Archipelago-wide survey of Philippine forest dragons (Agamidae: *Gonocephalus*): multilocus phylogeny uncovers unprecedented levels of genetic diversity in a biodiversity hotspot

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We utilize robust geographical genetic sampling, and phylogenetic analysis of a new multilocus dataset to provide the first inference of relationships among Philippine *Gonocephalus*, combined with estimates of putative species diversity, in this almost unknown island radiation. Our results reveal startling levels of undocumented diversity, genetically partitioned at a number of geographical levels across the archipelago. We present the first survey of genetic lineage diversity, coupled with an archipelago-wide clarification of geographical structure in a unique archipelago-endemic radiation. Philippine *Gonocephalus* have previously escaped the attention of biogeographers as a result of the taxonomic confusion associated with low numbers of preserved specimens in museum collections. With new vouchered material and genetic sampling from a comprehensive, archipelago-wide vertebrate biodiversity inventory, our findings join many recent studies in highlighting the unprecedented faunal diversity in one of the world's most unique biodiversity conservation hotspots. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2017, **120**, 410–426.

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INTRODUCTION

Recent advances in molecular-based methods of candidate species identification and delimitation have allowed for increasingly robust inferences of the diversity of fundamental units of biodiversity and evolutionary history (Marshall *et al.*, 2006; Leaché & Mulcahy, 2007), fostering in a new era in biodiversity studies aimed at accurately estimating the species diversity of traditionally-recognized taxonomic groups (Welton *et al.*, 2010a, b; Barret &

Freudenstein, 2011; Brown *et al.*, 2012), phylogenetically-defined focal clades (Wiens & Penkrot, 2002; Rissler & Apodaca, 2007; Setiadi *et al.*, 2011; Welton *et al.*, 2014), and/or geographical regions (Knowles & Carstens, 2007; Brown & Diesmos, 2009; Brown *et al.*, 2013). A number of coalescent-based species delimitation methods have become standards in biodiversity studies, with an increasingly diverse set of approaches and data types now comprising the focus of integrative models for empowering species concepts with real data (Yang & Rannala, 2010; Fujita *et al.*, 2012; Fujisawa & Barraclough, 2013; Grummer, Bryson & Reeder, 2013; Solis-Lemus, Knowles & Ané, 2015).

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To approach species delimitation comprehensively and objectively, an initial best practice 'first step' is the application of a number of available data types (e.g. morphology, DNA sequence variation, biogeographical expectations) to identify putative species clusters. This initial 'discovery' step can then be followed by tests of hypothesized species splits using independent loci or metrics of phenotypic or ecological variation (species 'delimitation' phase: Brown *et al.*, 2012; Barley *et al.*, 2013; Carstens *et al.*, 2013; Welton *et al.*, 2013), which must then necessarily be followed by formal taxonomic revision (Siler & Brown, 2010; Welton *et al.*, 2010b) if we are to adequately recognize and enumerate biological diversity (Mace, 2004).

In the present study, we apply a number of molecular-based methods to identify candidate species boundaries and lineage diversity in a subset of the South-east Asia agamid genus *Gonocephalus*. These conspicuous, medium-bodied lizards ironically have been absent from contemporary studies addressing patterns of diversification, despite having a distribution spanning three Biodiversity Hotspots (Indochina, Sundaland, and Philippines: Myers *et al.*, 2000).

GONOCEPHALUS

The genus *Gonocephalus* comprises 16 recognized species (Uetz & Hošek, 2016) and is distributed throughout South-east Asia from the Thai-Malay Peninsula and Greater Sunda islands to the Philippines (Teynié *et al.*, 2004; Ananjeva, Orlov & Nguyen, 2007; Manthey, 2010; Grismer, 2011). Species of *Gonocephalus* are medium-sized, conspicuous (i.e. elaborate phenotypes, with brightly coloured scalation, ornamental crests along the neck and dorsum, and enlarged heads; Grismer, 2011) components of riparian forest environments across South-east Asia, when suitable arboreal perch sites (e.g. small saplings and understory tree trunks) are available. Given their extravagant ornamentation and conspicuous phenotypes, it is ironic that this group has largely escaped the scrutiny of herpetologists. To date, only six molecular phylogenetic studies have included samples of *Gonocephalus* (Honda *et al.*, 2000, 2002; Macey *et al.*, 2000; Schulte *et al.*, 2004; Pyron, Burbrink & Wiens, 2013; Grismer *et al.*, 2016). At the time of these studies, the taxon *G. robinsonii* was assigned to the genus *Gonocephalus*, although this problematic, non-monophyletic taxonomic inclusion in the genus (see below) was rectified by Denzer *et al.* (2015), who erected a novel genus for the taxon (*Malayodracon*).

Honda *et al.* (2002) examined karyotype variation within *Gonocephalus* and used ribosomal RNA to infer the relationships among just four of the species [*Gonocephalus miotympanum* (now synonomized with *Gonocephalus bornensis*; Manthey & Denzer, 1992), *Gonocephalus grandis*, *Gonocephalus chamaeleontinus*, and *Malayodracon robinsonii*]. Macey *et al.* (2000) and Schulte *et al.* (2004) incorporated just a single sample (*G. grandis*), whereas Honda *et al.* (2000) and Pyron *et al.* (2013) included only four taxa [*G. chamaeleontinus*, *G. miotympanum* (*G. bornensis*), *G. grandis*, and *M. robinsonii*, and *G. chamaeleontinus*, *Gonocephalus kuhlii*, *G. grandis*, and *M. robinsonii*, respectively] to investigate higher-level relationships among agamids. These three previous studies incorporating multiple *Gonocephalus* taxa, regardless of the molecular markers and analyses applied [mitochondrial DNA (mtDNA) (Honda *et al.*, 2000), ribosomal RNA (Honda *et al.*, 2002) or mtDNA and nuclear loci (nuDNA; Pyron *et al.*, 2013)], recovered *M. robinsonii* as a distantly related lineage to a well-supported and otherwise monophyletic *Gonocephalus* clade.

Manthey & Denzer (1991) conducted the only genus-wide survey of morphological variation, and included all of the taxa recognized at the time of their study. Their work resulted in the designation of five phenotypically cohesive 'morpho-groups' (the generalized distribution of each taxon follows in parentheses): (1) the *belli* Group consisting of *Gonocephalus bellii* (Thai-Malay Peninsula), *Gonocephalus beyschlagi* (Sumatra), *G. bornensis* (Borneo), *Gonocephalus interruptus* (Philippines), *Gonocephalus liogaster* (Borneo, Thai-Malay Peninsula), *Gonocephalus semperi* (Philippines), and *Gonocephalus sophiae* (Philippines); (2) the *grandis* Group consisting only of *G. grandis*; (3) the *chamaeleontinus* Group consisting of *Gonocephalus abbotti* (Thai-Malay Peninsula), *G. chamaeleontinus* (Thai-Malay Peninsula, Sumatra, Natuna Islands), *Gonocephalus doriae* (Borneo), and *G. kuhlii* (Java, Sumatra); (4) the *megalepis* Group consisting of *Gonocephalus klossi* (Sumatra), *Gonocephalus lacunosus* (Sumatra), and *Gonocephalus megalepis* (Sumatra); and (5) the *robinsonii* Group consisting of *M. robinsonii* (Thai-Malay Peninsula; Denzer *et al.*, 2015) and *Gonocephalus mjobergi* (Borneo). Although the inter-relationships among these groups were not posited, the groupings themselves provide reasonable hypotheses for sub-generic affinities for *Gonocephalus*. Given the morphological variation within the genus, marked sexual dimorphism in most species, the preference for forested habitats, and a distribution spanning one of the most biologically diverse and geologically complex regions on the planet, *Gonocephalus* represents an untapped system for studying patterns of faunal diversification, regional adaptation, and

historical biogeography of South-east Asia (Woodruff, 2010; Lohman *et al.*, 2011; Brown *et al.*, 2013).

