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Taxonomic Status of the Sumatran Pitviper *Trimeresurus (Popeia) toba* David, Petri, Vogel & Doria, 2009 (Squamata: Viperidae) and Other Sunda Shelf Species of the Subgenus *Popeia*

Elijah Wostl,[1,4] Irvan Sidik,[2] Wahyu Trilaksono,[2] Kyle J. Shaney,[1] Nia Kurniawan,[3] and Eric N. Smith[1]

[1]*Amphibian and Reptile Diversity Research Center and Department of Biology, University of Texas at Arlington, Arlington, Texas USA*
[2]*Laboratory of Herpetology, Museum Zoologicum Bogoriense, Research Center for Biology, Indonesian Institute of Sciences–LIPI, Widyasatwa Loka Jl. Raya Jakarta Bogor km 46, Cibinong, West Java, Indonesia*
[3]*Department of Biology, Universitas Brawijaya, Jl. Veteran, Malang, East Java, Indonesia*

Abstract.—**The pitviper *Trimeresurus (Popeia) toba* was described on the basis of slight morphological differences between six specimens collected in northern Sumatra and the other recognized species of *Trimeresurus (Popeia)* from the Sunda Shelf. In January 2014, we collected two additional specimens of *T. (P.) toba* from Sumatra and located a third unexamined specimen at the Museum Zoologicum Bogoriense. We compared molecular and morphological data generated from these specimens with existing data for *T. (P.) toba* and the other Sunda Shelf *Trimeresurus (Popeia)*. Our findings indicate that *T. (P.) toba* is indistinguishable from *T. (P.) barati*, the other species that occurs on Sumatra. Additionally, with the exception of *T. (P.) nebularis*, all currently recognized species of *Trimeresurus (Popeia)* from the Sunda Shelf are minimally divergent and the morphological characters used to diagnose the individual species broadly overlap. For these reasons, we conclude that all should be considered a single species, *T. (P.) sabahi*.**

Abstrak.—**Ular bandotan toba, *Trimeresurus* (*Popeia*) *toba* telah dideskripsikan berdasarkan perbedaan kecil pada morfologi antara enam spesimen dari Sumatera Utara dan spesies lain yang dikenali sebagai *Trimeresurus* (*Popeia*) dari Paparan Sunda. Pada bulan Januari 2014, kami mengumpulkan dua spesimen tambahan *T.* (*P.*) *toba*, dan satu spesimen ketiga yang belum diperiksa di Museum Zoologicum Bogoriense. Kami membandingkan data molekuler dan morfologi yang dihasilkan dari spesimen-spesimen baru terhadap data yang ada dari *Trimeresurus* (*Popeia*) Paparan Sunda lainnya. Temuan kami menunjukkan bahwa *T.* (*P.*) *toba* tidak dapat dibedakan terhadap *T.* (*P.*) *barati*, spesies lain yang diketahui dari Sumatera. Selain itu, dengan pengecualian pada *T.* (*P.*) *nebularis*, saat ini semua spesies *Trimeresurus* (*Popeia*) dari Paparan Sunda berbeda sedikit dan karakter morfologi yang digunakan untuk menentukan individu spesies secara luas tumpang tindih. Untuk alasan ini, kami menyimpulkan bahwa semuanya harus dianggap merupakan spesies tunggal, *T.* (*P.*) *sabahi*.**

The *Trimeresurus (Popeia) popeiorum* (Squamata, Viperidae) species complex of Southeast Asia has a convoluted taxonomic past. Initially thought to be composed of a single widespread species, in the past decade they have been placed in a new genus, *Popeia* (Malhotra and Thorpe, 2004), and several populations have been afforded species status on the basis of morphological differences. Several authors have subsequently relegated *Popeia* the status of subgenus (David et al., 2009, 2011; Sumontha et al., 2011), an arrangement that we provisionally follow in this paper.

Regenass and Kramer (1981) were the first to recognize divisions in this seemingly uniform group by proposing that populations from Sumatra and Borneo are distinct subspecies: *Trimeresurus popeiorum barati* and *Trimeresurus popeiorum sabahi* respectively. Vogel et al. (2004) posited that *T. (P.) popeiorum* was composed of five distinct species: (1) *Trimeresurus popeiorum* from India, Laos, Myanmar, and Thailand; (2) *T. fucatus* from the Malay Peninsula and southern Thailand; (3) *T. nebularis* from the Cameron Highlands of Peninsular Malaysia; (4) *T. sabahi* from Borneo; and (5) *T. barati* from Sumatra. In 2006, the number of *Trimeresurus (Popeia)* species recognized from the Sunda Shelf increased to five with the description of *T. (P.) buniana* from Pulau Tioman, Malaysia (Grismer et al., 2006). In that same year, Sanders et al. (2006) re-examined the taxonomy of the *T. (P.) popeiorum* complex and concluded that specimens from throughout the Sunda Shelf, excluding the Cameron Highlands of the Malaysian Peninsula, were composed of a single widespread species, *T. (P.) sabahi*.

Trimeresurus (Popeia) toba was first described on the basis of the morphological analysis of six specimens, three collected in 1891 by Elio Modigliani and three without a given collector or date of collection (David et al., 2009). According to the original description, *T. (P.) toba* is distinguishable from all *Trimeresurus (Popeia)* species in the region by the combination of several morphological characters: 21 dorsal scale rows at midbody, the lack of a postocular stripe, a unicolored (white) or absent lateral stripe, unkeeled (or nearly so) temporal and occipital scales, the number of ventral scales, eye color, and pattern (David et al., 2009).

