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Did geckos ride the Palawan raft to the Philippines?

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ABSTRACT

Aim We examine the genetic diversity within the lizard genus *Gekko* in the Philippine islands to understand the role of geography and geological history in shaping species diversity in this group. We test multiple biogeographical hypotheses of species relationships, including the recently proposed Palawan Ark Hypothesis.

Location Southeast Asia and the Philippines.

Methods Samples of all island endemic and widespread Philippine *Gekko* species were collected and sequenced for one mitochondrial gene (NADH dehydrogenase subunit 2) and one nuclear gene (phosducin). We used maximum likelihood and Bayesian phylogenetic methods to derive the phylogeny. Divergence time analyses were used to estimate the time tree of Philippine *Gekko* in order to test biogeographical predictions of species relationships. The phylogenetic trees from the posterior distribution of the Bayesian analyses were used for testing biogeographical hypotheses. Haplotype networks were created for the widespread species *Gekko mindorensis* to explore genetic variation within recently divergent clades.

Results Both maximum likelihood and Bayesian phylogenetic analyses indicated that Philippine *Gekko* species are a diverse clade with a long history in the archipelago. Ancestral range reconstruction and divergence time analyses suggest a Palawan microcontinental origin for this clade, coinciding with Palawan's separation from Asia beginning 30 Ma, with subsequent diversification in the oceanic Philippine islands. The widespread species *G. mindorensis* and *G. monarchus* diversified in the late Miocene/early Pliocene and are potentially complexes of numerous undescribed species.

Main conclusions The view of the Philippine islands as a 'fringing archipelago' does not explain the pattern of species diversity in the genus *Gekko*. Philippine *Gekko* species have diversified within the archipelago over millions of years of isolation, forming a large diverse group of endemic species. Furthermore, the Philippine radiation of gekkonid lizards demonstrates biogeographical patterns most consistent with stochastic colonization followed by *in situ* diversification. Our results reveal the need to consider deeper time geological processes and their potential role in the evolution of some Philippine terrestrial organisms.

Keywords

Biogeography, Buruanga Peninsula, diversification, *Gekko*, islands, lizards, Palawan Ark Hypothesis, Philippines, rafting, Southeast Asia.

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INTRODUCTION

Southeast Asia's complex geological history and highly variable geography and ecology provide an ideal template for testing

the effects of geographical change on phylogenetic processes (Brown & Guttman, 2002; Evans *et al.*, 2003; Jansa *et al.*, 2006; Esselstyn *et al.*, 2009). More specifically, the Philippine archipelago, spanning components of the Sundaland–Eurasian and

Philippines sea plates (Rangin, 1990; Yumul *et al.*, 2003, 2009a; Dimalanta & Yumul, 2004), has been subjected to numerous geological processes since the beginning of the Mesozoic, such that the current distribution of land has resulted from a combination of volcanism, strike-slip fault activity and sea plate collisions (Rangin, 1990; Yumul *et al.*, 2003, 2009a; Dimalanta & Yumul, 2004). Additionally, the region's floral and faunal assemblages have been further shaped by repeated climatological oscillations, primarily since the onset of the Pleistocene (Heaney, 1986; Hall, 1996, 1998; Rohling *et al.*, 1998; Karns *et al.*, 2000; Siddall *et al.*, 2003), wherein fluctuations in sea level caused dramatic changes in the size, connectivity and presumably the ecological characteristics of islands.

Faunal demarcations in the Philippines have traditionally been explained by the geography of Pleistocene aggregate island complexes (PAICs: Heaney, 1985; Heaney *et al.*, 1998, 2005; Brown & Diesmos, 2002, 2009; for review see Siler *et al.*, 2010). During glacial periods, decreased sea levels (100–140 m below current levels) resulted in increased land-positive area and greater connectivity between adjacent islands previously separated by shallow water. The differential biotic exchange presumably conferred by the cyclical island formation and fragmentation remains a useful paradigm for explaining the distribution of biotic diversity in the Philippines.

Despite its elegant simplicity and general applicability, the PAIC model of diversification has received only mixed support from analyses of the evolutionary relationships within numerous taxa (for review see Siler *et al.*, 2010, 2011a). However, this model remains an explanatory, heuristic tool for exploring and understanding many of the evolutionary processes underlying the accumulation of biodiversity in the Philippines and in the Indo-Malayan archipelago as a whole (Dickerson, 1928; Kloss, 1929; Delacour & Mayr, 1946; Inger, 1954; Dickinson *et al.*, 1991; Heaney & Regalado, 1998; Inger & Voris, 2001; Brown & Guttman, 2002; Diesmos *et al.*, 2002; Catibog-Sinha & Heaney, 2006; Esselstyn & Brown, 2009). The numerous taxa that contradict a primarily PAIC-driven model of diversification highlight the need for caution when applying a generally applicable philosophy to the diversification processes of a mega-diverse region such as the Philippines (Esselstyn & Brown, 2009). Recent studies have opened the door to new hypotheses concerning mechanisms of island colonization, range expansion and speciation in the Philippine archipelago (Blackburn *et al.*, 2010; Esselstyn *et al.*, 2010).

The Palawan Ark Hypothesis (Blackburn *et al.*, 2010) represents a departure from the PAIC model of diversification and community assembly, and considers the influence of deeper time plate tectonics and species' history of rafting across oceanic basins on emergent islands. Following its separation from Asia beginning around 30 Ma (Hall, 1996, 1998; Zamoros & Matsuoka, 2004; Yumul *et al.*, 2009a,b), the Palawan microcontinental block moved south-eastward, eventually colliding with the Philippine mobile belt during the Miocene (*c.* 20–16 Ma; Hall, 2002; Zamoros *et al.*, 2008; Yumul *et al.*, 2009b). Although the association between

Palawan Island and Asia prior to the opening of the South China Sea has been well documented (Hall, 1996, 1998; Zamoros & Matsuoka, 2004; Yumul *et al.*, 2009a,b), only recently have components of the eastern and central Philippine islands been confirmed to be associated with the Palawan microcontinental block (Zamoros & Matsuoka, 2004; Zamoros *et al.*, 2008). Recent studies have shown that the north-west peninsula of Panay Island (Buruanga Peninsula: now part of the central Philippine islands), Carabao Island, south-west Mindoro Island and the Romblon Island Group were part of the North Palawan terrane, which migrated with the Palawan microcontinental block following its separation from Asia (Zamoros & Matsuoka, 2004; Zamoros *et al.*, 2008; but see Hamilton, 1979; Holloway, 1982). Although studies of onshore limestone (Holloway, 1982; Yumul *et al.*, 2009a) and carbonate deposits (Yumul *et al.*, 2009a) suggest that components of the present-day land-positive members of the Palawan microcontinental block were submerged, possibly up until the late Oligocene to early Miocene (Saldivar-Sali *et al.*, 1981; Mitchell *et al.*, 1986), little evidence in support of its complete submergence has been presented (Blackburn *et al.*, 2010). On the contrary, several studies have suggested the possibility that at least some portion of the Northern Palawan Block was land-positive as early as the late Eocene (Taylor & Hayes, 1980; Mitchell *et al.*, 1986; for review see Blackburn *et al.*, 2010). The unique biogeographical patterns observed for endemic Palawan vertebrates, as well as inferred ancient divergences pre-dating the Palawan microcontinental block's breakup from Asia, have led researchers to suggest the possibility of an ark-driven mechanism of isolation and island colonization, namely the Palawan Ark Hypothesis (Blackburn *et al.*, 2010).

An insular radiation of gekkonid lizards

Enhanced survey efforts and careful scrutiny of morphological and molecular data from recognized species has resulted in a dramatic increase in the recognized diversity of Philippine species in the genus *Gekko*. Eleven species of *Gekko* are considered endemic to the Philippine archipelago (*G. athymus*, *G. carusadensis*, *G. crombota*, *G. ernstkelleri*, *G. gigante*, *G. mindorensis*, *G. palawanensis*, *G. porosus*, *G. romblon*, *G. rossi* and *G. coi*; Brown *et al.*, 2011). Recent phylogenetic studies suggest that as many as 14 species may soon be recognized (including three new endemic unrecognized taxa in the Babuyan Islands; Brown *et al.*, 2011). The diversity of Philippine *Gekko* represents a considerable range in body size, general appearance and ecological attributes (Brown *et al.*, 2011). Two species (*G. mindorensis* and *G. monarchus*) have long been considered widespread, with distributions spanning much of the archipelago, including disjunct island populations from multiple PAICs (Brown & Alcala, 1978). Interestingly, five endemic Philippine species (*G. athymus*, *G. ernstkelleri*, *G. palawanensis*, *G. romblon*, *G. coi*) all occur within the Palawan microcontinental block (Zamoros *et al.*, 2008). To date, no studies have explicitly tested the validity of many island endemic species of *Gekko* from a phylogenetic perspective.

