

Population Genetic Structure and Revised Geographic Range for the Tridactyl Skink (*Brachymeles muntingkamay*) from Luzon Island, Philippines

The genus *Brachymeles* represents a unique radiation of semi-fossorial (burrowing) lizards, all but two of which are endemic to the Philippines in Southeast Asia (Siler et al. 2011a, 2012b). Although all species can be described as slender, brown, and small- to medium-sized, this radiation represents one of the best systems for studying the evolution of limb reduction and loss; body forms within the genus range from pentadactyl to externally limbless (Siler and Brown 2011). Over the last decade, a renewed effort at understanding evolutionary patterns within *Brachymeles* has resulted in the discovery of 21 new species previously unknown to science or masquerading within the range of a previously recognized, widespread, and morphologically diverse species (Siler 2010; Siler and Brown 2010, 2011; Siler et al. 2009, 2010a,b, 2011a,b,c,d, 2012a,b). Although the dense survey efforts leading to these discoveries have filled in many gaps in our understanding of this radiation, there still remain species with unique body plans that are poorly represented in collections and are far less understood (Siler 2010; Siler et al. 2011c,d, 2012a).

Many of the species representing unique digit classes within the genus are micro-endemics, with recognized ranges currently limited to single sites. Among these taxa, several low-elevation species have persisted despite nearly complete deforestation across their limited ranges on Mindanao and Cebu islands (*B. cebuensis*, *B. pathfinderi*); until rediscovered in 2009, *B. pathfinderi* had not been observed for more than 80 years (Siler et al. 2011c). Other taxa include several mid-to-high elevation species (*B. elerae*, *B. muntingkamay*, *B. wrighti*), with *B. elerae* and *B. wrighti* long represented in museum collections by only a few specimens (Siler et al. 2011d). All three of these species occur in the mountainous terrain of northern Luzon Island in the Philippines. Interestingly, all micro-endemic taxa are non-pentadactyl, with finger counts ranging from 3–5 and toe counts ranging from 2–4.

Brachymeles muntingkamay Siler, Rico, Duya, and Brown, 2009 is one of 36 recognized lizard species of *Brachymeles*, and is one of only four species known to possess three digits on the fore- and hind limbs. This unique species was discovered only recently in mid- to high-elevation forest on Mt. Palali in the northern portions of Luzon Island, and has since been presumed to be endemic to this single, isolated mountain peak in

the Caraballo Mountain Range of central Luzon. During a recent survey around the caldera of Mt. Cagua in the extreme northeastern portion of Luzon Island, a second montane population of this species was discovered.

From 7–23 July 2011, seven adults and one juvenile of *Brachymeles muntingkamay* were collected during herpetological field surveys in mid- to high-elevation forest on Mt. Cagua, in extreme northeast Luzon Island, Philippines (Fig. 1). Here we report on this newly discovered population of *B. muntingkamay* from Mt. Cagua, located in the northern extreme of the Sierra Madre Mountain Range. The new population is not only geographically disjunct from the type locality of *B. muntingkamay*, but is located in a completely disparate mountain range on the island. With this new discovery, we present data on the genetic identity and structure between the two geographically disjunct populations, provide new photographic and morphological data for the species, revise the known geographic range of *B. muntingkamay*, and discuss the implications of this discovery for future research involving other unique (and possibly rare) mid- to high-elevation vertebrate species in the Philippines.

Materials and Methods.—Field survey protocols follow Brown et al. (1996, 2000), Diesmos et al. (2005), Siler et al. (2011e), and McLeod et al. (2011). Vouchered specimens collected in the field were deposited in the University of Kansas Natural History Museum (KU) herpetological collections and the National Museum of the Philippines (PNM). We examined fluid-preserved specimens for variation in qualitative and mensural characters. Sex was determined by gonadal inspection, and measurements were taken to the nearest 0.1 mm with digital calipers by CDS. X-rays were taken with a company cabinet X-ray on Kodak MIN-R 2000 film exposed at 5 milliamperes and 30 volts for 1 minute 15 seconds. Meristic and mensural characters were chosen based on Siler et al. (2009): snout–vent length, total length, tail length, paravertebral scale-row count, supralabial count, infralabial count, supraciliary count, and supraocular count. Additionally, we counted the number of presacral vertebrae from x-ray images of specimens. A summary of representative diagnostic characters highlighting the minor variation observed between mountain populations is provided in Table 1.

Genetic samples of *B. muntingkamay* from the recently surveyed site in the Municipality of Gonzaga were used to investigate the northeastern population's identity, as well as its genetic similarity with the type populations of topotypic *B. muntingkamay* from Mt. Palali, central Luzon (Fig. 1). The dataset included newly collected sequences of the mitochondrial gene NADH dehydrogenase subunit 2 (*ND2*), supplemented by published *ND2* sequence data from *B. elerae* and *B. boulengeri* for use as outgroup samples (Siler et al. 2011a). We follow the methods described in Siler et al. (2012c) for calculating haplotype networks. All Mt. Cagua specimens were initially identified on the basis of body and head scalation patterns (Siler et al. 2009).