In January 2014, we collected two additional specimens of *T. (P.) toba* (male and female) from near Sipirok, Kabupaten Tapanuli Selatan, Sumatera Utara Province, Sumatra, ~70 km south of the type locality. The specimens conform to the original description of *T. (P.) toba* in regard to the number of dorsal scales at midbody. Given the proximity of the collection site to the type locality and a morphological diagnostic match, we have little doubt that the specimens are referable to *T. (P.) toba* as it is currently understood. In addition to the specimens collected in January, we examined a previously unreported specimen of *T. (P.) toba* found in the herpetological collection of the Museum Zoologicum Bogoriense of the Indonesian Institute of Sciences (MZB.Ophi.2158). This specimen also originates from Kabupaten Tapanuli Selatan, though it was collected in November 1996.

To date, these three represent the only contemporary specimens of *T. (P.) toba*. Vogel et al. (2014) provide a photo of a live *T. (P.) toba* from Sumatera Barat Province; however, the

[4]Corresponding Author; E-mail: ewostl@uta.edu
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TABLE 1. List of specimens used in this paper and Genbank accession numbers.

Species	Locality	Coordinates	Specimen #	ND4	Cyt-b
Trimeresurus (Trimeresurus) insularis	East Java, Indonesia	not available	AM A109	AY352833	AY352767
Trimeresurus (Popeia) "barati"	Bengkulu Province, Sumatra, Indonesia	not available	AM B361	AY371837	AY371801
T. (P.) "barati"	Lampung Province, Sumatra, Indonesia	−5.28721°N 104.55401°E	UTA-R 61639	KP939319	KP899261
T. (P.) "barati"	Lampung Province, Sumatra, Indonesia	−5.42364°N 104.6922°E	MZB.Ophi.5199	KP939320	KP899262
T. (P.) "barati"	Jambi Province, Sumatra, Indonesia	−2.26013°N 101.49512°E	UTA-R 61640	KP939321	KP899263
T. (P.) "barati"	Jambi Province, Sumatra, Indonesia	−2.25985°N 101.49493°E	MZB.Ophi.5197	KP939322	KP899264
T. (P.) "buniana"	Pulau Tioman, Malaysia	not available	AM B519	AY371853	AY371818
T. (P.) "fucata"	Nakhon si Thammarat, Thailand	not available	AM A202	AY371840	AF171904
T. (P.) "fucata"	Nakhon si Thammarat, Thailand	not available	AM A203	AY059588	AY371796
T. (P). nebularis	Cameron Highlands, Malaysia	not available	AM B346	AY371850	AY371810
T. (P). popeiorum	Phongsali Province, Laos	not available	AM B196	AY059590	AY059571
T. (P). popeiorum	Mon State, Myanmar	not available	CAS 222195	AY371841	AY371806
T. (P). popeiorum	Bago State, Myanmar	18.88328°N 95.87914°E	CAS 205847	AY371855	AY371816
T. (P). popeiorum	West Thailand	not available	AM B52	AY371836	AY371800
T.(P.) "sabahi"	Sabah, Borneo, Malaysia	not available	AM B341	AY371834	AY371803
T.(P.) "sabahi"	Borneo (East Malaysia)	not available	AM B344	AY371842	AY371815
T. (P.) "toba"	Sumatera Utara Province, Sumatra, Indonesia	01.68455°N 99.34737°E	UTA-R 61641	KP939323	KP899265
T. (P.) "toba"	Sumatera Utara Province, Sumatra, Indonesia	01.61736°N 99.22556°E	MZB.Ophi.5342	KP939324	KP899266

species allocation is questionable. The specimen is from Padang Panjang, ~50 km from the type locality of *T. (P.) barati* and possesses a bicolored lateral stripe, a characteristic of *T. (P.) barati* (David et al. 2009).

We also collected a small series of *T. (P.) barati*, two from Jambi Province, Sumatra, and two from Lampung Province, Sumatra, and examined a fifth specimen, also in the MZB collection, that was obtained near Padang, Sumatera Barat Province.

Herein we use molecular data from these new specimens to assess the validity of *T. (P.) toba* and its closest relatives. We also combine the morphologic data collected from these specimens with data already published for this species complex to illustrate the lack of diagnostic characters. Our results are in agreement with the much larger analysis of this group by Sanders et al. (2006) and we refer readers to that study for a thorough morphometric analysis that includes specimens from the type series of both *T. (P.) buniana* and *T. (P.) toba*. We build on it by providing molecular data for *T. (P.) toba* that was unavailable at the time of their study.

MATERIALS AND METHODS

Sampling.—Specimens used in this analysis were collected during herpetological surveys undertaken in Sumatra in May–June 2013 and January–February 2014. Specimens were photographed alive and then euthanized. Dorsal, ventral, and lateral photographs were taken of the specimens postmortem with a scale for size reference. Specimens were preserved in 10% formalin until they could be transferred to 70% ethanol. Before preservation either muscle or liver tissue was collected from each specimen and stored in 1.5 mL of cell lysis buffer.

