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Invasive range expansion of the small carpenter bee, *Ceratina dentipes* (Hymenoptera: Apidae) into Hawaii with implications for native endangered species displacement

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Abstract The small carpenter bee, *Ceratina* (*Neoceratina*) *dentipes*, is an invasive species throughout the South Pacific and has been identified as one of three alien *Ceratina* species established in Hawaii. Invasive bees are thought to be exacerbating declines of Hawaii's native yellow-faced bees (*Hylaeus*) but, outside of the honey bee (*Apis mellifera*), relatively little empirical work has been done to track the origins and avenues of introduction for these species. Extant research addressing *C. dentipes* is limited, but suggests that it is a rapidly moving invasive, morphologically similar to both native and non-native bees within its introduced ecosystems, and may be capable of parthenogenic reproduction. *Ceratina dentipes* thus represents a potentially serious conservational challenge for terrestrial ecosystems throughout the Pacific. Here, we perform the first comprehensive phylogeographic assessment of *C. dentipes*, drawing from invasive populations across Hawaii and the South Pacific, and from native populations in Southeast Asia. Bayesian phylogenetic reconstruction and analyses of molecular variation both indicate that *C. dentipes* is

very recently introduced in Hawaii. Analyses also reveal that invasive populations of *C. dentipes* likely originated in Borneo or mainland Southeast Asia before being widely and rapidly dispersed, most likely through anthropogenic transport.

Keywords Phylogeography · Island biogeography · Phylogeny · Pollinator ecology

Introduction

Plant-pollinator mutualisms are among the most biologically important and sensitive terrestrial systems. Because pollinators and angiosperms are highly codependent for reproductive success (Klein et al. 2007; Brittain et al. 2012; Klatt et al. 2015) they may be strongly affected by changes in ecosystem composition (Brown and Paxton 2009), such as through the introduction of new plant or animal species (Traveset and Richardson 2006, 2011; Potts et al. 2010). Alien pollinators may have particularly negative impacts on native pollination systems (Santos et al. 2012; Albrecht et al. 2016), and may cause native species losses through pathogen spread or competition over shared niches or resources (Aizen et al. 2011; Vanbergen et al. 2018).

Bees are often among the chief pollinators in many terrestrial ecosystems (Michener 2007; Brittain et al. 2012). Depending on species, bees normally demonstrate either generalist or specialist foraging behavior.

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Introduced generalists, such as *Apis mellifera*, utilize both native and invasive floral resources, often without providing efficient pollination services to either group (Santos et al. 2012; Cane and Trepedino 2017). Specialists, by comparison, rely on one or just a few species from which they may be uniquely well-adapted to gather pollen (Michener 2007). Because of this, specialist pollinators may be particularly susceptible to competitive exclusion by invasive taxa (Traveset and Richardson 2011); while relatively flexible generalists are often better equipped to tolerate or even benefit from species introductions (Drossart et al. 2017).

Limitation in physical size, resource accessibility, or biological diversity may leave an ecosystem susceptible to exploitation by invasive species (With 2002; Stachowicz and Tilman 2005; Sax et al. 2007). As such, island ecosystems are often significantly impacted by species introductions (Reaser et al. 2007; Graham et al. 2017). The Hawaiian archipelago is one of the most isolated island systems in the world, but has become burdened with a long list of invasive plants and animals (Loope and Mueller-Dombois 1989; Snelling 2003; Plentovich et al. 2009), including at least 19 species of introduced bees (Snelling 2003; Magnacca et al. 2013). Alien insects now dominate plant-pollinator networks in some Hawaiian ecosystems (Shay et al. 2016) and may be exacerbating native species loss (Pimentel et al. 2004; Sax and Gaines 2008), including at least seven species of endemic yellow-faced bees (*Hylaeus*; Magnacca 2007a, b) recently listed as endangered (US Fish and Wildlife Service 2016). Among Hawaii's introduced bee taxa are three species of small carpenter bee, genus *Ceratina*, which have collectively come to occupy the entirety of the archipelago. Some foundational work has been done to initiate management efforts of the small green carpenter bee [*C. (Pithitis) smaragdula*; Ali et al. 2016; Shell and Rehan 2017], but *C. (Neoceratina) dentipes* and *C. (Ceratinula) arizonensis* remain largely unaddressed outside of biodiversity surveys (Daly and Magnacca 2003; Snelling 2003; Magnacca and King 2013).

Ceratina dentipes has a broad native distribution which stretches across Southeast Asia (SE Asia; Friese 1914; van der Vecht 1952; Tadauchi and Tasen 2009; Groom et al. 2017). This wide range includes many large oceanic expanses which could indicate some natural capacity for long distance dispersal (LDD) via island