The newly collected *B. muntingkamay* specimens include: KU 330086, 330087, 330089–93, seven adult females; KU 330088,

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juvenile. All specimens were collected on Mt. Cagua, Barangay Magrafil, Municipality of Gonzaga, Cagayan Province, Luzon Island, Philippines (18.22538°N, 122.11445°E; 694 m elev.; WGS 84; Fig. 1). Voucher information corresponding to data from novel sequences deposited in GenBank, and haplotype labels corresponding to those shown in Figure 1, are included in Table 2.

Results and Discussion.—The haplotype network reveals some degree of haplotype diversity within and between the two sampled populations of *Brachymeles muntingkamay* (Fig. 1). Both populations are recovered as genetically distinct, albeit with low genetic divergence observed between the two allopatric populations (uncorrected pairwise sequence divergence 1.2–1.4%; Fig. 1). Intraspecific genetic structure within both sampled populations appears similar based on the few individuals sampled for this study.

Meristic and mensural characters of the newly discovered population are consistent with the recognized diagnostic characters for *Brachymeles muntingkamay* (Table 1). Character variation observed between the Mt. Palali and Mt. Cagua populations includes (Table 1): 1) presacral vertebrae 42 at Mt. Cagua (versus 42 or 44 at Mt. Palali); 2) supraciliaries 6 at Mt. Cagua (versus 6 or 7 at Mt. Palali); 3) supraoculars 5 at Mt. Cagua (versus 5 or 6 at Mt. Palali); and 4) prefrontals separated at Mt. Cagua (versus in medial contact at Mt. Palali). All other diagnostic characters for *B. muntingkamay* (Siler et al. 2009) were observed to be consistent (CDS, pers. obs.).

TABLE 1. Summary of representative meristic and mensural characters among adult specimens from the two disjunct populations of *Brachymeles muntingkamay*. Sample size, body length, and tail length are included for reference (snout–vent length, total length given as range over mean \pm standard deviation).

	Mt. Palali	Mt. Cagua
Sample size	12 female	7 female
Number of digits (fore/hind)	3/3	3/3
Snout–vent length	61.8–81.3 (73.6 \pm 5.9)	61.8–81.3 (73.6 \pm 5.9)
Total length	107.4–136.0 (124.0 \pm 8.6)	109.7–116.4 (113.3 \pm 2.9)
Tail length/snout–vent length	50–79 (65 \pm 10)	53–85 (64 \pm 14)
Presacral vertebrae number	42, 44	42
Paravertebral scale–row count	85–90	87–88
Supralabial count	6 (12)	6 (7)
Infralabial count	6 (12)	6 (7)
Supraciliary count	6 (10) 7 (2)	6 (7)
Supraocular count	5 (11) 6 (1)	5 (7)
Prefrontal contact	Present	Absent

TABLE 2. Summary of specimens corresponding to genetic samples included in the study. Haplotype identification numbers (ID) correspond to those shown in Figure 1. KU = University of Kansas Natural History Museum.

Voucher	Locality	Haplotype ID	Genbank Accession Numbers (ND2)
KU 330086	Mt. Cagua, Barangay Magrafil, Municipality of Gonzaga, Cagayan Province, Luzon Island, Philippines	5	JX966377
KU 330087	Mt. Cagua, Barangay Magrafil, Municipality of Gonzaga, Cagayan Province, Luzon Island, Philippines	4	JX966378
KU 330088	Mt. Cagua, Barangay Magrafil, Municipality of Gonzaga, Cagayan Province, Luzon Island, Philippines	5	JX966375
KU 330089	Mt. Cagua, Barangay Magrafil, Municipality of Gonzaga, Cagayan Province, Luzon Island, Philippines	5	JX966376
KU 330090	Mt. Cagua, Barangay Magrafil, Municipality of Gonzaga, Cagayan Province, Luzon Island, Philippines	5	JX966373
KU 330092	Mt. Cagua, Barangay Magrafil, Municipality of Gonzaga, Cagayan Province, Luzon Island, Philippines	5	JX966374
KU 308813	Mt. Palali, Barangay Maddiangat, Municipality of Quezon, Nueva Vizcaya Province, Luzon Island, Philippines	3	HQ907478
KU 308865	Mt. Palali, Barangay Maddiangat, Municipality of Quezon, Nueva Vizcaya Province, Luzon Island, Philippines	2	JX966372
KU 308866	Mt. Palali, Barangay Maddiangat, Municipality of Quezon, Nueva Vizcaya Province, Luzon Island, Philippines	3	HQ907476
KU 308922	Mt. Palali, Barangay Maddiangat, Municipality of Quezon, Nueva Vizcaya Province, Luzon Island, Philippines	2	JX966371
KU 308923	Mt. Palali, Barangay Maddiangat, Municipality of Quezon, Nueva Vizcaya Province, Luzon Island, Philippines	2	HQ907477
KU 308953	Mt. Palali, Barangay Maddiangat, Municipality of Quezon, Nueva Vizcaya Province, Luzon Island, Philippines	1	JX966370