Molecular Methods.—Deoxyribonucleic acid (DNA) was extracted from the tissue using "serapure" (Rohland and Reich, 2012) magnetic beads. Twenty-five milliliters of the lysis buffer containing digested tissue was combined with 25 μL of water and 5 μL of proteinase K and incubated at 57°C for 1 h to ensure that digestion was complete. After the incubation period, the tissue sample was mixed with 1.8 μL of serapure beads for every 1 μL of sample. The remaining steps of DNA extraction followed the procedures for cleaning polymerase chain reaction products with AMPure® magnetic beads (Agencourt®, Bioscience, Beverly, Massachusetts).

The mitochondrial gene nicotinamide adenine dinucleotide (reduced) dehydrogenase subunit 4 (*ND4*) was amplified using the forward primer ND4 (5′-CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC-3′) and the reverse primer LEU (5′-CAT TAC TTT TAC TTG GAT TTG CAC CA-3′; Arévalo et al., 2004). The *ND4* thermal cycle profile consisted of an initial denaturation at 94°C for 3 min followed by 30 cycles of a 30-sec denaturation at 94°C, a 45-sec annealing phase at 52°C, and a 1-min extension at 72°C. This was followed by a final 7-min extension at 72°C.

The mitochondrial gene cytochrome b (*cyt-b*) was amplified with the forward primer GLUDGE (5′-TGA CTT GAA RAA CCA YCG TTG-3′) and the reverse primer ATRCB3 (5′-TGA GAA GTT TTC YGG GTC RTT-3′; Parkinson et al., 2002). The thermal cycle used to amplify *cyt-b* consisted of an initial denaturation at 95°C for 2 min followed by two cycles of a 1-min denaturation at 95°C, a 1.5-min annealing step at 48°C, and a 1-min extension at 68°C. This was followed by 35 cycles of a 25-sec denaturation at 95°C, a 1.5-min annealing step at 50°C, and a 1-min extension at 72°C. This was followed by a final 10-min extension at 72°C. The product from each reaction was cleaned using serapure (Rohland and Reich, 2012) magnetic beads following the procedure for AMPure magnetic beads. Sequencing reactions in both primer directions were performed using standard protocols associated with BigDye® terminator chemistry (Applied Biosystems, Foster City, California) at the University of Texas at Arlington (UTA) genomics core facility (Arlington, Texas; gcf.uta.edu). Sequences were assembled and cleaned using the program Sequencher® 4.8 (Genecodes, Ann Arbor, Michigan) and individually aligned using MEGA 6.0 (Tamura et al., 2013). The data set was augmented with sequences available on Genbank from Indonesia, Laos, Malaysia, Myanmar, and Thailand. Table 1 contains a complete list of specimens used in this study along with Genbank accession numbers and locality data.

TABLE 2. Morphological characters used to distinguish among southern-clade *Trimeresurus (Popeia)* species (Vogel et al., 2004; Grismer et al., 2006; Sanders et al., 2006; David et al., 2009) Note broad overlap in all. The "#" column represents the number of individuals used in original description followed by the number of additional specimens examined by authors in parentheses. VEN = ventral scales, SC = subcaudal scales, O&T = occipital and temporal scales (−, +, and ++, represent unkeeled, keeled, and strongly keeled, respectively), POS = postocular stripe.

	Sex	#	VEN	SC	DSR	TL/TTL	HL/SVL	EP/EN	O&T	POS	LS	Eye color	Dorsal pattern	Tail pattern
T. (P.) barati	F	5(2)	146–159	55–63	17–19	0.15–0.18	0.054–0.064	0.55–0.62	−, +	absent	thin and faint or absent	yellow–green/ deep red	uniform green	distinct
T. (P.) buniana	F	1	170	61	21	0.22	0.055	0.22–0.33		absent	white/bicolored	turquoise with maroon center	uniform green	
T. (P.) fucata	F	19	151–170	59–73	19–21	0.16–0.19	0.051–0.063	0.51–0.66	+, ++	absent	white	yellow–green/ yellow/copper	uniform green	indistinct-distinct
T. (P.) sabahi	F	3	148–156	59–65	21	0.17–0.18	0.051–0.070	0.52–0.61	−	absent	white or yellow	deep red-orange	uniform green	distinct
T. (P.) toba	F	4(2)	147–156	57–64	19–21	0.15–0.18	0.057–0.059	0.24–0.26	−, +	absent	bicolored, thin and faint, or absent	deep orange	uniform green	distinct
T. (P.) barati	M	12(2)	142–153	62–73	17–19	0.19–0.23	0.050–0.061	0.55–0.62	−, +	absent	uniform–bicolored	yellow–green/ deep red	uniform green	distinct
T. (P.) buniana	M	3	170–174	76–78	21	0.22–0.23	0.044–0.049	0.22–0.33		present	bicolored	gold	banded	
T. (P.) fucata	M	36	156–171	69–84	19–21	0.20–0.24	0.046–0.059	0.51–0.66	−, +	white, bicolored, or absent	bicolored	yellow–green/ yellow copper	uniform green banded	indistinct-distinct
T. (P.) sabahi	M	7	147–157	69–76	21	0.19–0.24	0.05–0.058	0.52–0.61	−, +	absent	bicolored	deep red–orange	uniform green	distinct
T. (P.) toba	M	2(1)	153–159	73–77	19–21	0.20–0.23	0.052	0.27	−, +	absent	thin,white bicolored	deep orange	uniform green	distinct

Phylogenetic Analysis.—To account for variation in the length of available sequences, three different data sets were generated: one consisting of all available sequences of *Trimeresurus (Popeia)* trimmed to remove missing data; a second composed of all available sequences, including gaps and missing data; and a third that attempted to preserve sequence length and remove missing data by incorporating fewer sequences. The different data sets produced similar but not identical results, with differences appearing in the placement of a few individuals of nontarget taxa and slight variation in support values. The same topology of the taxa of interest, *Trimeresurus (Popeia)* from the Sunda Shelf, was recovered regardless of the data set used.