In the Philippines, three species of *Gonocephalus* are currently recognized: *G. interruptus* (Boulenger, 1885), *G. semperi* (Peters, 1867), and *G. sophiae* (Gray, 1845). Unfortunately, the type localities (Gray, 1845; Peters, 1867; Boulenger, 1885) for these taxa were reported only as 'Philippines', a problematic situation given the insular nature of the country (comprising more than 7000 islands) and the ever increasing levels of documented vertebrate endemism corresponding to individual islands or island groups (Brown & Diesmos, 2009; Brown *et al.*, 2013). Taylor (1922) restricted the type locality of *G. interruptus* to Mindanao Island, stating that the '*type of this species was collected in Mindanao and presented to the British Museum by G. Taylor …*' Taylor did not provide a source for this information (Taylor, 1922). Additionally, Taylor hinted at the problematic nature of the characters used to define the Philippine species, even going so far as to refer to Peter's 1867 description as '*very meager*' (Taylor, 1922: 135). The original descriptions, as well as the study by Taylor (1922), were based on very low numbers of specimens (Gray, 1845: one adult and two young specimens; Peters, 1867: four specimens; Boulenger, 1885: one specimen) with questionable provenance, leading to the taxonomic confusion present today. Taylor (1922) later noted a single specimen of *G. interruptus* from western Mindanao (Pasananka = Pasonanca, Zamboanga) appearing to exhibit characters of *G. semperi*, and even suggested that '*… this and Peters' species will have to be united*'. Contemporary morphological analyses comparing the phenotypes of the type material with that of specimens of known provenance has not yet been forthcoming but will be required to determine the specific provenances of type material for the recognized taxa (Dixon & Kluge, 1964; Bauer & Russell, 1986). Although no Philippine samples have been included in molecular phylogenetic studies to date, the work of Manthey & Denzer (1991) provides a reasonable hypothesis for the sub-generic affinities between Philippine and non-Philippine taxa (i.e. affinity to members of the *G. bellii* Group).

The combination of Philippine *Gonocephalus* being such a poorly understood group and the Philippines increasingly being recognized as a hotspot of terrestrial vertebrate diversity (Catibog-Sinha & Heaney, 2006; Brown *et al.*, 2013) renders Philippine *Gonocephalus* a particularly intriguing system for a survey of genetic variation. The past several decades have seen a resurgence in studies aimed at understanding the evolutionary history and patterns of diversification across a wide range of vertebrate taxa endemic to the country, resulting in the description of at least 56 reptile (Uetz & Hošek, 2016) and 14 amphibian (Diesmos *et al.*, 2015; Frost, 2015) species over the last 15 years alone (Brown *et al.*, 2012, 2013). This trend will likely apply to Philippine *Gonocephalus* given the genus' largely riparian ecology, presumably low vagility, and reliance on primary or secondary growth forest.

In the present study, we utilize a novel multilocus phylogenetic dataset to identify candidate lineages that may correspond to the named Philippine taxa (*G. interruptus*, *G. semperi*, and *G. sophiae*), and additional lineages (putative species) in need of future delimitation analyses and comprehensive taxonomic revision. We clarify geographical patterns of lineage diversification in *Gonocephalus*, and assess the degree to which observed patterns correspond to climate-driven partitioning along Pleistocene Aggregate Island Complexes (PAICs) (Heaney, 1985; Brown & Diesmos, 2002, 2009). This work represents the first exploration of phylogenetic evidence in support (relative to the morphological groupings of Manthey & Denzer, 1991) of the taxonomic recognition of isolated insular lineages of Philippine *Gonocephalus*, and our results stand as reasonable hypotheses for the assessment of underestimated species diversity in this enigmatic island archipelago clade.

MATERIAL AND METHODS

TAXON SAMPLING AND DATA COLLECTION

Our combined dataset consists of an alignment of 4732 bases for 146 samples (see Supporting information, Appendix S1). Of the ingroup samples, 91 individuals were sampled from the Philippines; the remaining individuals ($N = 55$) were sampled from populations in West Malaysia and Sarawak (Borneo) (Fig. 1; see also Supporting Information, Appendix S1). This sampling represents eight of the 16 recognized species of *Gonocephalus*, including: *G. interruptus*, *G. semperi*, and *G. sophiae* (Philippines); *G. abbotti*, *G. belli*, *G. chamaeleontinus*, and *G. grandis* (West Malaysia); and *G. bornensis* (Sarawak, Borneo). We sequenced one mitochondrial gene, NADH dehydrogenase subunit 2 (ND2), five protein-coding nuclear loci: brain-derived neurotrophic factor (BDNF), diacylgloceral lipase-α (DGL-α), β-nerve growth factor (NGFB), prostaglandin E receptor 4 (PTGER4), and synuclein, α-interacting protein (SNCAIP), and one anonymous nuclear locus (L54) (Alföldi *et al.*, 2011). To explore the monophyly of our sampled ingroup taxa (Honda *et al.*, 2002; Pyron *et al.*, 2013), and root our phylogenetic inferences, we included eight agamid samples as outgroups: *Acanthosaura capra*, *Acanthosaura lepidogaster*, *Ceratophora aspera*, *Cophotis ceylanica*, *Japalura polygonata*, *Lyriocephalus scutatus*, and *Salea horsfieldii*.
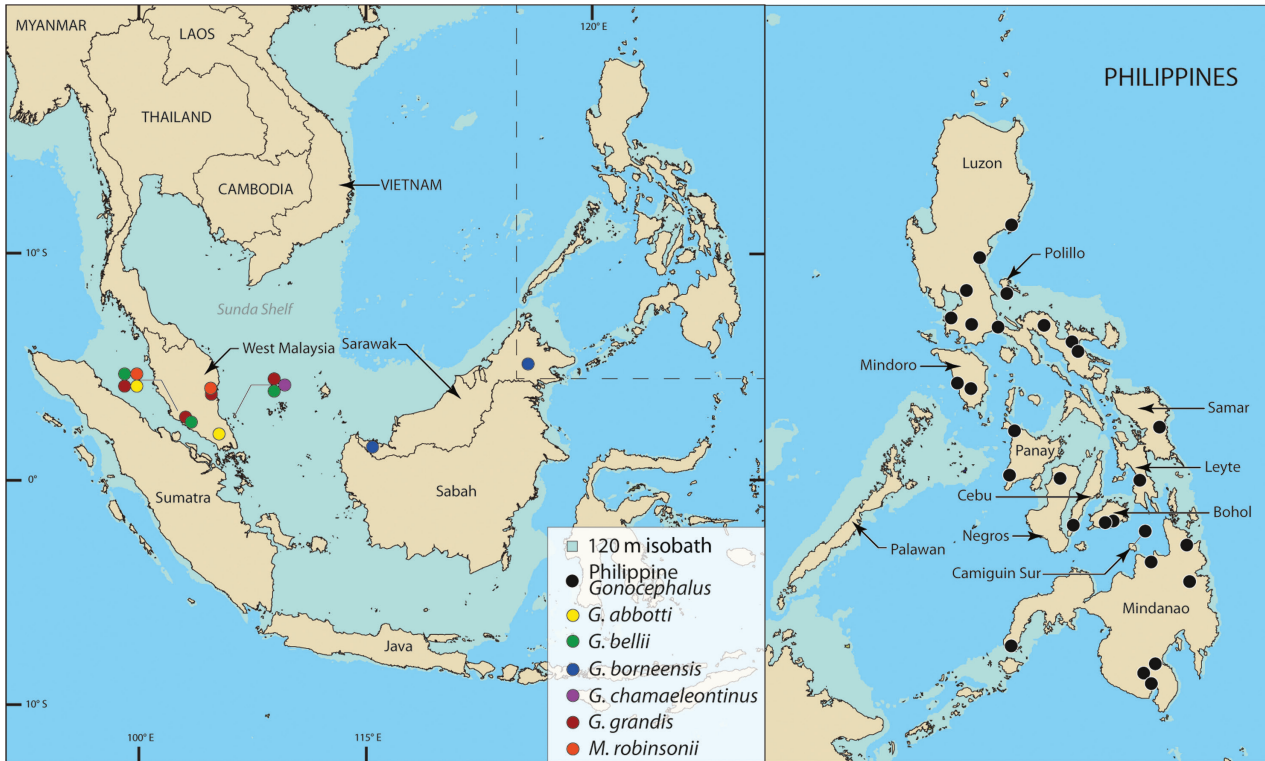
Figure 1. Distribution of *Gonocephalus* species and *Malayodracon robinsonii* samples included in the present study (left), as well as the location of Philippine samples (right).