Patterns of diversification and biogeographical histories of range-restricted and widespread species

Phylogeny and diversification patterns have now been investigated in groups of mammals (Steppan *et al.*, 2003; Heaney *et al.*, 2005; Jansa *et al.*, 2006; Roberts, 2006a,b; Esselstyn & Brown, 2009; Esselstyn *et al.*, 2009; Esselstyn & Oliveros, 2010), frogs (Brown & Guttman, 2002; Brown *et al.*, 2009b, 2010), birds (Miranda *et al.*, 1997, 1998; Jones & Kennedy, 2008; Oliveros & Moyle, 2010) and lizards (Siler *et al.*, 2010, 2011a,b); however, much remains to be learned from phylogenetic studies with regard to how Philippine biodiversity has been produced, structured, partitioned and maintained (Brown & Diesmos, 2009; Esselstyn *et al.*, 2010). The earliest views of the archipelago involved characterization of distribution of its vertebrates as indicative of a 'fringing' archipelago (Dickerson, 1928; Darlington, 1957; Myers, 1962; Brown & Alcalá, 1970; Diamond & Gilpin, 1983). According to this interpretation, island archipelagos adjacent to mainland sources for faunal exchange were often characterized by an 'immigrant pattern' of faunal distributions in which species were distributed along possible migration routes, with various groups dropping out with distance from the source, according to each group's relative dispersal ability (Taylor, 1928; Darlington, 1957; Myers, 1962; Brown & Alcalá, 1970; Diamond & Gilpin, 1983).

Towards the end of the 20th century, the expected immigrant patterns and the concept of a 'fringing' archipelago

were largely replaced by a 30+ year paradigm emphasizing island endemism, former island connectivity (Leviton, 1963; Heaney, 1985) and glacial–interglacial cycles of sea level fluctuations (Heaney, 1985; Heaney *et al.*, 1998, 2005; Brown & Diesmos, 2002, 2009; Evans *et al.*, 2003; Roberts, 2006a,b; Esselstyn & Brown, 2009; Siler *et al.*, 2010, 2011a). However, the earlier hypotheses can still be seen in works emphasizing entryways for dispersal and routes of colonization into the Philippines (Diamond & Gilpin, 1983; Brown & Guttman, 2002; Esselstyn *et al.*, 2004; Jones & Kennedy, 2008; Brown *et al.*, 2009b; Oliveros & Moyle, 2010).

In this study we investigate the prevalence of cryptic genetic diversity in Philippine *Gekko*, evaluate historically accepted species boundaries, and compare diversification patterns between island endemic and widespread species from phylogenetic and population-genetic perspectives. Additionally, we test whether patterns of extant diversity are consistent with predictions derived from the Palawan Ark Hypothesis (Blackburn *et al.*, 2010).

MATERIALS AND METHODS

Taxon sampling and data collection

Ingroup sampling included 153 individuals collected from 38 localities, with all 11 currently recognized species of *Gekko* from the Philippines (Fig. 1 and Appendix S1 in Supporting

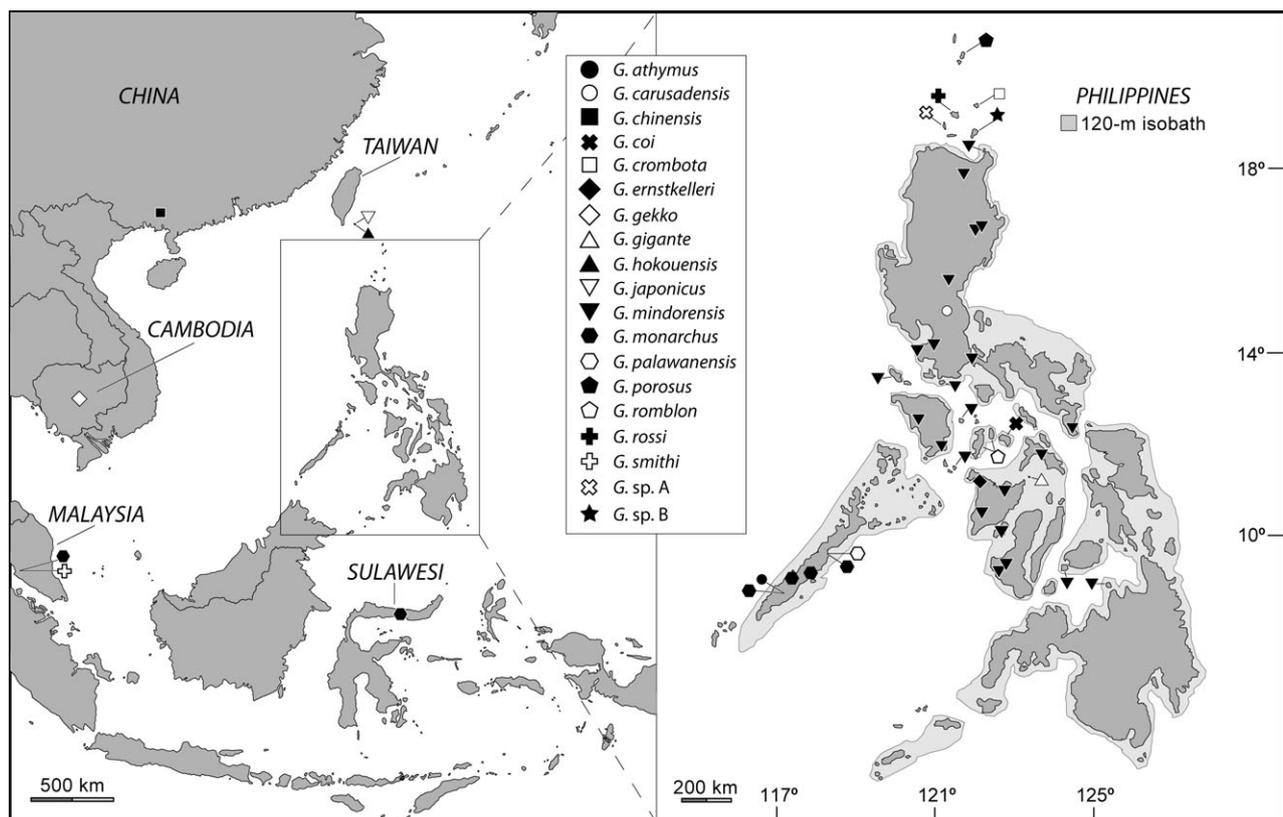


Figure 1 Distribution of *Gekko* samples in Southeast Asia used for this study. Species-specific locality markers correspond to the internal figure key.

Information). To assess the monophyly of the Philippine species, as well as investigate appropriate outgroup taxa, samples of *Gekko* species from the Asian mainland, Peninsular Malaysia and Sulawesi were included, as well as six *Cyrtodactylus* species, *Lepidodactylus herrei* and *Ptychozoon kuhli* (Appendix S1).

We sequenced the mitochondrial gene NADH dehydrogenase subunit 2 (*ND2*) and components of three flanking transfer RNA genes (tRNA^{trp}, tRNA^{ala}, tRNA^{asn}) using the primers and protocols of Brown *et al.* (2009a) in 172 voucher specimens. For 91 of these samples, we also sequenced the nuclear phosphocin gene (*PDC*) using the primers and protocols of Jackman *et al.* (2008). Polymerase chain reaction (PCR) and sequencing protocols followed Siler *et al.* (2010). All sequences were deposited in GenBank (Appendix S1).

Sequence alignment and phylogenetic analyses

Initial alignments were produced in MUSCLE 3.8.31 (Edgar, 2004) and manual adjustments were made in MACCLADE 4.08 (Maddison & Maddison, 2005). To assess phylogenetic congruence between the mitochondrial and nuclear data, we inferred the phylogeny for each subset independently using likelihood and Bayesian analyses. Following the observation of no strongly supported incongruence between datasets, we concatenated data for subsequent analyses. Exploratory analyses of the combined dataset of 172 individuals (including 81 lacking *PDC* sequences) and a reduced dataset of 91 individuals (no missing data) supported identical relationships, and we therefore chose to include all available data (172 individuals) for subsequent analyses of the concatenated *ND2* + *PDC* dataset. Alignments and resulting topologies are deposited in Dryad (doi:10.5061/dryad.7f327q53).

Partitioned Bayesian analyses were conducted in MRBAYES 3.1.2 (Ronquist & Huelsenbeck, 2003). The mitochondrial dataset was partitioned by codon position for the protein-coding region of *ND2* and the three flanking tRNAs (tRNA^{trp}, tRNA^{ala}, tRNA^{asn}) were analysed as a single subset, as was *PDC*. The Bayesian information criterion (BIC; Schwarz, 1978), as implemented in MODELTEST 3.7 (Posada & Crandall, 1998), was used to select the best model of nucleotide substitution for each partition (Table 1). Because the TN93 model is not implemented in MRBAYES 3.1.2, the best scoring

Table 1 Models of evolution of Philippine species of *Gekko* selected by Bayesian information criterion (BIC) and applied for partitioned, model-based phylogenetic analyses.

Partition	BIC model	Number of characters
<i>ND2</i> , 1st codon position	HKY + I + Γ	346
<i>ND2</i> , 2nd codon position	HKY + Γ	346
<i>ND2</i> , 3rd codon position	GTR + Γ	346
All tRNAs (Trp, Ala, Asn)	HKY + Γ	223
Phosducin	TN93 + I + Γ	419

ND2, NADH dehydrogenase subunit 2 gene.

mode that is implemented (K80 + I + G) was used instead. A rate multiplier model was used to allow substitution rates to vary among subsets, and default priors were used for all model parameters. We ran eight independent Markov chain Monte Carlo (MCMC) analyses, each with four Metropolis-coupled chains, an incremental heating temperature of 0.02, and an exponential distribution with a rate parameter of 25 as the prior on branch lengths (Marshall *et al.*, 2006; Marshall, 2010; Siler *et al.*, 2010, 2011a). All analyses were run for 25 million generations, with parameters and topologies sampled every 5000 generations. To assess chain stationarity, all sampled parameter values and log-likelihood scores from the cold Markov chain were plotted against generation time and compared among independent runs using TRACER 1.4 (Rambaut & Drummond, 2007). Finally, we plotted the cumulative and non-overlapping split frequencies of the 20 most variable nodes, and compared split frequencies among independent runs using Are We There Yet? (AWTY; Wilgenbusch *et al.*, 2004). Although all samples showed patterns consistent with stationarity after 2.5 million generations (i.e. the first 10%), we conservatively discarded the first 20% of samples as burn-in.

