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Testing for multiple invasion routes and source populations for the invasive brown treesnake (*Boiga irregularis*) on Guam: implications for pest management

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Abstract The brown treesnake (*Boiga irregularis*) population on the Pacific island of Guam has reached iconic status as one of the most destructive invasive species of modern times, yet no published works have used genetic data to identify a source population. We used DNA sequence data from multiple genetic markers and coalescent-based phylogenetic methods to place the Guam population within the broader phylogeographic context of *B. irregularis* across its native range and tested whether patterns of genetic variation on the island are consistent with one or multiple introductions from different source populations. We also modeled a series of demographic scenarios that differed in the effective size and duration of a population bottleneck immediately following the invasion on Guam, and measured the fit of these simulations to the observed data using approximate Bayesian computation. Our results exclude the possibility of serial introductions from different source populations, and instead verify a single origin from the Admiralty Archipelago off the north coast of Papua New Guinea. This finding is consistent with the hypothesis that *B. irregularis* was accidentally transported to Guam during military relocation efforts at the end of World War II. Demographic model comparisons suggest that multiple snakes were transported to Guam from the source locality, but that fewer than 10 individuals could be responsible for establishing the population. Our results also provide evidence that low genetic diversity stemming from the founder event has not been a hindrance to the ecological success of *B. irregularis* on Guam, and at the same time offers a unique 'genetic opening' to manage snake density using classical biological approaches.

Keywords *Boiga irregularis* · Invasive species · Phylogeny · Biological control · Founder event

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Introduction

Non-indigenous invasive species have been known for decades to be one of the greatest drivers of ecosystem collapse on islands, with invasive mammals receiving the most attention to date (Courchamp et al. 2003). Recently, a number of invasive snake species have gained public attention due to their increasing impacts on both island and mainland areas [e.g. Pythons and

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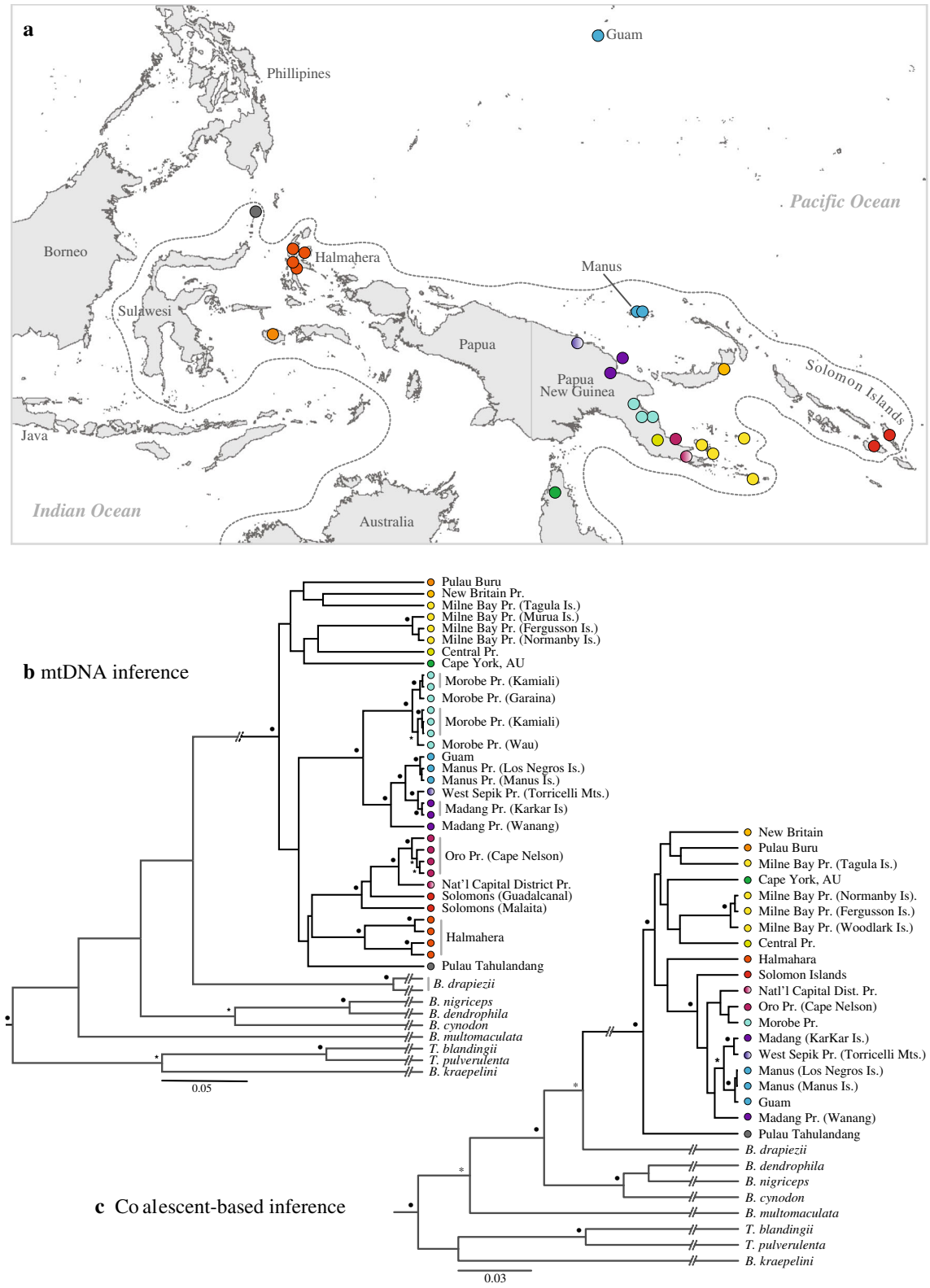
Boa constrictor in Florida, USA (Reed and Rodda 2009; Dorcas et al. 2012), *B. constrictor* in Cozumel, Mexico (Martinez-Morales and Cuarón 1999; Vázquez-Domínguez et al. 2012) Puerto Rico, USA (Reynolds et al. 2013) and Aruba, Dutch West Indies (Quick et al. 2005), the Habu of Japan (Sawai et al. 1999), kingsnakes in Gran Canaria, Spain (Cabrera-Pérez et al. 2012), wolf snakes in Mauritius and Christmas Island, Australia (Smith et al. 2012)], with the brown treesnake (*Boiga irregularis*) on the Pacific island of Guam representing a textbook example of all that can go wrong when an introduced predator becomes established in a non-indigenous environment (see reviews in Kraus 2009).

