Levu Highlands
MSAPB332-11_ABJ008 L. (H.) fijiensis Viti Levu Highlands
MSAPB298-11_ABE010 L. (H.) fijiensis Viti Levu Coastal
MSAPB326-11_ABJ002 L. (H.) fijiensis Viti Levu Highlands
1
0.71
0.74
0.63
0.99
0.76
0.99
0.84
1
A
B
C
*
D
E

Figure S1. MrBayes consensus phylogram based on mtDNA (COI) dataset. Dataset partitioned by codon position run under a GTR + I + Γ substitution prior, sampling every 1000th iteration with a burnin of 15 000 trees and a total of 5 x10^7 iterations. Bayesian posterior probability values are given for key node, with nodes under 0.50 collapsed. Bars labeled A-E indicate clades described in Figure 1. An asterisk indicates a discrepancy between the two trees.

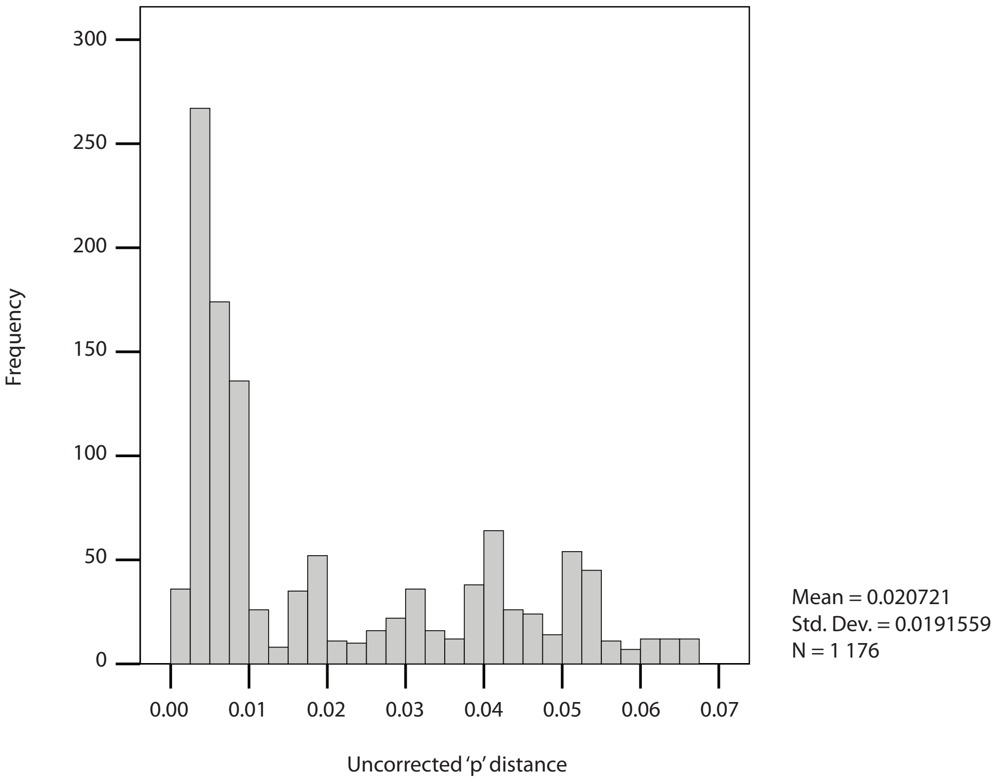

Figure S2. Genetic distances of Fijian *Homalictus* haplotypes. Histogram displaying genetic divergences (uncorrected p-distances) within Fijian *Homalictus* haplotypes calculated from 49 unique COI sequences of the analysed dataset.