Partitioned maximum likelihood (ML) analyses were conducted in GARLI 2.0.1019 (Zwickl, 2006) for all three datasets under the same partitioning strategy used in Bayesian analyses and the BIC-selected substitution models for each subset (Table 1). We performed 10 independent search replicates with starting trees generated using random-order stepwise additions with 100 attachments per taxon. Each replicate was terminated when there were no topological improvements in log-likelihood > 0.01 for more than 10,000 generations, and no overall improvement in log-likelihood > 0.001 for more than 500 generations. To assess clade support, we ran 1000 bootstrap replicates under the same settings, except with one tree search per bootstrap replicate.

Topological hypotheses

We tested the following taxonomic and geographical based hypotheses to address the following questions concerning the patterns of *Gekko* diversification (Table 2). (1) Is the radiation of species of the genus *Gekko* in the Philippines monophyletic? (2) Are the Philippine populations of the widespread species *G. monarchus* monophyletic? (3) Are populations within the historically accepted distributions (Brown & Alcalá, 1978) of *G. mindorensis* and *G. monarchus* monophyletic? (4) Does the PAIC model of diversification explain the patterns of genetic diversity found in widespread species of Philippine *Gekko*? (5) Are the patterns of genetic diversity in widespread species of Philippine *Gekko* similar to those noted in other lineages (Siler *et al.*, 2010)?

We estimated the probability of each hypothesis within a Bayesian framework. The topological hypotheses for these questions are outlined in Table 2, with hypotheses 1–4 derived from taxonomic questions and hypotheses 5–8 derived from PAIC predictions; the remaining hypotheses (9–12) have been observed in other taxa and are, in part, derived from

Table 2 Description of topology tests conducted on Philippine species of *Gekko* using Bayesian methods.

Hypothesis	Description of constraint	Posterior probability	
Higher-level tests of Philippine <i>Gekko</i> diversity			
<i>Taxonomy-based hypotheses</i>			
H ₁	Monophyly of Philippine <i>Gekko</i>	All samples of Philippine <i>Gekko</i> populations	0.0
H ₂	Monophyly of Philippine <i>G. monarchus</i> populations	All <i>G. monarchus</i> samples from Palawan	0.0
H ₃	Monophyly of <i>G. mindorensis</i> , <i>sensu</i> Brown & Alcala (1978)	All <i>G. mindorensis</i> samples from Mindoro, Calagna-an, Caluya, Carabao, Negros, Panay, Guimaras, Cebu, Mactan, Semirara, Sico gon and Masbate islands	0.0
H ₄	Monophyly of <i>G. monarchus</i> , <i>sensu</i> Brown & Alcala (1978)	All <i>G. monarchus</i> samples from Indonesia, Malaysia and Palawan Island (Philippines) and all <i>G. mindorensis</i> samples from Mindanao, Camiguin Sur, Panglao and Luzon islands	0.0
Fine-scale tests of diversification patterns of <i>G. mindorensis</i>			
<i>Hypotheses consistent with the PAIC model of diversification</i>			
H ₅	Monophyly of Luzon PAIC populations	All <i>G. mindorensis</i> samples from Luzon, Palau and Verde islands	0.0
H ₆	Monophyly of Negros–Panay PAIC populations	All <i>G. mindorensis</i> samples from Masbate, Negros and Panay islands	0.0
H ₇	Monophyly of Mindoro PAIC populations	All <i>G. mindorensis</i> samples from Mindoro Island	0.0
H ₈	Monophyly of Mindanao PAIC populations	All <i>G. mindorensis</i> samples from Camiguin Sur and Panglao islands	1.0
<i>Hypotheses consistent with patterns observed in previous studies</i>			
H ₉	Monophyly of Luzon PAIC + Lubang Island populations	All <i>G. mindorensis</i> samples from Lubang, Luzon, Palau and Verde islands	0.0
H ₁₀	Monophyly of Mindoro PAIC + Lubang Island populations	All <i>G. mindorensis</i> samples from Mindoro and Lubang islands	0.0
H ₁₁	Monophyly of Mindanao + Negros–Panay PAIC populations	All <i>G. mindorensis</i> samples from Camiguin Sur, Masbate, Negros, Panay and Panglao islands	0.0
H ₁₂	Monophyly of Luzon + Mindoro PAIC populations	All <i>G. mindorensis</i> samples from Luzon, Mindoro, Palau and Verde islands	0.0

PAIC, Pleistocene aggregate island complexes.

expectations based on geological history and/or island proximity. We used the proportion of 16,004 post burn-in trees consistent with each hypothesis as an estimate of the posterior probability of the hypothesis.

Haplotype networks for *Gekko mindorensis*

We explored hierarchical relationships at the population level using the program *tcs* 1.18 (Clement *et al.*, 2000) to link haplotypes into a statistical parsimony network. *tcs* uses the relationship of inferred ancestral (interior) haplotypes relative to younger (tip) haplotypes to make inferences about spatial patterns of genetic variation.

Timing of diversification

To estimate an ultrametric, time-calibrated phylogeny, we used a Bayesian relaxed molecular clock model implemented in *BEAST* 1.6.1 (Drummond & Rambaut, 2007). The absence of a fossil record for *Gekko* or closely related members of the family Gekkonidae prevents the use of fossil-based node-age calibrations within this group. However, Gamble *et al.* (2011)

recently used five fossil/biogeographical calibrations to estimate the timing of divergences among higher-level gecko relationships. Their estimate of the 95% highest posterior density (HPD) for the divergence time between *Cyrtodactylus* and *Gekko* was 70.13–97.77 Ma (exact numbers provided by authors). To calibrate our analysis, we used a gamma prior distribution – $\Gamma(139.7, 0.5969)$ – for the root node age that places 95% of the prior probability between 70.13 and 97.77 Ma. To assess the sensitivity of this prior, we also performed analyses using a uniform prior distribution – $U(70.13, 97.77)$ – for comparison. We partitioned the alignment into five subsets (*ND2* codons, tRNAs and *PDC*), applying a separate BIC-selected nucleotide substitution model (Table 1) to each. We allowed branch-specific rates of substitution to vary across the tree according to uncorrelated lognormal distributions (UCLDs) (Drummond *et al.*, 2006). We applied separate UCLDs to the mitochondrial and nuclear data. For both UCLD relaxed-clocks, we used an exponential prior distribution with a mean of 0.01 for the standard deviation. We used a Yule process tree prior and left all remaining priors at default values. We ran four independent analyses of 50 million generations, sampling the parameter

values every 20,000 generations. We assessed stationarity of the chains by plotting parameter values and likelihood scores of all four chains over generations, and confirming congruence among consensus trees across the four chains. We conservatively discarded the first 500 samples as burn-in and combined the remaining 2000 samples across all four independent MCMC chains.

We recognize that any approach using secondary calibration points is subject to a variety of sources of error (Graur & Martin, 2004). However, we proceeded with the analysis presented here to provide an estimate of the temporal framework for diversification of Philippine *Gekko*, which may be re-evaluated in future analyses if more rigorous calibrations become available.

Biogeographical reconstructions

To explore whether there is statistical support for historical biogeographical patterns within Philippine species of the genus *Gekko*, we compared empirically observed (extant) species distributions to estimates of ancestral distributions using the program BAYESTRAITS 1.0 (Pagel, 1994; Pagel & Lutzoni, 2002). Using LOGCOMBINER (BEAST 1.6.1; Drummond & Rambaut, 2007), the set of chronograms resulting from divergence dating analyses was sampled at a lower frequency, resulting in a set of 1000 chronograms. Each topology was reduced to a single lineage per species for biogeographical reconstructions. For *G. monarchus*, we included a representative from Palawan Island. Given the sampling included in this study and the geological history of the Philippines, the assumption that populations of *G. monarchus* on Palawan Island represent the initial source for the species is the most parsimonious choice. This approach requires only two subsequent dispersal events to explain populations in Malaysia and Sulawesi, rather than a minimum of three dispersal events under a scenario where the source population occurs in Malaysia or Sulawesi.

In BAYESTRAITS, we ran MCMC chains for 25 million generations, sampling every 5000 generations. We discarded the first 50% of samples as burn-in, leaving 2500 samples to summarize the posterior probabilities of ancestral character states. The 'AddNode' command of BAYESTRAITS was used to specify all nodes in the chronograms for visualization of the posterior probabilities of character states at each node.

RESULTS

Taxon sampling, data collection and sequence alignment

The complete, aligned matrices contain 21 and nine samples of non-Philippine species for the mitochondrial and nuclear datasets, respectively. Following initial unrooted analyses, and assuming the root of the tree does not lie within Philippine *Gekko*, we polarized the tree using samples of gecko species in the genus *Cyrtodactylus*. Within each dataset, variable charac-

ters were observed as follows: 890 out of 1263 for the mitochondrial data and 34 out of 419 for the nuclear data.

