ARTICLES

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Genetic Identity, Geographic Ranges, and Major Distribution Records for Frugivorous Monitor Lizards of Luzon Island, Philippines

Some of Southeast Asia's most enigmatic reptile species include the arboreal, frugivorous monitor lizards of the central and northern Philippines (Auffenberg 1988; Gaulke and Curio 2001; Welton et al. 2010). Comprised of just three known species, this morphologically, ecologically, and phylogenetically distinct group has even been assigned to its own subgenus, *Philippinosaurus* (Mertens 1962; Pianka et al. 2004) on the basis on cranial features and dentition.

Knowledge on the biology of the Philippines' frugivorous monitor lizards has been slow to accumulate. The first known species (*Varanus olivaceus*, of Luzon, Polillo, and Catanduanes islands; Fig. 1) has been shrouded in mystery and confusion from the time that it first became known to science (Gray 1845; Hallowell 1857; Mertens 1942a–c, 1959, 1962; Taylor 1922). For more than a century it was thought to be extinct, until a rediscovery made possible the first thorough taxonomic description (Auffenberg 1976). Later Auffenberg (1988) performed extensive studies of its diet, natural history, habitat, home range ecology, and demographic population structure. Unfortunately, no additional studies on this elusive and poorly known species have been published in peer-reviewed, scientific literature.

In 2001, Gaulke and Curio (2001) published the description of an exceptionally rare and severely threatened second species in this group, *V. mabitang*. The biology of this species has been studied in some detail and we now have a general appreciation of its diet, ecology, geographic range (Gaulke et al. 2002, 2005; Gaulke 2005, 2010; Gaulke and Demegillo 2008) and conservation status ("Vulnerable;" IUCN 2011) on Panay Island.

A third species, *V. bitatawa*, was described in 2010 (Welton et al. 2010; Siler et al. 2010) in the central and northern Sierra Madre Mountain Range of Luzon Island (Fig. 1). Although the study by Welton et al. (2010) demonstrated the morphological, ecological, and phylogenetic distinctiveness of *V. bitatawa*, no additional data on its distribution, natural history, or conservation status have been forthcoming.

For the past several years, as part of extensive biodiversity studies aimed at understanding the patterns of distribution of Luzon's amphibians and reptiles (Brown et al. 1996, 2000, *in press*; Devan-song and Brown 2012; McLeod et al. 2011; Siler et al. 2010), we have accumulated additional distribution records and genetic samples for *Varanus olivaceus* and *V. bitatawa*. Because little to no new information for the Luzon faunal region has been provided, aside from the studies of Auffenberg (1988) and Welton et al. (2010), our new findings constitute major range extensions for both species. Additionally, to circumscribe geographic ranges and species boundaries, we present new genetic data from the northern and western-most records for both species. Our results greatly extend the known ranges of both species, bolster species boundaries, contribute to our current understandings of genetic divergence between the taxa, and provide new opportunities for studies of natural history and conservation genetics of these rare, endemic Philippine forest monitors.

Materials and Methods.—Field survey protocols have been reported in Brown et al. (1996, 2000), Diesmos et al. (2005), Siler et al. (2011), and McLeod et al. (2011). We obtained vouchered (preserved specimens deposited in the National Museum of the Philippines, PNM, and Biodiversity Institute, University of Kansas, KU) genetic samples from new localities for *V. olivaceus* and *V. bitatawa*, and supplemented Welton et al.'s (2010) mitochondrial dataset with new genetic sequences from the NADH dehydrogenase subunit 1 (*ND1*) and flanking tRNAs (Welton et al. 2010). Because we prefer to use specimens from known localities whenever possible, we did not include Ast's (2001) *V. olivaceus* sequence (obtained from a zoo animal), which is of unknown provenance. We follow the same methods reported in Welton et al. (2010), including primer identity and use, PCR amplification and sequencing protocols, purification techniques,

