

**MULTILOCUS PHYLOGENY AND BAYESIAN ESTIMATES OF SPECIES
BOUNDARIES REVEAL HIDDEN EVOLUTIONARY RELATIONSHIPS AND
CRYPTIC DIVERSITY IN SOUTHEAST ASIAN WATER MONITORS**

(GENUS *VARANUS*)

BY

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Abstract

Recent advances in conceptual, numerical, and methodological approaches in phylogenetic systematics have enabled increasingly robust approaches to the question of species delimitation in empirical studies of biodiversity. As the diversity of lines of evidence available to systematists has increased, the inferential power of species delimitation methods has also expanded. Here we showcase a model system in a data-rich, comparative approach to evaluating methods of species delimitation among the abundant and conspicuous monitor lizards (*Varanus*). The water monitors (*Varanus salvator* Complex), a widespread lineage distributed throughout Southeast Asia and southern India, have been the subjects of numerous taxonomic treatments, drawing particular attention to the possibility of undocumented species diversity in the Philippines. Despite these taxonomic changes reliance on purportedly diagnostic differences in morphological characters, no attention has been given to the genetic underpinnings of currently recognized species diversity in Philippine water monitors. We collected a 5-gene dataset, estimated the phylogeny of the *Varanus salvator* Complex, and inferred species boundaries using a Bayesian coalescent approach. Our results contradict previous systematic and taxonomic hypotheses and reveal surprising affinities between Philippine and non-Philippine lineages. We reject previous traditional taxonomic treatments, and simultaneously uncover levels of cryptic diversity never alluded to in past studies. In general, our results suggest that a combination of both phenotypic and genetic data will be most informative to taxonomists, systematists, and biodiversity specialists when attempting to estimate species diversity. We advocate the use of multilocus datasets for testing the validity of recognized evolutionary lineages and estimating species boundaries, and recommend reserving taxonomic changes for cases in which multiple lines of evidence, namely molecular and morphological, agree.

Acknowledgements

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Introduction

The Aristotelian practice of delineating and naming species (Linnaeus 1735) has evolved over the past five centuries to be an essential (Sites and Marshall 2003; Wiens 2007) subdiscipline of phylogenetic systematics (Doyle 1995; Wiens and Penkrot 2002; Hey *et al.* 2003; Sites and Marshall 2004; Leaché and Mulcahey 2007; Barrett and Freudenstein 2011). Although early methods to classify and delimit species primarily utilized small numbers of morphological differences between putative species (Merrell 1981), more recent approaches have embraced the need for consideration of not only diagnostic morphological characters, but also inferences of evolutionary history (Marshall *et al.* 2006; Leaché and Mulcahey 2007). In fact, the application of increasingly diverse lines of evidence to delimit boundaries between evolutionary lineages has become paramount in biodiversity studies aimed at accurate estimations of species diversity (Wiens and Penkrot 2002; Rissler and Apodaca 2007; Knowles and Carstens 2007; Brown and Diesmos 2009; Setiadi *et al.* 2011; Welton *et al.* 2010a, 2010b; Barrett and Freudenstein 2011).

Taking a more pluralistic approach to taxonomy and species delimitation has been the focus of many recent studies (Sites and Marshall 2004; Dayrat 2005; Esselstyn 2007; Padial and de la Riva. 2009; Barrett and Freudenstein 2011). Of the many new approaches developed to investigate species boundaries, the Bayesian species delimitation of Yang and Ranalla (2010) has ignited both enthusiasm (Leaché and Fujita 2010; Setiadi *et al.* 2011; Spinks *et al.* 2012) and concern (Bauer *et al.* 2010). The approach provides a mechanism for testing species boundaries in a rigorous and objective Bayesian framework with genetic data. An ideal model system for exploring these methods would be a small, but relatively diverse clade with a long history of differing taxonomic perspectives, disparate types of data previously applied, and undocumented

evolutionary relationships. One such system is the water monitors of Southeast Asia (*Varanus salvator* Complex).

Despite being some of the most abundant and conspicuous reptiles of Southeast Asia, the species diversity of monitor lizards remains highly contested (Pianka *et al.* 2004). Types of data applied to species boundaries in this group have ranged from general morphology and color patterns, to detailed investigations of meristic and mensural characters (Mertens 1942a–c; and most subsequent taxonomic works). Additionally, morphology of genitalia and lungs (Böhme 1988; Card and Kluge 1995; Böhme and Ziegler 1997, 2005; Ziegler and Böhme 1997; Becker *et al.* 1989) and karyotype (King and King 1975) and allozyme variation (reproductive structure and lung morphology (Branch 1982;), along with karyotype (Holmes *et al.* 1975; King *et al.* 1991; Baverstock *et al.* 1993) have been used to estimate diversity within *Varanus*. Most recently, DNA sequence data have been used to gain insight into phylogenetic relationships (Ast 2001; Welton *et al.* 2010a), historical biogeography (Fuller *et al.* 1998; King *et al.* 1999; Schulte *et al.* 2003), and body size evolution (Pianka 1995; Collar *et al.* 2011). Although the genus is best known for the Komodo Dragon (*Varanus komodoensis*), 73 species are currently recognized, 13 of which have been described in the past decade (The Reptile Database 2012).

Although external morphology may have been the predominant source of data in many past studies, exclusive reliance on this line of evidence may limit our ability to accurately assess species diversity if morphological characters are conservative, or character differences are slight enough to escape recognition by taxonomists, or if speciation is not accompanied by morphological divergence (Harris and Sá-Sousa 2002; O’Conner and Moritz 2003; Boumans *et al.* 2007). Other than body size trends and general color pattern, the use of morphology has not resulted in the identification of clear, of discrete, non-overlapping, character state differences

among all recognized species (Gaulke 1991, 1992; Koch *et al.* 2007, 2010a). To date, no study has applied extensive geographic sampling, genetic data, or robust statistical methods to estimate species diversity and clarify lineage boundaries in this widespread vertebrate group.

Here, we provide the first molecular study of the systematic relationships of this unique assemblage of Southeast Asian lizards. We apply a series of multilocus, phylogeny-based, population genetic, and Bayesian species delimitation approaches to test more conservative (Mertens 1950; Gaulke 1991, 1992) versus more liberal (Koch *et al.* 2007, 2010a) taxonomic assessments. Our results contradict past approaches based solely on one data type, and illustrate how a more integrative approach can provide a data-rich, objective perspective that both evaluates past assessments of species diversity and also identifies additional candidate lineages (possible new species) for future scrutiny by taxonomists.

Materials and methods

Sample collection

Our dataset consists of 81 *Varanus salvator* Complex samples representing natural populations at 56 localities. These include 70 samples from 45 localities in the Philippines, eight samples from three localities in Indonesia, and a single sample each from Myanmar, West Malaysia, and Singapore. Our sampling includes eight of the 12 currently recognized, named taxonomic units within the *V. salvator* Complex (Table 1, Appendix; genetic material for *V. salvator salvator*, *V. s. ziegleri*, *V. s. andamanensis*, and *V. rasmussenii* currently is unavailable). In order to assess the monophyly of Philippine taxa and the *V. salvator* Complex, we incorporated samples representing 53 of the 94 described taxonomic units (species and subspecies) within the genus *Varanus*, as well as samples from two closely-related outgroups, *Heloderma* and *Lanthonotus*

(Appendix; Caldwell 1999; Lee and Caldwell 2000; Ast 2001; Evans *et al.* 2005; Conrad *et al.* 2011).

Table 1. Taxonomic history of the *Varanus salvator* Complex illustrating the historical uncertainty of species level diversity within the group.

One species (Laurenti 1768)	<i>Varanus (Stellio) salvator</i>
Four species (Boulenger 1885)	<i>V. cumingi</i> , <i>V. nuchalis</i> , <i>V. salvator</i> , <i>V. togianus</i>
One species with five subspecies (Mertens 1942a–c)	<i>V. salvator</i> , <i>V. s. cumingi</i> , <i>V. s. marmoratus</i> , <i>V. s. nuchalis</i> , <i>V. s. togianus</i>
One species with eight subspecies (Mertens 1963; Gaulke 1991; Böhme 2003)	<i>V. salvator</i> , <i>V. s. andamanensis</i> , <i>V. s. bivittatus</i> , <i>V. s. cumingi</i> , <i>V. s. komaini</i> , <i>V. s. marmoratus</i> , <i>V. s. nuchalis</i> , <i>V. s. togianus</i>
Seven species with five subspecies (Koch <i>et al.</i> 2007, 2010a; Koch and Böhme 2010)	<i>V. cumingi</i> , <i>V. cumingi samarensis</i> , <i>V. marmoratus</i> , <i>V. nuchalis</i> , <i>V. palawanensis</i> , <i>V. rasmusseni</i> , <i>V. togianus</i> , <i>V. salvator</i> , <i>V. s. andamanensis</i> , <i>V. s. bivittatus</i> , <i>V. s. macromaculatus</i> , <i>V. s. zieglerei</i>

Sequencing of DNA

Genomic DNA was extracted from liver or muscle tissue stored in 95% ethanol following a guanidine thiocyanate extraction protocol (Esselstyn *et al.* 2008). Polymerase chain reactions (PCR) and cycle sequencing reactions for the mitochondrial region used published nested primers (Table 2; Ast 2001). We screened a suite of candidate loci from recent studies of higher-level squamate relationships (Table 2; Townsend *et al.* 2008; Alföldi *et al.* 2011) for intraspecific variability, and among those that amplified easily, selected the four most variable for this study (Supplemental Table 1). We visualized amplified PCR product in 1.5% agarose gels, and purified products with 2 µl of a 20% dilution of ExoSAP-IT (US78201, Amersham Biosciences, Piscataway, NJ) using a thermal profile of 31 min at 37°, followed by 15 min at 80°. We used ABI Prism BigDye Terminator chemistry (v3.1; Applied Biosystems, Foster City, CA) in cycle sequencing reactions, and purified products with Sephadex Medium (NC9406038, Amersham

Biosciences, Piscataway, NJ) in CentriSep 96 spin plates (CS-961, Princeton Separations, Princeton, NJ). Sequencing products were then analyzed with an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems). Sequencing products were assembled and edited using Geneious (v3.0; Drummond *et al.* 2011).

Table 2. Loci and associated primers sequenced for this study. Thermal profiles for PCR and cycle sequencing reactions vary only by annealing temperature (55–58°) across primers and samples.

Locus/Gene	Primer	Source	Sequence (5'–3')
ND1	L3827	Sorenson <i>et al.</i> 1999; Ast 2001	GCAATCCAGGTCGGTTTCTATC
	H4644.VS2	Sorenson <i>et al.</i> 1999; Ast 2001	TCRAATGGGGCTCGGTTKGTTC
	L4500	Sorenson <i>et al.</i> 1999; Ast 2001	GTTGCMCAAACCATCTCHTAYGAA
	H5191	Sorenson <i>et al.</i> 1999; Ast 2001	GGGGTATGGGCCCGATAGC
ND2	L4951	Sorenson <i>et al.</i> 1999; Ast 2001	CCTCCTCTGAAAACAATTTCTCCC
	H5766	Sorenson <i>et al.</i> 1999; Ast 2001	GGATGAGAAGGCTAGGATTTTKCG
	L5601	Sorenson <i>et al.</i> 1999; Ast 2001	TGACTMCCAGAAGTHCTTCAAGG
	H5760	Sorenson <i>et al.</i> 1999; Ast 2001	GATGAGGAGTGCTATTGGGGC
	H6681	Sorenson <i>et al.</i> 1999; Ast 2001	GGTATAGGGTGCCGATGTCTTTGT
DGL- α	DGLf	Alföldi <i>et al.</i> 2011	ATGCTATTGTGGGCATTGCT
	DGLr	Alföldi <i>et al.</i> 2011	TGTTGGGTCAAAGACGCATA
L52	L52f	Alföldi <i>et al.</i> 2011	TCCTGTTCCACATATTCAGCA
	L52r	Alföldi <i>et al.</i> 2011	AATGCATTTGTCTGGAAGGC
L74	L74f	Alföldi <i>et al.</i> 2011	ACAGAAGGGGTGGTTCTGG
	L74r	Alföldi <i>et al.</i> 2011	TGTCATTGGTATTGATCTTGGC
PRLR	PRLR.F1	Townsend <i>et al.</i> 2008	GACARYGARGACCAGCAACTRATGCC
	PRLR.R3	Townsend <i>et al.</i> 2008	GACYTTGTGRACTTCYACRTAATCCAT

Sequences from mitochondrial gene regions (mtDNA: NADH Dehydrogenase Subunit 1 and 2: ND1, ND2), and associated flanking tRNAs (tRNA^{leu}, tRNA^{ile}, tRNA^{gln}, tRNA^{met}, tRNA^{trp}, tRNA^{ala}, and tRNA^{asn}), were isolated for 81 ingroup (*V. salvator* Complex) and 14 outgroup samples and combined with Ast's (2001) dataset. We also sequenced four nuclear loci (nDNA: two anonymous loci [Alföldi *et al.* 2011; primers deposited at Dryad: doi:XXXX], and the prolactin receptor [PRLR] and diacylglycerol lipase-alpha [DGL- α] genes): DGL- α (80 ingroup, 9 outgroup samples), anonymous nuclear locus L52 (63, 15), anonymous nuclear locus L74 (66, 17), and PRLR (59, 9). All novel sequences were deposited in GenBank (accession Nos. XXXX (ND1–ND2); XXXX (DGL- α); XXXX (L52); XXXX (L74); XXXX (PRLR)).

Sequence alignment and phylogenetic analyses

We produced initial alignments in Muscle (v3.7; Edgar 2004), with manual adjustments in Se-Align (v2.0a9; Rambaut 2002; submitted at Dryad: doi:XXXX). In order to assess phylogenetic congruence between mitochondrial and nuclear data, we inferred phylogenies for each locus independently under both Maximum Likelihood (ML) and Bayesian frameworks. We found weakly supported nDNA topologies, but high support for mtDNA lineages (Supplemental Figure

Table 3. Estimated models of evolution by data partition, as inferred by jModeltest, and applied for partitioned model-based analyses. tRNA partition includes tRNA^{leu}, tRNA^{ile}, tRNA^{gln}, tRNA^{met}, tRNA^{trp}, tRNA^{ala}, and tRNA^{asn}.

Partition	AIC model
ND1 1 st position	JC
ND1 2 nd position	GTR+ Γ
ND1 3 rd position	GTR+ Γ
ND2 1 st position	GTR+ Γ
ND2 2 nd position	GTR+I+ Γ
ND2 3 rd position	GTR+I+ Γ
tRNAs	GTR+I+ Γ
DGL- α	GTR+I+ Γ
L52	HKY+I+ Γ
L74	K80+I+ Γ
PRLR	HKY

1). Due to the absence of well-supported topological incongruence between mtDNA and nDNA trees, we conducted subsequent analyses using a combined, partitioned, concatenated dataset. Following a number of recent studies (Brandley *et al.* 2005; Siler and Brown 2010; Wiens *et al.* 2010) we treated each nuclear locus as a distinct partition, and partitioned mitochondrial DNA by coding region (ND1, ND2), codon position, and tRNAs (tRNA^{leu}, tRNA^{ile}, tRNA^{gln}, tRNA^{met}, tRNA^{trp}, tRNA^{ala}, and tRNA^{asn}). We used the Akaike information criterion (AIC), as implemented in jModeltest (v0.1.1; Posada 2008), to select the most appropriate model of nucleotide substitution for each of the eleven partitions (Table 3).