For each of these taxa, published mtDNA sequences were available on GenBank (see Supporting information, Appendix S1).

SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSIS

Initial alignments were produced with MUSCLE, version 3.831 (Edgar, 2004), as implemented in GENEIOUS, version 5.5.6 (Drummond *et al.*, 2011), with manual adjustments made in MESQUITE, version 2.75 (Maddison & Maddison, 2011) to ensure a proper reading frame and the absence of internal stop codons.

In accordance with a number of recent phylogenetic studies on agamids (Smith *et al.*, 2011; Pepper *et al.*, 2014; Siler *et al.*, 2014b), we partitioned our dataset by nuclear locus, and by codon position within mitochondrial genes. We used JMODELTEST2, version 2.1.4 (Guindon & Gascual, 2003; Darriba *et al.*, 2012) to assess the best-fit model of sequence evolution for each locus (Table 1) and for each codon position within the mitochondrial gene. The Bayesian information criterion was used for model selection in an effort to accommodate variable sample sizes among lineages.

We first estimated the phylogeny for each locus independently using RAxML-VI-HPC, version 7.5.4 (Stamatakis, 2006) to investigate potential conflict among

loci. Because of the absence of well-supported topological conflicts, subsequent analyses were conducted under three partitioning strategies, two of which treated each nuclear locus as a separate partition but with the mitochondrial gene treated either as a single partition or with each mtDNA codon position modeled separately, and the last partitioning approach involving the combination of the data into two partitions (one nuclear, one mitochondrial). Maximum likelihood (ML) analyses were conducted in RAxML-VI-HPC, version 7.5.4 (Stamatakis, 2006). Because of implementation restrictions, we applied the most complex model of evolution (GTR+I+Γ) to all subsets and ran 1000 replicate ML inferences, each initiated with a random starting tree utilizing the rapid hill-climbing algorithm of Stamatakis *et al.* (2007) and Stamatakis, Hoover & Rougemont (2008). Clade support was then assessed with 1000 bootstrap pseudoreplicates.

We estimated uncalibrated phylogenetic relationships in a Bayesian framework with BEAST, version 1.8.1 (Drummond & Rambaut, 2007; Drummond *et al.*, 2012). We applied both Yule and Birth-Death tree priors, with five exploratory analyses of 10 million generations run for each to assess potential variability in parameter space. A final 500 million generation analysis was run under each tree prior, with sampling every 50 000 generations. TRACER, version 1.5 (Rambaut &

Table 1. Partitioning scheme and models of evolution for each locus inferred by JMODELTEST2 (Guindon & Gascual, 2003; Darriba *et al.*, 2012)

Partition	AIC-preferred model	BIC-preferred model	Model implemented (RAxML)	Model implemented (BEAST/*BEAST)
ND2 (first position)	GTR+I+Γ	HKY + Γ	GTR+I+Γ	HKY+Γ
ND2 (second position)	GTR+I+Γ	HKY+I+Γ	GTR+I+Γ	HKY+I+Γ
ND2 (third position)	GTR+I+Γ	GTR+I+Γ	GTR+I+Γ	GTR+I+Γ
PTGER4	HKY	HKY	GTR+I+Γ	HKY
NGFB	GTR+Γ	K80+Γ	GTR+I+Γ	HKY+Γ
BDNF	HKY+I	K80+I	GTR+I+Γ	HKY+I
DGL-α	GTR+Γ	K80+I	GTR+I+Γ	HKY+I
SNCAIP	GTR	HKY	GTR+I+Γ	HKY
L54	HKY+I	HKY+I	GTR+I+Γ	HKY+I

AIC, Akaike information criterion; BIC, Bayesian information criterion.
Codon position for each locus within a partition is in parentheses.

Drummond, 2007) was used to assess run stationarity and reasonable burn-in duration (conservatively, 20%) and to ensure that effective sample sizes (ESS) were > 200. All alignments, input.xml files and prior information have been deposited at Data Dryad (doi: 10.5061/dryad.1225k).

Candidate species discovery and species tree estimation

In addition to assessing the support for monophyly of lineages in phylogenetic inferences, we employed three coalescent-based approaches for defining putative species or visualizing significant population-level genetic diversity using molecular data.

First, we estimated haplotype diversity and population genetic structure for mitochondrial and concatenated nuclear datasets using the NeighborNet algorithm in SPLITSTREE, version 4.12.8 (Huson & Bryant, 2006). For nuclear loci, we explored the effect of using a standardized distance matrix created in POFAD, version 1.03 (Joly & Bruneau, 2006), which uses multiple loci to infer allelic variation resulting from population dynamics (Posada & Crandall, 2001; Cassens, Mardulyn & Milinkovitch, 2005; Zarza, Reynoso & Emerson, 2008). With these networks, the underlying patterns of spatially partitioned genetic variation and equally parsimonious inferences can be illustrated effectively (Cassens *et al.*, 2003).

Second, we analyzed our mtDNA dataset with MEGA5, version 5.2.2 (Tamura *et al.*, 2011) to assess mean pairwise genetic distances between populations. Lineages were identified on the basis of monophyly and relative divergence (ML and Bayesian analyses; Figs 2, 3), with corresponding distinct clusters inferred with network analyses (Fig. 4), resulting in the identification of as many as 12 Philippine lineages, or candidate species.

Finally, based on the results of the above analyses, we estimated the uncalibrated species tree for the sampled species of the *belli* Group (Philippine lineages + *G. belli* and *G. bornensis*) with *BEAST, version 1.8.1 (Drummond & Rambaut, 2007; Drummond *et al.*, 2012) using (1) only phased nuclear data (phased with DNASP, version 5.0; Librado & Rozas, 2009) or (2) phased nuclear and mitochondrial data. Sequences were assigned to one of 14 a priori species/putative species based on the results from phylogenetic, network, and genetic divergence analyses. To aid in computational efficiency, we reduced the full dataset down to two samples per lineage. We ran *BEAST with nuclear data alone, as well as with all data divided into single concatenated nuclear and mitochondrial partitions. We applied the best-fitting model of nucleotide substitution for the concatenated nuclear dataset using JMODELTEST, version 2.1.4 (Guindon & Gascual, 2003; Darriba *et al.*, 2012) (Table 1) and the most complex model previously inferred for the mitochondrial data. We utilized a strict clock and a Yule tree prior for all analyses. We ran a single 1.0×10^9 generation-analysis for each dataset, sampling every 100 000 generations. TRACER, version 1.5 (Rambaut & Drummond, 2007) was used to assess run stationarity and reasonable burn-in length (conservatively, 20%) and to ensure that ESS values were > 200.