Phylogenetic analyses

Analyses of the combined data (*ND2* + *PDC*) resulted in topologies with high bootstrap support (ML) and posterior probabilities (Fig. 2), with topologies congruent across all analyses. Interestingly, *Ptychozoon kuhli* is recovered as sister to all species in the genus *Gekko* included in this study (Fig. 2). *Gekko hokouensis*, *G. japonicus* and *G. chinensis* make up a well-supported clade sister to all Philippine gekkonid species (Fig. 2). Within this clade, *G. hokouensis* is recovered as the sister species to *G. japonicus* (Fig. 2, Clade A), with no analyses recovering *G. hokouensis* as nested within the Philippine radiation of *Gekko* species (or even closely related to sampled populations). Given the possibility that the chosen root for analyses (*G. gekko*), and the outgroup sampling strategy, may influence outgroup relationships, additional taxa should be obtained for a more exhaustive analysis of gekkonid relationships before definitive conclusions may be drawn concerning outgroup relationships.

Among the diversity of species found in the Philippines, the large and morphologically distinct Palawan Island endemic *G. athymus* was recovered in all analyses to be the sister to all other Philippine species (Fig. 2, Clade B). Most analyses resulted in moderate support for the position of *G. ernstkelleri* in the phylogeny (Fig. 2, Clade C); however, the species was sometimes recovered as part of a three-clade polytomy with Clades D and E (Fig. 2). With the exception of *G. palawanensis*, all other range-restricted, island (or island group) endemics were recovered as part of a single clade in all analyses (Fig. 2, Clade D). Sampled island populations of *G. romblon* on Romblon and Tablas islands were observed as part of a genetically structured clade, with an apparent deep divergence between the recently described (Brown *et al.*, 2011) Sibuyan Island endemic species *G. coi* (Fig. 2, Clade D). No genetic structure was observed between populations of *G. gigante* from North Gigante and South Gigante islands (Fig. 2, Clade D). The newly discovered *G. carusadensis* from Luzon Island was recovered as the sister to all island endemics from the Babuyan and Batanes island groups off the north coast of Luzon (Fig. 2, Clade D). Interestingly, no analyses supported the monophyly of species from the Babuyan Island Group, as *G. rossi* from Calayan Island was always recovered as sister to *G. porosus* from the Batanes Island Group (Fig. 2, Clade D).

The smallest Philippine species of *Gekko*, *G. palawanensis*, was recovered as the sister species to the widespread (as currently recognized) species *G. mindorensis* and *G. monarchus* (Fig. 2, Clade E). The three species known to occur on Palawan Island are not recovered as each other's closest relatives, but rather part of three distinct clades (Fig. 2, Clades B, E, G).

The two widely distributed species, *G. mindorensis* (a Philippine endemic) and *G. monarchus* (a more widespread Southeast Asian species), were recovered as reciprocally monophyletic clades in all analyses (Fig. 2, Clade F). However, northern and southern populations of *G. monarchus* from

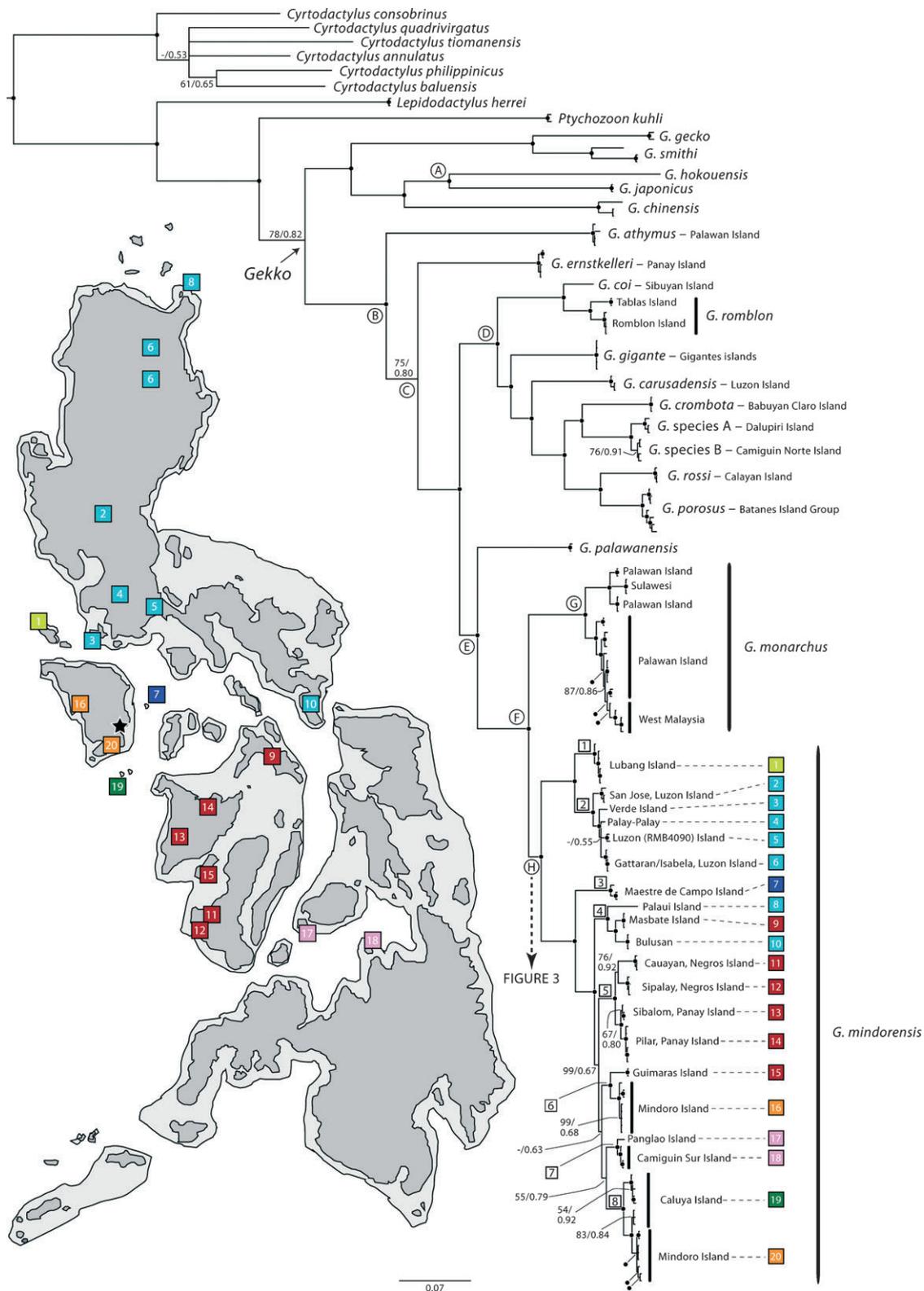


Figure 2 Hypothesized relationships of Philippine species of *Gekko*, illustrated by the maximum clade credibility tree resulting from Bayesian analyses. Nodes supported by $\geq 95\%$ Bayesian posterior probabilities (PP) and $\geq 70\%$ maximum likelihood bootstrap proportion (MLBP) were considered significantly supported. Terminals are labelled with taxonomic names and sampling localities (Appendix S1). Alpha and numerical labels correspond to clades referred to in the text. Sampling localities among the eight well-supported clades of *G. mindorensis* are indicated by coloured, numerically labelled boxes, each shown on the map of the Philippines. The scale bar represents the number of nucleotide substitutions per site.

Palawan Island were never recovered as a monophyletic group (Fig. 2, Clade G). The northern populations on Palawan Island were always recovered as part of a genetically structured clade with populations of *G. monarchus* from Sulawesi (Fig. 2, Clade G). In contrast, the southern populations on Palawan Island were recovered as part of a well-supported, and genetically structured, clade, which includes populations of *G. monarchus* from Peninsular Malaysia (Fig. 2, Clade G). Although the previously hypothesized distribution of *G. monarchus* in the Philippines (Brown & Alcalá, 1978) included island populations from Palawan, the Mindanao (Camiguin Sur and Panglao islands) and Luzon (Luzon, Verde and Palauí islands) PAICs, we have confirmed only Palawan populations to be morphologically diagnosable as *G. monarchus* and only Palawan populations were recovered as part of the *G. monarchus* clade (Fig. 2, Clade G).

The widespread endemic Philippine species *G. mindorensis* was always recovered as the sister taxon to the widespread non-endemic taxon *G. monarchus* (Fig. 2, Clade H). Populations from across the Philippines make up a highly structured group with eight well-supported clades that do not adhere to commonly accepted faunal boundaries (Fig. 2, Clades 1–8). With the exception of populations from the Mindanao PAIC (Fig. 2, Clade 7), no analyses supported the monophyly of populations from Philippine PAICs. *Gekko mindorensis* sampled near the type locality on Mindoro Island (Fig. 2, Clade 8, Haplotype Group 20) was always recovered as sister to populations from the geographically proximate, deep-ocean island of Caluya. Surprisingly, the population of *G. mindorensis* sampled from western Mindoro Island was never recovered as part of Clade 8, but rather as the sister population to specimens collected on Guimaras Island in the Negros–Panay PAIC (Fig. 2, Clade 6). Populations from Negros and Panay islands, both part of the Negros–Panay PAIC, were always recovered as a monophyletic group (Fig. 2, Clade 5). Interestingly, the Masbate Island (Negros–Panay PAIC) population was recovered as part of a well-supported clade including populations from the southern tip of the Bicol Peninsula on Luzon Island and Palauí Island off the north-eastern coast of Luzon, both recognized as members of the Luzon PAIC (Fig. 2, Clade 4). The deep-ocean islands of Lubang and Maestre de Campo both have genetically distinct populations of *G. mindorensis* (Fig. 2, Clades 1 and 3). The genetically distinct population from Lubang Island is always recovered as the sister to a clade of populations from across Luzon Island (Fig. 2, Clade 2). Although all analyses supported eight distinct clades making up the *G. mindorensis* complex, three nodes regularly received lower support, making it difficult to infer the relationships among Clades 4–8 (Fig. 2).