We conducted partitioned maximum likelihood (ML) analyses using the program

RAxMLHPC (v7.0; Stamatakis 2006) for the combined dataset. We applied the more complex model (GTR + I + Γ) to all subsets, and 1000 replicate ML inferences were performed for the analysis. Each inference was initiated with a random starting tree and used the rapid hill-climbing algorithm of Stamatakis *et al.* (2007, 2008). Clade support was assessed with 1000 bootstrap pseudoreplicates. Partitioned Bayesian analyses in MrBayes (v3.1.2; Ronquist and Huelsenbeck 2003) were conducted with a rate multiplier to allow substitution rates to vary among subsets. Default priors were used for all model parameters except branch lengths, which were adjusted on subsequent runs to facilitate run convergence (Brown *et al.* 2010; Marshall *et al.* 2006b; Marshall 2010). We ran four independent MCMC analyses, each with four Metropolis-coupled chains set at the default heating scheme. Analyses were run for 40 million generations, sampling every 5,000 generations. We assessed stationarity by plotting all sampled parameter values and log-likelihood scores from the cold Markov chains from each independent run against generation time using Tracer (v1.4; Rambaut and Drummond 2007). We also compared split frequencies among independent runs for the 20 most variable nodes using Are We There Yet? (AWTY, Wilgenbusch *et al.* 2004). We conservatively discarded the first 20% of samples as burn-in.

Population structure

We estimated haplotype diversity and population genetic structure for mitochondrial and nuclear datasets, initially analyzing each locus independently. Nuclear data were phased for each locus using the program PHASE (v2.1; Stephens *et al.* 2001; Stephens and Scheet 2005). Statistical parsimony allelic networks were estimated using the program TCS (v1.21; Clement *et al.* 2000), which utilizes a 95% connection significance criterion. For comparison, concatenated nuclear

and mitochondrial data were analyzed with the NeighborNet algorithm in SplitsTree (v4.11.3; Huson and Bryant 2006), which attempts to account for the uncertainty associated with both sampling and systematic errors. In addition to analyzing the raw, concatenated nuclear and mitochondrial data, we explored the effect of using a standardized distance matrix for nuclear loci (created with the program POFAD [v1.03; Joly and Bruneau 2006]), which facilitates the use of multiple loci and allows for inference of population dynamics which have resulted from the presence of allelic variation (Posada and Crandall 2001; Cassens *et al.* 2005; Zarza *et al.* 2008). Resulting networks can effectively illustrate equally parsimonious inferences and underlying patterns of spatially partitioned genetic variation (Cassens *et al.* 2003).

We applied the program Structure's Bayesian clustering method (v2.3.3; Pritchard *et al.* 2000; Falush *et al.* 2003, 2007; Hubisz *et al.* 2009) to our phased nuclear data to estimate population structure, identify allelic variants, possible migrants, and individuals with an admixture of genetic ancestry. Using this method, allelic composition is reported as posterior mean estimates of inferred allelic populations (Cabria *et al.* 2011). In the absence of prior knowledge of relationships, and given monitor lizards' inherent capability for dispersal across both terrestrial and marine barriers (Hoogerwerf 1954; Gaulke 1991; Rawlinson *et al.* 1992), we used the most flexible "admixture" model for all analyses. We varied the *a priori* estimate of populations from a single, panmictic population distributed across all of Southeast Asia ($K = 1$), to a maximally partitioned ($K = 32$) series of populations including all islands (and/or biogeographic subregions within large islands) represented in our sampling. We ran analyses for 5 million iterations, discarding a burn-in of 500,000. We selected the preferred number of populations based on the mean value of the log likelihood for each value of K . To distinguish between samples that exhibited mixed versus pure allelic composition, we used a 90%

composition threshold (Pritchard *et al.* 2000) and visualized results with the program Distruct (v1.1; Rosenberg 2004).

Bayesian Species Delimitation

We approached questions of taxonomic diversity on the basis of three evolutionary hypotheses. In order to provide an objective starting point for the program Bayesian Phylogenetics and Phylogeography (BP&P; Yang and Ranalla 2010), we first evaluated support for lineages based on the phylogenetic estimate derived from our concatenated dataset. We then estimated the phylogeny using the multi-species coalescent model implemented in *BEAST (v1.7.0; Drummond and Rambaut 2007; Heled and Drummond 2010). For *BEAST analyses, we applied separate GTR + γ nucleotide substitution models and lognormal-distributed relaxed clock models to the nuclear and mitochondrial subsets. Both discrete gamma distributions of among site rate variation had six rate categories. We arbitrarily set the mean rate of the mitochondrial relaxed clock hyper-parameter to 1.0, and estimated the rate of the nuclear relaxed clock relative to the mitochondrial clock; we used a uniform prior (U(0, 2.0)) on the mean of the lognormal-distributed nuclear relaxed-clock hyper-parameter. We used an exponentially distributed prior (Exp(20)) on the standard deviation of both lognormal-distributed relaxed-clock hyper-parameters and default priors for both GTR models. Gene trees were estimated independently (conditional on the species tree) for each of the five loci, using random starting trees and the ploidy levels (autosomal versus mitochondrial) set appropriately. We used a Yule process prior on the species tree and constrained the effective population size along each branch to be constant. We used the default (1/x) priors for the Yule process birth rate and mean effective population size and ran two independent analyses for 100 million generations, sampling every

20,000 generations. We assessed the stationarity and convergence of the MCMC chains by plotting all parameters' likelihood, prior, and posterior scores over generations using Tracer (Rambaut and Drummond 2007), discarded a 20 million generation burn-in, and confirmed run convergence and sufficient sample sizes ($ESS > 200$ for all parameters).

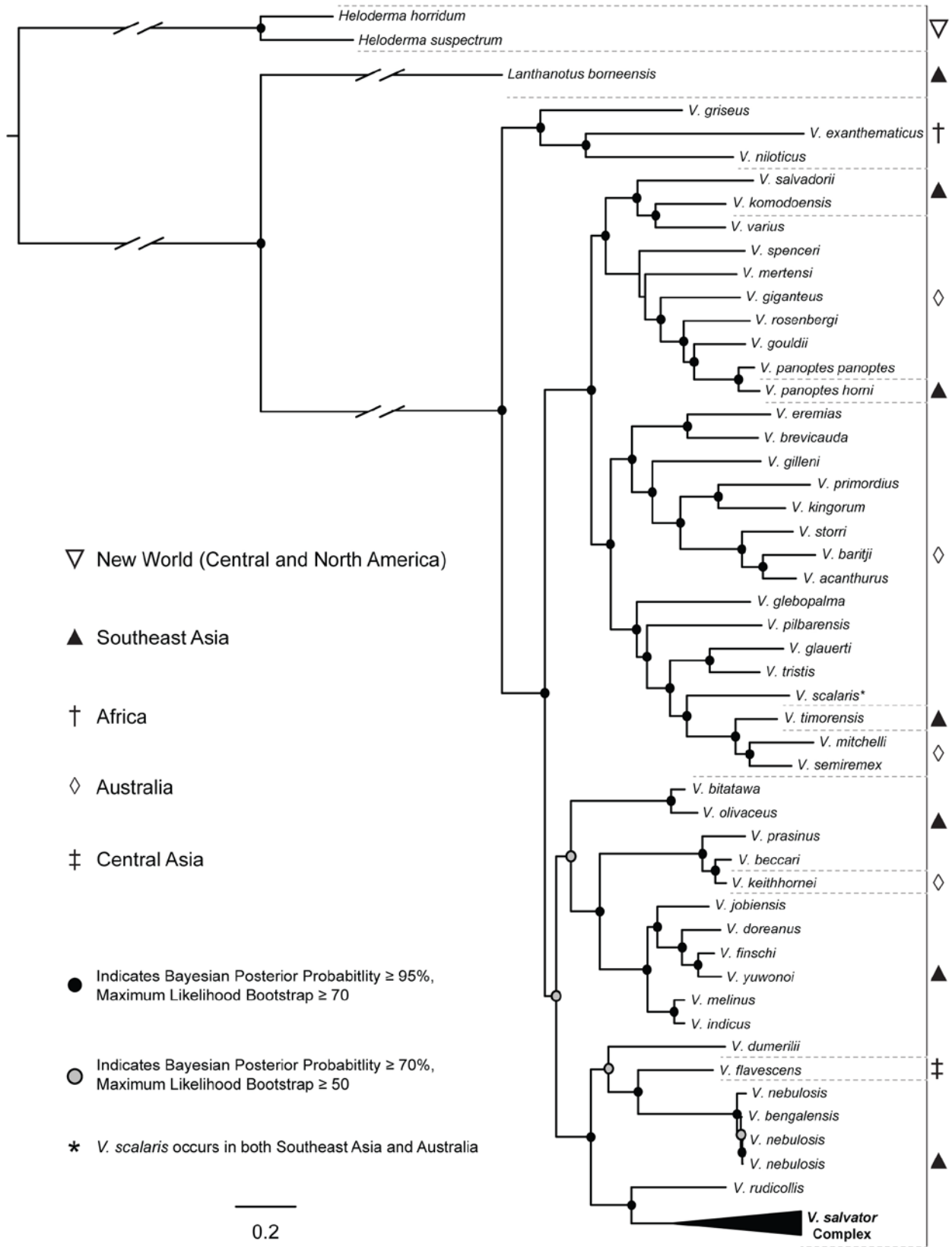
To evaluate current taxonomy and explore species boundaries we used the program BP&P with starting topologies estimated from our *BEAST species trees and phylogenetic estimate. With these user-specified guide trees, BP&P estimates the probability of splits between terminal taxa, assuming no admixture following speciation. We applied BP&P using phased data nuclear data, including all nine putative lineages. Individual runs using the rjMCMC algorithm evaluated subtrees created through the collapsing of nodes present on the guide tree, without branch swapping. All analyses were run for 500,000 generations, sampling every 50 generations, discarding a burn-in of 10,000. We used the "0" algorithm with the fine-tuning parameter $\epsilon = 15$, and explored the effect of lower (5, 10) and greater (20, 25) values of ϵ (Yang and Rannala 2010), with no major impact on results. In order to assess the effect of priors on the ancestral population size (θ) and the root age (τ), three different prior regimes were tested for each topology (Leaché and Fujita 2010). The prior settings reflect: (1) a relatively large ancestral population with shallow divergences ($\theta = 1, 10; \tau = 2, 2000$), (2) a relatively large ancestral population with deep divergences ($\theta = 1, 10; \tau = 1, 10$), both with a prior mean = 0.1 and variance = 0.01, and (3) a relatively small ancestral population and shallow divergences ($\theta = 2, 2000; \tau = 2, 2000$), both with a prior mean = 0.001 and variance = 5×10^{-7} . The first of these three settings is expected to be the most conservative, generally favoring models with fewer species (Leaché and Fujita 2010).

Results

Sampling and phylogenetic inference

Our complete, aligned matrices include 146 ND1–ND2 (2531bp), 89 DGL- α (651 bp), 86 L52 (545 bp), 90 L74 (185 bp), and 74 PRLR sequences (541 bp), respectively. Variable/parsimony-informative characters are: 1610/1460 (mtDNA); 32/16 (DGL- α); 37/18 (L52); 8/4 (L74); and 32/14 (PRLR). We rooted our tree with *Heloderma* based on accepted superfamily Varanoidea relationships (Caldwell 1999; Lee and Caldwell 2000; Townsend *et al.* 2004; Evans *et al.* 2005; Wiens *et al.* 2010; Conrad *et al.* 2011).

Analyses of the combined mitochondrial and nuclear datasets resulted in topologies with high bootstrap support (ML) and posterior probabilities (Bayesian; Figs. 1, 2). The inferred topologies were congruent across analyses, and generally, our results support those of Ast (2001) and Collar *et al.* (2011), strongly support the monophyly of the *V. salvator* Complex, and indicate that Philippine species are paraphyletic with respect to non-Philippine lineages. Within the Philippines, eight major, well-supported clades of water monitors were recovered (BS \geq 70%, PP \geq 0.95; Fig. 2A–H). Many clades correspond well to Southeast Asian biogeographical regions (Clade A: Mindanao faunal region; C: Sulawesi; E: Palawan Island; F: Mindoro faunal region; G: Bicol faunal region; H: Visayan faunal region + Romblon Island Group); others contain samples from multiple regions (D: Sumatra, Java, Myanmar, Singapore). The most surprising general results were our findings of the paraphyletic nature of *V. marmoratus* and the inference of all non-Philippine species nested within the large Philippine clade. Our results indicate a close relationships between *V. palawanensis* and *V. cf. marmoratus* from Mindoro faunal region, and a sister relationship between *V. nuchalis* and *V. cf. marmoratus* from the Bicol faunal region (Fig. 2).



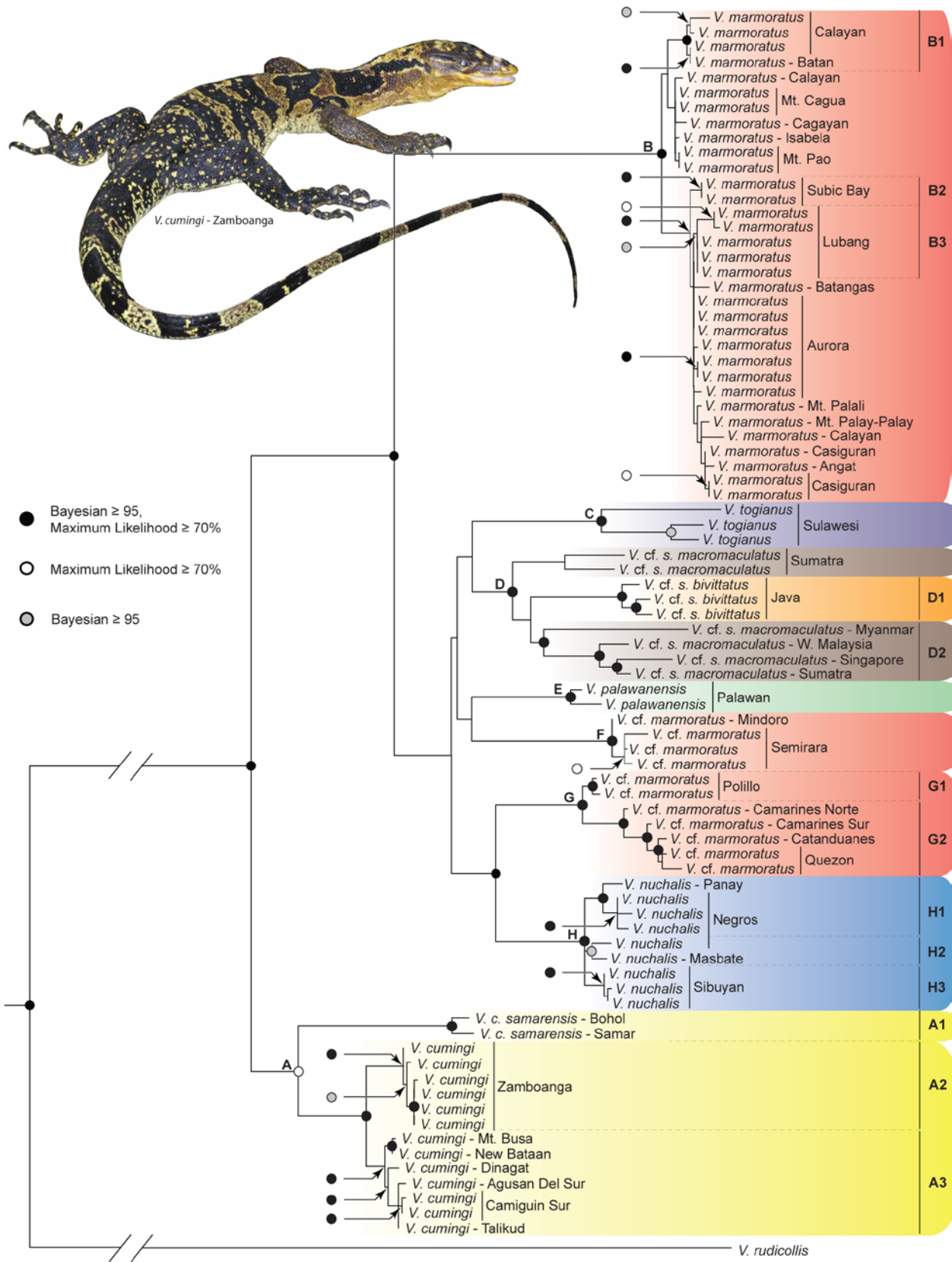


Figure 1. Maximum Likelihood estimate of species level relationships within Varanidae. Likelihood bootstrap and Bayesian posterior probability nodal support is indicated with shaded circles (see key).