LUKE J. WELTON, CAMERON D. SILER
Biodiversity Institute and Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, Kansas 66045, USA
ARVIN C. DIESMOS
Herpetology Section, Zoology Division, National Museum of the Philippines, Padre Burgos Avenue, Ermita 1000, Manila, Philippines
MAE LOWE L. DIESMOS
Department of Biological Sciences, College of Science, University of Santo Tomas, España 1015, Manila, Philippines
RONALDO D. LAGAT, RUBIE M. CAUSAREN
Biological Sciences Department, De La Salle University—Dasmariñas DBB-B, Dasmariñas 4115, Cavite, Philippines
RAFE M. BROWN*
Biodiversity Institute and Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, Kansas 66045, USA; e-mail: rafe@ku.edu
**Corresponding author*

alignment methods, targeted gene region, and methods for phylogenetic analysis (additional details are provided in the supplemental information accompanying Welton et al. [2010]). Thus, we added four additional sequences to the same *ND1* data set presented in Welton et al. (2010) and re-ran the same analyses, including the new genetic samples reported here. All specimens were initially identified on the basis of patterns of coloration (Fig. 2) and scalation (Auffenberg 1976, 1988; Welton et al. 2010). The *V. olivaceus* specimens included PNM 9726, from the boundary between the Municipalities of Polillo and Burdeos, Polillo Island; KU 322186 from the Municipality of Presentacion, Camarines Sur Province; KU 329517 from Angat Dam Watershed, Bulacan Province; PNM 9780 from Mt. Palaya-Palay, Cavite Province. The *V. bitatawa* samples include PNM 9719 (holotype), from the Municipality of Casiguran, Aurora Province; KU 322188 (paratype), the Municipality of San Mariano, Isabela Province; KU 330730, Barangay Magrafil, Municipality of Gonzaga, Cagayan Province; and KU 330636, Barangay Santa Clara, Municipality of Santa Clara, Cagayan Province. New mitochondrial gene sequences collected in this study are deposited in Genbank (KU Catalog numbers/Genbank Accession numbers: KU 329517/JQ413241, PNM 9780/JQ413242, KU 330636/JQ413243, KU 330730/JQ413244).