Native to Indonesia, coastal Australia, and islands throughout northwest Melanesia (Fig. 1a), *B. irregularis* is presumed to have been introduced to Guam in military cargo during the late 1940s, where it has had devastating and lasting effects on the trophic structure, economics and human health on the island (Savidge 1987; Fritts and Rodda 1998; Rodda and Savidge 2007; Rogers et al. 2012). Rodda et al. (1992) hypothesized that the invasive population originated from Manus, the largest island within the Admiralty Archipelago, based on the estimated timing of the snake's arrival on Guam, the morphological similarity between *B. irregularis* on Guam and the Admiralty Islands, and the location of staging areas for military equipment salvage in the western Pacific at the end of World War II. However, no published studies have used genetic data to identify the source population (but see Rawlings 1995), and large Allied military bases in other parts of Papua New Guinea (including the Bismarck Archipelago), the Solomon Islands, and elsewhere in Melanesia were established within the native range of *B. irregularis*. Transportation of cargo and military personnel to and from these locations would have provided ample opportunity for incidental snake movement, as shown for invasive Pacific island populations of the scincid lizard *Carlia ailanpalai* (Austin et al. 2011). Under this scenario, the Guam population could represent an amalgam of *B. irregularis* populations from different parts of the native range, allowing them to avoid complications associated with a genome-wide bottleneck that might otherwise result from a single source founder event. This phenomenon has been detected in the house gecko (*Hemidactylus frenatus*) and in the brown anole (*Anolis sagrei*), where invasive populations exhibit greater genetic diversity than single populations within the native range as a consequence of repetitive introductions from different areas, and may be providing them with a competitive advantage in their introduced ranges (Kolbe et al. 2004; Tonione et al. 2011).

Fig. 1 **a** Map of sampling locations (Pr. = Province of Papua New Guinea). *Dashed line* encompasses the native range of *B. irregularis*. **b** Tree estimated from mitochondrial haplotypes only. **c** Coalescent-based tree inferred from individual genealogies from all nuclear and mitochondrial genes (i.e. *BEAST). Bullets indicate posterior probabilities >0.95; asterisks >0.90