Figure 2. Maximum Likelihood estimate of phylogenetic relationships within the *Varanus salvator* Complex. Likelihood bootstraps and Bayesian posterior probability nodal support is indicated with shaded circles (see key).

Population structure

TCS identified 61 and 44 unique haplotypes from mtDNA and nDNA, respectively (Fig. 3; Table

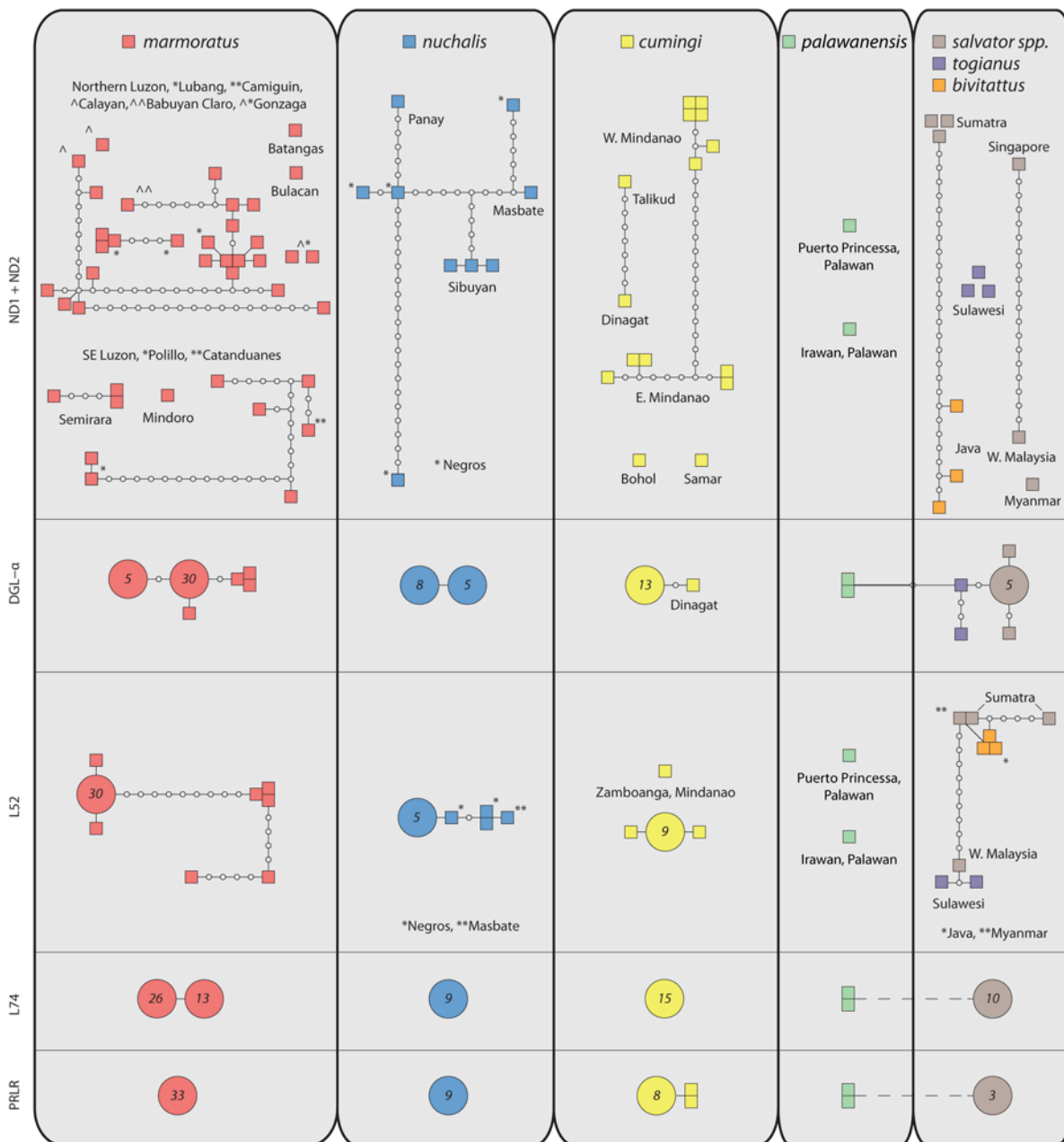


Figure 3. Mitochondrial and nuclear (ND1–ND2, DGL-α, L52, L74, PRLR) statistical haplotype networks inferred by TCS (Clement *et al.* 2000).

4). Haplotype diversity is highest within *V. marmoratus*, with entirely unique variants (distinct networks or individual samples) corresponding to well-supported clades identified in phylogenetic analyses (Figs. 2, 3). In mtNDA networks TCS recovered a significant distinction between populations from central and northern Luzon, the Bicol faunal region, and the Mindoro faunal region. TCS also identified unique haplotypes in populations from small islands surrounding Luzon as well as the various faunal subregions within Luzon. Within *V. nuchalis*, unique haplotypes are recovered for Panay, Masbate, Sibuyan, and Negros islands (with four distinct haplotypes; Fig. 3). Both analyses identified distinct haplotypes for *V. cumingi* on smaller islands of the Mindanao faunal region and marked divergence between east and west Mindanao Island (Fig. 3). Distinct haplotypes were also detected in *V. togianus* (n = 3), *V. salvator bivittatus* (3), Sumatran *V. s. macromaculatus* (3), and a single haplotype across Myanmar, Malaysia, and Singapore (Fig. 3). As expected, haplotype diversity was significantly lower in nuclear loci; only in L52 does the partitioning of genetic diversity correspond to major geological components of Southeast Asia's major landmasses (Fig. 3). *Varanus marmoratus* and *V. nuchalis* exhibit the highest proportions of unique haplotypes (79.4 and 76.5 % unique, respectively; Table 4).

Our SplitsTree analyses recovered similar patterns of genetic variation, with greater distinctiveness of sampled taxa apparent in mtDNA (Fig. 4; Table 4), including 13, well-supported clusters (>70 BS; Fig. 4). These represent samples from: (A) Samar and Bohol islands (*V. c. samarensis*); (B) western Mindanao Island (*V. c. cumingi*); (C) eastern Mindanao Island (*V. c. cumingi*); (D) northern Luzon Island, Lubang Island, and the Batanes and Babuyan island groups (*V. marmoratus*); (E) Sulawesi Island (*V. togianus*); (F) Mindoro and Semirara islands (*V. cf. marmoratus*); (G) Bicol Peninsula (Luzon Island), and Polillo and Catanduanes islands (*V. cf.*

Table 4. Summary of haplotype diversity within the *Varanus salvator* Complex as inferred by TCS and SplitsTree. TCS results are presented by locus (Fig. 3), while those of SplitsTree are indicative of mitochondrial analyses alone (Fig. 5). * Haplotypes which are distinct from the typical *V. salvator* form as inferred by TCS. ^ Haplotypes which are shared with the typical *V. salvator* form. ** Taxa which are further partitioned by SplitsTree, with clusters corresponding to geographic distributions of lineages.

Taxon	ND1+ND2	DGL- α	L52	L74	PRLR	% Unique	Distinct mtDNA cluster
<i>V. cumingi</i>	8	2	4	1	2	70.6	+**
<i>V. c. samarensis</i>	2	1	1	1	1	33.3	+
<i>V. marmoratus</i>	19	4	5	2	4	79.4	+
<i>V. marmoratus</i> (Mindoro)	3	3	1	1	1	44.4	+**
<i>V. marmoratus</i> (Bicol)	7	3	3	2	1	56.3	+
<i>V. nuchalis</i>	9	1	4	1	1	76.5	+
<i>V. palawanensis</i>	2	1	1	1^	2^	50.0	+
<i>V. s. bivittatus</i>	3	1	1*	1	1	42.9	+
<i>V. s. macromaculatus</i>	6	3	5	1	1	62.5	+**
<i>V. togianus</i>	3	2*	2*	1	1	66.7	+

marmoratus); (H) Panay, Negros, and Sibuyan islands (*V. nuchalis*); (I) Palawan Island (*V. palawanensis*); (J) western Malaysia and Sumatra (*V. s. macromaculatus*); (K) Myanmar (*V. s. macromaculatus*); (L) Java (*V. bivittatus*); and (M) Sumatra (*V. s. macromaculatus*; Fig. 4).

Analyses of the concatenated nuclear data recovered two poorly supported clusters: one containing *V. marmoratus*, *V. cf. marmoratus* (Bicol and Mindoro faunal region), and *V.*

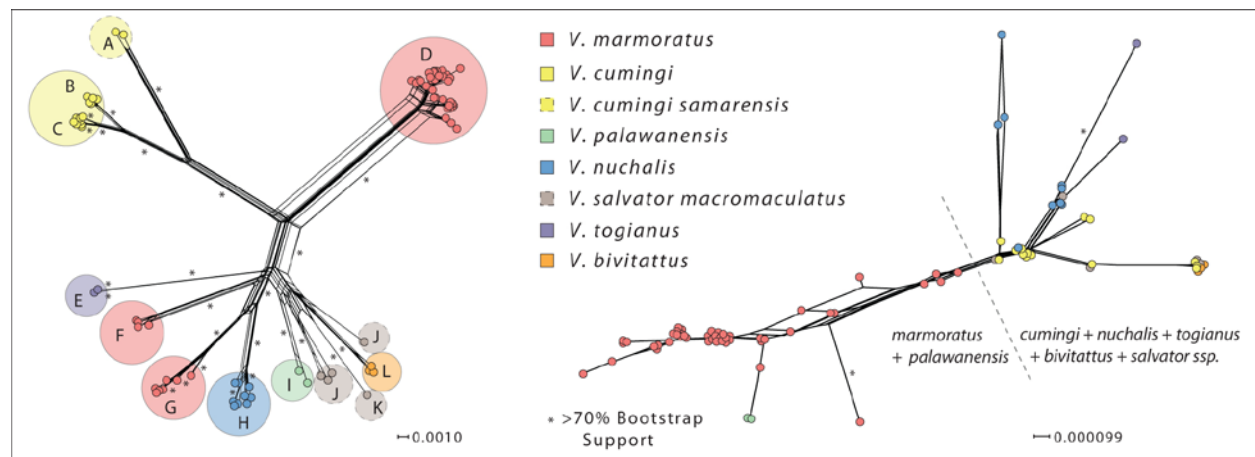


Figure 4. Mitochondrial and nuclear haplotype networks inferred by SplitsTree (Husan and Bryant 2006). Clusters correspond to (Philippines, unless noted): A) Samar and Bohol islands; B) western Mindanao Island; C) eastern Mindanao Island; D) northern Luzon, Batan, Calayan, and Lubang islands; E) Sulawesi Island, Indonesia; F) Mindoro and Semirara islands; G) the Bicol Peninsula of southeastern Luzon, and Catanduanes and Polillo islands; H) Masbate, Negros, Panay, and Sibuyan islands; I) Palawan Island; J) Western Malaysia, and Sumatra Island, Indonesia; K) Myanmar; L) Java Island, Indonesia; and M) Sumatra Island, Indonesia.

palawanensis samples, and another containing all remaining samples. Our SplitsTree inference based on the standardized distance matrix from POFAAD did not differ significantly in structure (Supplementary Figure 2).

Structure analyses support the presence of six genetically distinct populations ($K = 6$) with a maximal value of the log likelihood of the data (-187.0 ; greater and lesser values of K exhibited increased variance and lower posterior probabilities). These inferred populations consist of individuals from (1) central Luzon, Lubang and Calayan islands (*V. marmoratus*); (2) northern Luzon Island and Batan Island (*V. marmoratus*); (3) Palawan (*V. palawanensis*), and the Mindoro and Bicol faunal regions (*V. cf. marmoratus*); (4) Panay, Negros, and Sibuyan islands (*V. nuchalis*); (5) Mindanao, Camiguin Sur, Talikud, and Samar islands (*V. cumingi* and *V. c. samarensis*); and (6) Java (*V. bivittatus*), Sumatra, and Myanmar (*V. s. macromaculatus*). All six genetic groupings contain individuals of little admixture, in which they are assigned to a single deme, or with part of their allelic composition derived from multiple demes (Fig. 5; Table 5).

Table 5. Allelic deme distribution within the *Varanus salvator* Complex. Assignment to demes, versus admixed allelic ancestry, is based on a 90% composition threshold. Deme names correspond to proportional distribution among taxa sampled.