The latter data set, in which the number of sequences was reduced to preserve sequence length and remove missing data, was used for all analyses and interpretation. This data set includes all species of *Trimeresurus (Popeia)* for which genetic data are available, though in many cases representation is reduced to a single sequence.

A preliminary Bayesian analysis of sequences representing the individual genes was conducted and the results were examined for well-supported divergent topologies. None was detected and the sequences for the individual genes were concatenated into a single representative sequence.

The final concatenated data set consists of 980 base pairs from 18 individual specimens from throughout the Sunda Shelf. We did not include *T. (P.) phuketensis* Sumontha, Kunya, Pauwels, Nitikul, and Punnadee, 2011 because of lack of molecular data. *Trimeresurus (Trimeresurus) insularis* was used as an outgroup taxon.

The uncorrected pairwise distance between each sequence was compared using MEGA 6.0 (Tamura et al., 2013). The program PartitionFinder (Lanfear et al., 2012) was used to find the best partitioning scheme and best-fit model of molecular evolution for each codon of each gene using the Bayesian information criterion.

Six independent Bayesian analyses of the concatenated data set were conducted in Mr. Bayes 3.2.2 (Ronquist et al., 2012) using three heated chains and one cold chain each for 10 million generations with sampling occurring every 1,000 generations. To assess adequate mixing, autocorrelation, and to determine the appropriate number of trees to discard as burn-in, we used the program TRACER (Rambaut et al., 2014). The program ARE WE THERE YET (Wilgenbusch et al., 2004) was used to assess the convergence of the separate analyses onto a single topology.

A maximum likelihood (ML) tree was generated using the RaXML GUI (Silvestro and Michalak, 2012) with partitions set to each gene and codon position. Support for the ML tree was calculated using 1,000 bootstrap replicates. Phylogenetic trees were visualized using the program FigTree 1.4.0 (Rambaut, 2012).

Morphology.—We examined two specimens of *T. (P.) toba* collected in January 2014 and one collected in November 1996. We also examined five specimens of *T. (P.) barati,* two collected in Lampung Province, Sumatra, two collected in Jambi Province, Sumatra approximately 66 km from the collection locality of the paratype, and one museum specimen collected from the vicinity of Padang, in Sumatera Barat Province (MZB.Ophi.1736). Characters examined are those identified as being important for distinguishing species in the *Trimeresurus (Popeia)* species complex of the Sunda Shelf (Vogel et al., 2004; Grismer et al., 2006; Sanders et al., 2006; David et al., 2009) These include the number of ventral scales; the number of subcaudal scales; the number of dorsal scale rows at midbody (DSR); the number of

TABLE 3. Morphometric values for examined *Trimeresurus (Popeia) toba* and *T. (P.) barati* specimens. All measurements are in millimeters. VEN = number of ventral scales, SC = subcaudals, SL = supralabial scales R/L, IL = infralabial scales R/L, SR3SL = number of scales between third supralabial and eye R/L, CS = cephalic scales between supraocular scales.

Species	Specimen	Sex	SVL	VEN	SC	DSR	TL	TTL	HL	SL	IL	SR3SL	CS	EP	EN
T. (P.) barati	MZB.Ophi.5199	F	512	159	60	19	93	605	28.4	9/9	12/12	0/0	9	1.26	6.28
T. (P.) barati	UTA-R 61640	F	287	147	63	19	62	349	18.3	10/10	12/12	0/0	11	0.9	4.4
T. (P.) barati	MZB.Ophi.1736	F	327	143	58	19	78	405	19.9	9/9	11/11	0/0	9	1.44	4.76
T. (P.) toba	MZB.Ophi.5342	F	517	154	61	21	106	623	30.3	10/11	12/12	0/1	11	1.7	7.03
T. (P.) toba	MZB.Ophi.2158	F	544	147	57	19	118	662	31.3	10/10	12/12	0/0	10	1.73	6.72
T. (P.) barati	UTA-R 61639	M	492	153	72	19	139	631	24.8	9/9	11/11	0/0	9	1.74	5.53
T. (P.) barati	MZB.Ophi.5197	M	574	147	70	19	166	740	31.6	10/10	12/12	0/0	9	1.63	6.92
T. (P.) toba	UTA-R 61641	M	385	159	77	21	94	479	20.1	10/9	11/12	1/0	11	1.26	4.62

supralabial scales; the number of infralabial scales; the number of cephalic scales between supraoculars; the number of scales between the third supralabial and the subocular scales; eye–pit distance (EP); eye–nostril distance (EN); tail length (TL) divided by the total length (TTL); head length (HL) divided by the snout-vent length (SVL); whether the occipital and temporal scales are unkeeled (−), keeled (+), or strongly keeled (++); color and nature of the postocular stripe; color and nature of the lateral stripe (LS); dorsal pattern, and tail pattern. These data were compared with the existing data available for each species. Table 2 provides the known range of values for these characters for the currently recognized species of *Trimeresurus (Popeia)* of the Sunda Shelf, and Tables 3 and 4 provide the values of these characters for specimens examined by the authors. Summary statistics are expressed as mean ± SD.