While several techniques have proven successful at locally controlling snake abundance on Guam (Engeman and Linnell 1998; Engeman et al. 1998; Campbell 1999; Savarie et al. 2001; Clark and Savarie 2012), their overall density is still far beyond that observed on islands within the snake's native range and the prospects for these techniques to eradicate or significantly reduce the population's size on an island-wide scale have yet to be fully assessed (Savidge 1991; Rodda et al. 1999). Furthermore, they are costly and require continuous labor to maintain effectiveness (Colvin et al. 2005). One technique that has received limited attention to date is the use of classical biological management (but see Fritts and Scott 1985; Telford Jr 1999; Nichols 2000; Dobson and Altizer 2001; Caudell et al. 2002a, b; Richmond et al. 2011), where parasites or pathogens that infect *B. irregularis* populations in the native range would be used to reduce the population density on Guam. This limited attention is likely due to the inherent risks associated with the technique, its track record of unintended consequences, and a general skepticism on its probability for success (particularly with regard to available data on the effects of haemoparasite infection in *B. irregularis*: Caudell et al. 2002b; Jakes et al. 2003). Genetic confirmation of source populations, identifying routes of invasion, and characterization of post-invasion demography represent critical first steps toward evaluating the prospects for this form of management (Roderick and Navajas 2003; Hufbauer and Roderick 2005; Estoup and Guillemaud 2010; Lawson Handley et al. 2011).

To this end, we used DNA sequence data from mitochondrial and nuclear loci to place the Guam population within the broader phylogeographic context of *B. irregularis* populations spanning the native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Nat'l Capital District Pr.
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Pulau Tahulandang
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B. dendrophila
B. cynodon
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range. Our objective was to provide genetic confirmation of invasion routes and to characterize the genetic variation of the invasive population, building on previous work based on morphology, historical military records, and the timing of the snake's arrival and spread on Guam (Savidge 1987; Rodda et al. 1992; Whittier et al. 2000). We were also interested in modeling the demography of the population during the early phases of the invasion to better understand the severity and duration of the initial bottleneck. Sudden, severe decreases in effective population size and low density of individuals can lead to evolutionary changes that are outside the realm of natural selection, and in some cases these changes may enhance the capacity for population establishment (Keller and Taylor 2008; Estoup and Guillemaud 2010; Lucek et al. 2012). Loss of genetic diversity, the source of that diversity and low density of snakes during the early establishment phase also has implications for the prospects of classical biological management, a subject that has yet to be explored from a phylogenetic perspective for this system.

Materials and methods

Geographic sampling and genetic marker choice

We collected mitochondrial and nuclear DNA sequence data from 65 *B. irregularis* from different locations in the native and introduced range (Fig. 1a). We also included samples from eight species of the mainly Asian *Boiga* complex to use as outgroups. These included *B. nigriceps*, *B. dendrophila*, *B. cynodon*, *B. multomaculata*, *B. kraepilini*, *B. drapiezii*, *Toxicodryas* (formerly *Boiga*) *blandingii*, and *T. pulverulenta.* Outgroup taxa were represented by single samples, with the exception of *B. drapiezii* ($n = 2$). For Guam, we sequenced individuals from eight sites spanning the entire island for the main phylogenetic analyses (described below). We also generated mitochondrial sequence data for a larger sample of 24 individuals from Guam to test for the presence of multiple mitochondrial haplotypes at these same eight locations. Locality information for all specimens is provided in the Electronic Supplemental Material.

We extracted DNA from liver tissue using a Qiagen® DNeasy Blood and Tissue Kit and sequenced the mitochondrial NADH dehydrogenase subunit 4 gene (ND4) and three upstream tRNA genes, and four nuclear genes; intron 5 of the TATA-box binding protein (*Bi*TBP); the recombination activation gene subunit 1(RAG-1); the proto-oncogene *Cmos*; and the myosin heavy chain type II (MyHC-2). We followed a standard PCR amplification protocol and sequenced each marker in two directions using Big Dye v3.1 chemistry on an ABI 3730xl DNA Analyzer at Genewiz (La Jolla, CA). We provide additional details on PCR protocols and primer sequences in the ESM.

Where individuals were heterozygous for length polymorphisms (i.e. insertion-deletions, or 'indels') at a given nuclear locus, we used the software INDELLIGENT (Dmitriev and Rakitov 2008) to resolve the allele phases. This method yields accurate reconstructions when the allele sequences are similar, the indel is small relative to the length of the fragment, and multiple indels, if present, are well spaced. Our samples met each of these criteria (except for the *Bi*TBP gene, where we recovered a single large indel in all but one *B. irregularis*; see summary statistics in Table S1), and in no cases were the number of resolved positions for a given sequence any less than 96 % as determined by the output from INDELLIGENT. For site-specific polymorphisms, we used a predictive method implemented in the software package PHASE to resolve allele states (Stephens et al. 2001; Stephens and Donnelly 2003). If the phase of a polymorphic site could not be inferred with a posterior probability above 0.90, that site was coded using the International Union of Pure and Applied Chemistry (IUPAC) nucleotide ambiguity codes and treated as missing data in the phylogenetic analyses.