Taxon	% Single Population Assignment	Inferred Allelic Population	Primary Demes of Admixture
<i>Varanus cumingi</i> (n = 13)	69.2	<i>cumingi, salvator</i>	<i>nuchalis</i> and <i>palawanensis</i>
<i>V. c. samarensis</i> (n = 2)	50.0	<i>cumingi</i>	<i>nuchalis</i> and <i>palawanensis</i>
<i>V. marmoratus</i> (n = 31)	80.6	<i>marmoratus</i> 1 and 2	<i>marmoratus</i> 1 and 2, and <i>salvator</i>
<i>V. marmoratus</i> (n = 4) Mindoro faunal region	50.0	<i>palawanensis</i> and <i>cumingi</i>	<i>marmoratus</i> 1, <i>cumingi</i> , <i>nuchalis</i> , and <i>palawanensis</i>
<i>V. marmoratus</i> (n = 6) Bicol faunal region	50.0	<i>palawanensis</i>	<i>marmoratus</i> 1, <i>cumingi</i> , <i>nuchalis</i> , and <i>palawanensis</i>
<i>V. nuchalis</i> (n = 9)	100.0	<i>nuchalis, cumingi,</i> and <i>marmoratus</i> 2	n/a
<i>V. palawanensis</i> (n = 2)	50.0	<i>palawanensis</i>	<i>marmoratus</i> 1 and 2, and <i>palawanensis</i>
<i>V. salvator bivittatus</i> (n = 3)	100.0	<i>salvator</i>	n/a
<i>V. s. macromaculatus</i> (n = 5)	80.0	<i>salvator</i> and <i>cumingi</i>	<i>Marmoratus</i> 2, <i>nuchalis</i> , <i>palawanensis</i> , and <i>salvator</i>
<i>V. togianus</i>	0.0	n/a	<i>Marmoratus</i> 2, <i>cumingi</i> , <i>nuchalis</i> , <i>palawanensis</i> , and <i>salvator</i>

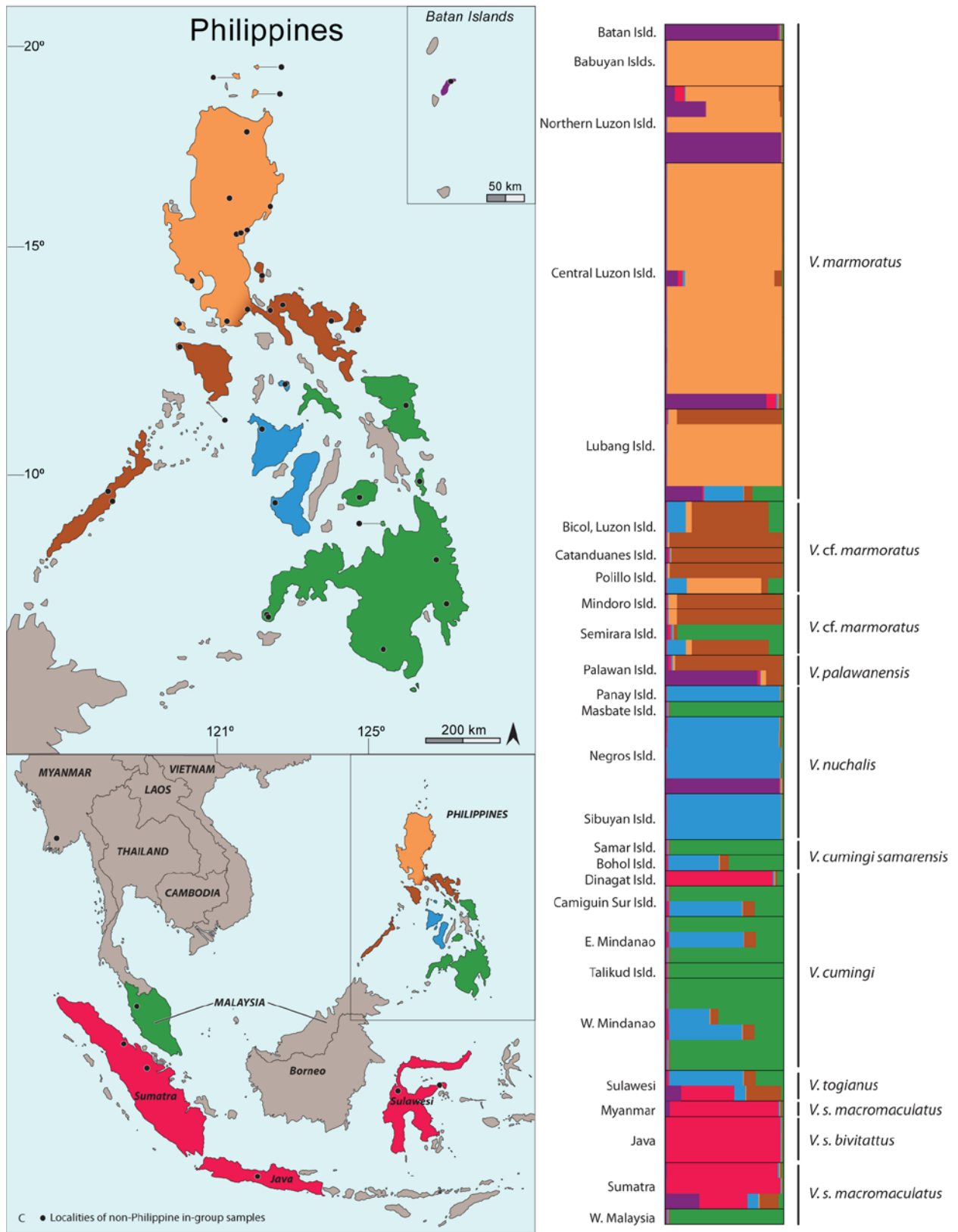


Figure 5. Distruct (Rosenberg 2004) visualization of Structure analyses and summarized geographic distribution of major *Varanus* demes (Pritchard *et al.* 2000) for K=6 allelic population.

Species delimitation

Most of our *BEAST analyses (12 runs under 6 settings) yielded similar species relationships. In posterior samples, two topologies were preferred at nearly identical frequencies (consensus trees calculated from different subsamples of the same chain, or from independent chains, yielded either topology; Supplemental Figure 3). These two topologies differ slightly in that *V. cf. marmoratus* (Mindoro Island) and *V. palawanensis* are recovered either as sister species within a clade of *V. cf. marmoratus* (Bicol faunal region), true *V. marmoratus* (northern and central Luzon), and *V. nuchalis* (Fig. 6B), or as consecutive outgroups to a clade comprised of *V. cf. marmoratus* (Bicol faunal region), true *V. marmoratus* (Luzon), *V. nuchalis*, and, *V. salvator* (Fig. 6C; Supplemental Figure 3). Given the alternative placements of the Mindoro faunal region lineage, we estimated species boundaries in BP&P under both species tree topologies, as well as under the topology recovered by our concatenated gene tree analysis. BP&P analyses were consistent across runs for all three topologies, with high support for most described species. The split distinguishing populations of *V. c. cumingi* and *V. c. samarensis* received weak support (speciation probability [sp] = 0.05–0.44; Fig. 6), and the split between *V. cf. marmoratus* from the Mindoro faunal region and *V. palawanensis* was only moderately supported (sp = 0.73–1.0; Fig. 6A–B). However, the split distinguishing *V. cf. marmoratus* (Bicol faunal region) from its inferred closest relative was always recovered with high probability (sp = 0.99–1.0; Fig. 6). In both instances of lower split support, speciation probabilities were highest under the assumptions of small ancestral populations and recent divergences (Fig. 6A, B); in topology C, all lineages except for *V. c. samarensis* received high support (sp = 1.0; Fig. 6).

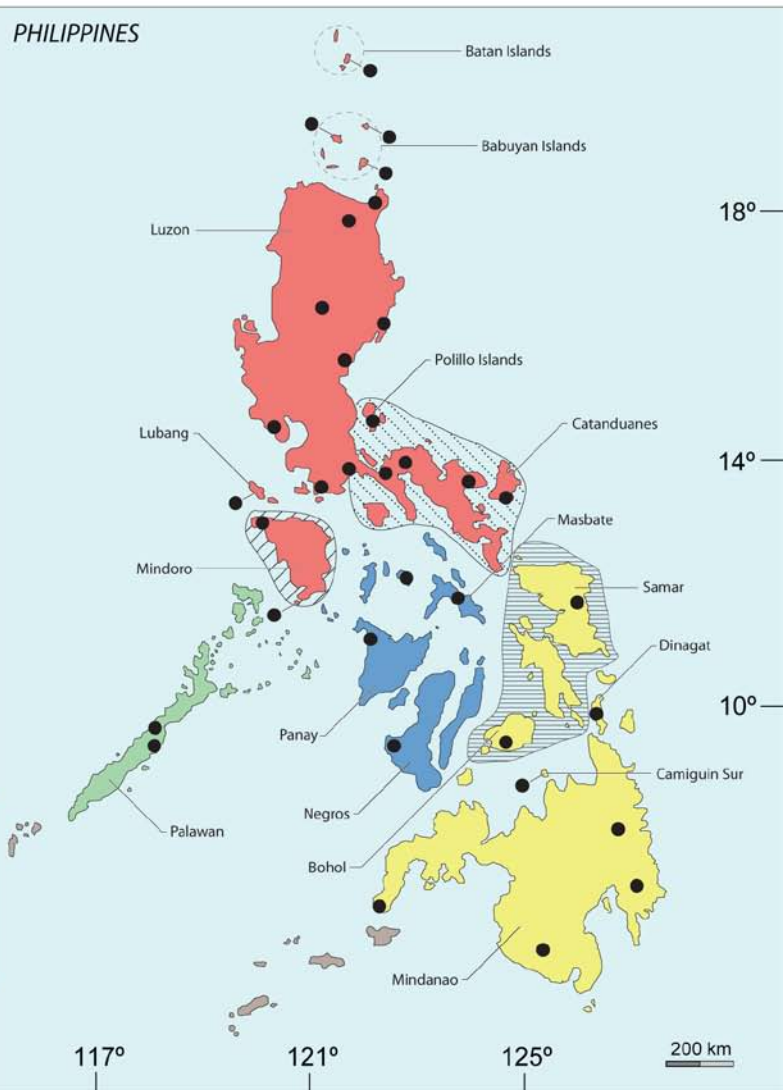
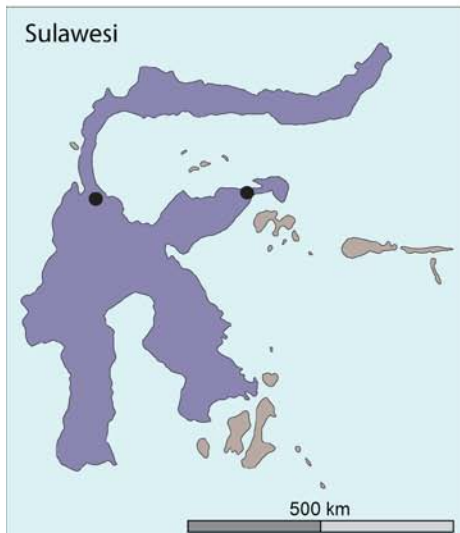
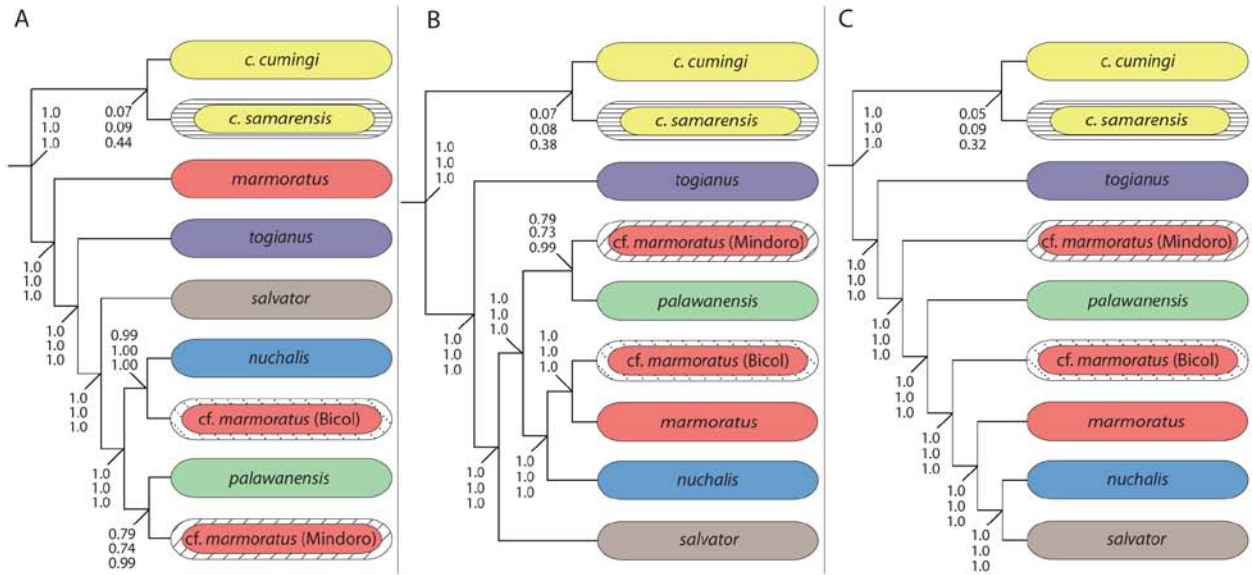


Figure 6. Bayesian species delimitation for the *Varanus salvator* Complex, with topologies inferred from: A) concatenated phylogenetic analyses, and (B, C) preferred *BEAST species tree reconstructions. Speciation probabilities depicted at nodes correspond to three sets of priors to explore the effects of ancestral population size and depth of divergences between putative species (See Materials and Methods for details).

Discussion

Since its original description nearly two and a half centuries ago (Laurenti 1768), *Varanus salvator* has undergone numerous taxonomic revisions resulting in increased recognition of diversity from a single species (Laurenti 1768), to four species (Boulenger 1885), back to a single species (*V. salvator*) with five (Mertens 1942a–c) or eight (Mertens 1963; Gaulke 1991,

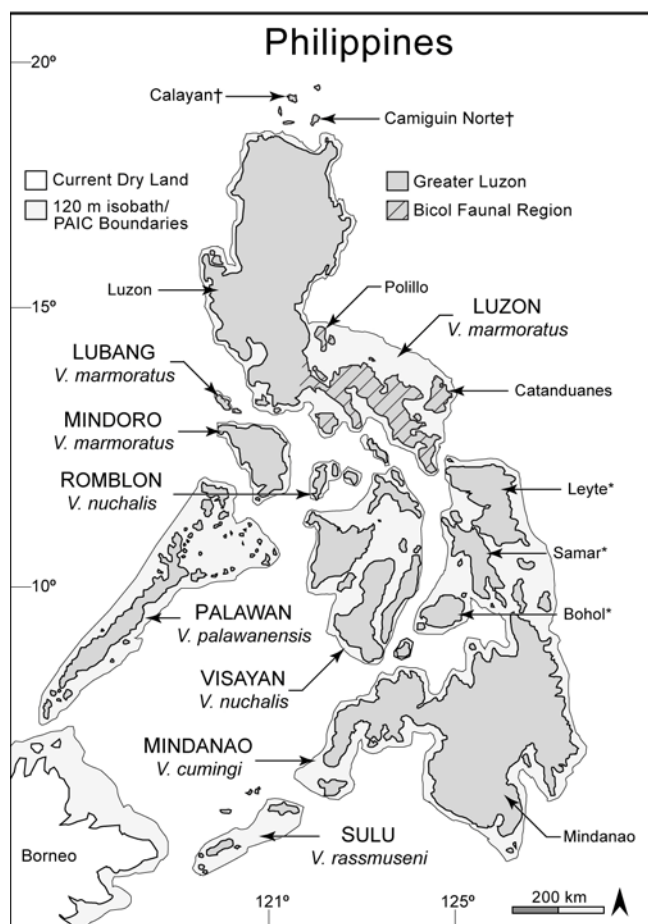


Figure 7. Recognized faunal regions and island groups of the Philippines, including distributions of species within the *Varanus salvator* Complex.

1992; Böhme 2003) subspecies, and most recently, to six species and six subspecies (Koch and Böhme 2010; Koch *et al.* 2007, 2010a, b). Nearly half of the 12 currently recognized, named taxa are endemic to the Philippines (Table 1), and their distributions approximately correspond to recognized faunal regions (Fig. 7; Brown and Diesmos 2009). We consider traditional, morphology-based taxonomy as a reasonable basis for hypotheses of species diversity if character-based diagnostic definitions of morphologically distinguishable units are provided. In the

absence of conflicting data, traditionally defined species are, of course, considered valid under currently accepted guidelines for taxonomic nomenclature (ICZN 1999). However, with respect to water monitors, past studies have been limited by their reliance on combinations of color pattern, body size and meristic characters (scale counts), as well as relatively small sample sizes available in museum collections. Although these types of data can provide useful diagnostic characters (Mertens 1942a–c), recent treatments of the *Varanus salvator* Complex have been unable to incorporate statistical analyses of large sample sizes, and have not incorporated historical biogeography (but see Gaulke 1991) or underlying genetic variation.