hopping (Groom et al. 2014a; Rehan and Schwarz 2015). However, *C. dentipes* has recently been collected as far west as the island of Mauritius, and south and east throughout the South Pacific into the Cook Islands and French Polynesia (Kuhlmann 2006; Rehan et al. 2010; Groom et al. 2014a), suggesting it may be a model “tramp” species, particularly well-suited to anthropogenic dispersal (Groom et al. 2017). Like all small carpenter bees, *C. dentipes* forms its linear nesting burrow by digging into the soft pith of woody stemmed plants (Okazaki 1992), and may occasionally form multifemale nests (Rehan et al. 2009). *Ceratina dentipes* appears to be a generalist forager as it visits many different floral species across its range (Krombein 1951; Soh and Ngiam 2013; da Silva et al. 2015). Individuals have also been observed foraging alongside *Hylaeus* in Hawaii (Snelling 2003), suggesting there may be niche overlap between *C. dentipes* and some *Hylaeus*. Conspicuously, only female specimens have been collected in Hawaii to date, suggesting that invasive populations of *C. dentipes* may be capable of parthenogenic reproduction (Snelling 2003; Daly and Magnacca 2003; Magnacca and King 2013). Further, *C. dentipes* is highly similar in appearance to other small black bee species across its native range (Krombein 1951; Hirashima 1971; Shiokawa 2009) and may be easily mistaken for both *C. arizonensis* and some *Hylaeus* in Hawaii (Daly and Magnacca 2003). Though reliable keys exist (Snelling 2003), this morphological monotony has made previous identification of *C. dentipes* (sensu stricto) challenging (Krombein 1951; Cronin 2004; Shiokawa 2009) and could confound both research and conservation efforts which seek to apply methods based on identifications in the field. As *C. dentipes* thus presents a potentially considerable conservation challenge, we undertake this study to (1) determine the biological origins of *C. dentipes* on Hawaii and (2) assess the population structure, phylogeography, and invasive history of *C. dentipes* across its current distribution.

Methods

Sample collection, DNA extraction and sequencing

Following Shiokawa (2009), we re-identified to species *Ceratina (Neoceratina)* samples originally

determined as *C. dentipes* collected in Borneo (BO), Hawaii (HA), Samoa (SA), Thailand (TH), Vietnam (VI), the Philippines (PH), and the Solomon Islands (SO) (herein collectively referred to as the *C. dentipes* species group), and selected two *Ceratina arizonensis* individuals collected in California for use as an outgroup (Table S1). *Ceratina arizonensis* occur alongside and bear great resemblance to *C. dentipes* in Hawaii but are expected to be highly genetically distinct from this group. As such, their use should simultaneously root individuals belonging to our *C. dentipes* species group while standing as a useful molecular distinction of invasive species identities in Hawaii. Where possible, we determined the sex of each sampled individual by counting tergal segments along the abdomen (6 segments = female; 7 segments = male). We then removed three legs from each individual and extracted DNA from these tissues by phenol–chloroform isolation. Voucher specimens are currently deposited at the University of New Hampshire Insect Collections. Genetic material was then amplified at the COI region following the methods of Hebert et al. (2003a, b), employing the Lep1 (F + R) primer pair (Lep1F, 5'-ATTCAACCAATCATAAAGATATTGG-3'; Lep1R, 5'-TAAACTTCTGGATGTCCAAAAAATCA-3'). PCR reactions were assembled as follows: 7.2 µl double distilled H₂O, 2.0 µl 10 × buffer, 2.0 µl MgCl₂ [25 mM], 1.0 µl Lep1-F [10 mM], 1.0 µl Lep1-R [10 mM], 0.4 µl dNTPs [2 mM], 0.4 µl Taq [5U/µl], 6.0 µl DNA [6 ng/µl] for a 20-µl total reaction volume. Reactions were performed in an Eppendorf Mastercycler gradient thermocycler following cycling settings from Shell and Rehan (2016): 94 °C for 1 min; followed by six cycles of 94 °C for 1 min, 45 °C for 90 s and 72 °C for 75 s; followed by 36 cycles of 94 °C for 1 min, 51 °C for 90 s and 72 °C for 75 s; followed by a final extension period of 72 °C for 5 min. Amplification success was confirmed via electrophoresis (3 µl sample load, 1% agarose gel, 87V run, 45 min GelRed bath stain). Remaining reaction volumes were then loaded into 96-well plates (twin.tec, semi-skirted, blue, cat. No. 951020362), and submitted to Eurofins Genomics in Louisville, Kentucky, for PCR cleanup and Sanger sequencing.

Sequence quality and alignment

Ninety-five COI sequences were visually inspected and manually edited for base call accuracy using BioEdit software (Hall 1999). This new barcode dataset (GenBank accession numbers MG815683–MG815777) was then combined with 32 published *C. dentipes* sequences from individuals collected in Vietnam (VI), Samoa (SA), Fiji (FJ), and Vanuatu (VA) (Groom et al. 2014a). This set of ingroup sequences was then aligned via ClustalW using default settings (Thompson et al. 1994) and trimmed to a consensus region of 593 unambiguous, gap-free base pairs. We processed the two new *C. arizonensis* sequences (MG815778 and MG815779), as well as one published *Ceratina bispinosa* sequence in identical fashion (Rehan et al. 2010), and confirmed that this consensus dataset was free of *Wolbachia* contamination via BLASTn database search. We then used these two outgroups to root the *C. dentipes* species group during molecular phylogenetic analyses. In total, 127 *C. dentipes* species group sequences, including 39 newly sequenced *C. dentipes* individuals from Maui, Hawaii, and 56 individuals from across SE Asia and the South Pacific were compiled for analyses (Fig. 1; Tables S1, S2).