Phylogeographic analysis

We performed phylogenetic analyses using various data partitioning schemes in BEAST v1.7.5 (Drummond et al. 2012). Best-fit models of evolution for different data partitions (i.e. genes, codon position, and indels) were identified based on Bayesian Information Criterion (BIC) scores in jMODELTEST 2.3 (Posada 2008). We conducted two different Bayesian analyses in BEAST, one restricted to the mtDNA dataset only and one combining both the mtDNA data and all nuclear gene copies. Data for this study are available at: Dryad (doi:10.5061/dryad.5c6f6).

For the mtDNA analysis, we assigned separate nucleotide substitution models to each codon position and the combined tRNA genes. We implemented a Yule Process tree prior and conducted two runs of 3×10^7 steps each, retaining every 1,000th sample from the posterior distribution. We combined data from both runs after verifying convergence statistics in Tracer v1.4.1 (Rambaut and Drummond 2007) and Awty (Nylander et al. 2008), and summarized the information from the posterior distribution of trees on the maximum clade credibility tree. We considered those branches with posterior probabilities greater than 0.90 to be strongly supported.

For the second analysis, we used the combined gene dataset and the coalescent-based inference method *Beast to generate a summary tree from the different gene genealogies sampled across all individuals (Heled and Drummond 2010). The *Beast method accounts for differences in the time to coalescence among genes and assumes that any discordance in the genealogies is a consequence of retained ancestral polymorphism and not gene flow (Rannala and Yang 2003), an assumption that is almost certainly met with island snake populations. We prefer this approach over non-coalescent, concatenated-gene methods because inspection of the individual nuclear gene genealogies revealed substantial shared polymorphism across our sampling locations, an artifact that can mislead phylogenetic inference. The *Beast approach also allowed us to take greater advantage of the information from heterozygous sequences. To perform these analyses, we specified groups of individuals from different geographic areas (i.e. islands or sites in close proximity; Fig. 1) as terminal taxa and unlinked the substitution and clock models for each gene. We did not model partition the ND4 gene by codon position because doing so substantially increased the duration of this analysis, and the results were qualitatively no different from the partitioned, single gene analysis. We performed four separate runs of 4×10^7 steps using a Yule Process tree prior for all data partitions, retained samples every 1,000th step, and combined the log files from each run after verifying convergence statistics as described above.

Demography of the invasion

We used approximate Bayesian computation (ABC) in the software *DIYABC* v2.0 (Cornuet et al. 2014) to model five different invasion scenarios that varied in the effective size of the founder population and then compared the fit of those different scenarios to the observed data. Our model assumed the following: An effective number of snakes (*Ns*) were established from a single source population of effective size *N2* that was introduced to Guam *T1* generations ago. After *db* generations following the introduction, the bottlenecked population *Ns* then increased to the current effective population size *N1* (Fig. 2). A similar ABC approach has been used to good effect in modeling the demography of founder events in *Megastigmus* seed wasps (Auger-Rozenberg et al. 2012), *Sirex* woodwasps (Boissin et al. 2012), four bumblebee species in the genus *Bombus* (Lye et al. 2011), and yellow-eyed penguins in the genus *Megadyptes* (Lopes and Boessenkool 2010).

Our main analysis modeled five scenarios that differed only in the priors imposed on *Ns*, which were constrained as follows: (*Ns* ~U[2–10], [11–30], [31–50], [51–70], and [71–100]). The smallest of these values assumed that the effective size could have consisted of 10 or fewer snakes during the bottleneck phase, or as many as 100. Because of high individual variation and temporal fluctuation in age of sexual maturity (largely owing to shifts in prey availability on Guam), a precise estimate for generation time in these snakes is not feasible (B. Lardner and J. Savidge, personal communication). Thus we used a uniform

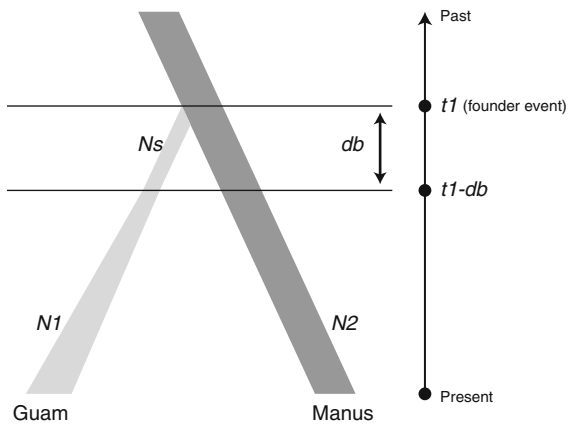

Fig. 2 Diagram of the main demographic scenario modeled in *DIYABC* (times and population sizes are not to scale). Estimated parameters are in italics: *N1*: Guam effective population size, *N2*: Manus effective population size, *Ns*: effective size of the founder population, *db*: duration of the bottleneck, *t1* timing of the introduction event (in generations)