Our sampling of individuals from throughout the range of all but one currently recognized Philippine water monitor species allows for comprehensive genetic analyses of the *V. salvator* Complex across the archipelago with a variety of analytical approaches. This sampling includes the major, geographically proximate populations of water monitors occurring outside of the Philippines (i.e., islands of the Sunda Shelf, Sulawesi, and Asian mainland). The absence of available samples of *V. s. salvator* (Sri Lanka), *V. s. andamanensis* (Andaman islands), *V. s. komaini* (Thailand), *V. s. zieglerei* (Obi Island, Indonesia), *V. s. macromaculatus* (Borneo), and *V. rassmuseni* (Tawi-Tawi Island, Philippines) precludes systematic inferences for those taxa at present. However this does not hinder our primary goals of inferring phylogenetic affinities, population structure, and species boundaries among Philippine populations.

Phylogenetics and Population Structure

Although the focal group of this study is the Philippine assemblage of water monitors, our results underscore the necessity of geographically broad sampling in order to accurately estimate

evolutionary relationships and species-level diversity within widespread species complexes. Within the Philippines, the monophyly of all but one of the five described taxonomic units were supported in phylogenetic analyses (*V. c. cumingi*, *V. c. samarensis*, *V. nuchalis*, and *V. palawanensis*). In contrast, the taxon *V. marmoratus*, recovered here as a paraphyletic assemblage, represents three distinct, biogeographically discrete, well-supported clades that are not each other's closest relatives. The two newly discovered lineages include a clade from Mindoro faunal region, and one from the Bicol faunal region and Polillo and Catanduanes islands (Fig. 7). The first of these is sister to *V. palawanensis*, and although this relationship is not strongly supported, the geographic proximity of the Palawan and Mindoro faunal regions provides plausible biogeographic evidence for a close, presumably dispersal-mediated relationship, which has been observed in many other vertebrates (Brown and Guttman 2002; Evans *et al.* 2003; Brown *et al.* 2009; Esselstyn *et al.* 2010; Siler *et al.* 2012). The second lineage is inferred to be sister to *V. nuchalis* from the Visayan faunal region. This relationship is both novel and somewhat surprising, in that this lineage does not share phylogenetic affinities with the rest of Luzon. Biogeographically, however, the Bicol and Visayan faunal regions are geographically proximate, increasing the probability of contemporary gene flow between these regions. The recovery of novel phylogenetic relationships among Philippine water monitors once again highlights the dynamic nature of the Philippine archipelago (Brown and Diesmos 2009)—many vertebrate groups have diversified via apparently complex combinations of vicariance (possibly via sea level oscillation), dispersal, and *in situ* diversification across habitat barriers and ecological gradients (Esselstyn and Brown 2009; Esselstyn *et al.* 2009; Linkem *et al.* 2010; Siler *et al.* 2010, 2012; Welton *et al.* 2010a,b, in press).

The phylogenetic relationships of taxa outside of the Philippines reveals well supported monophyletic lineages corresponding to major landmasses (i.e., samples from Java [*V. s. bivittatus*] and Sulawesi [*V. togianus*]). However, mixed affinities are evident on Sumatra, with one sample closely related to *V. s. bivittatus* (Java) and two samples related to *V. s. macromaculatus* (Malaysia and Singapore). The relatively close proximity of Sumatra to the Asian mainland most likely increases the potential for gene flow between these two regions.

Estimates of haplotype diversity depicted in networks, and analyses of population structure, in part mirrored conclusions from our phylogenetic inferences. Of the taxonomic units sampled, all were supported as distinct in mitochondrial haplotype analyses (Figs. 3, 4; Table 4). The combined results of haplotype network analyses reveal that proportions of unique haplotypes in excess of 50% correspond to recognized species, while values below that are indicative of lower taxonomic units (i.e., subspecies, populations; Table 4). The lineage of *V. cf. marmoratus* from the Mindoro faunal region exhibits a proportion of unique haplotypes below this apparent threshold (44.4% unique), while the lineage from the Bicol faunal region exhibits a proportion greater than the threshold value (56.3% unique; Table 4). Affinities recovered by SplitsTree overwhelmingly correspond to expectations based on biogeography (Figs. 4, 7).

Our Structure analyses, while not recovering support for all described taxa, did reveal allelic admixture among currently recognized species, subspecies, and well-supported lineages—suggesting either gene flow or persistence of ancestral polymorphisms. Within *V. nuchalis* and *V. cumingi*, individuals possessed alleles predominant in *V. marmoratus* and *V. salvator* subspecies, respectively. The presence of taxon-specific alleles within populations of other species is intriguing, and involves a single individual in each case identified here. This situation is apparent

within the Philippines, as well as throughout portions of Southeast Asia, with predominately Philippine alleles present in Sulawesi, Peninsular Malaysia, and Myanmar. Although historical and contemporary natural processes of dispersal likely have contributed to these patterns, it is also possible that more recent, human mediated dispersal has occurred as well. Water monitors are frequently transported between islands, traded as bush meat, marketed in both legal and illegal pet trade (Gaulke 1998), and likely transported in agricultural shipments (personal observations). Due to relatively high dispersal ability and a general propensity for human-aided translocation, the potential for accidental or intentional introduction of water monitors is particularly high.

Our analyses indicate the strong possibility of dynamic historical and contemporary gene flow among populations of the *Varanus salvator* Complex. Phylogenetic and population genetic analyses support the distinctiveness, to varying degrees, of all eight taxonomic units sampled, and underscore the utility of employing multiple analytical techniques to mitochondrial and nuclear data in order to bolster support for phylogenetic inferences. However, formal taxonomic recognition of all entities detected here is complicated by the possibility of high levels of gene flow among putative taxa, and varying, non-equivalent levels of genetic divergence between named taxa.

Species Delimitation and Conservation

With a few exceptions, Philippine water monitor species are phenotypically distinct (corresponding to named taxa; Table 6), and possess geographical ranges circumscribed by the well-characterized biogeographical regions. However, questions concerning the manner in which

the putative species are now diagnosed persist. Past studies (Gaulke 1991; Koch *et al.* 2007, 2010a) have identified characteristics (body size, coloration, scale counts) that were argued to be diagnostic of each putative species in unique combinations. However, to date, no non-overlapping, discrete, (taxonomically diagnostic) character state differences between Philippine species have been identified (Table 6). In the case of the most recent study (Koch *et al.* 2010a), even multivariate analyses of continuous morphological variables showed overlap along major principal components between *V. marmoratus* and the newly described *V. palawanensis*, and yet it was argued that body size and color pattern provided sufficient justification for the recognition of the latter as a distinct species.

In this study we have attempted to use multiple lines of evidence (monophyly in our multilocus phylogenetic estimate, unique haplotype and diagnostic allelic diversity, statistically-supported clustering in network analyses, Bayesian species delimitation, consideration of morphology, and the biogeographic inference of allopatry) to re-consider species boundaries in Philippine taxa and infer the presence of additional evolutionary units. The majority of our analyses support the continued recognition of most named taxa (Table 6) and suggest that the distinct lineages of the Mindoro and Bicol faunal regions will likely warrant recognition if analyses of morphological data corroborate their genetic distinctiveness. However, recognition of lower taxonomic entities (subspecies) was only partially supported by phylogenetic and haplotype analyses, which similarly failed to corroborate elements of current species-level taxonomy suggesting that the status of some of these lineages may require reconsideration. For example, structure analyses did not distinguish between *V. palawanensis* and the Mindoro and Bicol faunal region populations of *V. cf. marmoratus*. Additionally, BP&P analyses provided

Table 6. Summary of morphological data used previously (Gaulke 1991; Koch *et al.* 2007, 2010a) to delimit species, and support from phylogenetic (monophyly), unique haplotype diversity, population genetics (TCS haplotypes and NeighborNet clusters), Bayesian species delimitation analyses, and biogeographic distribution of taxa. Morphological data summarized from Gaulke (1991, 1992) and Koch *et al.* (2007, 2010a). PN corresponds to phylogenetic networks produced in SplitsTree.

	<i>V. c. cumingi</i>	<i>V. c. samarensis</i>	<i>V. marmoratus</i>	<i>V. nuchalis</i>	<i>V. palawanensis</i>	<i>V. salvator macromaculatus</i>	<i>V. s. bivittatus</i>	<i>V. togianus</i>
Monophyly	+	+	–	+	+	–	+	+
Haplotype	+	+	–	+	+	+	+	+
PN Cluster (>70%)	+	+	–	+	+	+	+	+
BP&P	+	–	–	+	+/-	–	–	+
Allopatry	+	+	+	+	+	+	+	+
Body color (dorsal)	5–6 transverse bands of yellow ocelli over black background, with occasional yellow paravertebral stripe	5–8 transverse bands of yellow ocelli over black background	Variably, 4–6 transverse bands of light ocelli over black background	Variably, 4 transverse bands of light ocelli over black background, with occasional light paravertebral stripe	Up to 8 transverse bands of light ocelli, over mostly dark background, mottled with brightly bordered scales	n/a	n/a	n/a
Head color (dorsal)	Predominantly yellow-gold, with black temporal streak occasionally bordered by below by lighter streak	Predominantly black, with symmetrical yellow markings	Predominantly black, with 1 or 2 indistinct cross-bands on snout	Predominantly black, but with occasional light markings	Predominantly dark, but occasionally with light markings or light temporal streak	n/a	n/a	n/a
Occipital Scales	46–57	47–51	48–59	50–61	46–65	49–65	47–63	44–58
Nuchal Scales	26–33	21–30	24–33	19–30	24–32	29–50	28–52	31–42
Scales around midbody	121–150	130–152	115–145	136–169	129–178	135–178	101–175	120–142
Dorsal Scales	114–136	114–127	101–123	94–138	116–182	125–172	123–189	117–172
Ventral trunk scales	77–85	81–78	80–92	84–94	78–95	75–95	75–97	77–82
Scales around base	82–108	90–109	85–107	84–110	98–119	88–126	79–128	87–112

of tail								
Scales around tail, 1/3 from base	42–55	46–55	48–65	43–55	48–70	41–82	51–71	54–63
Narial position	1.9–2.26 times closer to tip of snout than to eye	2.0–3.29 times closer to tip of snout than to eye	1.85–2.6 times closer to tip of snout than to eye	1.78–2.13 times closer to the tip of the snout than to eye	1.69–2.20 times closer to tip of snout than to eye	2.0–2.8 times closer to tip of snout than to eye	1.67–2.88 times closer to tip of snout than to eye	1.82–2.70 times closer to tip of snout than to eye

only variable support for splits between these taxa and no support for the hypothesized split between *V. c. cumingi* and *V. c. samarensis*. The lack of clear support for the recognition of the species *V. palawanensis* and the subspecies *V. c. samarensis* may weaken the case for continued recognition of these taxa. However, given their apparent phenotypic distinctiveness (body size and color pattern, respectively; Gaulke 1991, 1992; Koch *et al.* 2010a), their continued recognition may be warranted—or at least favorable, given conservation concerns.

The substantial level of putative species diversity in Philippine water monitors bolsters the archipelago's designation as a biodiversity hotspot and a global conservation priority (Brown and Diesmos 2009; Welton *et al.* 2010a). Our identification of multiple unrecognized evolutionary lineages of water monitors has implications for the conservation of large-bodied vertebrates in the archipelago (Welton *et al.* 2010a, in press), and our identification of apparently cryptic monitor lineages from the Mindoro and Bicol faunal regions suggests the existence of additional species diversity or, at the very least, evolutionary significant units for conservation.

Monitor lizards are frequent attractions at zoological parks, and are commonly encountered in both legal and illegal animal trade (Gaulke 1998; Yuwono 1998; Schlaepfer *et al.* 2005; Cota *et al.* 2009) where they are harvested for skin and bush meat (Shine *et al.* 1996; Shine and Harlow 1998; Fa *et al.* 2000; Stuart 2004; Pernetta 2009; Welton *et al.* in press). Monitor lizards represent a particularly compelling group for studies relating to conservation, trade, and sustainable harvest given that they represent a commercially important component of local Asian vertebrate faunas (Shine *et al.* 1996; Koch *et al.* 2010a), are a heavily exploited vertebrate group (Mace *et al.* 2007; Shine *et al.* 1996; Shine and Harlow 1998; Schlaepfer *et al.* 2005), and are important components of the diet of many indigenous cultures (Mittermeier *et al.* 1992; Nash 1997; Stewert 2004; Welton *et al.* 2010a, in press). Given the ubiquitous presence of water

monitors in trade (Schlaepfer *et al.* 2005), we perceive an immediate need for identification and study of all lineages, some of which may be subject to differential harvesting pressures (Gaulke 1988; Welton *et al.* in press). Although water monitors are some of the most widely traded of vertebrates, efforts to conserve novel evolutionary lineages, such as those identified here, will be necessary for the long-term survival of these unique species in their natural habitats. The application of multiple analytical techniques to identify genetic components of biodiversity, coupled with statistically rigorous and objective methods of species delimitation can yield surprising results. In the present case, an integrative approach provided the opportunity to objectively evaluate diversity in a taxonomic group notorious for having a historically contentious past, while offering the potential to identify putative new species and important units for conservation (Leaché and Fujita 2010; Brown *et al.* in press).

References

- Alföldi J, Di Palma F, Grabherr M *et al.* (2011) The genome of the green anole lizard and a comparative analysis with birds and mammals. *Nature*, **477**, 587–591.
- Ast J (2001) Mitochondrial DNA evidence and evolution in Varanoidea (Squamata). *Cladistics*, **17**, 211–226.
- Barrett CF, Freudenstein JV (2011) An integrative approach to delimiting species in a rare but widespread mycoheterotrophic orchid. *Molecular Ecology*, **20**, 2771–2786.
- Bauer, AM, Parham JF, Brown RM *et al.* (2010) On the availability of new Bayesian-delimited gecko names and the importance of character based definitions of species. *Proceedings of the Royal Society of London B* doi:10.1098/rspb.2010.1330
- Baverstock PR, King D, King M *et al.* (1993) The evolution of species of the Varanidae:

- microcomplement fixation analysis of serum albumins. *Australian Journal of Zoology*, **41**, 621–638.
- Becker HO, Böhme W, Perry SF (1989) Die lungenmorphologie der warane (Reptilia: Varanidae) und ihre systematischstammesgeschichtliche bedeutung. *Bonner Zoologische Beitrage*, **40**, 27–56.
- Böhme W (1988) Zur genitalmorphologie der Sauria: funktionelle und stammesgeschichtliche aspekte. *Bonner Zoologische Monographien*, **27**, 1–176.
- Böhme W (2003) Checklist of the living monitor lizards of the world (family Varanidae). *Zoologische Verhandelingen*, **341**, 3–43.
- Böhme W, Ziegler T (1997) *Varanus melinus* sp. n., ein neuer Waran aus der *V. indicus*-Gruppe von den Molukken, Indonesien. *Herpetofauna*, **19**, 26–34.
- Böhme W, Ziegler T (2005) A new monitor lizard from Halmahera, Moluccas, Indonesia (Reptilia: Squamata: Varanidae). *Salamandra*, **41**, 51–59.
- Boulenger GA (1885) *Catalogue of the lizards in the British Museum (Natural History). Second edition. Vol. 2. Iguanidae, Xenosauridae, Zonuridae, Anguinae, Anniellidae, Helodermatidae, Varanidae, Xantusiidae, Teeidae, Amphisbaenidae*. Taylor and Francis, London, England.
- Boumans L, Vietes DR, Glaw F, Vences M (2007) Geographical patterns of deep mitochondrial differentiation in widespread Malagasy reptiles. *Molecular Phylogenetics and Evolution*, **45**, 822–839.
- Brandley MC, Schmitz A, Reeder TW (2005) Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of Scincid lizards. *Systematic Biology*, **54**, 373–390.