Brachymeles muntingkamay is now recognized to occur in separate and distinct geological units of Luzon Island: the Caraballo and the Sierra Madres mountain ranges (Fig. 1). The species occurs in primary- and secondary-growth forest at mid-elevations (Siler et al. 2009). All newly collected individuals from Mt. Cagua were observed within small, rotting logs. Interestingly, all individuals of *B. muntingkamay* observed at both localities are females. Given what is known now about species in the genus, we suspect that the absence of male specimens is simply a result of sampling effort, and future surveys conducted in these regions of Luzon Island will likely result in the observation of male individuals. Whether males of this species possess a unique behavior relative to females that has led to gender bias in collections remains unknown, and in-depth studies are needed on the natural history of this unique mid-elevation species. *Brachymeles muntingkamay* remains one of only three limbed, non-pentadactyl species that occur at higher elevations (incl. *B. elerae* and *B. wrighti*; Siler 2010; Siler et al. 2011d). The discovery of a high elevation, isolated population of *Brachymeles muntingkamay* more than 200 km from the type locality brings to light questions regarding the full distribution of this species.

While a few biotic surveys have been conducted within the intervening landscape (Brown et al. 2000, 2012, 2013; Siler et

al. 2011e), the presence of this species in intervening areas between Mt. Cagua and Mt. Palali has yet to be documented. Due to the limited access to suitable, mid- to high-elevation habitat, it is likely that additional populations may be present along central and southern portions of the Sierra Madre Mountain Range. However, this can only be validated with continued survey efforts, targeting higher elevation habitat in intervening areas (e.g., Cagayan and Isabela Provinces). Until such time, the distribution of this species is known only from two disjunct, mid-elevation localities. With this new data, we have re-evaluated this species against the IUCN criteria for classification, and find that it does not qualify for Critically Endangered, Endangered, Vulnerable, or Near Threatened status. *Brachymeles muntingkamay* is quite abundant on both Mt. Palali and Mt. Cagua, and we predict the species has a broad geographic distribution throughout the Sierra Madres Mountain Range at mid- to high elevations. We therefore classify this species as Least Concern LC (IUCN, 2011).

This discovery has major implications for conservation biology of reptiles in the mountains of the northern Philippines, with the possibility of underestimated ranges for mid- and high-elevation micro-endemic species, especially on topographically complex islands like Luzon and Mindanao. Just considering ground skinks, the newly discovered montane population of *B.*