prior bounded by 10 and 50 generations for the timing of the introduction event (*t1* ~U[10–50]), which spans a wide range of plausible values for generation time while placing the arrival of the first snakes sometime during the late 1940s (Rodda et al. 1992). We assumed that the bottleneck could have lasted up to 10 generations (*db* ~ U [2–10]). To assess the effect of this prior on estimates of *Ns* (which may be partly influenced by the duration of the bottleneck; Lye et al. 2011), we ran a separate series of analyses in which we fixed *db* to 2, 5, 8, and 10 generations and estimated *Ns* as a free parameter with a broad uniform prior (~U[2–100]). As no information is available on the effective sizes of either the Guam or the Manus populations, we used equal priors bounded by 10–100,000 for *N1* and *N2*. Additional information on mutation models and rates are provided in the ESM.

The first step of the approximate Bayesian analysis was to construct a reference table consisting of simulated datasets defined by the parameters in each scenario, with parameter values generated from their prior distributions. We assumed uniform prior probability for the different scenarios such that each had roughly equal representation in the reference table, which consisted of 5×10^6 simulated datasets. We then performed a pre-evaluation step using a principle components analysis (PCA) to ensure that at least one or more combinations of scenarios and priors could produce simulated datasets that are close enough to the observed data to perform model comparisons. The PCA was based on the space of parameter values and summary statistics for 100,000 simulated datasets generated from the prior distributions of parameters specified in the different scenarios.

After verifying model suitability, we then compared the fit of different scenarios by estimating their posterior probabilities. Starting with the reference table, we ranked the simulated datasets from each scenario in order of increasing distance to the observed data using the 'direct' and 'logistic' approaches (Beaumont et al. 2002; Miller et al. 2005; Cornuet et al. 2008). Distance between datasets was based on the normalized Euclidean distances between summary statistics estimated from the real and simulated sets. These statistics included the following: (single sample statistics) number of haplotypes, number of segregating sites, mean of the pairwise differences, variance of the number of pairwise differences, number of private segregating sites; (two sample statistics) number of distinct haplotypes in a pooled sample, number of segregating sites in a pooled sample, mean within sample pairwise distances, and mean of between sample pairwise differences.

Next, we measured confidence in the best-fit scenario by estimating the error for scenario 1. We limited this analysis to the two scenarios with the highest posterior probabilities during the model comparison step. We selected 500 datasets for the 'direct' approach and 20,000 for the 'logistic' approach and measured the proportion of times that the best-fit scenario had the highest posterior probability compared to the competing scenario. Finally, we performed an assessment of the goodness-of-fit of a model parameter posterior combination (Gelman et al. 1995). This analysis is similar to the PCA performed in the pre-evaluation step, only in this case the PCA includes datasets from the posterior predictive distribution. A good fitting model should produce a cloud of data points simulated from the priors, on top of which lies the observed data *within* a smaller cloud of datasets from the posterior predictions.

Results

Summary data for the various genetic markers and indices of diversity by sampling location are provided in the ESM. Our results show strong support for a sister species relationship between the Indonesian *B. drapiezii* and *B. irregularis,* with the northernmost sampled population of *B. irregularis* from Pulau Tahulandang, Sulawesi forming the sister to all remaining lineages within *B. irregularis* (Fig. 1).

On Guam, we recovered a single mtDNA haplotype among all sampled individuals ($n = 32$). This haplotype was identical to one of two others recovered from 12 snakes collected on Manus and Los Negros Islands (the two islands are separated by a narrow isthmus and are hereafter referred to as Manus); the two haplotypes differed by at most three segregating sites, with one recovered at substantially higher frequency on Manus ($f = 0.85$). Together, these haplotypes formed a well-supported clade that was sister to a second clade composed of populations in the Torricelli Mountains on the north coast of mainland Papua New Guinea and Karkar Island (~30 km off the north coast in the Madang Province). We did not recover the Manus/Guam haplotypes in any other sampling locations in the snake's native range.

In contrast to the complete lack of mtDNA variation on Guam, we detected some allelic differences for each of the nuclear genes; however, the differences involved single-site substitutions and no more than two alleles per locus were recovered from all of the Guam snakes combined. These variants reflect the retention of ancestral polymorphism from the source population rather than in situ evolution on Guam, given that 'Guam alleles' were detected on Manus for all nuclear genes. In fact, incomplete lineage sorting in the nuclear genes was a common theme across all samples—this was demonstrated by the relatively high nuclear diversity overall, but with few shared derived characters for diagnosing regional groups. This translated to less geographic structure for the individual nuclear gene trees (not shown) compared to the mtDNA gene tree, as expected given the smaller effective population size and the shorter coalescence time of mitochondrial genes. Nonetheless, when we combined the data from all loci and individuals and inferred the tree using the coalescence-based approach in *BEAST, we again recovered strong support for a Manus/Guam clade (although the relationships of other regional clades across this tree are less well-resolved than in the mtDNA tree).