RESULTS

Sampling, genetic diversity, and phylogenetic inference

Phylogenetic analyses of the combined, partitioned dataset resulted in a topology with a no fewer than 12 well-supported, divergent lineages of Philippine
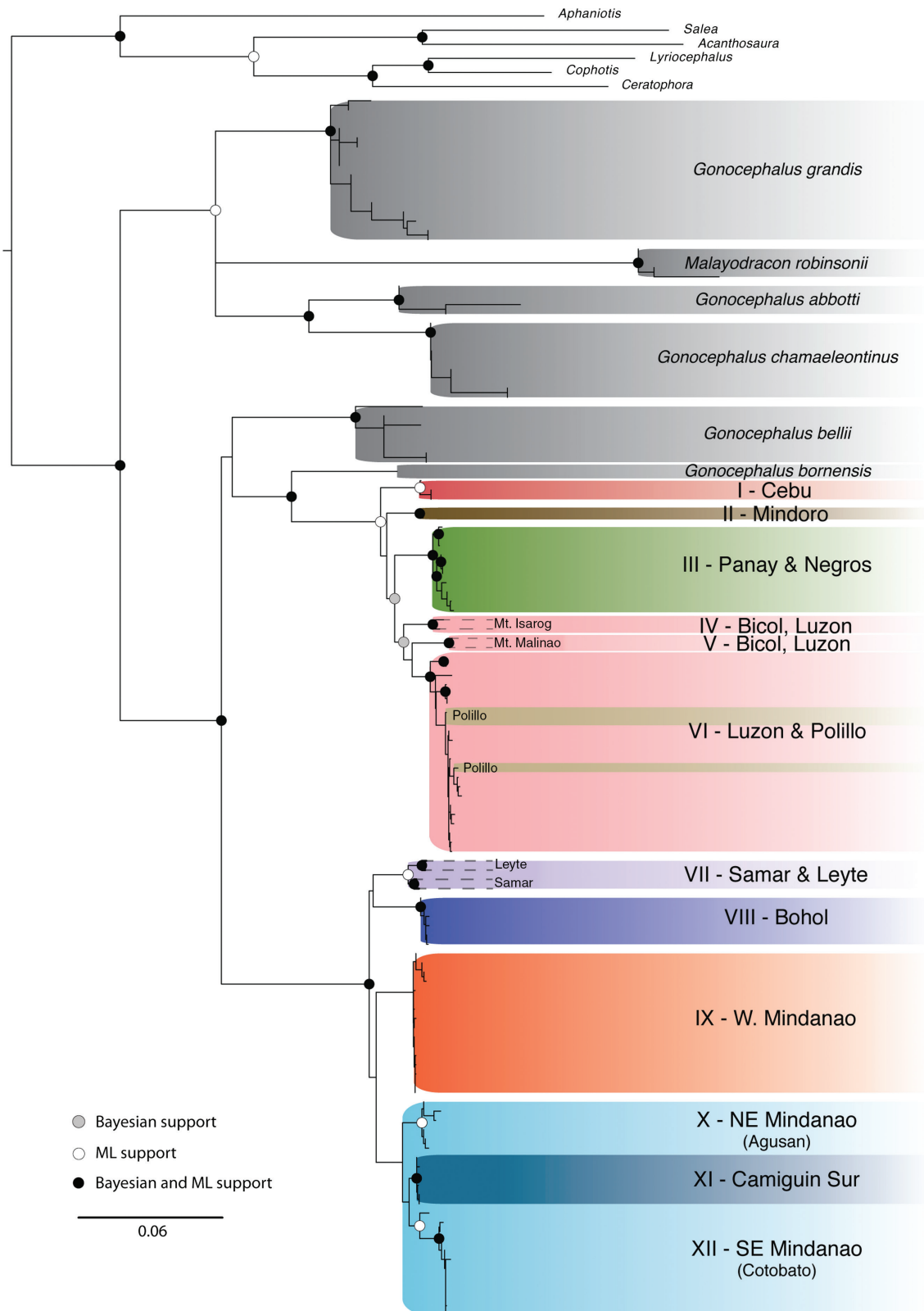
Figure 2. Maximum likelihood estimate of phylogenetic relationships among sampled species of *Gonocephalus* with bootstrap support indicated at nodes. Posterior probability nodal support from a separate Bayesian analysis (BEAST) is indicated in addition to bootstrap values (see key).
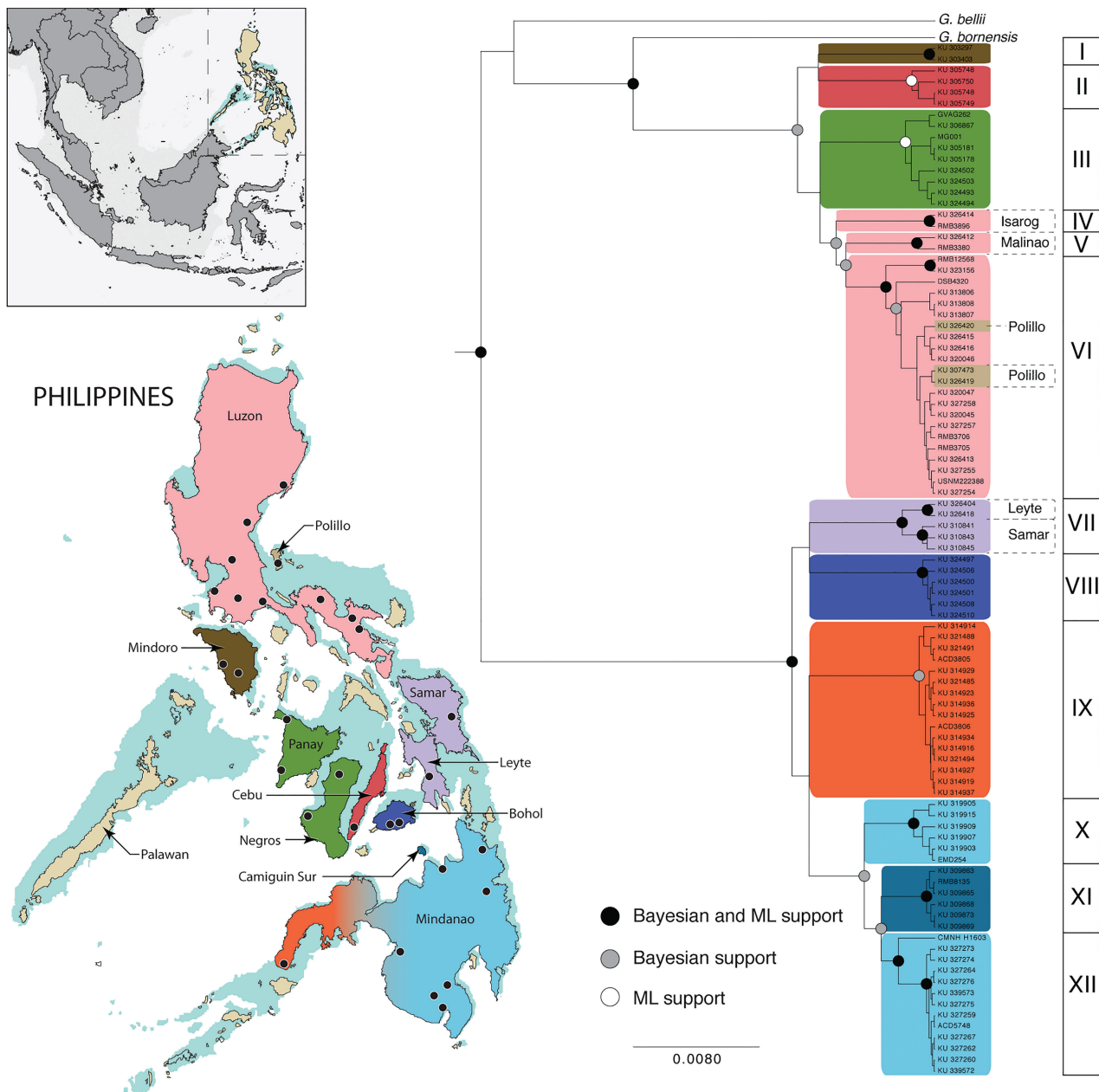
Figure 3. Inferred distribution of major Philippine lineages of *Gonocephalus* (with sampling localities; left) and maximum likelihood estimate of phylogenetic relationships (right), with posterior probability nodal support from a separate Bayesian analysis (BEAST) indicated at nodes (see key).