- Brown JM, Hedtke SM, Lemmon AR, Lemmon EM (2010) When trees grow too long: investigating the causes of highly inaccurate Bayesian branch-length estimates. *Systematic Biology*, **59**, 145–161.
- Brown RM, Diesmos A (2009) Philippines, Biology. In: *Encyclopedia of Islands* (eds Gillespie R, Clague D), pp 723–732. University of California Press, Berkeley.
- Brown RM, Guttman SI (2002) Phylogenetic systematics of the *Rana signata* complex of Philippine and Bornean stream frogs: reconsideration of Huxley's modification of Wallace's Line at the Oriental-Australian faunal zone interface. *Biological Journal of the Linnean Society*, **76**, 393–461.
- Brown RM, Siler CD, Diesmos AC *et al.* (2009) The Philippine frogs of the genus *Leptobrachium* (Anura; Megophryidae): phylogeny-based species delimitation, taxonomic revision, and descriptions of three new species. *Herpetological Monographs*, **23**, 1–44.
- Brown RM, Siler CD, Grismer LL, Das I, McGuire JA. In press. Phylogeny and cryptic diversification in Southeast Asian flying geckos. *Molecular Phylogenetics and Evolution*.
- Cabria MT, Michaux JR, Gómez-Moliner BJ *et al.* (2011) Bayesian analysis of hybridization and introgression between the endangered European mink (*Mustela lutreola*) and the polecat (*Mustela putorius*). *Molecular Ecology*, **20**, 1176–1190.
- Caldwell MW (1999) Squamate phylogeny and the relationships of snakes and mosasauroids. *Zoological Journal of the Linnean Society*, **125**, 115–147.
- Card W, Kluge AG (1995) Hemipeneal skeleton and varanid systematics. *Journal of Herpetology*, **29**, 275–280.

- Cassens I, Van Waerebeek K, Best PB *et al.* (2003) The phylogeography of dusky dolphins (*Lagenorhynchus obscurus*); a critical examination of network methods and rooting procedures. *Molecular Ecology*, **12**, 1781–1792.
- Cassens I, Mardulyn P, Milinkovitch MC (2005) Evaluating intraspecific ‘Network’ construction methods using simulated sequence data: Do existing algorithms outperform the global maximum parsimony approach? *Systematic Biology*, **54**, 363–372.
- Clement M, Posada D, Crandall K (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1660.
- Collar DM, Schulte JE, Losos JB (2011) Evolution of extreme body size in monitor lizards (*Varanus*). *Evolution* **65**, 2664–2680.
- Conrad JL, Ast JC, Montanari S *et al.* (2011) A combined evidence phylogenetic analysis of Anguimorpha (Reptilia: Squamata). *Cladistics*, **27**, 230–277.
- Cota M, Chan-Ard T, Makchai S (2009) Geographical distribution and regional variation of *Varanus salvator macromaculatus* in Thailand. *Biawak*, **3**, 134–143.
- Dayrat B (2005) Toward integrative taxonomy. *Biological Journal of the Linnean Society*, **85**, 407–415.
- Doyle JJ (1995) The irrelevance of allele tree topologies for species delimitation, and a non-topological alternative. *Systematic Botany*, **20**, 574–588.
- Drummond AJ, Ashton B, Buxton S *et al.* (2011) Geneious v5.4, available from <http://www.geneious.com/>
- Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.

- Edgar R (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, **32**, 1792–1797.
- Esselstyn JA (2007) Should universal guidelines be applied to taxonomic research? *Biological Journal of the Linnaean Society*, **90**, 761–764
- Esselstyn JA, Garcia J, Saulog M *et al.* (2008) A new species of *Desmalopex* (Pteropodidae) from the Philippines, with a phylogenetic analysis of the Pteropodimi. *Journal of Mammology*, **89**, 815–825.
- Esselstyn JA, Timm RM, Brown RM (2009) Do geological or climatic processes drive speciation in dynamic archipelagos? The evolution of shrew diversity on SE Asian islands. *Evolution*, **63**, 2595–2610.
- Esselstyn JA, Brown RM (2009) The role of repeated sea-level fluctuations in the generation of shrew (Soricidae: *Crocidura*) diversity in the Philippine Archipelago. *Molecular Phylogenetics and Evolution*, **53**, 171–181.
- Esselstyn JA, Oliveros CH, Moyle RG *et al.* (2010) Integrating phylogenetic and taxonomic evidence illuminates complex biogeographic patterns along Huxley's modification of Wallace's Line. *Journal of Biogeography*, **37**, 2054–2066.
- Evans BJ, Brown RM, McGuire JA *et al.* (2003) Phylogenetics of fanged frogs: testing biogeographical hypotheses at the interface of the Asian and Australian faunal zones. *Systematic Biology*, **52**, 794–819.
- Evans SE, Wang Y, Li C (2005) The early Cretaceous lizard genus *Yabeinosaurus* from China: resolving and enigma. *Journal of Systematic Palaeontology*, **4**, 319–335.
- Fa JE, Garcia Yuste JE, Castelo R (2000) Bushmeat markets on Bioko Island as a measure of hunting pressure. *Conservation Biology*, **14**, 1602–1613.

- Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, **164**, 1567–1587.
- Falush D, Stephens M, Pritchard JK (2007) Inference of population structure using multilocus data: dominant markers and null alleles. *Molecular Ecology Notes*, **7**, 574–578.
- Fuller S, Baverstock P, King D (1998) Biogeographic origins of goannas (Varanidae): a molecular perspective. *Molecular Phylogenetics and Evolution*, **9**, 294–307.
- Gaulke M (1991) Systematic relationships of the Philippine water monitors as compared with *Varanus salvator salvator*, with a discussion of possible dispersal routes. *Mertensiella*, **15**, 154–167.
- Gaulke M (1992) Taxonomy and biology of the Philippine monitors (*Varanus salvator*). *Philippine Journal of Science*, **121**, 345–381.
- Gaulke M (1998) Utilization and conservation of lizards and snakes in the Philippines. In: *Conservation, Trade and Sustainable Use of Lizards and Snakes in Indonesia*. (Ed. Erdelen W), pp. 137–142. *Mertensiella*, supplement to *Salmandra*, **9**.
- Harris JD, Sá-Sousa P (2002) Molecular phylogenetics of Iberian Wall Lizards (*Podarcis*): is *Podarcis hispanica* a species complex? *Molecular Phylogenetics and Evolution*, **23**, 75–81.
- Heled J, Drummond A (2010) Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution*, **27**, 570–580.
- Hey J, Waples RS, Arnold ML *et al.* (2003) Understanding and confronting species uncertainty in biology and conservation. *Trends in Ecology and Evolution*, **18**, 597–603.
- Holmes RS, King M, King D (1975) Phenetic relationships among varanid lizards based upon comparative electrophoretic data and karyotypic analyses. *Biochemical Systematic Ecology*, **3**, 257–262.

- Hoogerwerf A (1954) Notes on the vertebrate fauna of the Krakatau islands. *Treubia*, **22**, 319–348.
- Hubisz MJ, Falush D, Stephens M *et al.* (2009) Inferring weak population structure with the assistance of sample group information. *Molecular Ecology*, **9**, 1322–1332.
- Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution*, **23**, 245–267.
- International Commission on Zoological Nomenclature (1999) International Code of Zoological Nomenclature, Fourth Edition. International Trust for Zoological Nomenclature and The Natural History Museum, London, U.K.
- Joly S, Bruneau A (2006) Incorporating allelic variation for reconstructing the evolutionary history of organisms from multiple genes: an example from *Rosa* in North America. *Systematic Biology*, **55**, 623–636.
- King M, King D (1975) Chromosomal evolution in the lizard genus *Varanus* (Reptilia). *Australian Journal of Biological Science*, **28**, 89–108.
- King D, King M, Baverstock P (1991) A new phylogeny of the Varanidae. *Mertensiella*, **2**, 211–219.
- King D, Fuller S, Baverstock P (1999) The biogeographic origins of varanid lizards. In: *Mertensiella: advances in monitor research II* (eds Horn HG, Böhme W), pp 43–49. Rhine Brook: Deutsche Gesellschaft für Herpetologie und Terrarienkunde.
- Knowles LL, Carstens BC (2007) Delimiting species without monophyletic gene trees. *Systematic Biology*, **56**, 887–895.
- Koch A, Auliya M, Schmitz A *et al.* (2007) Morphological studies of South East Asian water monitors (*Varanus salvator* Complex): nominotypic populations and taxonomic overview.

- In: Advances in Monitor Research III* (eds. Horn HG, Böhme W, Krebs U). *Mertensiella*, **16**, 109–180.
- Koch A, Böhme W (2010) Heading east: a new subspecies of *Varanus salvator* from Obi Island, Maluku Province, Indonesia, with a discussion about the easternmost occurrence of Southeast Asian water monitor lizards. *Russian Journal of Herpetology*, **17**, 299–309.
- Koch A, Gaulke M, Böhme W (2010a) Unraveling the underestimated diversity of Philippine water monitor lizards (Squamata: *Varanus salvator* complex), with the description of two new species and a new subspecies. *Zootaxa*, **2446**, 1–54.
- Koch A, Auliya M, Ziegler T (2010b) Updated checklist of the living monitor lizards of the world (Squamata: Varanidae). *Bonn Zoological Bulletin*, **57**, 127–136.
- Laurenti JN (1768) *Specimen medicum exhibens synopsis reptilium emendatam cum experimentis circa venena et antidota reptilium austriacorum*. Joan Thomae, Vienna, Austria.
- Leaché AD, Fujita MK (2010) Bayesian species delimitation in West African forest geckos (*Hemidactylus fasciatus*). *Proceedings of the Royal Society B: Biological Sciences*, **277**, 3071–3077.
- Leaché AD, Mulcahey DG (2007) Phylogeny, divergence times and species limits of spiny lizards (*Sceloporus magister* species group) in western North American deserts and Baja California. *Molecular Ecology*, **16**, 5216–5233.
- Lee MSY, Caldwell MW (2000) *Adriosaurus* and the affinities of mosasaurs, dolichosaurs, and snakes. *Journal of Paleontology*, **74**, 915–937.
- Linnaeus C (1735) *Systema naturae, vie regna tria naturae systematice proposita per classes, ordines, genera, & species*. Leiden: Haak. pp. 1–12.

- Linkem CA, Hesed K, Diesmos AC *et al.* (2010) Species boundaries and cryptic lineage diversity in a Philippine forest skink complex (Reptilia; Squamata; Scincidae: Lygosominae). *Molecular Phylogenetics and Evolution*, **56**, 572–585.
- Mace GM, Possingham HP, Leader-Williams N (2007) Prioritizing choices in conservation. In: *Key Topics in Conservation Biology* (eds Macdonald D, Service K), pp 17–34. Blackwell Publishing Ltd. Oxford, UK.
- Marshall DC (2010) Failure of partitioned Bayesian phylogenetic analyses: lost in the land of long trees. *Systematic Biology*, **59**, 108–117.
- Marshall JC, Arevalo E, Benavides E *et al.* (2006a) Delimiting species: Comparing methods for Mendelian characters using lizards of the *Sceloporus grammicus* (Squamata: Phrynosomatidae) complex. *Evolution*, **60**, 1050–1065.
- Marshall DC, Simon C, Buckley TR (2006b) Accurate branch length estimation in partitioned Bayesian analyses requires accommodation of among-partition rate variation and attention to branch length priors. *Systematic Biology*, **55**, 993–1003.
- Merrell DJ (1981) *Ecological Genetics*. University of Minnesota Press. Minneapolis, MN
- Mertens R (1942a) Die familie der warane (Varanidae). *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft*, **462**, 1–116.
- Mertens R (1942b) Die familie der warane (Varanidae). *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft*, **465**, 117–234.
- Mertens R (1942c) Die familie der warane (Varanidae). *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft*, **466**, 235–391.

- Mertens R (1963) Liste der rezenten amphibian und reptilian: Helodermatidae, Varanidae, Lanthanotidae. In: *Das Tierreich* (eds Mertens R, Hennig W), pp. 1–26. Walter de Gruyter & Co., Berlin.
- Mittermeier RA, Carr JL, Swingland IR *et al.* (1992) Conservation of amphibians and reptiles. In: *Herpetology: Current Research on the Biology of Amphibians and Reptiles* (ed. Adler K) pp. 59–80. Proceedings of the First World Congress of Herpetology. Society for the Study of Amphibians and Reptiles, Oxford UK.
- Nash SV (1997) Fin, feather, scale and skin: observations on the wildlife trade in Lao PDR and Vietnam. *TRAFFIC Southeast Asia*, **1**, 1–45.
- O’Conner D, Moritz C (2003) A molecular phylogeny of the Australian skink genera *Eulamprus*, *Gnypetoscincus* and *Nangura*. *Australian Journal of Zoology*, **51**, 317–330.
- Padial JM, De la Riva I (2009) Integrative taxonomy reveals cryptic Amazonian species of *Pristimantis* (Anura). *Zoological Journal of the Linnaean Society*, **155**, 97–122.
- Pernetta AP (2009) Monitoring the trade: using the CITES database to examine the global trade in live monitor lizards (*Varanus* spp.). *Biawak*, **3**, 37–45.
- Pianka ER (1995) Evolution of body size: varanid lizards as a model system. *American Naturalist*, **146**, 398–414.
- Pianka ER, King DR, King RA (2004) Varanoid lizards of the world. Indiana University Press, Bloomington, IN.
- Posada D (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, **25**, 1253–1256.
- Posada D, Crandal KA (2001) Intraspecific gene genealogies: trees grafting into networks. *Trends in Ecology and Evolution*, **16**, 37–45.

- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.
- Rambaut A (2002) Se-AL: sequence alignment editor. Available from:
<http://iubio.bio.indiana.edu/soft/iubionew/molbio/dna/analysis/Pist/main.html>
- Rambaut A, Drummond A (2007) Tracer v1.4. Available from: <http://beast.bio.ed.ac.uk/Tracer>
- Rawlinson PA, Zann RA, Balen S *et al.* (1992) Colonization of the Krakatau islands by vertebrates. *GeoJournal*, **28**, 225–231.
- Rissler LJ, Apodaca JJ (2007). Adding more ecology into species delimitation: ecological niche models and phylogeography help define cryptic species in the black salamander (*Aneides flavipunctatus*). *Systematic Biology*. **56**, 924–942.
- The Reptile Database (2012) <http://www.reptile-database.org/> (accessed 1/2012).
- Ronquist F, Huelsenbeck J (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.
- Rosenberg NA (2004) Distruct: a program for the graphical display of population structure. *Molecular Ecology Notes*, **4**, 137–138.
- Schlaepfer MA, Hoover C, Dodd Jr. KD (2005) Challenges in evaluating the impact of the trade in amphibians and reptiles on wild populations. *Bioscience*, **55**, 256–264.
- Schulte JA, Melville J, Larson A (2003) Molecular phylogenetic evidence for ancient divergence of lizard taxa on either side of Wallace’s Line. *Proceedings of the Royal Society B: Biological Sciences*, **270**, 597–603.
- Setiadi MI, McGuire JA, Brown RM *et al.* (2011) Adaptive radiation and ecological opportunity in Sulawesi and Philippine fanged frog (*Limnonectes*) communities. *American Naturalist*, **178**, 221–240.