Topology tests

Among the taxonomy-based hypotheses, the Bayesian method provided no support (posterior probability approaching zero) for the monophyly of the genus *Gekko*, the monophyly of

G. monarchus, and the monophyly of the previously hypothesized distributions of the widespread species *G. mindorensis* and *G. monarchus*, *sensu* Brown & Alcalá (1978) (Table 2). All biogeography-based hypotheses that focused on *G. mindorensis* had posterior probabilities approaching zero, except for the hypothesis of monophyly for Mindanao PAIC populations of *G. mindorensis* (Table 2).

Haplotype networks

Haplotype networks for each clade of *G. mindorensis* reveal that most clades contain a few highly similar haplotypes that are restricted to a single PAIC or small island (Fig. 3). Clades that contain populations from larger islands have more haplotype diversity (e.g. Luzon and Negros–Panay; Fig. 3). Some clades have haplotypes occurring on multiple islands (Fig. 3, Clades 4, 6, 8), but only in Clade 8 do they form a network connecting samples from a PAIC and a distinct deep-ocean island. The small islands of Lubang and Maestre de Campo contain few haplotypes, but they are unique and do not form connections to other nearby islands (Fig. 3). Caluya has two sets of divergent haplotypes, one of which forms a network with the geographically proximate island of Mindoro (Fig. 3).

Timing of diversification

The four separate BEAST analyses converged on the same parameter space and were combined for further analysis using LOGCOMBINER (BEAST 1.6.1; Drummond & Rambaut, 2007). Due to the absence of internal calibrations, the confidence intervals for several node dates, especially those deeper in the chronogram, are broad (Fig. 4); however, the results still provide numerous interesting insights into the timing of diversification among lineages of *Gekko* in the Philippines.

The majority-rule consensus chronogram resulting from BEAST analyses using a gamma-distributed root age prior mirrors the ML and Bayesian topology (Fig. 4). The results when using a uniform root age prior were nearly identical (data not shown). The estimated mean rate for the mitochondrial subset was 1.55% Myr⁻¹, which is approximately threefold faster than the previously estimated mitochondrial substitution rate of 0.57% Myr⁻¹ in another group of geckos (Macey *et al.*, 1999; Fujita & Papenfuss, 2011). Not surprisingly, in some preliminary analyses using the 0.57% Myr⁻¹ rate as a calibration yielded divergence time estimates that were unrealistically old (data not shown). These results indicate that the radiation of *Gekko* species in the Philippines represents an old clade, having first entered the archipelago *c.* 38.9–21.2 Ma (95% HPD; Fig. 4, Nodes A, B). Diversification events on Palawan Island are inferred to have taken place during the island's approach to the rest of the Philippines, coincidental with its proximity to the Asian mainland during the opening of the South China Sea (Hall, 1996, 1998), supporting the Palawan Ark Hypothesis of Blackburn *et al.* (2010).

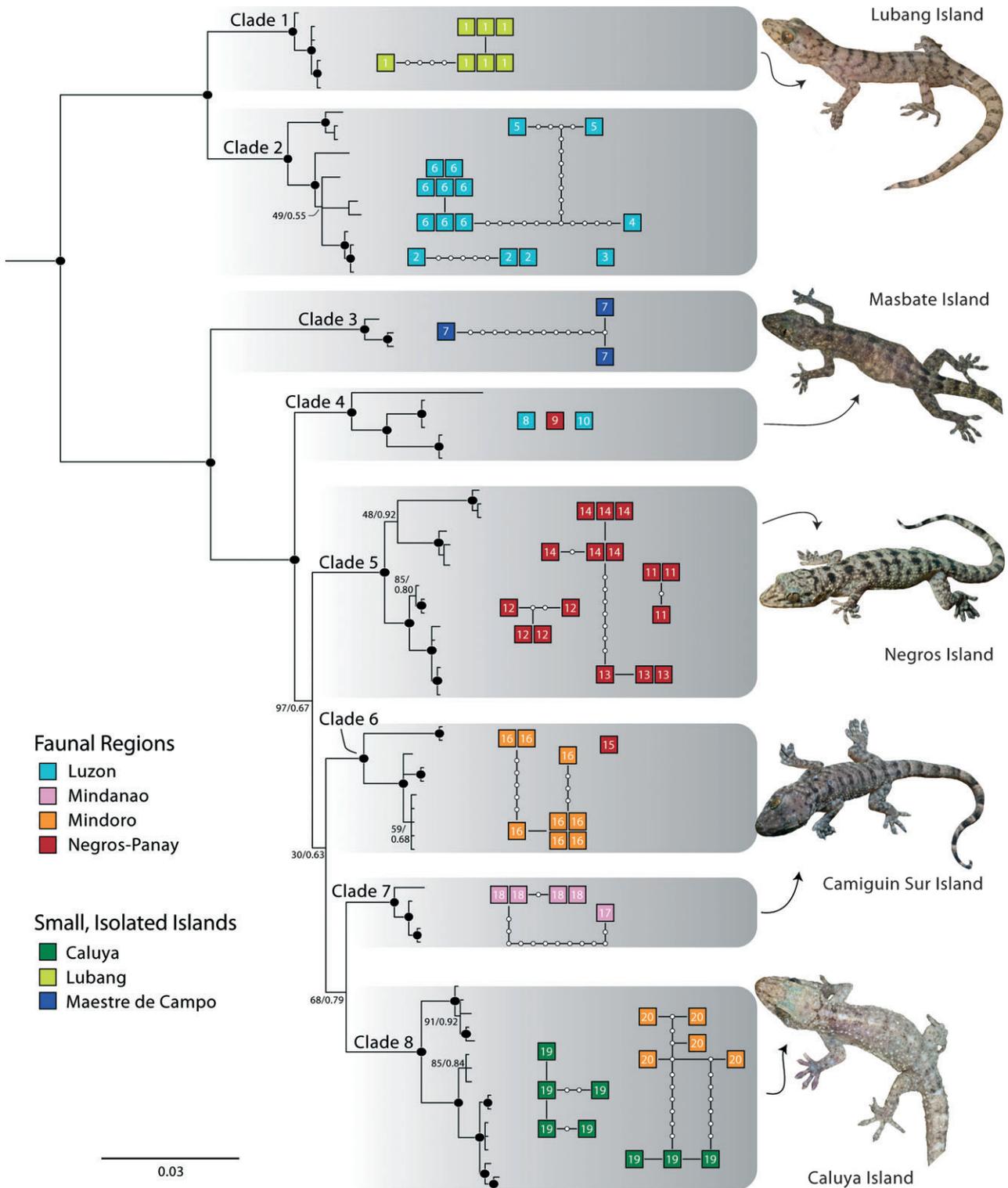


Figure 3 Expanded Clade H from Fig. 2 showing the hypothesized relationships among the eight well-supported clades of *Gekko mindorensis*. Nodes supported by $\geq 95\%$ Bayesian posterior probabilities (PP) and $\geq 70\%$ maximum likelihood bootstrap proportion (MLBP) were considered significantly supported and are marked by black dots. Each clade is shown with its corresponding minimum spanning, 95% connection probability, statistical parsimony haplotype network. Haplotype numeric labels and colours correspond to Philippine Pleistocene aggregate island complexes (PAICs) and small, deep-ocean islands (refer to Fig. 2). Photographs showing the variation in morphology across members of the eight clades are provided for reference. The scale bar represents the number of nucleotide substitutions per site.