Molecular phylogenetic analysis

Bayesian phylogenetic analyses of the *C. dentipes* species group were implemented in BEAST v.1.8.4 (Drummond et al. 2012). The most suitable substitution model was determined as general time reversible with invariant sites (GTR + I; Tavaré 1986) using the Bayesian Information Criterion (BIC) in jModelTest v.2.1.7 (Darriba et al. 2012). We applied this model set in BEAST under a lognormal relaxed molecular clock to allow for variable rates among multiple species lineages. A Markov Chain Monte Carlo (MCMC) was run for one billion iterations, sampling every 100,000th under a coalescent Bayesian Skyline tree model (Drummond et al. 2005). This analysis was run in triplicate to ensure estimation reliability and resulting log files were inspected in Tracer v.1.6 (Rambaut et al. 2014) to assess suitability of estimated sample size (ESS > 200). TreeAnnotator was then used to select a maximum clade credibility (MCC) tree from a bank of ten thousand logged clade simulations

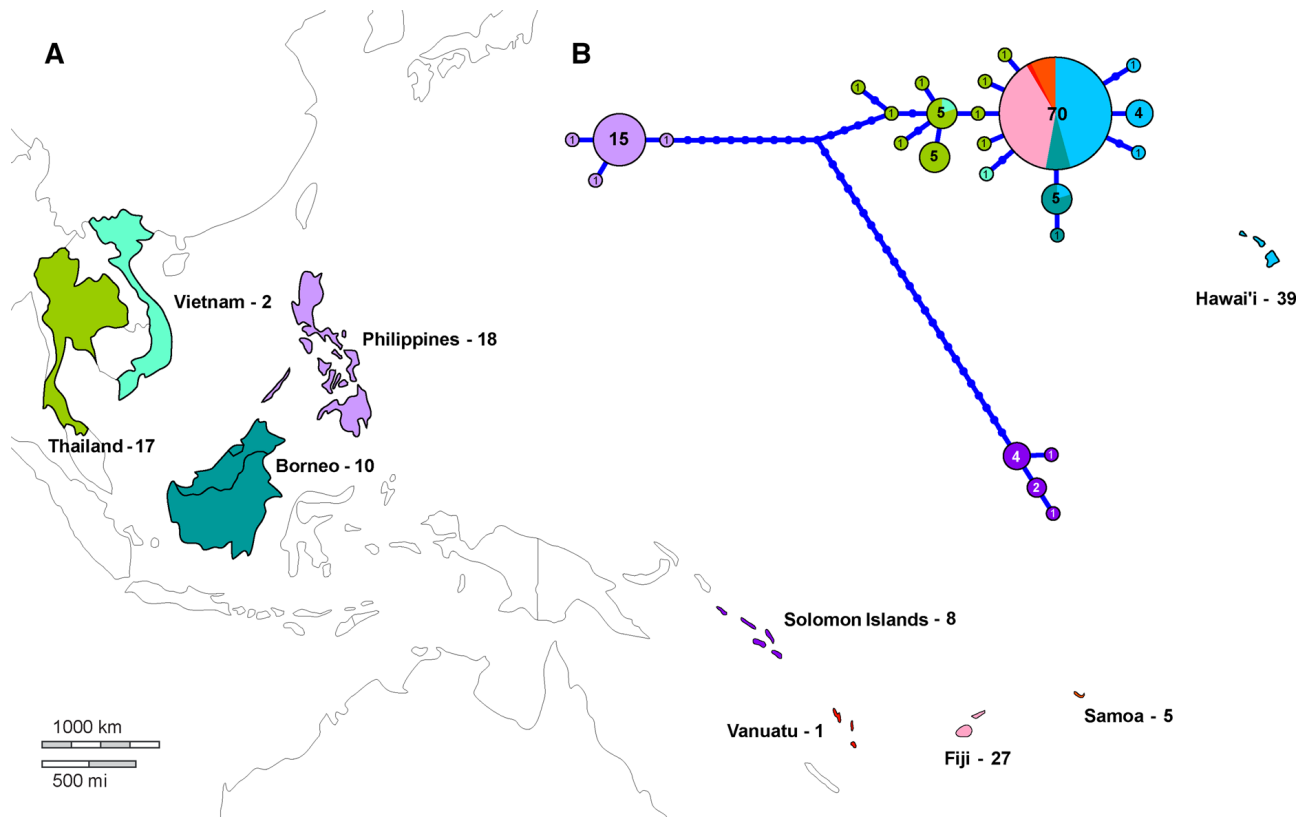


Fig. 1 **a** Geographic range distribution of the *Ceratina dentipes* species group sampled for molecular analyses, along with the total number of individuals sampled from each country. Scale bars indicate approximate distance. **b** Minimum spanning tree (MST) displaying haplotype variation among regional populations. Each circle represents a unique haplotype; the number at

the center of each circle represents the total number of individuals which share that haplotype. Haplotypes color coded to match mapped geographic regions as follows: Thailand—light green; Vietnam—light teal; Borneo—teal; Philippines—light purple; Solomon Islands—purple; Vanuatu—red; Fiji—pink; Samoa—orange; and Hawai'i—light blue

following a 30% burn-in of the first three thousand trees.