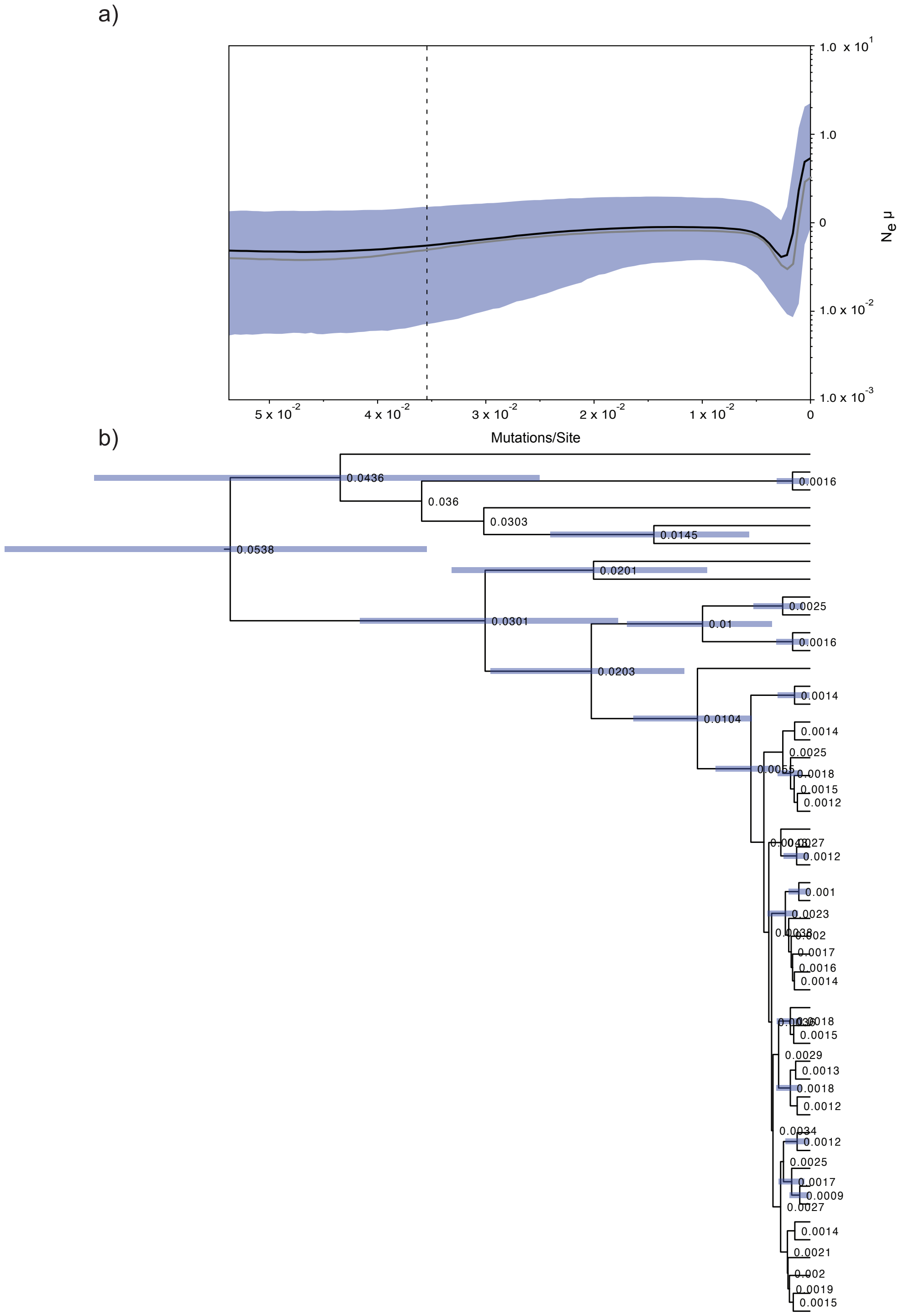
a)
1.0 x 10^1
1.0
0
1.0 x 10^-2
1.0 x 10^-3
Ne μ
5 x 10^-2
4 x 10^-2
3 x 10^-2
2 x 10^-2
1 x 10^-2
0
Mutations/Site
b)
0.0436
0.0016
0.036
0.0303
0.0145
0.0538
0.0201
0.0025
0.0301
0.01
0.0016
0.0203
0.0014
0.0104
0.0014
0.0025
0.0055
0.0018
0.0015
0.0012
0.0048
0.0027
0.0012
0.001
0.0023
0.0038
0.002
0.0017
0.0016
0.0014
0.0036
0.0018
0.0015
0.0029
0.0013
0.0018
0.0012
0.0034
0.0012
0.0025
0.0017
0.0009
0.0027
0.0014
0.0021
0.002
0.0019
0.0015

Figure S3. Enforced rate random local clock BSP reconstruction and tree. (a) Bayesian Skyline Plot (BSP) from a random local clock analysis implemented in BEAST. The heavy black line indicates the mean plot values, thin grey line represents median values, and the shaded area represents upper and lower confidence intervals for mean estimates. The y-axis can be interpreted as N_e x mutation rate/site, and if mutations rates are assumed to be constant, this can be interpreted as a measure of relative N_e over time. The x-axis is in units of mutations per site. (b) Maximum credibility tree from the BSP analysis. Node-ages estimated under an enforced strict clock rate of 1.0 mutations/site/generation. Age estimates given in mutations per site, and purple bars indicate upper and lower 95% credibility limits.