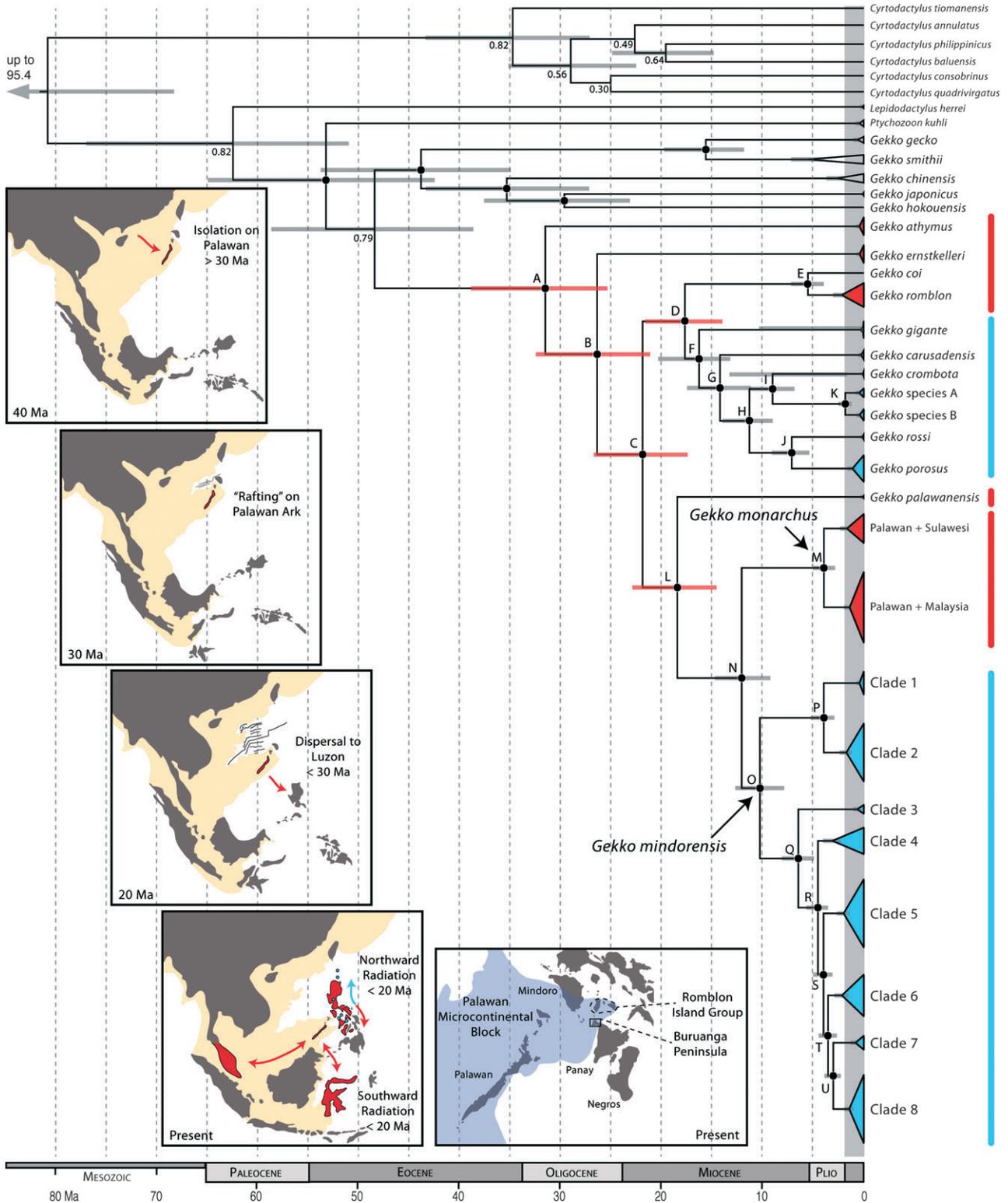


Figure 4 The 50% majority-rule consensus tree of Philippine species of *Gekko* with median node ages from the BEAST posterior Markov chain Monte Carlo (MCMC) sample. Bars at nodes represent the 95% highest posterior density of the node age. The letters at internal nodes correspond to those in Appendix S2. Clades have been collapsed for clarity, and represent unique species, or in the case of the widespread *Gekko mindorensis* and *G. monarchus*, well-supported clades. The Pleistocene is indicated by a vertical grey bar. Red confidence intervals highlight inferred diversification events consistent with the Palawan Ark Hypothesis. The inset figures illustrate the proposed Palawan Ark Hypothesis (Blackburn *et al.*, 2010) and location of the Palawan microcontinental block, based on reconstructions of geological history of Southeast Asia. Tan regions represent submarine continental margins. Large, coloured arrows indicate proposed dispersal routes; small grey contour lines indicate sea-floor spreading.

With few exceptions, our results indicate that all diversification events giving rise to the widespread species *G. monarchus* and *G. mindorensis* pre-date the Pleistocene and occurred during the Pliocene and Miocene (Fig. 4, Nodes L–U). With the exception of Clade 4, all clades of *G. mindorensis* (Fig. 4) were observed to have 95% HPD intervals that extended into the Pleistocene. In comparison, there is evidence for a northward progression of diversification events beginning around 21.9 Ma (95% HPD, 26.8–17.4 Ma; Fig. 4, Nodes C–K), originating in the central Philippine islands. These early divergences gave rise to the remaining recognized, range-restricted species. The 95% HPD intervals corresponding to the diversification events that gave rise to the numerous endemic lineages on islands in the extreme northern Philippines (Babuyan and Batanes island groups) coincide roughly with the published ages of these deep-ocean islands (Yang *et al.*, 1996).

Historical biogeography

The focus of our ancestral state reconstruction analyses was to explore whether we could reconstruct the geographical range

for the ancestral populations with surprisingly old inferred divergence dates (Fig. 5, Nodes A, B, C, D, L). The results of analyses of ancestral areas are never significantly impacted by placing restrictions on the ancestral character states among outgroup taxa and the node giving rise to all Philippine species in the genus *Gekko* (not shown). Without *a priori* knowledge of the true patterns of diversification within the region, we conservatively chose a model allowing for equal rates of transition among three major biogeographical regions: (1) mainland Asia and the Sunda Shelf, (2) Palawan microcontinental block, and (3) oceanic Philippines. The reconstructed ancestral ranges for the nodes of interest were slightly to moderately ambiguous (Fig. 5); however, despite these results, ancestral range reconstructions at several focal nodes received moderate to high posterior probabilities (PP) (Fig. 5). The analyses recover moderate to high support (0.85–0.94 PP) for all Philippine species of *Gekko* to be derived from an ancestral Palawan microcontinental lineage (Fig. 5). Additionally, the two widespread species (*G. monarchus* and *G. mindorensis*) and *G. palawanensis* are supported to be derived from an ancestral Palawan microcontinental lineage (0.87 PP; Fig. 5).

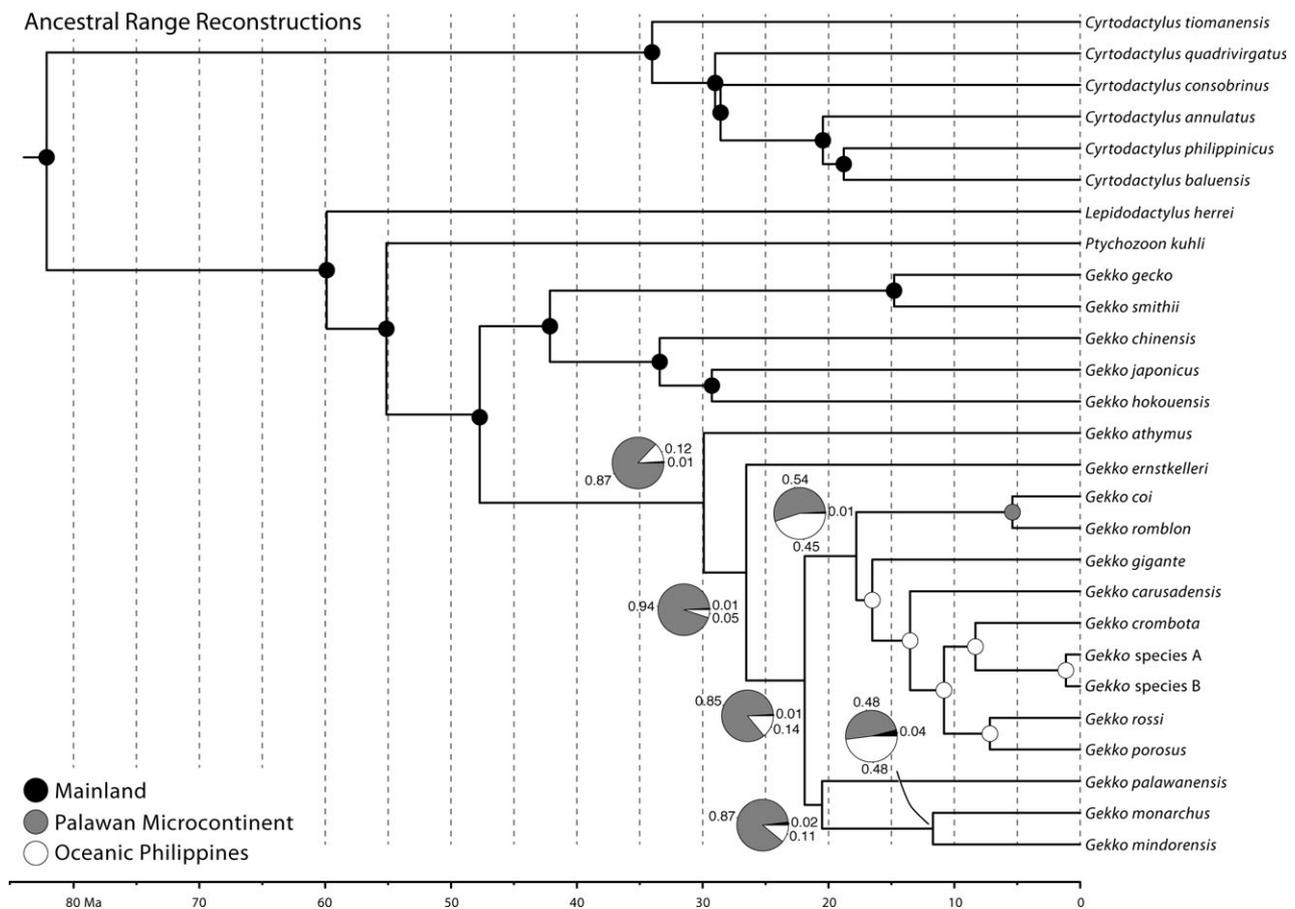


Figure 5 Maximum clade credibility chronogram and estimated biogeographical reconstructions of Philippine species of *Gekko*. Ancestral state reconstructions are indicated at each node. Small circles indicate unambiguous reconstructions of a character state (posterior probability ≥ 0.95), coloured according to the hypothesized state. Large pie graphs represent ambiguous reconstructions, with shades and percentages summarizing the posterior probabilities for each region.