Molecular clock estimations

Time to most recent common ancestor (tMRCA) within the *C. dentipes* species group was estimated using two methods: (1) uncalibrated Bayesian analysis, using average nucleotide variation across all sites; and (2) calibrated timetree analysis, with tMRCA predicted using an estimated mutation rate and observed fixed nucleotide differences (FNDs) between well-supported clades determined during initial Bayesian analysis. Both analyses were run using model settings supported for initial phylogenetic reconstruction. Given our *Ceratina* sample set's average AT content of $\sim 74\%$, we used a mitochondrial mutation rate of $6.2\text{E}-8$ $\mu\text{site/gen}$, as has been applied in previous studies (Groom et al. 2014b; Shell and Rehan

2016). We simulated our uncalibrated Bayesian analyses in BEAST using a lognormal relaxed molecular clock set to our estimated mutation rate ($\pm 1.0\text{E}-6$ rate standard deviation). The 'treeModel.rootHeight' prior was left unassigned such that root node height and branch lengths would be determined by averaged genetic distance proportional to age, given our estimated mutation rate across all bases. MCMC was again run for one billion iterations, sampling every 100,000th under a coalescent Bayesian Skyline tree model. This analysis was performed in triplicate to ensure estimation reliability and consistency. TreeAnnotator was then used to select the maximum clade credibility (MCC) tree from a bank of ten thousand logged clade simulations following a 30% burn-in of the first three thousand trees.

For our calibrated tMRCA runs, we calculated the approximate time required to accrue the observed FNDs detected among our study populations, given

our estimated mutation rate across 593 sites and assuming two generations per year as has been observed across tropical *Ceratina* (Rehan et al. 2009, 2015). We constructed a time tree in MEGA7 (Kumar et al. 2016) via the RelTime maximum likelihood method (Tamura et al. 2012) using model settings and tree topology supported by our uncalibrated BEAST analysis, and setting calibration reference points at both the root node of the ingroup (Solomon Islands + Philippines), and at the MRCA between the Philippines and the mainland SE Asia + Pan-Pacific clades.

Haplotype diversification estimation

To construct a calibrated MCC tree for the ingroup dataset, we used the HKY + I substitution model (Hasegawa et al. 1985) as supported by jModelTest under the BIC. We ran a coalescent Bayesian skyline tree model under a lognormal relaxed clock set to our estimated mutation rate assuming two generations per year. We set our treeModel.rootHeight prior using dates determined via the FND RelTime analysis. MCMC runs were again performed in triplicate (each for 250 million iterations, sampling every 25,000th) and resulting log files were inspected in Tracer to confirm acceptable ESS values. An MCC tree was selected following a 30% burn-in, and was annotated in FigTree to display posterior probability (PP) and 95% highest posterior density (95HPD) values. We then generated a lineage through time (LTT) plot and Bayesian Skyline plot (BSP) to assess ancestral haplotype diversification and changes in effective population size respectively.

Population genetic structure and haplotype diversity analyses

We ran all analyses of population genetic structure in Arlequin v 3.5.1.2 (Excoffier and Lischer 2010). We performed an analysis of molecular variance (AMOVA) and ran Tajima's D and Fu's F_S tests of neutrality to assess genetic variation within and among ingroup populations (based on 5000 simulations). Tajima's D and Fu's F_S values each test whether observed allelic diversity is significantly different than expected given population size; significant values ($p_D < 0.05$; $p_{FS} < 0.02$) indicate that a population is either experiencing expansion (positive values) or

contraction (negative values). Wright's F -statistics and exact tests of sample differentiation were performed to determine pairwise F_{ST} s, FNDs, and average percent sequence divergence (% seq div) among all groups. Sequences were initially partitioned by collection origin and analyzed via full pairwise comparison. Population structure was then reassessed using partitions based on the four major clades recovered with strong statistical support during molecular phylogenetic reconstructions. Results of tests of neutrality were taken only from analyses handling the four well-supported clades. We used Haploview (Salzburger et al. 2011) to assemble a minimum spanning tree (MST) for all ingroup data, color-coding individuals by collection region.

Results

Notably, all *C. dentipes* individuals collected in Hawaii were identified as female (Table S1). BEAST analyses of haplotype data recovered outgroup *Ceratina* (*Ceratinula*) *arizonensis* as basal to the *Ceratina* (*Neoceratina*) species group (PP = 1.00) and *Ceratina* (*N.*) *bispinosa* as sister to the ingroup clades (PP = 0.83; Fig S1). Within the *C. dentipes* species group, four clades were supported as monophyletic: Solomon Islands [determined as *C. (Neoceratina) spinipes*, PP = 1], Philippines (determined as *C. nr. dentipes*, PP = 1), mainland SE Asia (MSE Asia; determined as *C. dentipes sensu stricto* from Thailand and Vietnam; PP = 0.80), and Pan-Pacific, a widely-distributed set of *C. dentipes s. s.* individuals from Hawaii, Fiji, Thailand, Vietnam, Samoa, Vanuatu, and Borneo (PP = 0.51; Figs. 2; S3). The Solomon Islands clade was strongly supported as basal to the rest of the *C. dentipes* species group (PP = 1). The Philippines clade was recovered as sister to the MSE Asia + Pan-Pacific clade (PP = 1); and the MSE Asia group was well-supported as sister to the Pan-Pacific clade (PP = 1; Fig. 2).