Sex was determined by dissection of the tail and examination for the presence of hemipenes. Ventral scales were counted according to Dowling (1951). The first subcaudal was defined as the first scale posterior to the vent that came into contact with the scale opposite. Mid-dorsal scales were counted halfway between the tip of the snout and the vent. SVL and TL were measured to the nearest millimeter. HL was recorded as the distance from the posterior edge of the mandible to the tip of the rostral scale. The EP was measured from the anterior margin of the eye to the posterior border of the facial pit. EN was measured from the anterior margin of the eye to the posterior margin of the external nares. All measurements except SVL and TL were taken with digital calipers. Color of the LS was assessed while the animal was alive (not possible for MZB.Ophi.2158 and MZB.Ophi.1736). These data are illustrative, and used only for comparison with data that have already been published for the group. We lack both the numerical and geographic sampling for a more comprehensive morphometric analysis.

RESULTS

Molecular Analysis.—Our analysis revealed a strongly supported divide between specimens of *Trimeresurus (Popeia)* from mainland Southeast Asia and the Cameron Highlands of the Malay Peninsula (*T. [P.] popeiorum* and *T. [P.] nebularis*, respectively) and the other *Trimeresurus (Popeia)* species from the Sunda Shelf (*T. [P.] barati*, *T. [P.] buniana*, *T. [P.] fucatus*, *T. [P.] sabahi*, and *T. [P.] toba*). These results are largely the same as those of Sanders et al. (2006). We retain the terminology used in that study and refer to these two clades as the northern clade and southern clade, respectively. ML failed to support a northern clade, potentially because our northern clade samples consisted of few individuals with relatively high levels of molecular divergence. The within-group mean pairwise distance of the northern clade specimens, 0.05 substitutions per site, is as high as the average pairwise distance between the northern and southern clades. The relationship between the northern and southern clades is beyond the scope of our study; we defer to other studies (Sanders et al., 2006) that include more genes and strongly support this division.

Both analyses that we conducted showed that specimens from Sumatra (including both *T. [P.] barati* and *T. [P.] toba*), Borneo, and Pulau Tioman form a minimally divergent and unresolved polytomy. Both analyses support this monophyletic yet unresolved group as being sister to populations from south Thailand, although the support is low. As expected, specimens from the same locality are strongly supported as being most closely related to each other. Figure 1 shows a graphical

TABLE 4. Coloration and pattern of examined specimens.

Species	Specimen	Sex	Postocular stripe	LS	Eye color	Tail pattern
Trimesurus (Popeia) barati	MZB.Ophi.5199	F	absent	very thin and faint, yellowish	deep orange/red	clean edged
T. (P.) barati	UTA-R 61640	F	absent	present; thin; white on parts of scale rows 1+2	yellow/green/brown	clean edged
T. (P.) barati	MZB.Ophi.1736	F	absent	absent	n/a	mottled
T. (P.) toba	MZB.Ophi.5342	F	absent	absent	deep orange/red	clean edged
T. (P.) toba	MZB.Ophi.2158	F	absent	faint/absent	n/a	clean edged
T. (P.) barati	UTA-R 61639	M	absent	bicolored; off white/beige below light white bluish and indistinct above ~one scale wide	deep orange/red	clean edged
T. (P.) barati	MZB.Ophi.5197	M	absent	thin, faint, beige/off white	yellow/green/brown	mottled
T. (P.) toba	UTA-R 61641	M	absent	present; thin; bicolored; lower brown/red upper blue/white	orange/red	mottled