DISCUSSION

Taxon sampling

Our widespread geographical sampling across the range of most Philippine species has allowed for robust estimation of phylogenetic relationships and phylogeographical resolution of the geographical basis of inter-population genetic diversity. While our attempt to sample outgroup taxa widely resulted in well-supported relationships, we have not included samples for much of the gekkonid species diversity known to occur in southern mainland Asia (Manthey & Grossman, 1997; Nguyen *et al.*, 2009; Rösler *et al.*, 2011). However, our results are consistent with respect to a recent higher-level phylogenetic analysis of the genus *Gekko* (Rösler *et al.*, 2011). In this study, the Philippine radiation of species in the genus *Gekko* were supported to be sister to a clade of four groups of gekkonid lizards (*G. gecko* group, *G. japonicus* group, *G. petricolus* group, *G. vittatus* group; Rösler *et al.*, 2011).

Phylogenetic relationships and natural history of Philippine *Gekko*

Although we focus on diversity within Philippine *Gekko*, our results provide interesting insight into historical taxonomic anomalies. Fourteen species of *Gekko* (three of which remain undescribed) are considered to be endemic to the archipelago (Brown *et al.*, 2011); however, past treatments have included *G. hokouensis* in the Philippine fauna (Brown & Alcala, 1978). *Gekko hokouensis* is recovered as the sister species to *G. japonicus* (Fig. 3, Clade A) and never recovered as part of the Philippine radiation, a finding supported by recent analyses of species relationships within the genus *Gekko* (Rösler *et al.*, 2011). We suggest that *G. hokouensis* be removed from future considerations of the archipelago's species of *Gekko*.

The earliest-diverging lineages in the Philippine clade appear to have given rise to *G. athymus* (Palawan Island) and *G. ernstkelleri* (Panay Island), with the caveat that the phylogenetic position of *G. ernstkelleri* is not well supported. Subsequent diversification events appear to have been centred on the northern portions of the archipelago, with five species forming a clade from the extreme northern islands of the Babuyans and Batanes, including *G. species A* (Dalupiri Island), *G. species B* (Camiguin Norte Island), *G. species C* (unsampled here; from Fuga Island; see Brown *et al.*, 2009a), *G. crombota*, *G. rossi* and *G. porosus* (Oliveros *et al.*, 2011). A second major clade is composed of the Palawan species *G. palawanensis* and *G. monarchus* (with extra-archipelago populations on the Malaysian Peninsula and Sulawesi) sister to a central-northern archipelago clade, currently assigned to a single species, *G. mindorensis*.

Our analyses include extremely robust geographical sampling, accumulated from 20 years of survey work throughout the archipelago. Although additional species discoveries are expected (see discussion in Brown *et al.*, 2011), we anticipate that the major clades of Philippine *Gekko* have now been

characterized. Assuming this is correct, one interesting pattern that emerges is the distinction between small-island endemics, within-island geographically localized microendemisms, and geographically widespread taxa. Our analyses have confirmed the genetic distinctiveness of the isolated small-island endemics (*G. coi*, *G. crombota*, *G. gigante*, *G. porosus*, *G. rossi* and the additional three undescribed species from the Babuyans-Batanes and Romblon Province) and the newly discovered morphologically divergent limestone karst specialists, *G. carusadensis* and *G. ernstkelleri* (Brown *et al.*, 2011). Like the small-island endemic *G. gigante*, these species appear to be isolated by ecologically unique subterranean habitats and overlying nutrient poor limestone forests (Poffenberger & Smith-Hanssen, 2004; Clements *et al.*, 2006). At the opposite end of the spectrum of ecological specialization, this study reveals the widespread nature of the presumptive ecological generalists *G. mindorensis* and *G. monarchus*. Distributed throughout the Luzon, Mindoro and Visayan PAICs (Fig. 3), *G. mindorensis* occurs in a wide range of habitats from pristine forests, to isolated rock formations, to highly disturbed regenerated forests, to caves and agricultural areas (Brown & Alcala, 1978; C.D.S., pers. obs.). On Palawan and the Malay Peninsula, *G. monarchus* is a widespread, highly abundant ecological generalist found in a variety of habitats. In contrast, the Sulawesi population appears to be far less abundant, behaviourally unique (extremely secretive) and restricted to large boulders in riparian habitats (R.M.B., pers. obs.).

Although previously considered widespread in parts of the western (Palawan), northern (Luzon) and southern Philippines (Samar and Mindanao) (Brown & Alcala, 1978), specimens matching the description of *G. monarchus* (C.D.S., R.M.B. & L. Grismer, pers. obs.) are recovered only on Palawan Island in the Philippines (with apparent non-Philippine populations derived from dispersal events to the Malaysian Peninsula and Sulawesi, Indonesia). Although it is conceivable that in our two decades of sampling we missed *G. monarchus* on the islands of Luzon, Samar and Mindanao, we find it more likely that earlier records of this species were based on locality errors and/or misidentifications of *G. mindorensis* specimens.

Diversification patterns and species diversity in *G. mindorensis* and *G. monarchus*

Cryptic diversity, defined as the presence of two or more species when only one has been identified by morphological characters, has been documented as a global phenomenon (Bickford *et al.*, 2007; Pfenninger & Schwenk, 2007), and numerous recent studies suggest this also characterizes some Philippine gekkonid lineages (for review see Brown *et al.*, 2011). Dense population sampling across the range of *G. mindorensis* and *G. monarchus* revealed substantial genetic structure and distinct clades of previously undocumented diversity. *Gekko monarchus* samples segregate into two non-sister clades, with populations from northern and southern Palawan closely related to populations on Sulawesi and Peninsular Malaysia, respectively (Fig. 3, Clade G). *Gekko*

monarchus has a much wider distribution in Asia than is represented by the sampling in this study (Manthey & Grossman, 1997), and the inclusion of additional samples may reveal the widespread species to be yet another example of a cryptic species complex. A strange pattern of population genetic diversity is observed for *G. mindorensis*, with analyses recovering eight well-supported highly divergent clades that partition genetic diversity across the species' range (Figs 3 & 4). Past consideration of the range of morphological variation exhibited by specimens from the full range of *G. mindorensis* revealed only slight morphological variation (Brown & Alcalá, 1978). However, upon closer inspection of populations from distinct genetic clades (Figs 3 & 4), multiple diagnostic morphological characters are apparent (C.D.S. & R.M.B., pers. obs.). Thus, we suspect that what initially appeared to be a single widely distributed species, will probably be found to represent a complex of numerous cryptic species (C.D.S. & R.M.B., unpublished data). Taxonomic studies of other Philippine gekkonids (for review see Welton *et al.*, 2010) suggest that examination of non-traditional characters, such as variation in the number of post-cloacal lateral tubercles, patterns of snout scalation, and subtle variation in dorsal and lateral patterning on the head and the trunk, and in the ground and pattern body coloration, may be most useful for resolving remaining cryptic species boundaries.

The morphologically similar body plans within the species *G. mindorensis* and *G. monarchus* have led to confusion over species boundaries and the recognition of 'widespread' species with distributions across accepted faunal boundaries (Brown & Diesmos, 2009). The degree to which conservative, convergent or pleisiomorphic morphology has led to the underestimation of diversity within the genus is presently unknown.

One line of inference can be drawn by comparison to another densely sampled complex of widespread gekkonid species endemic to the Philippines. In another recent study, Siler *et al.* (2010) found similar patterns of highly divergent, geographically isolated genetic lineages circumscribed within the 'widespread' species in the genus *Cyrtodactylus* (*C. annulatus*, *C. agusanensis*, *C. philippinicus*). Analyses revealed all three species to be made up of deeply divergent, well-supported clades, often occurring in geographically isolated regions of the Philippines (Siler *et al.*, 2010). Taxonomic revisions following this study have resulted in the subsequent revision of *C. annulatus* and *C. agusanensis* into three and four morphologically and genetically distinct species, respectively (Welton *et al.*, 2010); an additional analysis of the *C. philippinicus* clade is presently underway. Other recent studies have revealed additional 'widespread' Philippine endemic reptiles to represent complexes of cryptic species, with few species actually possessing distributions that span recognized faunistic boundaries (Siler *et al.*, 2011a,b). Exceptions exist (Brown & Alcalá, 1970), with several of these possibly representing invasive species with suspected histories of human-mediated introductions (Diesmos *et al.*, 2006; Brown *et al.*, 2010). It is clear that the diversity of Philippine *Gekko* is considerably underestimated.