Uncalibrated Bayesian time tree estimation, based solely on average nucleotide variation and approximate mutation rate, suggests speciation events at 63 kya (95% CI 7.6–709 kya; Solomon Islands) and 26 kya (3.6–289 kya; Philippines + *C. dentipes s. s.* crown; Figs. S1, S2). Time tree estimation calibrated using observed FNDs indicated speciation events at 461 kya (434–486 kya; Solomon Islands) and 177 kya

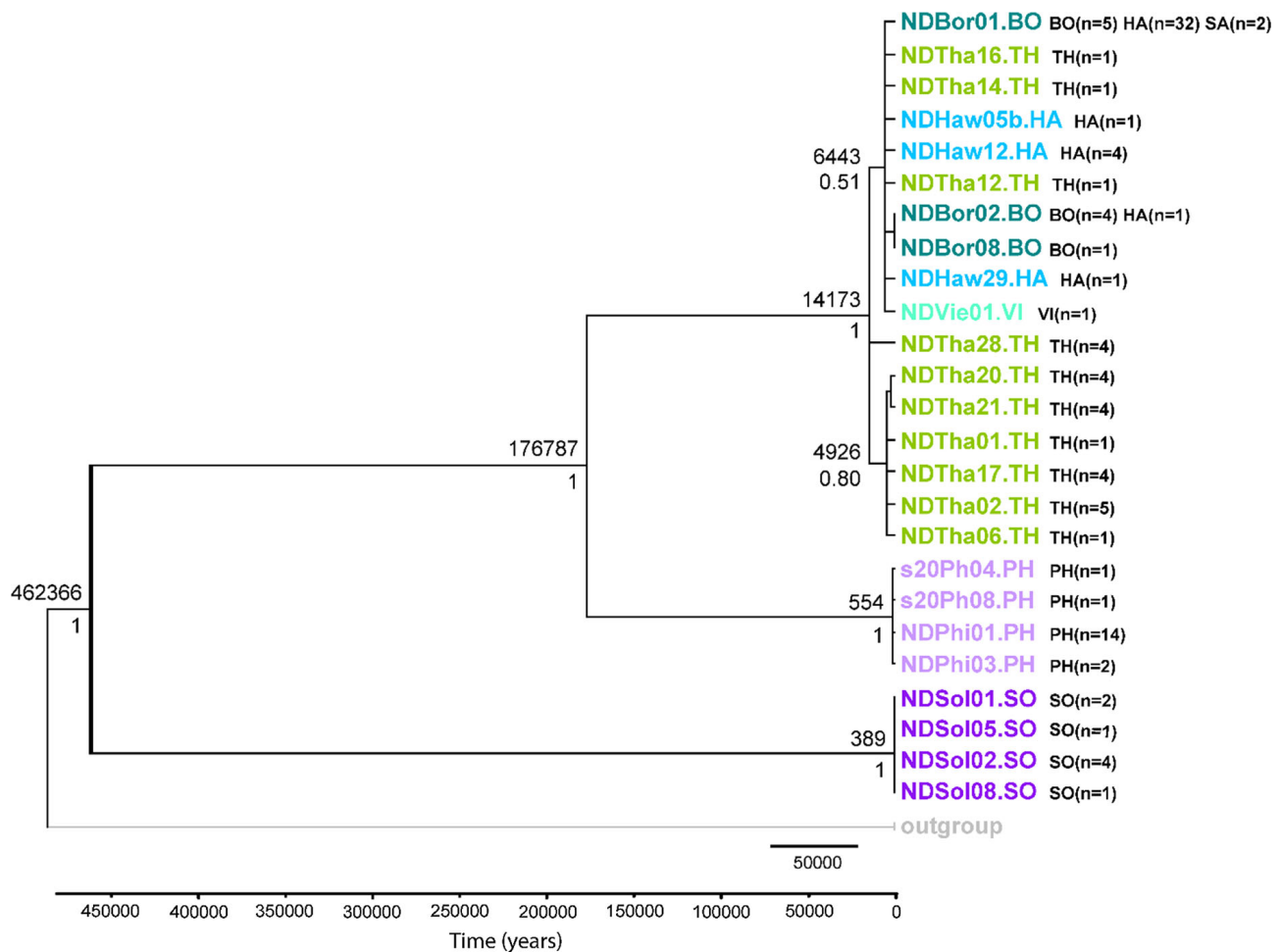


Fig. 2 Fixed nucleotide difference-calibrated time tree for unique *C. dentipes* species group haplotypes constructed using the RelTime maximum likelihood method in MEGA7. Haplotypes are color-coded by country of origin as in Fig. 1; total counts of individuals sharing highlighted haplotypes, along with

locations of origin, are indicated in black followed by collection location and sample size in parentheses. Node ages (above) and Bayesian posterior probability support values (below) are presented

(83–287 kya; Philippines + *C. dentipes* s. s. crown), with a crown age of 47 kya (19–88 kya) for the MSE Asia and Pan-Pacific clades (Figs. 2, S3, S4). Our BSP indicates a rapid increase in effective ingroup population size beginning around 25 kya (20–40 kya; Fig. S5a). Our LTT plot suggests haplotype diversity has followed a similar trend, with a rapid expansion beginning around 50 kya (25–75 kya; Fig. S5b).