Gonocephalus (Figs 2, 3). Although the inter-relationships among some of these populations could not be resolved, the monophyly of the lineages themselves is supported by likelihood bootstrap (≥ 70%) and/or posterior probabilities (≥ 0.95) (Figs 2, 3).

The support for relationships among all other *Gonocephalus* is similar to those in the Philippines (Fig. 2), with individual lineages supported as monophyletic but with little resolution deeper in the tree. *Malayodracon robinsonii* is recovered on a long

branch, nested among *G. grandis*, *G. abbotti*, and *G. chamaeleontinus* (Fig. 2). This relationship confounds previous systematic inferences but lacks statistical support, and is likely the result of limited taxon sampling (presented here) for non-Philippine and outgroup taxa. *Gonocephalus* (as currently recognized) is recovered as a paraphyletic group (inclusive of *M. robinsonii*), with two major divisions: a clade comprising Sundaland taxa and a clade with Sundaic species nested within and among Philippine
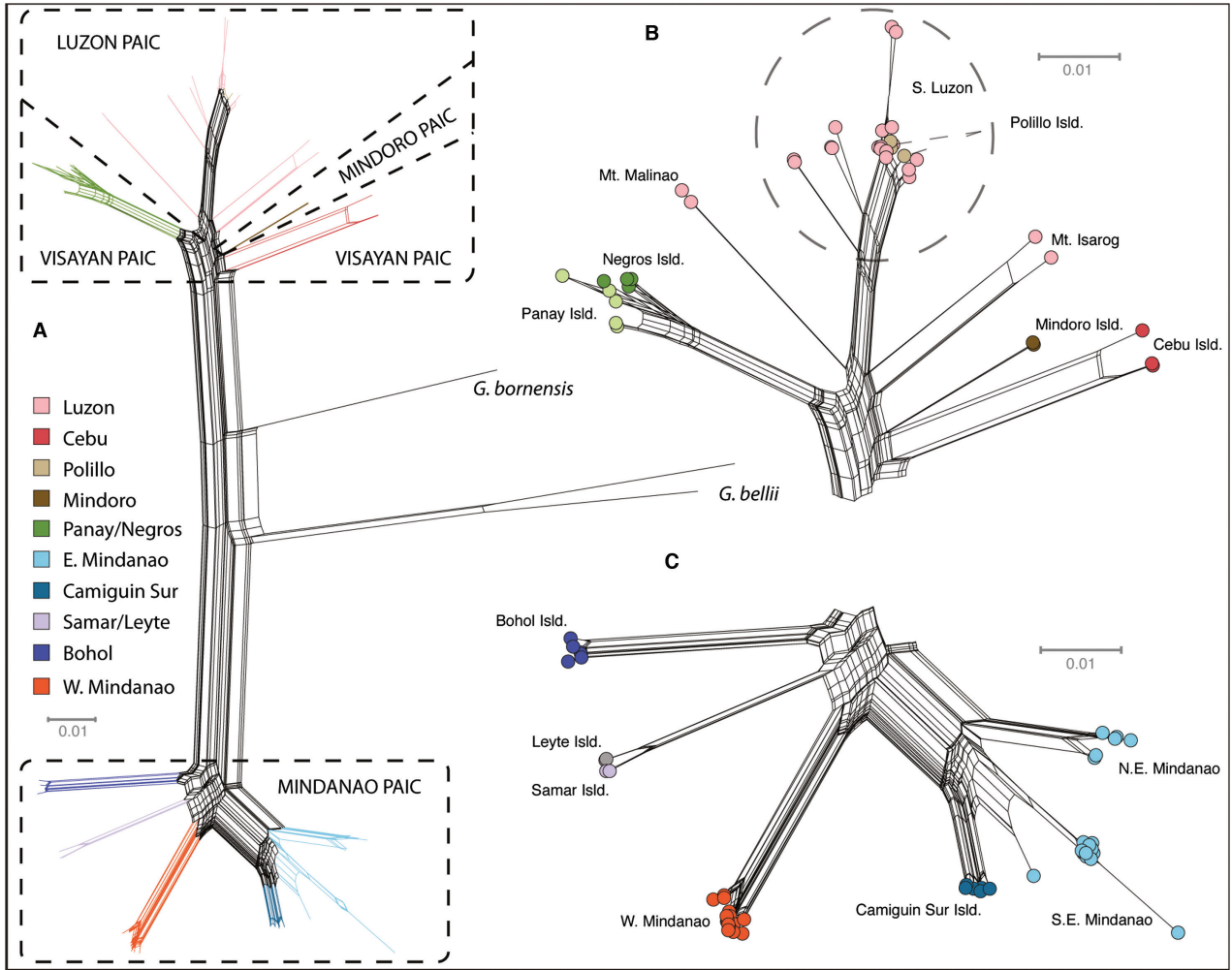
Figure 4. A, complete phylogenetic network for Philippine *Gonocephalus* and the closely-related *Gonocephalus bellii* and *Gonocephalus bornensis*, based on the mitochondrial NADH dehydrogenase subunit 2 (ND2) gene. Enlarged views of phylogenetic networks for (B) northern and (C) southern Philippine lineages.

lineages. This second, predominantly Philippine clade presumably contains the named taxa *G. interruptus*, *G. sophiae*, and *G. semperi* (with the caveat that these available names have not yet been assigned to specific, diagnosable lineages); it also consists of potentially at least nine other equally-divergent unnamed clades/putative undescribed species arranged into two primary groups: a north-central subclade and a southern subclade. *Gonocephalus bellii* and *G. bornensis* are recovered as sequentially diverging lineages at the base of the north-central Philippine subclade, although the placement of *G. bellii* lacks statistical support (Figs 2, 3). The other primary division within *Gonocephalus*, the exclusively Sundaic clade, is comprised of *G. abbotti*, *G. chamaeleontinus*, and *G. grandis* (Fig. 2).

Gonocephalus bellii is inferred as the sister lineage to a clade including *G. bornensis* and the north-central Philippine clade (lineages from Cebu, Luzon, Mindoro, Negros, Panay, and Polillo islands; Fig. 3, clades I, II, and III). Interestingly, samples from Polillo Island fail to form a monophyletic unit but, instead, are recovered in multiple places within a larger 'Luzon' clade (Fig. 3, clade IV). Additionally, the isolated volcanoes on the Bicol Peninsula of Luzon, Mt Isarog and Mt Malinao, each form their own divergent monophyletic lineage (Fig. 3, clade IV).

The remaining southern clade is exclusively Philippine, and consists of major lineages from Bohol, Camiguin Sur, Leyte, Mindanao, and Samar islands. Samar and Leyte form a clade that is sister

to Bohol but with no statistical support (Fig. 3, clades V and VI), and all three are sister to lineages from Mindanao and Camiguin Sur islands. Camiguin Sur is nested among three divergent Mindanao clades (north-east, south, and west). Although each of these represents a well-supported monophyletic group, we only found moderate support for relationships among these lineages (Fig. 3, clades VIII, IX, and X).