We rejected all topologies predicted from a PAIC-based model of diversification, as well as all hypotheses derived from patterns observed in other taxa. Many recent studies employing robust sampling, multi-locus phylogenies and statistical tests capable of rejecting numerous correlates of the PAIC model have generated similar results; however, many studies have simultaneously found support for some patterns expected by the PAIC model (Evans *et al.*, 2003; Heaney *et al.*, 2005; Roberts, 2006a,b; Esselstyn & Brown, 2009; Siler *et al.*, 2010, 2011a). It is interesting to note that of the published phylogenetic studies on Philippine vertebrates, species of the genus *Gekko* appear to contradict a PAIC model of diversification the most, especially when considering the observed biogeographical patterns of *G. mindorensis* (Fig. 3). If the widespread species of *Gekko* have strong dispersal abilities, as has been observed in other gekkonid taxa (Brown & Alcalá, 1978; Fisher, 1997), this might explain phylogenetic patterns inconsistent with the expectations of the PAIC model. However, if *G. mindorensis* and *G. monarchus* are such good dispersers, why is there so much diversity? Another possible explanation would be that the divergent, well-supported clades of *G. mindorensis* and *G. monarchus* recovered in phylogenetic analyses (Fig. 3) represent complexes of morphologically similar species, as has been observed for numerous other widespread Philippine endemics (for review see Siler *et al.*, 2011b).

Ultimately, we suspect that the evolutionary history of many vertebrate clades in the Philippines is too complex to be explained by a single overriding biogeographical model. Nevertheless, we advocate continued tests of the many predictions derived from the PAIC model for several reasons. First, we acknowledge the predictive and heuristic value of the model for hypothesis testing; the simplicity and elegance of the model is an ideal first step for formulating hypotheses and testing them in a comparative context (Brown & Diesmos, 2002, 2009). We also note the idiosyncratic nature of the way in which various taxa have illustrated deviations from the model's expectations (Esselstyn & Brown, 2009; Linkem *et al.*, 2010; Siler *et al.*, 2010, 2011a) and emphasize the relative value of all of these for understanding the various roles of non-PAIC processes of diversification. Similarly, evidence for substantial *in situ* diversification via fine scale isolation of microendemics, local ecological adaptation, elevational species succession, or other within-island population genetic processes clearly results in diversity not explained by a pure PAIC model of diversification (Brown & Diesmos, 2002, 2009; Heaney *et al.*, 2005; Esselstyn & Brown, 2009; Linkem *et al.*, 2010; Siler *et al.*, 2010, 2011a).

More fine-scale investigations of diversity within *G. mindorensis* seem to indicate that for some clades of *G. mindorensis*, intra-island geography appears to have played a larger role in population diversification (e.g. Luzon Island; Fig. 2). Other species appear to have experienced recent and rapid dispersal across historically separate deep-water oceanic barriers. For example, *G. monarchus* appears to have recently dispersed between Palawan Island, Peninsular Malaysia and

Sulawesi, crossing Wallace's Line and dispersing through the Palawan Filter Zone (Esselstyn *et al.*, 2010; Fig. 4). Similar to patterns noted by Esselstyn & Brown (2009) and Siler *et al.* (2010), modern islands, as opposed to palaeo-islands, seem to best explain genetic diversity for Philippine geckos in the genus *Gekko*. However, based on available data, it seems likely that several factors, including sea-level fluctuations, geological uplift, plate tectonics, topography on large islands, clade age and clade dispersal ability, have played roles in determining the diversification trajectories of lineages. Combining all of these factors into a single predictive model would be challenging and, for the time being, testing models based solely on any one of these factors remains useful. The results of our study join a growing body of literature indicating that while PAICs have indeed influenced patterns of biodiversity partitioning, they are not the sole factor, and do not appear to be the predominant explanation for diversification in terrestrial vertebrates (Brown & Diesmos, 2009; Esselstyn & Brown, 2009; Linkem *et al.*, 2010; Siler *et al.*, 2010, 2011a).

Biogeographical patterns consistent with ark-driven diversification

Ancestral character state reconstructions and divergence dating methods are limited in that they provide only a statistical framework with which to investigate data. For ancestral range reconstructions, analyses are conducted often in the context of a reduced tree with branch lengths and a single character per terminus. Keeping these constraints in mind, we asked what our data tell us about the timing and origin of diversification events in Philippine *Gekko*.

If our inferences of phylogeny, ancestral ranges and timing of diversification events are correct, the results of this study suggest an important role for the Palawan microcontinental block in the spread and diversification of Philippine *Gekko*. Potentially the sister species to all remaining Philippine *Gekko* species, and with a lineage divergence as old as any others in the Philippine clade (Fig. 4, Node A), *G. athymus* and *G. ernstkelleri* are both phylogenetically and biogeographically distinct taxa that may represent the closest relatives of the lineages that initially invaded the Philippines. A forest specialist with a strictly crepuscular daily activity cycle (R.M.B., pers. obs.), *G. athymus* is not only behaviourally unique (remaining Philippine species are generally nocturnal but not strictly crepuscular), but also morphologically very distinctive and unlike other taxa in the archipelago (Brown & Alcalá, 1962, 1978; Brown *et al.*, 2009a). In contrast, *Gekko ernstkelleri* is a karst habitat specialist, found solely on rocky outcrops and within cave systems, a microhabitat preference shared among all range-restricted, island and intra-island endemic species of *Gekko* in the Philippines (C.D.S. & R.M.B., pers. obs.).

The phylogenetic positions of *G. athymus* and *G. ernstkelleri* suggest that ancestral members of the genus may have initially colonized the archipelago via the western island arc composed of Balabac, Palawan, Busuanga and Coron (Hall, 2002),

presumably from a Sundaic source (Dickerson, 1928; Inger, 1954). Entryway into the archipelago via the Palawan Island Arc adjacent to the Asian mainland has been identified in several studies (Diamond & Gilpin, 1983; Brown & Guttman, 2002; Jones & Kennedy, 2008; Brown *et al.*, 2009a; Oliveros & Moyle, 2010), although the importance of the Palawan Filter Zone as a potential source of ancient phylogenetic diversity and a springboard for initial diversification into the Philippines has not been appreciated until relatively recently (Brown & Guttman, 2002; Evans *et al.*, 2003; Blackburn *et al.*, 2010; Esselstyn *et al.*, 2010) due to a prevailing interpretation of Palawan as a young (e.g. < 5 Myr old) island treated as a faunal extension of northern Borneo (Steere, 1888; Everett, 1889; Heaney, 1985, 1986; Dickinson *et al.*, 1991; Esselstyn *et al.*, 2004).

It appears that nearly all species diversity in the Philippine clade is derived from one or more faunal exchanges between the Palawan microcontinental block and the remaining oceanic portions of the Philippines. The topology of our preferred phylogenetic estimate (Fig. 2) suggests that all range-restricted, island and intra-island endemic species in the central and northern Philippines (*G. carusadensis*, *G. coi*, *G. crombota*, *G. ernstkelleri*, *G. gigante*, *G. romblon*, *G. rossi*, *G. sp. A*, *G. sp. B* and *G. sp. C*) may be derived from an initial invasion of the Buruanga Peninsula, with subsequent northward dispersal and diversification (Fig. 4). In contrast, all widespread, microhabitat generalist species appear to be derived from lineages that may have evolved within the Palawan Island Arc (*G. athymus*, *G. monarchus*, *G. palawanensis*; Figs 4 & 5), whereas subsequent diversification consisted of southward dispersal events giving rise to Sundaic populations of *G. monarchus* and the establishment of the eight divergent lineages now referred to the widespread species *G. mindorensis* (Fig. 4).

CONCLUSIONS

Whatever the true pattern of colonization and subsequent diversification, our results stand in stark contrast to the expectations of distribution patterns derived from an earlier view of the Philippine fauna as a 'fringing archipelago' (Darlington, 1957; Brown & Alcalá, 1970). Earlier authors discussed the distribution of life in the Philippines as the presumed result of immigrant patterns, in which species groups are distributed along major migration routes (the eastern versus western island arcs of the Philippines). Furthermore, faunal dropouts were expected to be distributed linearly (and proportionally with distance from the source) along colonization routes in a manner consistent with each group's relative dispersal abilities (Dickerson, 1928; Taylor, 1928; Inger, 1954; Myers, 1960, 1962; Leviton, 1963; Brown & Alcalá, 1970). However, our study unequivocally demonstrates the preponderance of ancient, within-archipelago diversification that has given rise to an almost exclusively endemic clade of highly distinct and morphologically diverse Philippine species. Thus, Philippine geckos join a small but rapidly growing group of diverse vertebrate lineages in which

remarkable species diversity appears to have been produced by exclusively autochthonous processes on this small but remarkably dynamic island archipelago (Brown & Diesmos, 2009).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Summary of specimens corresponding to genetic samples included in the study.

Appendix S2 Median node ages and 95% highest posterior density in millions of years ago.

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BIOSKETCH

All authors are generally interested in the fields of historical biogeography, evolution and phylogenetics, especially in Southeast Asia. The authors are all involved in research programmes investigating the evolutionary history of various Southeast Asian amphibian and reptile lineages. By combining active field programmes with modern molecular and bioinformatic techniques, we hope to contribute to a synthetic understanding of the mechanisms driving diversification in Southeast Asia.

Author contributions: C.D.S. and R.M.B. conceived the ideas; C.D.S., R.M.B., L.J.W., C.W.L., J.C.S. and A.C.D. participated in fieldwork; C.D.S. collected the data; C.D.S. and J.R.O. analysed the data; C.D.S. led the writing; R.M.B., J.R.O., C.W.L., L.J.W. assisted in writing.

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