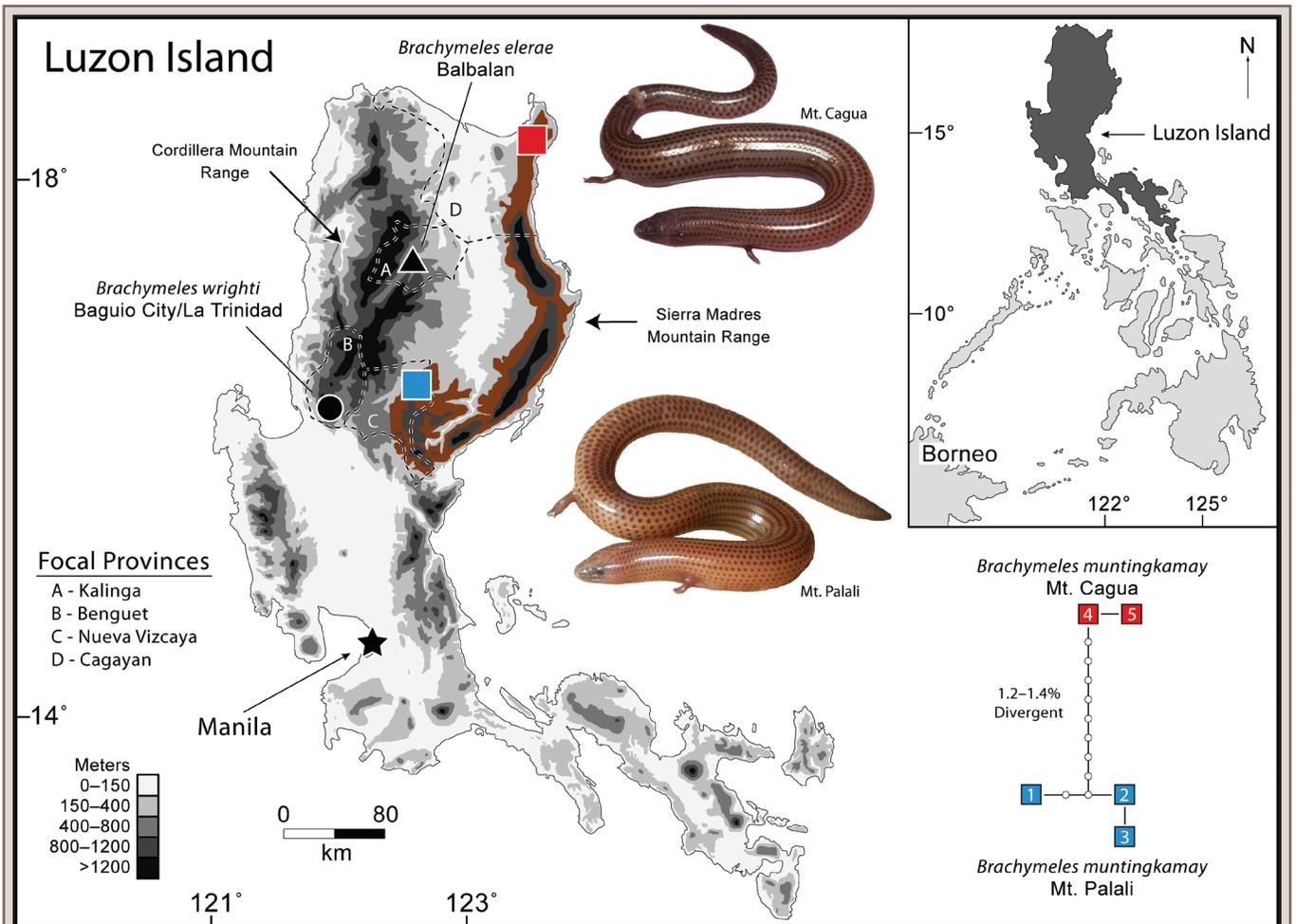


FIG. 1. Known localities of *Brachymeles muntingkamay*, with provincial boundaries for known populations of the three mid- to high-elevation species of *Brachymeles* on Luzon Island, Philippines. Hypothesized distribution of *B. muntingkamay* highlighted in brown. The minimum spanning, 95% connection probability, statistical parsimony haplotype network is shown depicting population-level genetic structure between and within the two sampled populations. Haplotype numeric labels and colors correspond to sampled individuals and localities (refer to Table 2). Photographs in life of *B. muntingkamay* from Mt. Cagua and Mt. Palali shown for comparison.

muntingkamay provides hope for future discoveries of the only two tetradactyl species in the genus *Brachymeles* (*B. elerae* and *B. wrighti*), as well as the poorly known Cordillera endemic species of *Eutropis* (*E. bontocensis*). At present there remain only two known specimens of *B. wrighti*, five specimens of *B. elerae*, and three specimens of *E. bontocensis* in museum collections. Unfortunately, both *B. wrighti* and *E. bontocensis* have not been observed in over 80 years, and our three focused surveys of the type localities for *B. wrighti* have resulted in no new records. Although such sparse data on the distribution of these species prevents a complete assessment of their geographic distribution, renewed survey efforts throughout the Cordillera Mountain Range may result in the discovery of additional populations of these vulnerable species. Fortunately, recent studies have indicated that many species of *Brachymeles* appear to be resilient to habitat degradation (Siler 2010; Siler and Brown 2010; Siler et al. 2011b,c,d, 2012a,b), and we remain optimistic that future surveys will result in similar discoveries of montane populations.

Acknowledgments.—We thank the Protected Areas and Wildlife Bureau (PAWB) of the Philippine Department of Environment and Natural Resources (DENR) for facilitating permits necessary for this and related studies and we are particularly grateful to M. Lim, C. J. L. De Leon, Custodio, and A. Tagtag for their logistical support of our research. The University of Kansas IACUC Facility approved all animal care and handling protocols. The Mt. Cagua Protected Area Management Board graciously endorsed our application to conduct field research in the Gonzaga area, Calayan Province. For assistance and companionship in the field, we thank J. Fernandez, P. Buenavente, V. Yngente, W. and J. Bulalacao, and C. Oliveros. We thank two anonymous reviewers for comments on an earlier draft of this manuscript. This work was supported by a U.S. National Science Foundation Biotic Surveys and Inventories grant (DEB 0743491).

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Note added in proof.—During the period of review and publication of this note, we have identified a third population of *B. muntingkamay* at an intervening locality at Nassiping, Isabela Province (Brown et al. 2013; pers. comm. with ACD) but no genetic material is available and its genetic relations remain unknown.