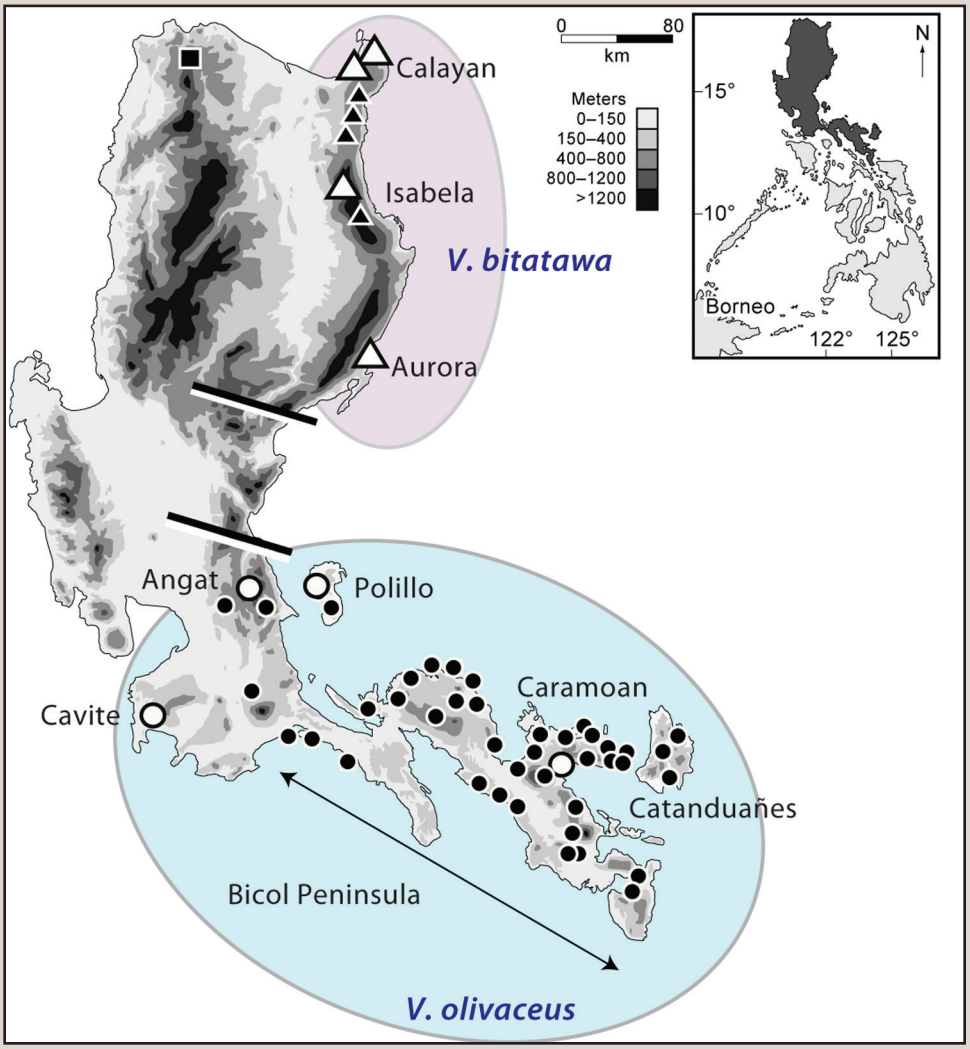

Fig. 1. Known distribution of Luzon's arboreal, frugivorous monitor lizard species. Circles denote known localities for *Varanus olivaceus*; triangles indicate vouchered localities for *V. bitatawa*. Small, closed symbols represent past records (Auffenberg, 1988; Welton, et al., 2010) and large, unshaded symbols are recent records for which genetic samples were available for the present study. The darkened square indicates the Municipality of Adams, where possible signs of an arboreal monitor lizard were observed in June 2011. Black bars indicate the approximate boundaries of the Mid-Sierra Filer Zone, as defined by Welton et al. (2010).

Results and Discussion.—Our record of *V. olivaceus* from the Angat Watershed of Bulacan Province constitutes the northernmost Luzon Island recorded occurrence for this species. Likewise the new specimen from Mt. Palay-palay, Cavite Province, represents the westernmost distributional record for *V. olivaceus*, and a major new range extension into the mountains of western Luzon, where no frugivorous or arboreal species of monitor lizard has ever been recorded (Auffenburg 1988; Devan-song and Brown 2012; Welton et al. 2010; Fig. 1). Finally, our new records of *V. bitatawa* from the Municipality of Gonzaga, Cagayan Province, constitute the northernmost records for this taxon.

Interestingly, despite targeted biodiversity surveys in the Municipalities of Maria Aurora and Mingan, Aurora Province (Siler et al. 2010), no known-locality records from within the 150 km gap, now recognized as the Mid-Sierra Filter Zone (Welton et al. 2010), have surfaced, although trade samples of uncertain provenance have been observed in markets in the town of Baler (ACD, pers. obs.). Although our new record of *V. olivaceus* from Angat Watershed narrows the distributional gap between the two species (Fig. 1), Welton et al.'s (2010) hypothesis that the Mid-Sierra Filter Zone may have led to the divergence of the two species still appears to be the best explanation for understanding the split between *V. olivaceus* and *V. bitatawa*. The coincident position of the three low-lying, arid river valleys (possibly serving as ecological barriers to dispersal for forest species; Welton et al. 2010), and the Lingayen-Dingalan geologic fault (possibly leading to physical isolation of species on different components of the Sierra Madre), within the Mid-Sierra Filter Zone (Defant et al. 1989; Yumul et al. 2003) are suggestive, but still do not provide definitive conclusions as to the exact mechanism of divergence between the two taxa.

The Mt. Palay-Palay, Cavite Province record of *V. olivaceus* was quite surprising, because no fruit-eating monitor species has ever been reported from western Luzon. This specimen could represent a natural occurrence of a population of *V. olivaceus* in

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Fig. 2. Photograph in life of (above) *Varanus olivaceus* (Angat Dam Watershed Reserve, Bulacan Province) and (below) *V. bitatawa* (Municipality of Gonzaga, Cagayan Province).

PHOTO BY RMB

Fig. 3. *Varanus bitatawa* (KU 330636) as bush meat, frequently consumed by local Agta townspeoples, in Barangay Santa Clara, Municipality of Gonzaga, Cagayan Province).