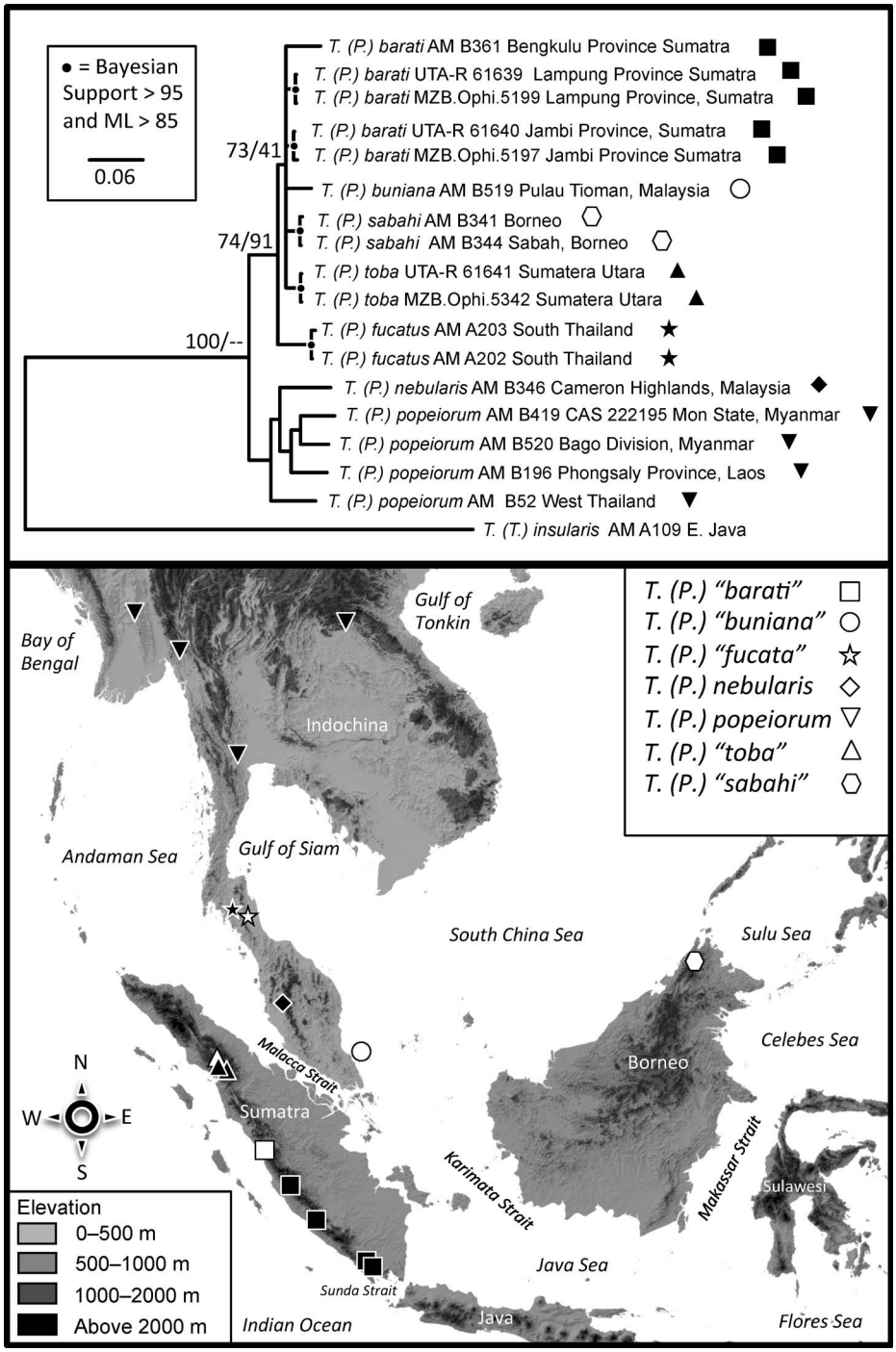

FIG. 1. Bayesian tree and map showing phylogenetic sampling in Southeast Asia. Posterior probabilities shown above the diagonal and ML bootstrap values below. Note the extremely shallow branch lengths within the southern clade. Type locality and species represented only by type material are represented by unfilled symbols.

representation of the phylogenetic relationships within this group on the Sunda Shelf.

The pairwise distances between specimens in the southern clade are low, ranging from 0.000 to 0.045 (0.023 ± 0.010). The greatest disparity is between a specimen of *T. (P.) fucatus* from southern Thailand and a specimen of *T. (P.) barati* from Bengkulu Province Sumatra. This specimen of *T. (P.) barati* (AM B361) is one of the most divergent of the southern clade specimens and differs from all other Sumatran specimens by 0.026–0.031 substitutions per site. In comparison, the other specimens from Sumatra differ by 0.000 to 0.019 substitutions per site. Unfortunately, this specimen is not available for detailed examination. It originates from the vicinity of Curup at an elevation of about 990 m above sea level, and is identified as *T. (P.) barati* solely on provenance. Excluding AM B361, pairwise distance within the southern clade ranges from 0.00 to 0.037 (0.021 ± 0.009).

When degenerate sites are excluded from the analysis, the pairwise distance within the southern clade is almost nil, 0.000–0.026 substitutions per site (0.010 ± 0.006). Once again, AM

TABLE 5. Uncorrected pairwise distances between *Trimeresurus (Popeia)* sp. based on 980 base pairs from the mitochondrial genes *cyt-b* and *ND4*. Below diagonal: pairwise distance of nucleotide sequence. Above diagonal: pairwise distance excluding degenerate sites. Specimens from the southern clade are in bold font.