Structural AMOVA analyses across our study populations revealed an overall $F_{ST} = 0.956$ ($p < 0.001$; Table 1). Pairwise comparisons of the MSE Asia and Pan-Pacific clades resulted in an $F_{ST} = 0.667$ ($p < 0.001$) despite low sequence divergence between these populations (zero FNDs and 0.23% seq div; $p < 0.001$). Analyses suggested that the Philippines population is isolated from both the

MSE Asia and Pan-Pacific groups (F_{ST} vs SE Asia = 0.925, F_{ST} vs Hawaii = 0.976; $p < 0.001$) and genetically distinct from each (FNDs = 13 and 16; % seq div = 2.6 and 2.7 respectively; $p < 0.001$). The Solomon Islands clade was found to be the most highly divergent from each other population. F_{ST} values were between 0.944 (MSE Asia) and 0.986 (Pan-Pacific; $p < 0.001$); and average percent sequence divergence was between 5.19 (MSE Asia) and 6.05 (Philippines; $p < 0.001$), with between 26 and 34 FNDs (Table 1). AMOVA results followed a similar pattern when performing full pairwise comparisons of sampled populations (Table S3). Tests of neutrality resulted in significantly negative values for the Pan-Pacific group (Tajima's $D = -2.00$, $p = 0.002$; Fu's $F_S = -14.61$, $p < 0.001$), the MSE

Table 1 Population structure of major *Ceratina dentipes* species group clades

	Pan-Pacific	MSE Asia	Philippines	Solomon Is.
Pan-Pacific	0.40 (0.07)	1.37 (0.23)	16.01 (2.70)	31.99 (5.39)
MSE Asia	0.667 (0)	2.17 (0.37)	15.35 (2.59)	30.80 (5.19)
Philippines	0.976 (16)	0.925 (13)	0.33 (0.06)	35.88 (6.05)
Solomon Is.	0.986 (30)	0.944 (26)	0.985 (34)	1.04 (0.17)

Diagonal indicates average pairwise differences within populations and value in parentheses indicates % sequence divergence within those populations; above diagonal are corrected average pairwise differences between populations and parentheses indicate % sequence divergence between those populations; below diagonal are pairwise FSTs, along with fixed nucleotide differences between populations in parentheses. Significance values ($p < 0.001$) indicated in bold. Fixation index over all loci $F_{ST} = 0.956$ ($p < 0.001$); there are 32 fixed differences among all populations

Asia population (Fu's $F_S = -4.68$, $p = 0.003$) and the Philippines population (Tajima's $D = -1.713$, $p = 0.026$; Fu's $F_S = -2.60$, $p = 0.002$; Table S4). Tajima's D and Fu's F_S were negative, but non-significant for the Solomon Islands population.

MST construction reflected results of both BEAST and AMOVA analyses and recovered three main haplotype sets made up of three distinct groups: Solomon Islands, Philippines, and a large cluster formed from both the MSE Asia and Pan-Pacific groups (Fig. 1b). Visual inspection of the MST reveals individuals from *C. dentipes*'s introduced range across Hawaii, Samoa, Fiji, and Vanuatu, all share haplotypes with individuals from Borneo and are within just two point mutations of individuals from elsewhere in MSE Asia.

Discussion

Here we present the first phylogeographic assessment of *Ceratina dentipes* with sequence data coverage across both its native and invasive distributions. We detect population sequence identities that are largely uniform between Pan-Pacific and mainland SE Asian distributions, confirming that invasive *C. dentipes* are very likely recently introduced on the Hawaiian archipelago. Significantly negative Tajima's D and Fu's F_S values indicate members of the Pan-Pacific clade are undergoing particularly rapid population expansion. We also identified two discrete and well-supported species groups, the Philippines (*C. nr. dentipes*) and Solomon Islands (*C. spinipes*) populations, which are genetically distinct from *C. dentipes* s.s. Bayesian and time tree analyses allow us to place an estimated date of *C. dentipes* speciation, and

provide an opportunity to consider the historical movement of this species group throughout SE Asia and into Oceania.