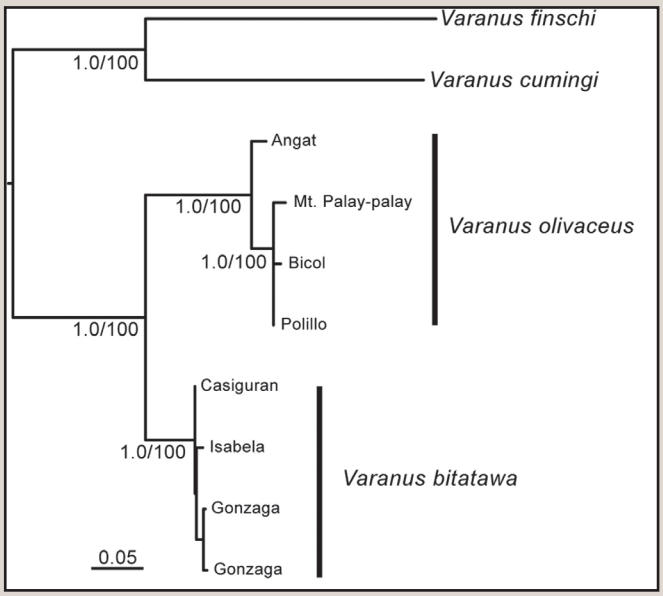

Fig. 4. Maximum likelihood phylogenetic estimate for Luzon frugivorous monitor lizards with support (Bayesian posterior probabilities/likelihood bootstraps) values plotted on selected nodes. Note presence of only minor intraspecific genetic variation within both species and the deep genetic split in *ND1* mitochondrial gene sequences between the two taxa.

the forests of southwestern Luzon, a relatively recent dispersal event (from eastern Luzon), or a human-mediated transplant. The fact that local hunters refer to *V. olivaceus* in Cavite Province with a unique common name, "Shabu," suggests to us that indigenous peoples in the area have a long cultural history with these animals. However, given the fact that illegally poached *V. olivaceus* individuals seized from pet traders have, in the past, been released in Mt. Palay-Palay National Park (C. Custodio, Protected Areas and Wildlife Bureau, Manila, pers. comm.), the possibility of an artificial transplant cannot be discounted. The lack of any appreciable genetic divergence between the Mt. Palay-Palay specimen and individuals from the Bicol faunal region and Polillo supports the interpretation of a recent dispersal event or transplant, but no strong conclusions can be derived from this study involving only a single locus and one mitochondrial gene fragment.

In the northernmost extent of the Sierra Madre Mountain Range, *V. bitatawa* is clearly a common species that constitutes an important food source for indigenous peoples' groups. During our three-week survey in the area in July–August 2010, we salvaged two specimens after they were snared in traps or hunted down by dogs belonging to Agta tribesmen (both specimens clearly were destined for immediate consumption by Agta family groups). Hunters interviewed in and around the Municipality of Gonzaga described *V. bitatawa* as a highly prized, commonly hunted, and valued trade item in the local bush meat market (Fig. 3).

To date, there are no substantiated records to suggest that arboreal, frugivorous monitor lizards are present in northwest Luzon. In 2011 we found characteristic scratch marks on fruiting *Pandanus* trees in the vicinity of Pagudpud, Ilocos Norte Province (Fig. 1; northern Cordillera Mountain Range) which suggests that *V. bitatawa* (or, possibly, some other arboreal and frugivorous species) may be present in the area (Brown et al., *in press*). Local residents at this site informed us of only one kind of *Varanus*, known locally as "Biawak," and described as a ground-dwelling species (clearly identifiable as *V. marmoratus*, the common Luzon water monitor). However, given the extreme secrecy of arboreal monitor lizards (Auffenberg 1988; Gaulke et al. 2002,

Table 1. Uncorrected pairwise sequence divergences (%, below diagonal) among individuals throughout the geographical ranges (sites in parentheses; see text) of *Varanus olivaceus* and *V. bitatawa*. *Varanus finschi* and *V. cumingi* were designated as outgroup in the phylogenetic analysis (Fig. 4).

		1	2	3	4	5	6	7	8	9	10
1.	*V. finschi*	—									
2.	*V. cumingi*	0.1550	—								
3.	*V. olivaceus* (Polillo)	0.1570	0.1651	—							
4.	*V. olivaceus* (Bicol)	0.1586	0.1663	0.0028	—						
5.	*V. olivaceus* (Bulacan)	0.1563	0.1642	0.0112	0.0146	—					
6.	*V. olivaceus* (Palay-palay)	0.1559	0.1697	0.0043	0.0043	0.0086	—				
7.	*V. bitatawa* (Aurora)	0.1551	0.1601	0.0374	0.0398	0.0396	0.0403	—			
8.	*V. bitatawa* (Isabela)	0.1606	0.1486	0.0622	0.0618	0.0442	0.0403	0.0034	—		
9.	*V. bitatawa* (Gonzaga–1)	0.1681	0.1486	0.0711	0.0740	0.0495	0.0389	0.0055	0.0078	—	
10.	*V. bitatawa* (Gonzaga–2)	0.1607	0.1461	0.0630	0.0650	0.0442	0.0431	0.0039	0.0061	0.0024	—

2005; Gaulke 2010; Welton et al. 2010), it remains distinctly possible that arboreal monitor lizard populations have yet to be discovered in the northern Cordillera Mountains.