Demographic estimates using approximate Bayesian methods

The model pre-evaluation using PCA showed that the observed data fell within the cluster of simulated data points based on the five demographic scenarios, indicating that it was appropriate to pursue comparative analyses. Model comparisons showed that scenario 1, where $Ns \sim U[1–10]$, had the highest posterior probability of the five tested (Fig. 3). Scenario 2 ($Ns \sim U[11–30]$) was ranked a distant second, with the remaining scenarios all having very low posteriors (Fig. 3). This was not unexpected because the model pre-evaluation step showed that a greater proportion of simulated parameter estimates fell below the observed values (either below 5 % or above 95 %) in scenarios 3–5, suggesting that the higher prior bounds on *Ns* resulted in successively worse fits to the real data. The measure-of-confidence step showed that scenario 1 had the highest posterior probability in 466 and 478 of 500 simulations using the direct and logistic approaches, respectively, indicating a type I error of 0.07 and 0.04 (i.e. probability with which scenario 1 was rejected even though it is the true scenario). Running the same analysis but simulating against scenario 2 to access type II error (i.e. probability of accepting scenario 1 when it is not the true scenario) gave 154 (or 139 for the logistic approach) over 500 in favor of scenario 1, or a type II error rate of 0.31 (0.28). Last, the goodness-of-fit test using the posterior predictions showed that simulated datasets based on scenario 1 produced parameter values and summary statistics that closely matched the observed data (Fig. 3), indicating that the model/posterior for scenario 1 provided a good fit to the observed data.

We found that the prior on *db* had a slight effect on the posterior estimate of *Ns*, which ranged from 2.4 to 10.1 (median of the posterior) across the range of priors tested (Table 1); however, the greatest change in the estimated *Ns* value was limited to models with the lowest constrained prior (i.e. $db = 2$ and 5). When $db \geq 5$, or when *db* was treated as a free parameter with uniform prior probability, the posterior for *Ns* was consistently between 9 and 10 snakes, indicating that the estimate was fairly robust to the choice of prior. These results suggest that while there was apparently a single source origin for *Boiga* on Guam, we cannot rule out the possibility that there were serial introductions from the same source on Manus Island.

Discussion

Our results exclude the possibility that the *B. irregularis* population on Guam represents a genetic composite of multiple sources from different geographic areas, and verifies the hypothesis of Rodda et al. (1992) in pinpointing the geographic origin as being on Manus Island. Our data also provide evidence that large genetic variation has not been a major contributing factor to the snake's immense ecological success on Guam, as observed in other examples of invasive reptile populations with serial introductions from different sources (Kolbe et al. 2004; Tonione et al. 2011). While large genetic variation may increase the capacity of an invasive population to adapt to new territories (Lee 2002; Kolbe et al. 2004; Facon et al. 2006), many examples of successful invaders with low genetic variability have been documented (Wares et al. 2005). In these cases, selectively advantageous alleles at specific loci or certain selected combinations of alleles at different loci may have engendered founder