CANDIDATE SPECIES DISCOVERY AND SPECIES TREE INFERENCE

Network analyses of the mitochondrial and combined datasets differed in their resolution, with the mitochondria-based networks yielding patterns of diversity similar to phylogenetic analyses (Fig. 4), but with the results of the combined dataset lacking any notable structure (not shown). These analyses identified 12 putative lineages in the Philippines, with the uncorrected pairwise distance among them ranging from 4% to 24% (mean = 14.1 and mode = 19%) (Table 2). Camiguin Sur and Samar-Leyte populations were the most divergent from one another, whereas lineages from southern and north-eastern Mindanao (Agusan and Cotobato) were the least divergent.

The topologies resulting from *BEAST species tree analyses were generally equivalent but with varying nodal support (posterior probability ≥ 95). Perhaps the most noteworthy finding is the inferred relationship between Philippine *Gonocephalus* and the other sampled members of the *bellii* Group (*G. bellii* and *G. bornensis*). Nuclear data alone recovered *G. bellii* as the sister taxon to the remaining lineages in the northern Philippines (Fig 5B). *Gonocephalus bornensis* was recovered nested within this Philippine clade, although its exact placement remains unresolved. The topology inferred from the combined nuclear and mitochondrial dataset placed *G. bornensis* as the first-branching lineage of a clade consisting of *G. bellii* sister to the northern Philippine lineages (Fig. 5A), although this relationship was only supported under a Yule tree prior (birth-death prior support = 0.602). Similarly, the lineage from Mt Malinao was strongly supported as distinct by the nuclear data alone, but was only supported under the birth-death tree prior when the mitochondrial data were included (Yule prior support = 0.665). Topologies recovered from the nuclear data alone were generally better resolved than those obtained with the inclusion of mitochondrial data. We recovered a species tree with a basal divergence between the north-central Philippine (+ *belli* and *bornensis*) lineages and a phylogentically distinct southern clade. Additionally, as in the concatenated phylogenetic inference, the inter-relationships among these lineages could not be resolved. Finally, the lineages from Bohol, Samar, and Leyte islands were recovered with consistent support across datasets and tree priors, although the phylogenetic affinities among these and lineages from Mindanao and Camiguin Sur remain unresolved (Fig. 5).

Table 2. Pairwise sequence divergence between recognized and putative species of *Gonocephalus* identified through phylogenetic and network analyses based on the mitochondrial ND2 gene

Taxon/population																
abbotti	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
bellii	0.25	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
bornensis	0.23	0.17	–	–	–	–	–	–	–	–	–	–	–	–	–	–
chamaeleontinus	0.15	0.24	0.21	–	–	–	–	–	–	–	–	–	–	–	–	–
grandis	0.39	0.33	0.37	0.38	–	–	–	–	–	–	–	–	–	–	–	–
Luzon	0.26	0.20	0.14	0.23	0.39	–	–	–	–	–	–	–	–	–	–	–
Mt Isarog	0.25	0.19	0.14	0.22	0.38	0.05	–	–	–	–	–	–	–	–	–	–
Mt Malinao	0.25	0.19	0.12	0.23	0.38	0.05	0.05	–	–	–	–	–	–	–	–	–
Mindoro	0.25	0.19	0.12	0.23	0.37	0.07	0.06	0.08	–	–	–	–	–	–	–	–
Negros-Panay	0.28	0.23	0.17	0.26	0.39	0.11	0.10	0.11	0.12	–	–	–	–	–	–	–
Cebu	0.24	0.18	0.13	0.23	0.38	0.07	0.06	0.08	0.06	0.12	–	–	–	–	–	–
Samar-Leyte	0.25	0.19	0.17	0.24	0.38	0.19	0.19	0.19	0.18	0.21	0.18	–	–	–	–	–
Bohol	0.25	0.18	0.17	0.23	0.38	0.18	0.19	0.18	0.17	0.20	0.17	0.07	–	–	–	–
Camiguin Sur	0.27	0.21	0.21	0.25	0.39	0.22	0.21	0.22	0.22	0.24	0.21	0.11	0.10	–	–	–
North-east Mindanao	0.24	0.18	0.16	0.22	0.38	0.18	0.18	0.18	0.17	0.21	0.17	0.07	0.07	0.07	–	–
South Mindanao	0.25	0.19	0.18	0.24	0.39	0.19	0.19	0.20	0.19	0.22	0.19	0.07	0.08	0.06	0.04	–
Zamboanga	0.24	0.18	0.17	0.23	0.38	0.19	0.19	0.18	0.18	0.21	0.18	0.06	0.07	0.11	0.08	0.08

DISCUSSION

Taxon sampling

The results of the present study provide the first inferences into the evolutionary relationships, patterns of diversification, and putative taxonomic diversity of the Philippine radiation of *Gonocephalus*. We recover no fewer than 12 divergent monophyletic lineages distributed on major landmasses across the archipelago. Perhaps most interesting is the distribution of these major lineages. Based on the sampling available, all of the Philippine lineages are recovered with allopatric distributions, or with only a single lineage identified at each sampling location. This is in stark contrast to the Sundaic species that are regularly encountered in syntopy/sympatry with congeners. This pattern may relate to Philippine *Gonocephalus* possessing more generalist ecological preferences/microhabitats or, alternatively, the

Philippine lineages may be too young to have come into secondary contact with congeners, and thus may never have been subject to selective pressures that might drive ecological and/or phenotypic uniqueness observed in the Sundaic forms.