- Siler CD, Brown RM (2010) Phylogeny-based species delimitation in Philippine loam-swimming skinks (Reptilia: Squamata: Scincidae: *Brachymeles*): taxonomic revision of the pentadactyl species groups and description of three new species. *Herpetological Monographs*, **24**, 1–54.
- Siler CD, Oaks JR, Esselstyn JA *et al.* (2010) Phylogeny and biogeography of Philippine bent-toed geckos (Gekkonidae: *Cyrtodactylus*) contradict a prevailing model of Pleistocene diversification. *Molecular Phylogenetics and Evolution*, **55**, 699–710.
- Siler CD, Oaks JR, Linkem CW *et al.* (2012) Did geckos ride the Palawan raft to the Philippines? *Journal of Biogeography* doi:10.1111/j.1365-2699.2011.02680.x.
- Sites JW, Marshall JC (2003) Delimiting species: A Renaissance issue in systematic biology. *Trends in Ecological Evolution*, **18**, 462–470.
- Sites JW, Marshall JC (2004) Operational criteria for delimiting species. *Annual Review of Ecology, Evolution, and Systematics*, **35**, 199–227.
- Shine R, Harlow PS (1998) Ecological traits of commercially harvested water monitors, *Varanus salvator*, in northern Sumatra. *Wildlife Research*, **25**, 437–447.
- Shine R, Harlow PS, Keogh JS (1996) Commercial harvesting of giant lizards: the biology of water monitors *Varanus salvator* in southern Sumatra. *Biological Conservation*, **77**, 125–134.
- Spinks PQ, Thomson RC, Hughes B *et al.* (2012) Cryptic variation and the tragedy of unrecognized taxa: the case of international trade in the spiny turtle *Heosemys spinose* (Testudines: Geoemydidae). *Zoological Journal of the Linnaean Society*, **164**, 811–824
- Stamatakis A (2006) RAxML-VI-HPC: Maximum Likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, **22**, 2688–2690.

- Stamatakis A, Blagojevic F, Nikolopoulos D *et al.* (2007) Exploring new search algorithms and hardware for phylogenetics: RAxML meets the IBM cell. *Journal of VLSI Signal Processing*, **48**, 271–286.
- Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology*, **57**, 758–771.
- Stephens M, Scheet P (2005) Accounting for decay of linkage disequilibrium in haplotypes inference and missing-data imputation. *American Journal of Human Genetics*, **76**, 449–462.
- Stephens M, Smith NJ, Donnelly P (2001) A new statistical method for haplotypes reconstruction from population data. *American Journal of Human Genetics*, **68**, 978–989.
- Stewart BL (2004) The harvest and trade of reptiles at U Minh Thuong National Park, southern Viet Nam. *TRAFFIC Bulletin*, **20**, 25–34.
- Townsend T, Larson A, Louis E, Macey JR (2004) Molecular phylogenetics of Squamata: the position of snakes, Amphisbaenians, and Dibamids, and the root of the squamate tree. *Systematic Biology*, **53**, 735–757.
- Townsend T, Alegre R, Kelley S *et al.* (2008) Rapid development of multiple nuclear loci for phylogenetic analysis using genomic resources: an example from squamate reptiles. *Molecular Phylogenetics and Evolution*, **47**, 129–142.
- Welton LJ, Siler CD, Bennet D *et al.* (2010a) A spectacular new Philippine monitor lizard reveals a hidden biogeographic boundary and a novel flagship species for conservation. *Biology Letters*, **6**, 654–658.
- Welton LJ, Siler C, Diesmos AC *et al.* (2010b) Philippine bent-toed geckos of the *Cyrtodactylus agusanensis* complex: multilocus phylogeny, morphological diversity, and description of three new species. *Herpetological Monographs*, **24**, 55–85.

Welton LJ, Siler CD, Diesmos AC *et al.* (In press) Genetic identity, geographic range, and major distribution records for frugivorous monitor lizards of Luzon Island, Philippines.

Herpetological Review.

Wiens JJ (2007) Species delimitation: new approaches for discovering diversity. *Systematic Biology*, **56**, 875–878

Wiens JJ, Penkrot TL (2002) Delimiting species based on DNA and morphological variation and discordant species limits in spiny lizards (Sceloporus). *Systematic Biology*, **51**, 69–91.

Wiens JJ, Kuczynski CA, Townsend T *et al.* (2010) Combining phylogenomics and fossils in higher-level squamate reptile phylogeny: molecular data change the placement of fossil taxa. *Systematic Biology*, **59**, 674–688.

Wilgenbusch JC, Warren DL, Swofford DL (2004) AWTY: A system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference. <http://ceb.csit.fsu.edu/awty>.

Yang Z, Rannala B (2010) Bayesian species delimitation using multilocus sequence data. *Proceedings of the National Academy of Sciences*, **107**, 9264–9269.

Yuwono FB (1998) The trade of live reptiles in Indonesia. In: *Conservation, Trade and Sustainable Use of Lizards and Snakes in Indonesia* (ed Erdelen, W), pp. 9–16. *Mertensiella*, supplement to *Salamandra*, **9**.

Zarza E, Reynolds VH, Emerson BC (2008) Diversification in the northern neotropics: mitochondrial and nuclear DNA phylogeography of the iguana *Ctenosaura pectinata* and related species. *Molecular Ecology*, **17**, 3259–3275.

Ziegler T, Böhme W (1997) Genitalstrukturen und paarungsbiologie bei squamaten reptilien, speziell den Platynota, mit bemerkungen zur systematic. *Mertensiella*, **8**, 1–210.

Appendix

Specimen voucher numbers, identification, and geographic location of samples for this study. AM, Australia Museum; AMNH, Australia Museum of Natural History; AZ, Australia Zoo; CAS, California Academy of Sciences; KU, University of Kansas; LSUH, La Sierra University; NZP, National Zoological Park of Indonesia; UF, University of Florida; UMMZ, University of Michigan Museum of Zoology; PZ, Philadelphia Zoo; QM, Queensland Museum; ROM, Royal Ontario Museum; and WAM, Western Australia Museum. *No voucher data available (see Ast 2001).

Taxon	Institution Number	Field Number	Country	Landmass	Locality
<i>H. horridum</i>	UMMZ 225052	UMFS 10134	Mexico	North America	N/A
<i>H. suspectum</i>	*	*	Mexico/United States	North America	N/A
<i>L. borneensis</i>	*	*	Indonesia	Borneo	N/A
<i>V. acanthurus</i>	AM R143881	N/A	Australia	Australia	Queensland
<i>V. baritji</i>	UMMZ 222676	AMH 51522	Australia	Australia	Northern Territory
<i>V. beccari</i>	UMMZ 227118	UMFS 10371	Indonesia	Aru Islands	N/A
<i>V. benglensis</i>	PZ 300941	N/A	Southern Asia	Southern Asia	N/A
<i>V. bitatawa</i>	KU 320000	LJW 0071	Philippines	Luzon Island	Aurora Province, Municipality of Casiguran
<i>V. bitatawa</i>	KU 322188	ACD 2796	Philippines	Luzon Island	Isabela Province, Municipality of San Mariano
<i>V. bitatawa</i>	KU 330730	RMB 15143	Philippines	Luzon Island	Cagayan Province, Municipality of Gonzaga
<i>V. bitatawa</i>	KU 330636	RMB 15075	Philippines	Luzon Island	Cagayan Province, Municipality of Gonzaga
<i>V. brevicauda</i>	AMH 46914	N/A	Australia	Australia	N/A
<i>V. cumingi samarensis</i>	KU 310870	CDS 2654	Philippines	Samar Island	Eastern Samar Province, Municipality of Taft
<i>V. c. samarensis</i>		CDS 4768	Philippines	Bohol Island	Bohol Province, Municipality of Bilar
<i>V. cumingi</i>	KU 315218	RMB 10490	Philippines	Mindanao Island	Zamboanga City Province, Municipality of Pasonanca
<i>V. cumingi</i>	KU 321814	RMB 11770	Philippines	Mindanao Island	Zamboanga City Province, Municipality of Pasonanca
<i>V. cumingi</i>	KU 315216	RMB 10255	Philippines	Mindanao Island	Zamboanga City Province, Municipality of Pasonanca
<i>V. cumingi</i>	KU 315220	RMB 9178	Philippines	Mindanao Island	Zamboanga City Province, Municipality of Pasonanca
<i>V. cumingi</i>	KU 315219	RMB 9131	Philippines	Mindanao Island	Zamboanga City Province, Municipality of Pasonanca
<i>V. cumingi</i>	KU 315217	RMB 9233	Philippines	Mindanao Island	Zamboanga City Province, Municipality of Pasonanca
<i>V. cumingi</i>		H 1627	Philippines	Mindanao Island	South Cotabato Province
<i>V. cumingi</i>		H 2627	Philippines	Mindanao Island	Mt. Putting Bato
<i>V. cumingi</i>	no voucher	INA 0003	Philippines	Dinagat Island	N/A
<i>V. cumingi</i>	UMMZ 225547	UMFS 10369	Philippines	N/A	N/A
<i>V. cumingi</i>	KU 309898	RMB 8093	Philippines	Camiguin Sur Island	Camiguin Province, Municipality of Mambajao
<i>V. cumingi</i>		CDSGS 08	Philippines	Camiguin Sur	Camiguin Province, Municipality of Mambajao

<i>V. cumingi</i>		H 1400	Philippines	Island	
<i>V. cumingi</i>	KU 314128	RMB 9500	Philippines	Talikud Island	Davao del Norte Province
<i>V. doreanus</i>	UMMZ 227117	UMFS 10296	Indonesia	Mindanao Island	Agusan del Sur Province, Municipality of Bunawan
<i>V. dumerilli</i>	UMMZ 227120	UMFS 10375	Indonesia	West Papua	N/A
<i>V. eremias</i>	AM R147247	N/A	Australia	Borneo	Kalimantan
<i>V. exanthematicus</i>	UMMZ 227735	UMFS 10959	N/A - trade	Australia	Northern Territory, Wauchope
<i>V. finschi</i>	no voucher	JF 129	Australia/Indonesia	Africa	N/A
<i>V. flavescens</i>	UF 7500	N/A	SW Asia	N/A	N/A
<i>V. giganteus</i>	no voucher	UMFS 10960	Australia	Asia	N/A
<i>V. gilleni</i>	AM R147264	N/A	Australia	Australia	N/A
<i>V. glauerti</i>	UMMZ 225504	UMFS 10370	Australia	Australia	N/A
<i>V. glebopalma</i>	UMMZ 218497	AM 45457	Australia	Australia	N/A
<i>V. gouldi</i>	AM R123634	N/A	Australia	Australia	New Wouth Wales, Bourke
<i>V. griseus</i>	UMMZ 221342	UMFS 10109	N/A	Asia	N/A
<i>V. griseus</i>	UMMZ 221342	UMFS 10109	N/A	Asia	N/A
<i>V. indicus</i>	AM R134948	AMH 36431	Solomon Islands	New Georgia Island	Mouth of Gobulu River near Tamaneke Village
<i>V. indicus</i>	AM 51525	N/A	Indonesia		
<i>V. indicus</i>	AM R137997	N/A	Papua New Guinea	Fergusson Island	Milne Bay District, Ilaila Village
<i>V. jobiensis</i>	UMMZ 211713	UMFS 9534	Indonesia	West Papua	N/A
<i>V. keithhorni</i>	QM 70792	J70792	Australia	Australia	Lockhart River, Cape York Peninsula, Queensland
<i>V. kingorum</i>	UMMZ 219012	AMH 46800	Australia	Australia	Northern Territory
<i>V. komodoensis</i>	NZP	N/A	Indonesia	Indonesia	N/A
<i>V. marmoratus</i>	KU 327752	ACD 6028	Philippines	Calayan Island	Cagayan Province, Municipality of Calayan
<i>V. marmoratus</i>	KU 304879	RMB 6000	Philippines	Calayan Island	Cagayan Province, Municipality of Calayan
<i>V. marmoratus</i>	KU 314033	RMB 9025	Philippines	Batan Island	Batanes Province, Municipality of Basco
<i>V. marmoratus</i>	KU 304880	RMB 6001	Philippines	Calayan Island	Cagayan Province, Municipality of Calayan
<i>V. marmoratus</i>	PNM	ACD 2575	Philippines	Luzon Island	Isabela Province, Municipality of Cabagan
<i>V. marmoratus</i>	KU 304595	RMB 5715	Philippines	Calayan Island	Cagayan Province, Municipality of Calayan
<i>V. marmoratus</i>	KU 326697	ACD 3414	Philippines	Luzon Island	Cagayan Province, Municipality of Gattaran
<i>V. marmoratus</i>		RMB 4519	Philippines	Luzon Island	Zambales Province, Municipality of Subic
<i>V. marmoratus</i>	TNHC 63000	RMB 4520	Philippines	Luzon Island	Zambales Province, Municipality of Subic
<i>V. marmoratus</i>		ACD 5863	Philippines	Luzon Island	Batangas Province, Municipality of Lipa City
<i>V. marmoratus</i>	KU 323437	RMB 12617	Philippines	Luzon Island	Aurora Province, Municipality of Baler