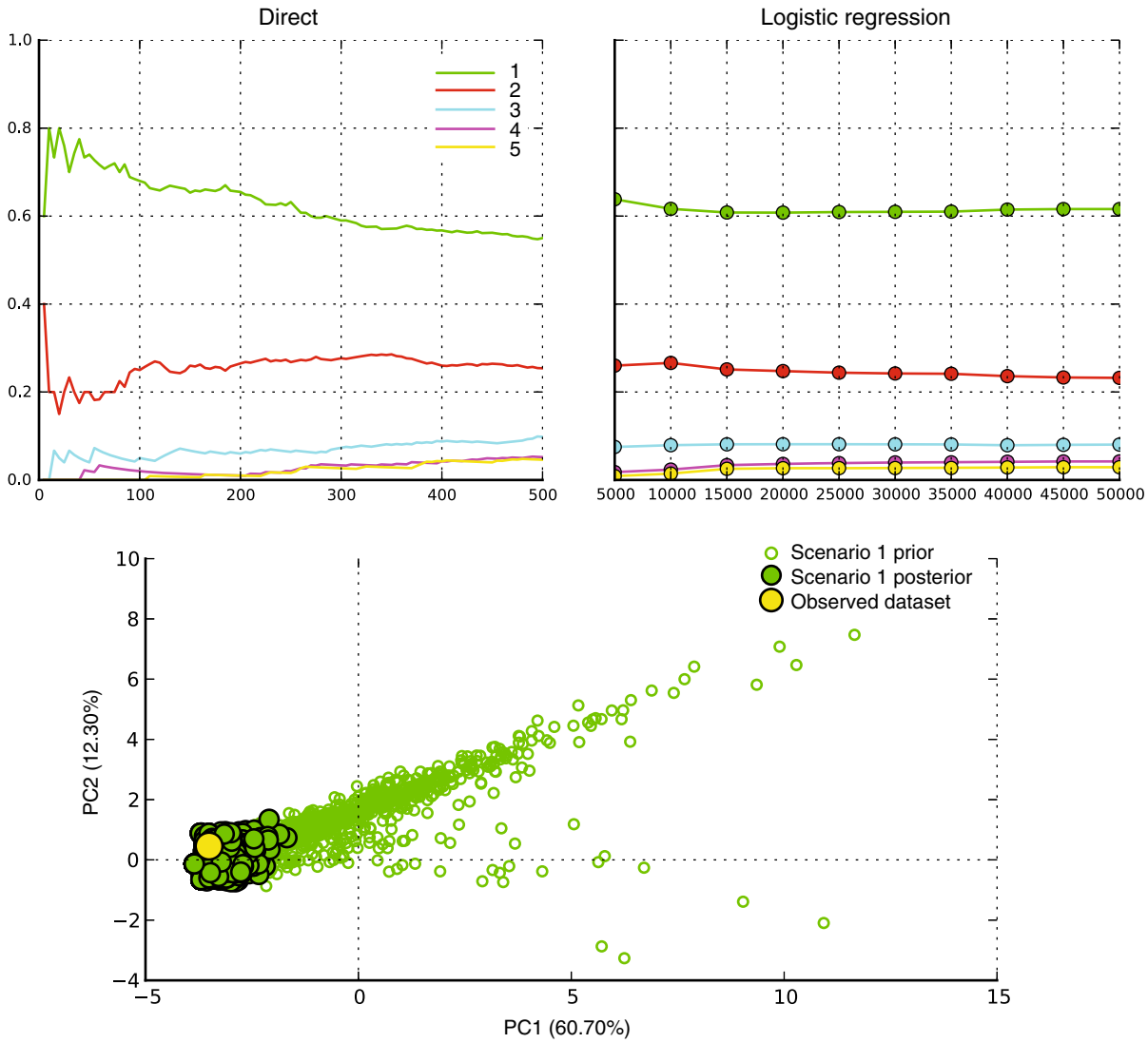

Fig. 3 Results of the *DIYABC* analyses. **a** Model comparison step using the direct approach (Miller et al. 2005). **b** Model comparison using the logistic regression approach (Beaumont et al. 2002). Scenarios 1–5 are color labeled (e.g. *S1* scenario 1, etc.). For both the direct and logistic approaches, scenario 1 represents the best fit to the observed data. **c** Posterior predictive check of model fit

individuals with genetic backgrounds that enhanced their establishment, despite having low genetic variability across the genome (Facon et al. 2006; Estoup and Guillemaud 2010). Whether this has happened here, or to what extent, may never be known, but we suspect that myriad ecological rather than genetic factors were more important determinants of invasion success for this species on Guam.

We considered the possibility that *B. irregularis* may have also been introduced to Manus Island as a consequence of similar incidental transport, given that Manus was a major military staging area during the Allied Pacific Campaign (Gailey 2004). However records for *B. irregularis* on Manus Island substantially predate World War II (Hediger 1933), and we did not recover the Manus/Guam haplotype in any other sampling areas in the native range. This suggests that the snake has been on Manus (or at least within the Admiralty Archipelago) long enough for the mutation process to have given rise to fixed variants, and we therefore assume that the establishment of *B. irregularis* in the Admiralty Archipelago occurred through

Table 1 Posterior estimates of the effective size of the founder population *Ns* on Guam

db	*Ns* (mode, median)	($Q_{0.050}$–$Q_{0.950}$)	Priors
*t*1–*db*	1.00, 10.10	(1.17–58.30)	(*t*1 ~ U[10–50]; *db* ~ U[1–12]; N_S ~ U[1–80])
*t*1–10	1.00, 9.33	(1.12–66.80)	(*t*1 ~ U[10–50]; N_S ~ U[1–100])
*t*1–8	1.00, 9.85	(1.04–67.70)	(*t*1 ~ U[10–50]; N_S ~ U[1–100])
*t*1–5	1.00, 7.55	(1.00–61.80)	(*t*1 ~ U[10–50]; N_S ~ U[1–100])
*t*1–2	1.00, 2.42	(1.00–30.10)	(*t*1 ~ U[10–50]; N_S ~ U[1–100])