Despite our extensive geographical sampling, there remain a number of major Philippine islands that have yet to be sampled or have not yet been sampled sufficiently, namely the many smaller, deep water islands (or smaller island complexes) of the archipelago, such as Siquijor, Camotes and Calamian islands, Ticao, Burias, and the Romblon Island Complex. Palawan, a very large island (Fig. 1) previously considered as a faunal extension of Sundaland (Heaney, 1985; Esselstyn *et al.*, 2010), has no recorded *Gonocephalus* lineages, which remains a glaring biogeographical anomaly. Given the isolation of these landmasses and the patterns of genetic diversity
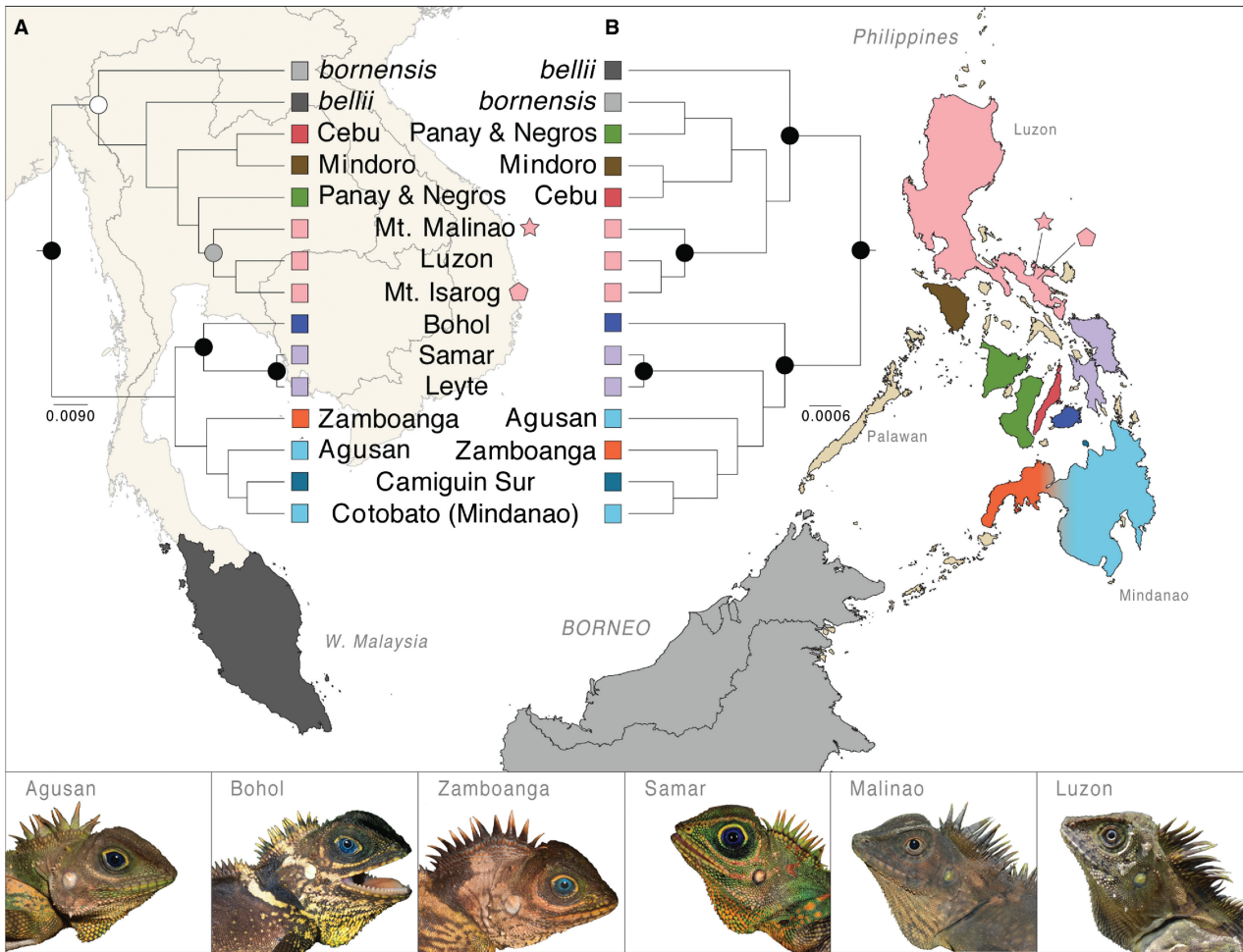

Figure 5. Coalescent-based species tree estimation for Philippine *Gonocephalus* and sampled members of the *bellii* Group. Nodes with black circles are indicative of posterior probabilities ≥ 95 for inferences derived from (A) nuclear + mitochondrial data, and (B) nuclear data only. Open and grey circles indicate support under Yule and birth-death tree priors, respectively. Bottom: representative images of Philippine *Gonocephalus*.

documented in the present study, it remains possible that these isolated islands may harbour additional diversity. Our results highlight the utility of surveys utilizing robust geographical sampling, even if employing only limited gene sampling, as an exploratory initial step to discovering geographical variants, population structure, highly divergent genetic lineages, and/or candidate species.

PHYLOGENETIC INFERENCE AND GENETIC DIVERSITY

The Philippine radiation of *Gonocephalus* comprises no fewer than a dozen divergent, well-supported lineages. Additionally, and perhaps most surprising, is the recovery of phylogenetic affinities between Philippine lineages and the Sundaic taxa *G. bellii* and *G. bornensis*. Our phylogenetic inference supports the presence of two deeply divergent clades in the Philippines: (1) a north-central clade containing both non-Philippine taxa *G. bellii* (West Malaysia) and *G. bornensis* (Borneo), and lineages from the Visayan (Cebu, Panay, and Negros) and Luzon (Luzon and Polillo) PAICs, and Mindoro Island; and (2) a southern clade containing lineages from the Mindanao PAIC (Mindanao, Samar, Leyte, Bohol, and Camiguin Sur). The inferred affinity with non-Philippine, Sundaic taxa, is not surprising given the geographical proximity between the Philippines and Borneo. However, the close relationship between Philippine taxa and *G. bellii* from Peninsular Malaysia is unexpected, and warrants future study.

The inability of our data to resolve the relationships among major lineages is likely a result of this radiation: (1) having diversified too rapidly to be confidently resolved (McGuire & Heang, 2001; Stanley *et al.*, 2011) or (2) having diversified gradually but representing a relatively young radiation in which the targeted portions of the genome have not yet accumulated the necessary changes sufficient to fully resolve relationships. It is somewhat unlikely that the application of more data to this system would yield additional systematic resolution given the inability of mitochondrial data, with its relatively rapid coalescent time and informativeness, to resolve these relationships (especially when coupled with nuclear loci). However, future studies would be best served to employ genomic-level data in an effort to resolve the phylogenetic affinities among Philippine *Gonocephalus*.

Philippine candidate species are all recovered at least 4% divergent from one another at the ND2 gene (most much more divergent), indicating allopatric circumscription of ranges in most cases. Such a finding is not surprising given the insular nature of most Philippine lineages. One interesting finding is the observation that the most proximate lineages geographically do not exhibit the lowest levels of divergence (Table 2). The isolated populations from the Isarog and Malinao volcanoes of the Bicol Peninsula (Luzon Island) are > 5% divergent from one another, despite being separated by < 35 km. This is in contrast to the least divergent lineages from north-eastern and southern Mindanao, which are divergent by approximately 4% and separated by several hundred kilometers.

Although any genetic distance threshold for putative species discovery is arbitrary, the amounts observed in the present study are in accordance with divergences shown to correspond to morphologically diagnosable species in other lizard genera, including other agamids (McGuire & Heang, 2001; Melville *et al.*, 2009; Siler *et al.*, 2014b). We only suggest attention to this threshold as a proxy for guiding future, more extensive investigations of taxonomic diversity.

CANDIDATE SPECIES DISCOVERY

We used multiple methods to discover and delimit candidate species with multilocus data. Across all analyses and dataset configurations (nuclear DNA vs. mtDNA + nuclear DNA), we inferred the presence of at least 12 divergent, monophyletic lineages. Given that three of these most likely will be shown to correspond to the three currently named taxa, the remaining nine represent novel discoveries of candidate species warranting further taxonomic study. Alternatively, there remains the possibility that the phylogenetic pattern observed in the present study is indicative of two widespread species: one with a north-central distribution in the archipelago and the other with an exclusively southern distribution. Additional studies incorporating morphological data and examination of the type material are required to sufficiently remedy the existing taxonomic confusion in this assemblage (*interruptus*, *semperi*, and *sophiae*), as well as test multiple hypotheses of taxonomic diversity.

Despite the inability of our species tree estimations to fully resolve the relationships among Philippine lineages or between those and lineages from Borneo (*G. bornensis*) and West Malaysia (*G. bellii*), the results unambiguously support our strong suspicion of some level of unrecognized diversity. We would expect that the discrepancies between the relationships inferred from nuclear-only and nuclear + mitochondrial topologies would most likely be a result of stochastic sorting of ancestral polymorphisms rather than introgression because of the geographical distance between *G. bellii*, *G. bornensis*, and the northern Philippine lineages. If introgression were the cause, one might expect the presence of a contact zone, or geographical ranges separated by relatively

short dispersal distances (Barbujani *et al.*, 1994), neither of which appear to be possible given the geographical ranges of the species involved. Similarly, the relationships inferred from the nuclear data alone are most plausible biogeographically, with species from Borneo (*G. bornensis*) most closely related to Philippine lineages, although it must be noted that our taxon sampling for the present study represents approximately half of the described diversity in the genus, and only five of the seven described species in the *bellii* Group.