Our preferred phylogenetic estimate (Fig. 4) suggests that specimens identified as *V. olivaceus* and *V. bitatawa* form clades of minimally divergent haplotypes and that the two species are sister lineages (Welton et al. 2010). Genetic divergence within each species was very low (*V. olivaceus* = 0.28–1.45; mean = 0.76%; *V. bitatawa* = 0.34–0.78; mean = 0.48%, Table 1) despite sampling from geographically distant extremes of the ranges of both species. The substantive genetic divergence between the two species (3.74–7.40; mean = 5.27%) (Fig. 4), and the topology of our inferred phylogenetic hypothesis provide further support for the recognition of two widespread, fruit-eating monitor lizard species, geographically partitioned on either side of Luzon's Mid-Sierra Filter Zone (Welton et al. 2010).

Although we have gathered important new data, bearing on the distribution, phylogeny and genetic identity of these poorly known monitor species, much work remains to be done. The IUCN currently lists *Varanus olivaceus* as "Vulnerable," with decreasing population trends "likely" (but unsubstantiated; IUCN 2011). Formal evaluations of the conservation status of *V. bitatawa* have yet to be undertaken. However, based on presumed habitat requirements (intact forest with the appropriate species of fruiting trees), and a clear conservation threat in the form of a substantial bush meat harvest, an informed, field data based, conservation status assessment for *V. bitatawa* would be desirable.

It should be emphasized, however, that for the continued existence of both species, the critical next step is actual field-based study of distribution patterns, natural history, diet, home range ecology, and habitat requirements throughout their known geographic ranges. Until these fundamental subjects receive at least some attention, effective conservation planning for these species will be very difficult, if not impossible (Brown et al., *in press*; IUCN 2011). Collection of these basic data would provide a solid foundation for an effective conservation action and management plan for Luzon's frugivorous monitor lizards and their unique forest habitats.

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Using a Species-Specific Habitat Model Helps Identify Unprotected Populations of the Federally Threatened Red Hills Salamander (*Phaeognathus hubrichti*)

The Red Hills Salamander (*Phaeognathus hubrichti*) was listed as federally threatened by the U.S. Fish and Wildlife Service in 1976 (IUCN endangered; IUCN 2011) in response to concerns of local herpetologists regarding the impact of habitat degradation on the few known populations at the time (e.g., Jordan and Mount 1975; Mount 1975; Schwaner and Mount 1970). Since listing, research has reported that removal of canopy trees during timber harvest has a negative effect on the remaining populations of *P. hubrichti* (Dodd 1991; Godwin 2008). The federal listing of *P. hubrichti* has provided a degree of protection against the effects of timber harvest in the form of habitat conservation plans (HCPs), administered by USFWS with large landholders. Because the majority of *P. hubrichti* habitat is managed for timber production (Bailey and Means 2004), HCPs are an essential component for species persistence. However, for conservation efforts to expand, extant populations of *P. hubrichti* must be identified. Due to the fossorial life history and patchy distribution of the species, identifying populations outside of known localities is a challenging task.

JOSEPH J. APODACA*
Department of Biological Sciences, University of Alabama, Tuscaloosa, Alabama 35487, USA
JESSICA A. HOMYACK
Weyerhaeuser NR Company, Vanceboro, North Carolina 28586-7606, USA
LESLIE J. RISSLER
Department of Biological Sciences, University of Alabama, Tuscaloosa, Alabama 35487, USA

**Corresponding author; current address: Department of Biological Sciences, Florida State University, Tallahassee, Florida 32306, USA; e-mail: JApodaca@bio.fsu.edu*

In 2006, a new population of *P. hubrichti* was discovered in Wilcox County, Alabama, which was not known to harbor any salamanders (M. Bailey, pers. comm.). Prior to this discovery, it was believed that *P. hubrichti* only inhabited the Tallahatta and Hatchetigbee formations within the Red Hills (Dodd 1991). Significantly, the Wilcox County population was found in an entirely different geologic formation, the Nanafalia. The extent of additional, and unprotected, populations outside of the known range is unclear.

Identifying additional populations within Wilcox County is important to the conservation of this narrowly endemic species,