Haplotype diversity and population structure

Population genetic analysis of *C. dentipes* provides an opportunity to directly compare native and invasive populations of *C. dentipes* from across an expansive known range (Fig. 1). Despite substantial geographic distance between mainland SE Asia and populations within the Pan-Pacific clade, we detected only minor sequence divergence between these groups. These results, taken together with the haplotype uniformity between invasive Hawaiian populations and *C. dentipes* from across Borneo and Melanesia (i.e. Solomon Islands, Vanuatu, and Fiji; Fig. 1b), suggest invasive populations moved rapidly eastward out of SE Asia (consistent with Friese 1914; van der Vecht 1952; Groom et al. 2014a). By contrast, the distinct *Neoceratina* species we identified in the Philippines and Solomon Islands appear to have remained relatively isolated to their respective regions (see Table S1). In a key to native and invasive bees on Hawaii, Snelling (2003) mentions that multiple species of *Neoceratina* had possibly been discovered across the South Pacific but had yet to be officially described. Krombein (1951), Hirashima (1971), Rejas (2008) and Shiokawa (2009) each also describe *C. (Neoceratina)* or the '*C. dentipes* species group' as a diverse and taxonomically challenging set. Given the need for taxonomic revision within this group, it is thus perhaps not unexpected, but no less significant, that we detected multiple species when ostensibly targeting just *C. dentipes* from across so wide a distribution. Notably, as *C. spinipes* was previously described only from

specimens collected in the Ryuku Islands (Shiokawa 2009), our detection of a population in the Solomon Islands, over 5200 km away, represents a substantial range extension for this group. It remains to be determined, however, whether this population is within *C. spinipes*' native distribution or may represent another significant dispersal event.

Prehistoric divergence and distribution

Estimating dates of divergence among our well-supported clades can help reveal the history of subgenus *Neoceratina* and the movement of *C. dentipes* prior to its Hawaiian invasion. Our time tree analysis indicates *C. dentipes* (Pan-Pacific and MSE Asia) is the most recently diverged of the detected clades (~ 47 kya), preceded by *C. nr. dentipes* in the Philippines (~ 177 kya) and *C. spinipes* in the Solomon Islands (~ 461 kya; Fig. 2, S3). These time points fall within the 95% CI windows supported by uncalibrated estimation (Fig. S2), and yield improved (i.e. more tightly constrained) 95% CIs for each major node (Fig. S4). These estimated dates of speciation also occur well after the Philippines, Indonesian and Melanesian island ranges had been established (i.e. post-Miocene epoch 5 Mya, Lohman et al. 2011) and thus lie within a geologically realistic timeframe. The genus *Ceratina* is known to have originated in Africa before travelling north into Eurasia and east and south across Asia into the Austral region (Rehan et al. 2010) from where it may have dispersed more widely via LDD events (Rehan and Schwarz 2015); suggesting a SE Asian origin for the subgenus *Neoceratina* (Tadauchi and Tasen 2009). Bayesian estimations of both effective population size and haplotype diversity indicate rapid population expansions and diversification beginning as early as 75 kya and continuing through present day (Fig. S5). Interestingly, this timeframe closely corresponds with well-supported date estimations of human dispersal into Indonesia (73 kya, Westaway et al. 2017) and Melanesia (40–30 kya, Sheppard 2011). The frequent and wide-ranging movement of early human settlers throughout SE Asia and Oceania could have contributed to the broad dispersal of *C. dentipes* and its relatives across their current native distributions, while simultaneously confounding the geographic point of origin for the *Neoceratina* lineage (Rehan et al. 2010).

Contemporary *C. dentipes* dispersal and invasive history

Our phylogeographic assessment of *C. dentipes* also provides an opportunity to consider a most likely avenue of ongoing invasive introduction in Hawaii. Though recently introduced, biodiversity surveys indicate *C. dentipes* has quickly established itself across the Hawaiian archipelago: after an initial detection on Maui in 1996, collections were subsequently made on the islands of Kauai, Oahu, Lanai, Molokai, and Hawaii (Snelling 2003). Previous research suggests invasive populations of *C. dentipes* may be capable of moving between Fiji, Vanuatu, and Samoa by LDD (Groom et al. 2014a), and invasive populations in Hawaii may be similarly able to move rapidly among islands in the archipelago. An invasive introduction into Hawaii via LDD is unlikely, however, as the oceanic currents and trade winds of the Pacific would likely prevent rafting or flight by *C. dentipes* out of SE Asia (Fig. S6; Ali et al. 2016; Tabata 1975).

Intentional introductions of alien bee species have historically been motivated by agricultural applications, typically aimed at facilitating the pollination of a crop of economic interest (Moritz et al. 2005). One of Hawaii's earliest invasive species, *A. mellifera*, was similarly introduced in 1857 for its pollination and honey production services (Howarth 1985; Roddy and Arita-Tsutsumi 1997; Moritz et al. 2005). As there is no record of an intentional introduction of *C. dentipes* on Hawaii, populations are likely to have instead been unwittingly introduced through anthropogenic traffic and trade, as has been suggested for many other invasive bees throughout the Pacific (Groom et al. 2014a, b, 2017). *Ceratina* preferentially occupy flowering woody plants (e.g. *Rhus* and *Rubus* species) in which they establish nesting burrows (identifiable by a minute entrance hole; Okazaki 1992; Rehan et al. 2009). As such, easily overlooked *C. dentipes* nests could be transported within cargo or luggage containing agricultural or ornamental plant matter. Hawaii receives heavy international traffic (Hawaii Tourism Authority 2016), and many major Pacific shipping routes thread their way through SE Asia, Melanesia and Polynesia before heading to the archipelago (Fig. 3). It is thus particularly notable that these routes of high-density shipping traffic effectively tie together the otherwise geographically isolated native and