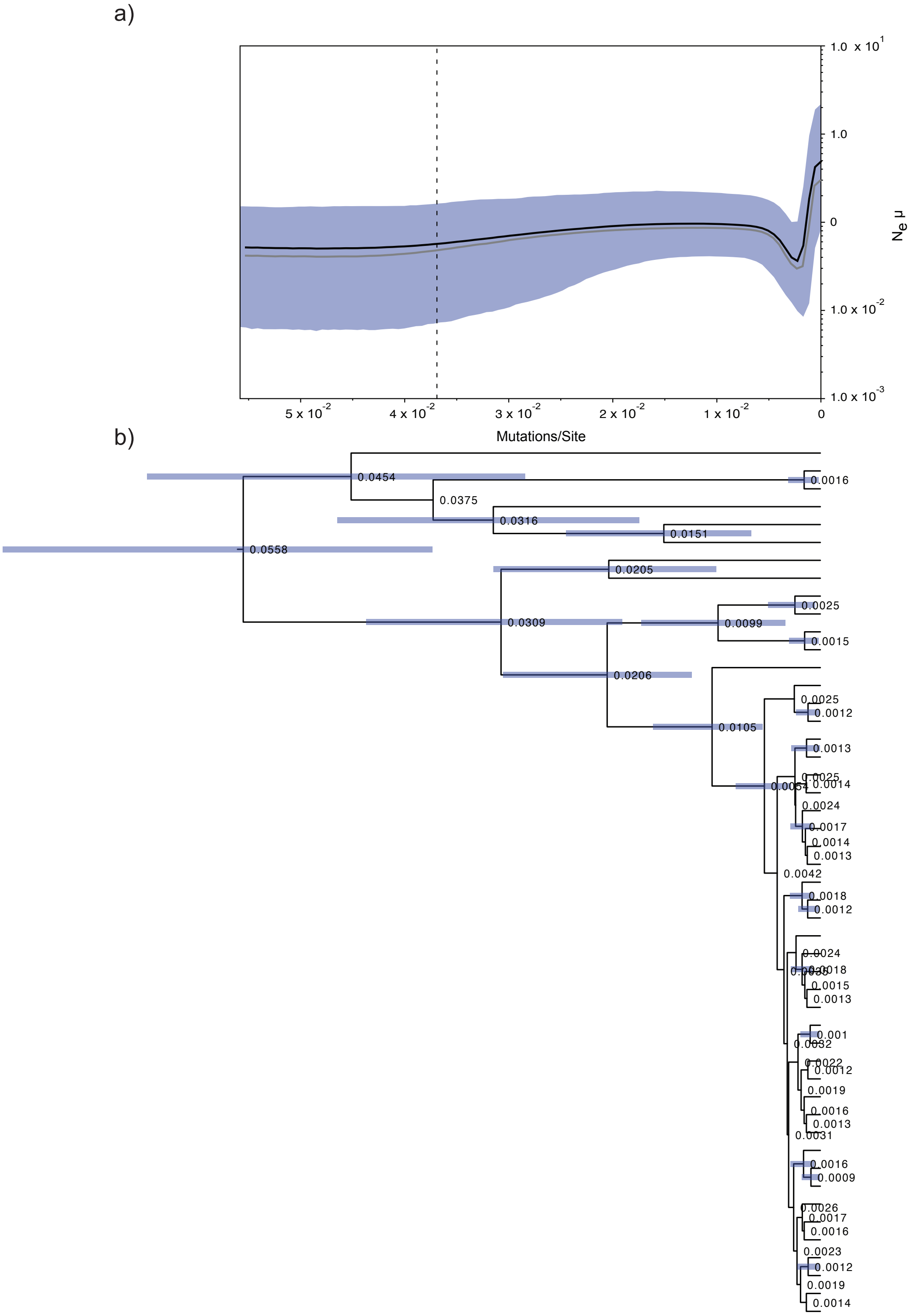
a)
1.0 x 10^1
1.0
0
1.0 x 10^-2
1.0 x 10^-3
Ne μ
5 x 10^-2
4 x 10^-2
3 x 10^-2
2 x 10^-2
1 x 10^-2
0
Mutations/Site
b)
0.0454
0.0016
0.0375
0.0316
0.0151
0.0558
0.0205
0.0025
0.0309
0.0099
0.0015
0.0206
0.0025
0.0012
0.0105
0.0013
0.0025
0.0054
0.0014
0.0024
0.0017
0.0014
0.0013
0.0042
0.0018
0.0012
0.0024
0.0035
0.0018
0.0015
0.0013
0.001
0.0032
0.0022
0.0012
0.0019
0.0016
0.0013
0.0031
0.0016
0.0009
0.0026
0.0017
0.0016
0.0023
0.0012
0.0019
0.0014

Figure S4. Enforced rate relaxed uncorrelated lognormal clock BSP reconstruction and tree. (a) Bayesian Skyline Plot (BSP) from a relaxed uncorrelated lognormal clock analysis implemented in BEAST. The heavy black line indicates the mean plot values, thin grey line represents median values, and the shaded area represents upper and lower confidence intervals for mean estimates. The y-axis can be interpreted as Ne x mutation rate/site, and if mutations rates are assumed to be constant, this can be interpreted as a measure of relative Ne over time. The x-axis is in units of mutations per site. (b) Maximum credibility tree from the BSP analysis. Node-ages estimated under an enforced strict clock rate of 1.0 mutations/site/generation. Age estimates given in mutations per site, and purple bars indicate upper and lower 95% credibility limits.