ID	Specimen	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	*Trimeresurus insularis* AM A109 E. Java		0.062	0.049	0.049	0.046	0.046	0.040	0.047	0.044	0.040	0.040	0.040	0.034	0.037	0.049	0.046	0.049	0.049
2	*Trimeresurus (Popeia) barati* AM B361 Bengkulu Province, Sumatra	0.132		**0.019**	**0.019**	**0.015**	**0.015**	**0.022**	**0.025**	**0.026**	0.031	0.034	0.037	0.037	0.031	**0.019**	**0.015**	**0.022**	**0.022**
3	*T. (P). barati* UTA-R 61639 Lampung Province, Sumatra	0.122	**0.029**		**0.000**	**0.003**	**0.003**	**0.009**	**0.013**	**0.013**	0.019	0.022	0.025	0.025	0.021	**0.006**	**0.003**	**0.009**	**0.009**
4	*T. (P). barati* MZB.Ophi.5199 Lampung Province, Sumatra	0.122	**0.029**	**0.000**		**0.003**	**0.003**	**0.009**	**0.013**	**0.013**	0.019	0.022	0.025	0.025	0.021	**0.006**	**0.003**	**0.009**	**0.009**
5	*T. (P). barati* UTA-R 61640 Jambi Province, Sumatra	0.126	**0.026**	**0.013**	**0.013**		**0.000**	**0.006**	**0.009**	**0.010**	0.015	0.018	0.022	0.021	0.018	**0.003**	**0.000**	**0.006**	**0.006**
6	*P .barati* MZB.Ophi.5197 Jambi Province, Sumatra	0.126	**0.026**	**0.015**	**0.015**	**0.002**		**0.006**	**0.009**	**0.010**	0.015	0.018	0.022	0.021	0.018	**0.003**	**0.000**	**0.006**	**0.006**
7	*T. (P.) buniana* AM B519 Pulau Tioman, Malaysia	0.120	**0.035**	**0.022**	**0.022**	**0.019**	**0.021**		**0.016**	**0.016**	0.015	0.019	0.022	0.015	0.012	**0.009**	**0.006**	**0.012**	**0.012**
8	*T. (P.) fucata* AM A202 Nakhon si Thammarat, Thailand	0.118	**0.042**	**0.030**	**0.030**	**0.027**	**0.027**	**0.036**		**0.000**	0.019	0.022	0.025	0.022	0.022	**0.013**	**0.009**	**0.013**	**0.013**
9	*T. (P.) fucata* AM A203 Nakhon si Thammarat, Thailand	0.117	**0.045**	**0.032**	**0.032**	**0.029**	**0.029**	**0.037**	**0.002**		0.019	0.022	0.025	0.022	0.022	**0.013**	**0.009**	**0.013**	**0.013**
10	*T. (P.) nebularis* AM B346 Cameron Highlands, Malaysia	0.123	0.069	0.059	0.059	0.057	0.059	0.058	0.059	0.061		0.009	0.012	0.015	0.009	0.019	0.015	0.015	0.115
11	*T. (P.) popeiorum* AM B196 Phongsali Province, Laos	0.121	0.069	0.058	0.058	0.054	0.056	0.058	0.057	0.058	0.054		0.009	0.012	0.012	0.022	0.018	0.018	0.018
12	*T. (P.) popeiorum* CAS 205847 Bago State, Myanmar	0.123	0.070	0.057	0.057	0.053	0.055	0.063	0.056	0.057	0.054	0.040		0.015	0.015	0.025	0.022	0.022	0.022
13	*T. (P.) popeiorum* CAS 222195 Mon State, Myanmar	0.115	0.059	0.052	0.052	0.050	0.050	0.050	0.053	0.054	0.048	0.044	0.037		0.012	0.025	0.021	0.025	0.025
14	*T. (P.) popeiorum* AM B52 west, Thailand	0.121	0.060	0.055	0.055	0.049	0.049	0.051	0.059	0.061	0.055	0.052	0.049	0.053		0.022	0.018	0.018	0.018
15	*T. (P.) sabahi* AM B341 Borneo, East Malaysia	0.128	**0.029**	**0.016**	**0.016**	**0.015**	**0.017**	**0.027**	**0.033**	**0.034**	0.060	0.059	0.056	0.049	0.054		**0.003**	**0.009**	**0.009**
16	*T. (P.) sabahi* AM B344 Sabah, Borneo	0.127	**0.029**	**0.016**	**0.016**	**0.015**	**0.017**	**0.027**	**0.032**	**0.034**	0.059	0.058	0.055	0.048	0.053	**0.001**		**0.006**	**0.006**
17	*T. (P.) toba* UTA-R 61641 Sumatera Utara Province	0.130	**0.031**	**0.016**	**0.016**	**0.017**	**0.019**	**0.029**	**0.030**	**0.032**	0.059	0.057	0.057	0.055	0.053	**0.020**	**0.020**		**0.00**
18	*T. (P.) toba* MZB.Ophi.5342 Sumatera Utara Province	0.130	**0.031**	**0.016**	**0.016**	**0.017**	**0.019**	**0.029**	**0.030**	**0.032**	0.059	0.057	0.057	0.055	0.053	**0.020**	**0.020**	**0.000**	

B361 is anomalous and differs from other Sumatran specimens by 0.015–0.022 substitutions per site. This is equivalent to or greater than the difference between many northern clade and southern clade specimens. If this specimen is excluded, variation in the southern clade drops to 0.000–0.016 substitutions per site (0.008 ± 0.004). Also notable in this analysis is a lack of difference between specimens from Borneo and some specimens from Sumatra (pairwise distance 0.000 substitutions per site) (Table 5).

Morphological Data.—Trimeresurus (Popeia) toba was recognized as being distinct from *T. (P.) barati* on the basis of the number of DSR, the relative TL of females, and the color of the LS in males (David et al., 2009). The two specimens collected in January 2014 from Kabupaten Tapanuli Selatan, Sumatera Utara Province conform to the original description of *T. (P.) toba* in regard to the number of DSR. The specimen collected in 1996, however, also from Kabupaten Tapanuli Selatan, possesses 19 scale rows and is referable to *T. (P.) barati* (David et al., 2009), despite being otherwise indistinguishable from proximally occurring *T. (P.) toba*.