Data show the mode, median, quantiles, and priors used to estimate *Ns*; *db* denotes the duration of the bottleneck in generations, and *t*1 is the timing of the founder event in generations in the past. In the first row, *db* was treated as a free parameter with uniform prior probability, whereas in the remaining analyses *db* was constrained to a single value to measure the effect of the prior on estimates of *Ns*

natural dispersal processes long ago. We note however, that our sampling is limited, and further collecting efforts in other parts of New Guinea could potentially reveal identical Manus/Guam haplotypes elsewhere.

Invasion demography

Our demographic models show that 10 or fewer snakes could have established the Guam population. Given the approach we implemented here, the estimated *Ns* value may not only reflect the effective size of the founder population, but also the effective size over a time frame at which the population was presumed to be bottlenecked (*t1–db* in the model). Thus, the number of transported snakes could have feasibly been fewer than 10, and it is possible that one or more could have been gravid females. The number of alleles recovered for the different nuclear genes are also consistent with this inference. We attempted to estimate a value for the timespan of the bottleneck *db*, however we were unable to generate a posterior sample that differed reasonably from the prior. This bias generally indicates that there is too little information in the data to estimate the parameter, which we have good reason to suspect here due to our relatively small sample size and other sources of uncertainty (i.e. generation time and effective population size). Nonetheless, we provide evidence that remarkably few snakes from a single small island off the north coast of Papua new Guinea gave rise to one of the most successful non-indigenous species invasions of modern times.

Another consideration related to the ability to estimate *db* is that the time since the founder event may be theoretically equal to the duration of the bottleneck *db*, such that the posterior can only reflect information contained in the prior. In other words, the current population is still bottlenecked with respect to the genome, even though the census size is large and snake density exceeds that in the native range (Rodda et al. 1992; Rodda and Savidge 2007). The absence of immigration from other locations in the native range means that only mutation and recombination can introduce new genetic variation to Guam snakes; however, so little evolutionary time has passed since the founder event that mutation can be safely eliminated as a source for new genetic variability. The lack of genetic rescue through immigration suggests that the population has persisted in a genetically precarious state since its arrival, and that life history traits and highly favorable ecological conditions on the island may be acting to buffer against the effects of this low genetic variability.

Phylogenetic applications in the development of classical biological control program on Guam

Efforts at controlling the *B. irregularis* population on Guam have gained ground and notoriety in recent years through the use of aerial baits (Clark and Savarie 2012), but the long-term success of the effort has yet to be fully realized and the need for population control and containment has not waned. We argue that while biological management is controversial, risky, and may ultimately prove ineffective, the results of our study signal a favorable genomic condition in the invasive population that plays to the advantage of classical biological approaches (Hufbauer and Roderick 2005; Fauvergue et al. 2012). For example, severe reduction in variability across the genome, particularly in genes regulating the immune response (e.g. major

histocompatibility genes, toll-like receptors, etc.), can compromise the ability to combat infection or disease, a phenomenon that is often of great concern for threatened and endangered species (O'Brien and Evermann 1988; Altizer et al. 2003; de Castro and Bolker 2005; Clark et al. 2011; Sutton et al. 2011). With a phylogeny serving as an evolutionary guideline, comparative phylogenomic analyses can now be used to better understand the extent to which the founder event may have diminished the Guam population's capacity to fight infection and disease (Seddon and Baverstock 1999; Hedrick et al. 2001; Ploshnitsa et al. 2012).

Reconstructing the phylogeography of *B. irregularis* also provides a straightforward starting point for reconnaissance work on naturally occurring, host-specific agents that satisfy criteria related to virulence and transmission (see Roderick and Navajas 2003 and Kang et al. 2007 for variants of this perspective). We have used this approach to begin parasite prospecting in different parts of the species' native range, targeting populations that vary in their phylogenetic distance to the Guam population and including the source on Manus (Richmond et al. 2011). The prospects for identifying suitable agents is enhanced by data showing that the Guam population has been purged of its former co-evolved parasites in the native range (Jakes et al. 2003; Richmond et al. 2011), which broadens the pool of parasites or pathogens that could serve as potential regulators. While there are considerable risks to this approach and its prospects for success should be viewed with healthy skepticism, classical biological control may provide an auxiliary means of reducing population density when combined with other techniques (e.g. aerial baits and bait trapping) that have proven successful on local scales (Dobson 1988; Cleaveland et al. 1999; Tompkins and Begon 1999).

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