BIOGEOGRAPHY

The most fundamental phylogenetic split within Philippine *Gonocephalus* conforms to a pattern observed in a number of other studies, in that populations from the Mindanao PAIC (Mindanao, Samar, Leyte, Bohol) form a distinct monophyletic evolutionary group sister to the central and northern island populations of the archipelago. This north-west–south-east split has been documented in birds (Anderson *et al.*, 2013; Hosner *et al.*, 2014), mammals (Heaney, 1986; Esselstyn & Brown, 2009), and numerous other herpetofaunal elements (McGuire & Alcala, 2000; Brown & Diesmos, 2002; Linkem *et al.*, 2010; Siler & Brown, 2010; Siler *et al.*, 2010, 2012, 2014b; Barley *et al.*, 2013; Welton *et al.*, 2013; Gonzalez *et al.*, 2014; Siler, Lira-Noriega & Brown, 2014a), and partly corresponds to patterns of island connectivity during the last glacial maxima (Brown & Diesmos, 2009). The recovery of the Sundaic taxa, *G. bellii* and *G. bornensis* (yielding the Philippine assemblage non-monophyletic), nested within predominantly Philippine lineages, stems from one of two possible scenarios: (1) the Philippines was invaded at two distinct times: one resulting in the lineages present in the northern and central portions of the archipelago, and the other affecting the southern landmasses; or (2) a single invasion of the archipelago followed by dispersal out of the Philippines, back to Borneo and Peninsular Malaysia. We refrain from rejecting either of these scenarios in favour of the other at present because of the lack of resolution inferred by our analyses. Rather, we emphasize that these biogeographical hypotheses should be evaluated with a more robust dataset in the future.

Interestingly, the patterns of diversification observed among Philippine *Gonocephalus* correspond to a variety of geological patterns. Distributions of major lineages fail to partition along Eastern and Western island arcs (Hall, 1996, 1998; Yumul *et al.*, 2003, 2009), a dominant pattern of diversification observed in Philippine radiations that has origins in Sundaland (Brown *et al.*, 2013; Brown & Siler, 2014). However, within these clades we do see partitioning

between eastern and western Mindanao (along the Cotabato Trench), and an apparent boundary along the Philippine Fault in central and south-east Luzon, separating the Bicol Peninsula from the rest of the island. Additionally, as previously noted, *Gonocephalus* has yet to be recorded from Palawan, a distributional pattern that would be considered anomalous under the classic Palawan-as-biogeographical-extension-of-Borneo perspective of the past (although many recent studies have rejected this: McGuire & Heang, 2001; Blackburn *et al.*, 2010; Esselstyn *et al.*, 2010; Siler *et al.*, 2012; Siler *et al.*, 2012; Brown *et al.*, 2013). This is potentially an artefact of Palawan having previously (approximately 10–20 Mya; Hall, 2013) been substantially more isolated (and possibly more difficult to colonize) as a result of its formerly more north-eastern position in the South China Sea (Hall, 2013). However, it is also conceivable that *Gonocephalus* has colonized Palawan, but has yet to be documented (or has gone extinct), highlighting the importance of continued faunal surveys throughout the understudied Palawan PAIC.

Within the two major clades, divergent lineages are distributed on individual islands (Cebu, Mindoro, Bohol), island complexes separated by shallow seas (Panay/Negros, Samar/Leyte), or individual geological components of larger islands [west, north-east, and south-east Mindanao, or the volcanoes of the Bicol Peninsula (Mts Isarog and Malinao) vs. the remainder of Luzon Island]. The close, population-level relationships of both Panay–Negros and Samar–Leyte reptiles has been documented in skinks (Linkem *et al.*, 2010; Barley *et al.*, 2013), geckos (Siler *et al.*, 2010, 2012, 2014a), monitor lizards (Welton *et al.*, 2013), and sailfin dragons (Siler *et al.*, 2014b), amongst others. At first glance, the expectation of pure PAIC-level faunal structuring would predict that the West Visayan PAIC islands of Panay, Negros, and Cebu (conjoined many times during the mid- to late-Pleistocene) would support minimally divergent and closely-related lineages, as would the Mindanao PAIC islands of Bohol, Samar, Leyte, and Mindanao. However, within these landmass amalgamations and recognized faunal regions (Brown & Diesmos, 2009), we see genetically highly distinctive populations from Cebu and Bohol islands. Additional historical events, bouts of colonization, and/or ecological factors may explain these non-intuitive patterns (Brown *et al.*, 2013; Hosner *et al.*, 2014).

The southern clade of Philippine *Gonocephalus* exhibits a north–south split between populations from Samar, Leyte, and Bohol vs. those from Mindanao and Camiguin Sur islands. Perhaps most interesting in this clade are the patterns of diversification within Mindanao itself. The affinity between Camiguin Sur and south-east Mindanao (Cotabato

coast and Davao) is somewhat surprising, although divergences between eastern and western Mindanao have been documented in a number of other taxa (Siler *et al.*, 2010; Welton *et al.*, 2013; Gonzalez *et al.*, 2014; Hosner *et al.*, 2014). However, it must be noted that the relationships inferred here are only moderately supported, and thus require more robust datasets and analyses to be fully resolved.

CONSERVATION

Philippine *Gonocephalus* have yet to be assessed for population viability or conservation priority and, although a number of non-Philippine species of *Gonocephalus* are commonly encountered in the international pet trade, there is no indication that this is the case for Philippine taxa. However, given the presumed low vagility and arboreal nature of *Gonocephalus*, and their ecological position as forest obligates, the genus likely represents yet another taxon that is negatively impacted by habitat loss and degradation. In particular, the Bicol Peninsula of Luzon has been identified as a conservation priority because of the unique genetic and taxonomic diversity in a number of other squamate groups (Welton *et al.*, 2013; Siler *et al.*, 2014b). We find similar genetic (and likely taxonomic) diversity within *Gonocephalus*, with divergent lineages inhabiting the dormant volcanoes of Mts Isarog and Malinao. This finding highlights the need for additional protected areas across the Bicol Peninsula, aimed at preserving what little primary and secondary growth forest remains. Of particular priority are the intact gallery forests and their adjacent riparian habitats, comprising environments necessary for *Gonocephalus* and a wide array of additional unrelated species.

The results of the present study reinforce the interpretation of the Philippines as a globally significant biodiversity hotspot. If the phylogenetically distinct diversity that we have discovered translates to taxonomic diversity, the Philippines may soon harbour more than half of the specific diversity within *Gonocephalus*. The continued study of Philippine *Gonocephalus* species boundaries, distributions, and phylogeny is a major priority because unresolved relationships and taxonomic confusion plague our ability to firmly interpret conservation priorities in this large clade.

Our survey of Philippine *Gonocephalus* genetic variation, by definition, requires additional, continued studies focusing on morphological variation to identify diagnosable phenotypic units, clarifying the group's taxonomic diversity. Because so many recent analyses have uncovered substantial, lineage-specific distributional, biogeographical, and phylogenetic differences between vertebrate groups (Esselstyn *et al.*, 2010; Brown *et al.*, 2013), it is imperative that future

conservation-related research efforts be directed towards utilizing the full diversity of land vertebrate taxa in the Philippines, such that summaries can effectively guide applied conservation actions. This can only be accomplished by extensive geographical sampling and integrative approaches for estimating species diversity, followed by a formal description of this diversity. We expect that future studies of *Gonocephalus*, incorporating improved taxonomic sampling from throughout the genus and utilizing genome-wide inferences, will clarify estimates of evolutionary history and taxonomic diversity in this diverse clade of Philippine lizards.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1. Sample identification, general collection locality, and GenBank accession numbers.

SHARED DATA

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.1225k (Welton LJ *et al.*, 2016).