The relative TL of females fails to differentiate the new specimens of *T. (P.) toba* from *T. (P.) barati*. According to David et al. (2009), *T. (P.) barati* females have a tail that constitutes 16.4–17.6% of the TTL, whereas *T. (P.) toba* females have a TL that constitutes 14.9–15.7% of the TTL. In the specimens of female *T. (P.) toba* that we examined, the tail is 17.0–17.8% of the TTL, outside of the known range for *T. (P.) toba* and overlapping with the range given for *T. (P.) barati* (David et al., 2009). The two *T. (P.) barati* females that we captured have tails that make up 15.3–17.7% of the TTL, overlapping the published values for both *T. (P.) barati* and *T. (P.) toba* (David et al., 2009).

The third characteristic used to distinguish *T. (P.) toba* from *T. (P.) barati*, the coloration of the LS in males, also does not prove to be an informative character. In the original description, male *T. (P.) toba* are stated to be readily distinguishable from *T. (P.) barati* on the basis of the LS: white and faint in *T. (P.) toba*, bicolored in *T. (P.) barati* (David et al., 2009). Of the two live male *T. (P.) barati* we examined the LS was bicolored in one (off-white/beige below and faint white/blue above) and a monochromatic beige/off-white in the other. The single male *T. (P.) toba* we examined had a thin bicolored LS: brown/red below and blue/white above (Fig. 2).

Our examination of pertinent characters of the newly collected specimens and subsequent comparison with the published values for the other southern clade *Trimeresurus (Popeia)* failed to reveal evidence of morphological distinction not only between *T. (P.) toba* and *T. (P.) barati*, but also between *T. (P.) toba*/*T. (P.) barati* and the other currently recognized southern-clade *Trimeresurus (Popeia)* (see Table 2). Given the relatively small sample size in the original descriptions and the broad overlap in given values for characters identified as being important in distinguishing the species, we can find little justification for the recognition of distinct species of southern-clade *Trimeresurus (Popeia)* on the basis of morphological characters. Sanders et al. (2006) came to the same conclusion using a much larger data set and thorough statistical tests.

DISCUSSION

The objective of this study was to investigate the taxonomic validity of *T. (P.) toba* using data from the first known contemporary specimens. Our findings indicate that this species is neither molecularly nor morphologically distinct from *T. (P.)*

FIG. 2. Male (A, UTA-R 61639) and female (B, MZB.Ophi.5199) *Trimeresurus (Popeia) "barati"* from Lampung Province, Sumatra. Male (C, MZB.Ophi.5197) and female (D, UTA-R 61640) *T. (P.) "barati"* from Jambi Province, Sumatra. Male (E, UTA-R 61641) and female (F, MZB.Ophi.5342) *T. (P.) "toba"* from Sumatera Utara Province, Sumatra.

barati. Additionally, we find little justification for the distinction of the other species of the southern-clade *Trimeresurus (Popeia)* complex. Our molecular analyses reveal the putative species *T. (P.) barati*, *T. (P.) buniana*, *T. (P.) fucatus*, *T. (P.) sabahi*, and *T. (P.) toba* to be largely undifferentiated. The observed molecular disparity reflects mitochondrial genes that evolve at a considerably higher rate than nuclear genes in vertebrates. We can only expect nuclear sequences to be markedly less divergent. Our comparison of the morphological characters used to describe these species shows that they are not diagnosable.

The only published character state that shows clear differentiation between the putative species is the ratio of the EP to the EN. Grismer et al. (2009) report this as 0.22–0.33 for *T. (P.) buniana* and 0.51–0.66 for all other species. The data for the other species either came from Vogel et al. (2004) or raw data provided by the same authors (Grismer et al., 2006). For the

specimens from Sumatra that we examined, this value ranges from 0.20 to 0.31. Grismer et al. (2006) explicitly state that they measured from the anterior margin of the eye to the posterior margin of the pit. An examination of photographs of the holotypes of *T. (P.) fucatus* and *T. (P.) nebularis* provided in Vogel et al. (2004) makes it clear that they derived this character by measuring from the anterior margin of the eye to the anterior margin of the pit. Therefore, this character state is not comparable between the different data sets.

This lack of divergence in this group is expected given the geologic history of the region. Borneo, Sumatra, and Peninsular Malaysia (including Pulau Tioman) have been part of a single continuous land mass for approximately 44% of the past 17,000 yr (Voris, 2000). The allopatric distribution of *Trimeresurus (Popeia)* on these land masses is a contemporary phenomenon. This is consistent with the findings of Inger and Voris (2001) that show that a large proportion of the snake fauna of Borneo, Sumatra, and the Malay Peninsula is shared among all three land masses.

Our results support that of Sanders et al. (2006) in recognizing the southern-clade *Trimeresurus (Popeia)* (*T. [P.] barati*, *T. [P.] buniana*, *T. [P.] fucatus*, *T. [P.] sabahi*, and *T. [P.] toba*) as a single species, *T. (P.) sabahi*. Doing otherwise obscures the close relationship between these populations in favor of recognizing a recently realized allopatric distribution of the same species.

Within this paper, we have used *Popeia* as a subgenus of *Trimeresurus*. We would be remiss not to mention that a consensus has not been reached on the appropriate designation of the genus-level name for this group. Our relegation of *Popeia* to subgenus is a compromise between highlighting the evolutionary and morphological distinctiveness of the group and maintaining taxonomic stability. Our usage is not in support of any one naming scheme, though we feel that as time passes and familiarity increases, *Popeia* will become widely recognized as the appropriate name for the genus.

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