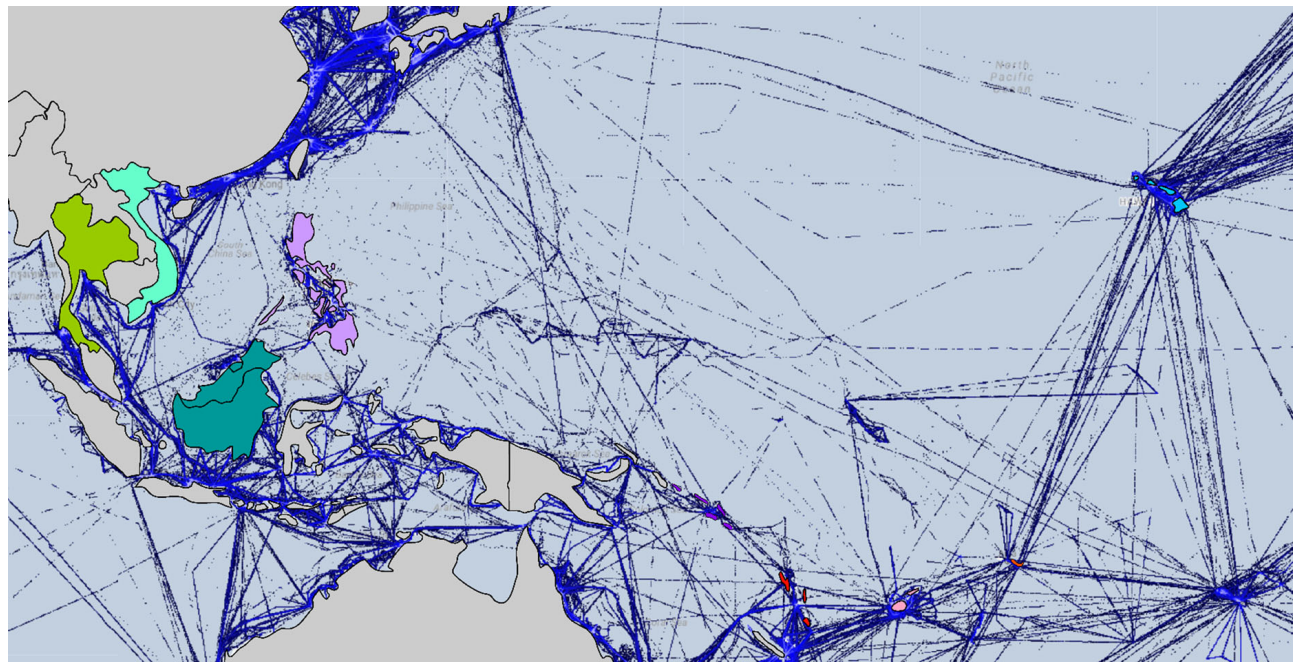


Fig. 3 Major shipping trade routes across the Pacific (map modified from www.MarineTraffic.com). Dark blue lines indicate routes of high density human traffic. Hawaii primarily receives its arrivals from the west coast of North America, and

from Samoa and French Polynesia, which receive heavy traffic from Southeast Asia. Countries sampled during this study are indicated by color as per Fig. 1; other regions are left in gray

invasive *C. dentipes* populations assessed during our study (Fig. 1b). This strongly implicates shipping traffic, originating in or passing through SE Asia, as the most probable candidate for the expansive invasive dissemination of *C. dentipes* throughout the South Pacific and Hawaii.

Potential invasive impacts

As it was likely introduced sometime in the late twentieth century, *C. dentipes* is potentially still in the process of becoming an invasive species in Hawaii. That said, *C. dentipes* was also the first of the alien *Ceratina* in Hawaii detected during sampling inland and at higher elevations (up to 820 m or more), suggesting it may be able to flexibly invade many different ecosystems (Snelling 2003). Despite consistent detection of *C. dentipes* in Hawaii, however, there is a paucity of empirical data detailing its impacts on Hawaiian fauna and flora. From what is known, *C. dentipes* and some *Hylaeus* spp. may nest within the same plants (e.g. *Scaevola taccada*; Graham 2015), creating the potential for nest site competition (Daly and Magnacca 2003). By contrast, as an invasive

generalist, *C. dentipes* may be as likely to pollinate invasive angiosperm species (e.g. *Pluchea carolinensis*) as it is to support native flora (e.g. *Gossypium tomentosum*, Pleasants and Wendel 2010; *Scaevola taccada* and *Sida fallax*, Snelling 2003). As such, additional work is needed to determine whether this group is having a net positive or negative impact on Hawaiian ecosystems, particularly on threatened *Hylaeus* populations. It is also worth considering that only female samples of *C. dentipes* have been collected in Hawaii to date, suggesting that the species is capable of parthenogenic reproduction (Snelling 2003; Daly and Magnacca 2003; Magnacca and King 2013). Parthenogenic reproduction may confound otherwise effective conservation applications (e.g. *Procambarus* ‘Marmorkrebs’; Jones et al. 2009) and has previously been documented in other invasive *Ceratina* (i.e. *C. dallatorreana*, Daly 1966).

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