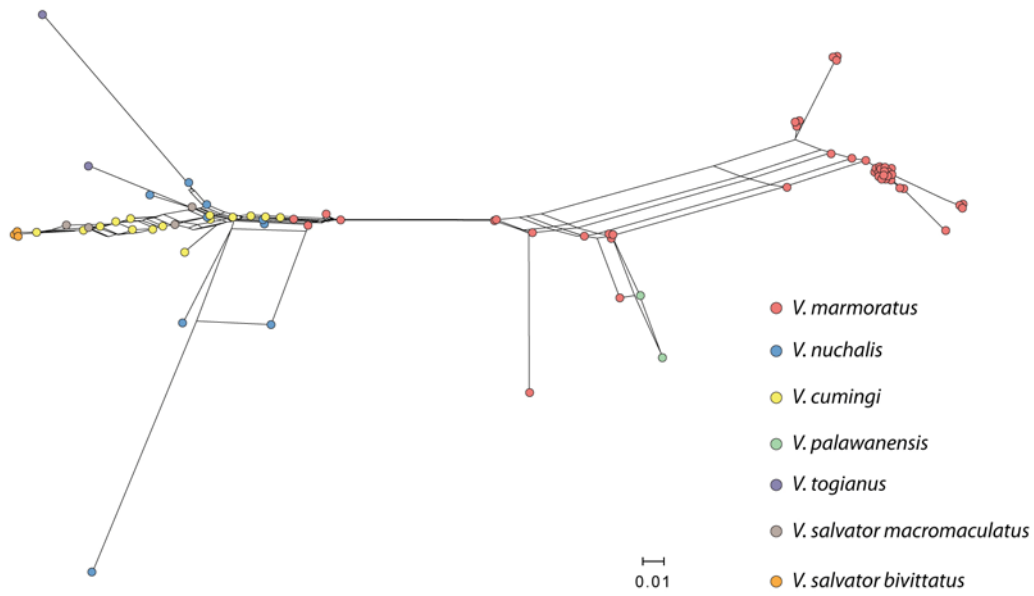
<i>V. marmoratus</i>	KU 323432	RMB 11922	Philippines	Luzon Island	Aurora Province, Municipality of Maria Aurora
<i>V. marmoratus</i>	KU 323433	RMB 12105	Philippines	Luzon Island	Aurora Province, Municipality of Maria Aurora
<i>V. marmoratus</i>	KU 305152	CDS 1680	Philippines	Lubang Island	Occidental Mindoro Province, Municipality of Lubang
<i>V. marmoratus</i>	KU 305159	CDS 1682	Philippines	Lubang Island	Occidental Mindoro Province, Municipality of Lubang
<i>V. marmoratus</i>	KU 322191	CDS 3912	Philippines	Lubang Island	Occidental Mindoro Province, Municipality of Lubang
<i>V. marmoratus</i>		CDS 3830	Philippines	Lubang Island	Occidental Mindoro Province, Municipality of Lubang
<i>V. marmoratus</i>	KU 305158	CDS 1681	Philippines	Lubang Island	Occidental Mindoro Province, Municipality of Lubang
<i>V. marmoratus</i>	KU 323362	RMB 12479	Philippines	Luzon Island	Aurora Province, Municipality of Baler
<i>V. marmoratus</i>	KU 323440	RMB 12800	Philippines	Luzon Island	Aurora Province, Municipality of San Luis
<i>V. marmoratus</i>	KU 323438	RMB 12801	Philippines	Luzon Island	Aurora Province, Municipality of San Luis
<i>V. marmoratus</i>	KU 323435	LJW 0065	Philippines	Luzon Island	Aurora Province, Municipality of Maria Aurora
<i>V. marmoratus</i>	KU 304853	RMB 5974	Philippines	Babuyan Claro Island	Cagayan Province, Municipality of Calayan
<i>V. marmoratus</i>		RMB 4290	Philippines	Luzon Island	Cavite Province, Mt. Palaypalay
<i>V. marmoratus</i>	KU 325842	RMB 13586	Philippines	Luzon Island	Nueva Vizcaya Province, Municipality of Quezon
<i>V. marmoratus</i>	KU 323434	LJW 0074	Philippines	Luzon Island	Aurora Province, Municipality of Casiguran
<i>V. marmoratus</i>	KU 323436	LJW 0072	Philippines	Luzon Island	Aurora Province, Municipality of Casiguran
<i>V. marmoratus</i>	KU 323439	LJW 0073	Philippines	Luzon Island	Aurora Province, Municipality of Casiguran
<i>V. marmoratus</i>	KU 326702	LJW 0075	Philippines	Polillo Island	Polillo Island
<i>V. marmoratus</i>	KU 326703	LJW 0077	Philippines	Polillo Island	Polillo Island
<i>V. marmoratus</i>	KU 313880	RMB 9910	Philippines	Luzon Island	Camarines Norte Province, Municipality of Labo
<i>V. marmoratus</i>	KU 306601	CWL 440	Philippines	Luzon Island	Camarines Sur Province, Municipality of Presentacion
<i>V. marmoratus</i>	KU 308216	CDS 2298	Philippines	Catanduanes Island	Catanduanes Province, Municipality of Bato
<i>V. marmoratus</i>	KU 306602	CWL 520	Philippines	Luzon Island	Quezon Province, Municipality of Calauag
<i>V. marmoratus</i>	KU 306603	CWL 521	Philippines	Luzon Island	Quezon Province, Municipality of Calauag
<i>V. marmoratus</i>	KU 308437	ELR 877	Philippines	Mindoro Island	Occidental Mindoro Province, Municipality of Paluan
<i>V. marmoratus</i>	KU 305161	CDS 715	Philippines	Semirara Island	Antique Province, Municipality of Caluya
<i>V. marmoratus</i>	KU 305163	CDS 648	Philippines	Semirara Island	Antique Province, Municipality of Caluya
<i>V. marmoratus</i>	KU 305151	CDS 714	Philippines	Semirara Island	Antique Province, Municipality of Caluya
<i>V. marmoratus</i>	KU 330731	RMB 15140	Philippines	Luzon Island	Cagayan Province, Municipality of Gonzaga
<i>V. marmoratus</i>	KU 329428	RMB 13827	Philippines	Luzon Island	Bulacan Province, Mun. of Dona Remedios Trinidad
<i>V. marmoratus</i>	KU 330132	RMB 14054	Philippines	Luzon Island	Ilocos Norte Province, Municipality of Adams
<i>V. marmoratus</i>	KU 330133	RMB 14342	Philippines	Luzon Island	Ilocos Norte Province, Municipality of Adams
<i>V. marmoratus</i>	KU 330729	RMB 14695	Philippines	Luzon Island	Cagayan Province, Municipality of Gonzaga
<i>V. melinus</i>	UMMZ 222681	UMFS 10164	Indonesia	Sula Islands	Sula Islands

<i>V. mertensi</i>	AM R123877	N/A	Australia	Australia	Western Australia, Mitchell Plateau, Mitchell Falls
<i>V. mitchelli</i>	UMMZ 210576	UMFS 8658	Australia	Australia	N/A
<i>V. nebulosis</i>	ROM 35017	N/A	Vietnam	mainland	Vin Phu, Tam Dao
<i>V. nebulosis</i>	LSUH	no voucher	Malaysia	Western Malaysia	Jerteh
<i>V. nebulosis</i>	KU	no voucher	Singapore	Singapore	Bukit Timeh
<i>V. niloticus</i>	UMMZ 221377	UMFS 10121	N/A	Africa	N/A
<i>V. nuchalis</i>		H 768	Philippines	Panay Island	Antique Province, Municipality of Culasi
<i>V. nuchalis</i>	KU 305157	CDS 305	Philippines	Negros Island	Negros Occidental Province, Municipality of Cauayan
<i>V. nuchalis</i>	KU 305153	CDS 304	Philippines	Negros Island	Negros Occidental Province, Municipality of Cauayan
<i>V. nuchalis</i>		CDS 4399	Philippines	Negros Island	Negros Occidental Province, Municipality of Silay City
<i>V. nuchalis</i>		RMB 3326	Philippines	Negros Island	Negros Oriental Province, Municipality of Valencia
<i>V. nuchalis</i>		CDS 5151	Philippines	Masbate Island	Masbate Province, Municipality of Masbate City
<i>V. nuchalis</i>	KU 305148	CDS 1375	Philippines	Sibuyan Island	Romblon Province, Municipality of Magdiwang
<i>V. nuchalis</i>	KU 305172	RMB 2963	Philippines	Sibuyan Island	Romblon Province, Municipality of Magdiwang
<i>V. nuchalis</i>	KU 305134	RMB 5145	Philippines	Sibuyan Island	Romblon Province, Municipality of Magdiwang
<i>V. olivaceus</i>	UMMZ 210202	UMFS 4637	Philippines	Luzon Island	N/A
<i>V. olivaceus</i>	KU 322186	LJW 0082	Philippines	Luzon Island	Camarines Sur Province, Municipality of Presentacion
<i>V. olivaceus</i>	KU 322187	LJW 0078	Philippines	Polillo Island	Quezon Province
<i>V. olivaceus</i>	KU 329517	DSM 1753	Philippines	Luzon Island	Bulacan Province, Mun. of Dona Remedios Trinidad
<i>V. palawanensis</i>		JAE 1339	Philippines	Palawan Island	Palawan Province, Municipality of Puerto Princessa
<i>V. palawanensis</i>	KU 309607	RMB 7730	Philippines	Palawan Island	Palawan Province, Municipality of Brooke's Point
<i>V. panoptes horni</i>	no voucher	UMFS 10157	Indonesia	New Guinea	S New Guinea
<i>V. p. panoptes</i>	UMMZ 210491	UMFS 8687	Australia	Australia	N/A
<i>V. pilbarensis</i>	WAM R132659	N/A	Australia	Australia	Western Australia (Pilbara region)
<i>V. prasinus</i>	UMMZ 225514	UMFS 10684	Australia/Indonesia	N/A	N/A
<i>V. primordius</i>	UMMZ 218495	AM 45455	Australia	Australia	Northern Territory
<i>V. rosenbergi</i>	AM R6154	N/A	Australia	Australia	N/A
<i>V. rudicollis</i>	UMMZ 210506	UMFS 9104	Indonesia	Borneo	N/A
<i>V. salvadorii</i>	UMMZ 225541	UMFS 10294	Indonesia	New Guinea	N/A
<i>V. salvator bivittatus</i>	UMMZ 227129	UMFS 11010	Indonesia	Java Island	Java Island
<i>V. s. bivittatus</i>	UMMZ 227122	UMFS 10671	Indonesia	Java Island	Java Island
<i>V. s. bivittatus</i>	UMMZ 227121	UMFS 10670	Indonesia	Java Island	Java Island
<i>V. salvator macromaculatus</i>	UMMZ 227119	UMFS 10374	Indonesia	Sumatra Island	Sumatra Island
<i>V. s. macromaculatus</i>	UMMZ 225562	UMFS 10944	Indonesia	Sumatra Island	Sumatra Island

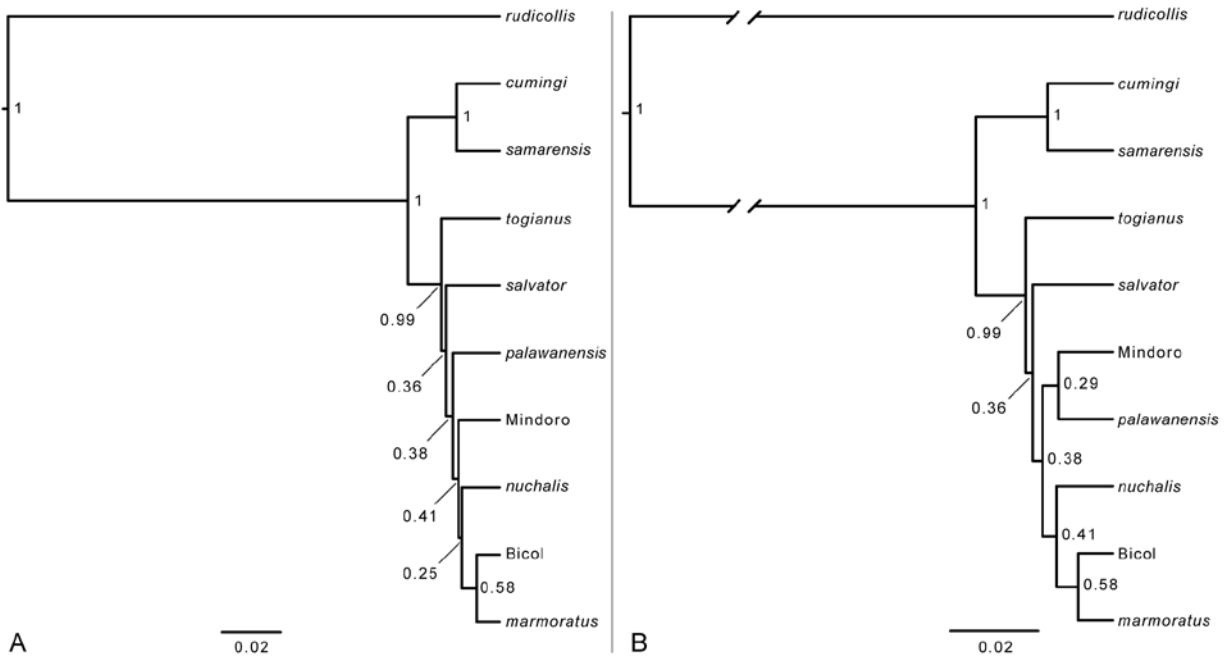
<i>V. s. macromaculatus</i>	LSUH	no voucher	Malaysia	Peninsular Malaysia	North Gerik
<i>V. s. macromaculatus</i>	no voucher	ACD 3585	Singapore	Singapore	N/A
<i>V. s. macromaculatus</i>	UMMZ 227128	UMFS 11009	Indonesia	Sumatra Island	Sumatra Island
<i>V. s. macromaculatus</i>	CAS 212011	JBS 4788	Myanmar	Asia	Ayeyarwade Division; Myaungmya District
<i>V. scalaris</i>	AM R138712	N/A	Australia	Australia	Northern Territory, Groote Eylandt
<i>V. scalaris</i>	UMMZ 218493	AM 45450	Australia	Australia	Northern Territory
<i>V. semiremex</i>	AZ-1	N/A	Australia	Australia	Queensland
<i>V. spenceri</i>	UMMZ 218500	AM 45454	Australia	Australia	N/A
<i>V. storri</i>	AM R143912	N/A	Australia	Australia	Queensland, Croydon
<i>V. timorensis</i>	WAM R132659	N/A	Indonesia	Timor	N/A
<i>V. togianus</i>	UMMZ 225543	UMFS 10298	Indonesia	Sulawesi Island	Sulawesi Island
<i>V. togianus</i>		RMB	Indonesia	Sulawesi Island	Sulawesi Island
<i>V. togianus</i>		BSI 1565	Indonesia	Sulawesi Island	Sulawesi Island
<i>V. tristis</i>	AM R143919	N/A	Australia	Australia	Queensland, Watsonville
<i>V. varius</i>	AM R133492	N/A	Australia	Australia	New South Wales, Border Ranges National Park
<i>V. yuwonoi</i>	UMMZ 225545	UMFS 10368	Indonesia	Halmahera	N/A

Supplemental Table 1. Individual Loci, original publications, and polymerase chain reaction (PCR) thermal profiles for each. Annealing temperature was varied to improve amplification for problematic samples.

Locus	Source	Thermal Profile
ND1, ND2	Sorenson <i>et al.</i> 1999; Ast 2001	4 min at 95°, followed by 35 cycles of 95° for 1 min, 55–58° for 1 min, and 72° for 2 min, and a final extension phase at 72° for 7 min
DGL- α	Alföldi <i>et al.</i> 2011	4 min at 95°, followed by 35 cycles of 95° for 1 min, 52–55° for 1 min, and 72° for 2 min, and a final extension phase at 72° for 7 min
L52	Alföldi <i>et al.</i> 2011	4 min at 95°, followed by 35 cycles of 95° for 1 min, 52–55° for 1 min, and 72° for 2 min, and a final extension phase at 72° for 7 min
L74	Alföldi <i>et al.</i> 2011	4 min at 95°, followed by 35 cycles of 95° for 1 min, 52–55° for 1 min, and 72° for 2 min, and a final extension phase at 72° for 7 min
prolactin receptor (PRLR)	Townsend <i>et al.</i> 2008	4 min at 95°, followed by 35 cycles of 95° for 1 min, 52–55° for 1 min, and 72° for 2 min, and a final extension phase at 72° for 7 min



Supplemental Figure 2. Distance-based (neighbor-joining) nuclear haplotype network as inferred by the NeighborNet algorithm of SplitsTree. Input standardized distance matrix created with the program POFAD.



Supplemental Figure 3. Bayesian estimates of the *Varanus salvator* Complex species tree as inferred by the program *BEAST. The two topologies were equally frequent in the posterior distribution of trees.