



A new species of shrew (Soricomorpha: *Crocidura*) from Java, Indonesia: possible character displacement despite interspecific gene flow

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We describe a new species of shrew (Soricomorpha, Soricidae, *Crocidura*) from Mt. Gede, West Java, Indonesia. Relative to other Javan shrews, the new species is small with a relatively thick, dark brown, medium-length tail. The new species is known only from the type locality at 1,611 and 1,950 m elevation on Mt. Gede. It occurs syntopically with at least 3 other *Crocidura* species: *C. orientalis, C. abscondita*, and *C. monticola*. Phylogenetically, the new species is nested among sympatric and allopatric populations of *C. monticola*. Although topological inferences differ slightly between the mtDNA gene tree and multilocus species tree, the new species renders *C. monticola* paraphyletic in all inferences when populations of the latter from across Java are analyzed. Despite the close relationship, the new species is absolutely larger (no overlap in measurement ranges) than the sympatric population of *C. monticola* for all 11 cranial characters measured and for tail length. The new species averages larger for all external and cranial measurements than *C. monticola* sampled from 4 mountains spread across Java, including the sympatric samples. The smaller external and cranial measurements of the Mt. Gede population of *C. monticola* compared to populations allopatric from the new species indicate possible character displacement. Population genetic analyses suggest this morphological divergence evolved despite asymmetric gene flow from the new species into the Mt. Gede population of *C. monticola* are now known from Java, 5 of which are endemic to the island.

Key words: cryptic species, gene flow, microendemism, molecular phylogeny, paraphyletic species, Soricidae, Sundaland, sympatry, taxonomy

Delimiting species and building a taxonomy that reflects evolutionary history represents a major challenge for biodiversity science (Bickford et al. 2007). The diversity of tropical hotspots, in particular, remains greatly underestimated, even for relatively well-characterized groups such as terrestrial vertebrates, including mammals (Costello et al. 2012, 2013; Engemann et al. 2015). Nevertheless, the now common use of genetic data to delimit species and infer relationships has resulted in dramatic improvements in our estimates of species- and populationlevel diversity in many groups. These efforts are enriching our understanding of diversification processes by revealing cases of speciation with gene flow (Davison et al. 2005; Via 2012), ecological speciation (Schluter 2009; Schafer and Wolf 2013), and paraphyletic species (Thulin et al. 2012; Ross 2014), all of which were considered theoretically plausible well before they could be documented with inferences from genetic data.

Shrews (Soricidae) have been particularly problematic for systematists because of their high diversity (~400 species), especially in tropical Africa and Southeast Asia, and their generally conservative morphology (Hutterer 2005;

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Kerbis Peterhans et al. 2009; Taylor et al. 2013; Demos et al. 2014, 2015). Early taxonomic assessments of *Crocidura* on the Southeast Asian continental shelf islands known as Sundaland (Sumatra, Borneo, Java, and many smaller islands) were hindered by a lack of adequate sampling. For example, of the 4 Javan *Crocidura* species recognized by Ruedi's (1995) revision, 3 were described from single specimens. A series of morphological revisions and faunal catalogs (Jenkins 1982; Corbet and Hill 1992; Ruedi 1995) examined available specimens, but they disagreed in the number of species of Javan *Crocidura* (1–4 species, 0–2 endemics). Recent efforts to inventory small mammals of Sundaland have made substantial improvements in the numbers of shrew specimens available, especially from Java (Ruedi 1995; Esselstyn et al. 2009, 2013, 2014; Omar et al. 2013; Demos et al. 2016).

Multilocus genetic analyses of these new specimens (Esselstyn et al. 2013; Demos et al. 2016) formed the basis for establishing the endemism of all but perhaps 1 species of Javan Crocidura, including a new species from western Java (Esselstyn et al. 2014). Currently, the shrew fauna of Java is known to include a pair of distantly related, small-bodied Crocidura (C. monticola Peters, 1870 and C. maxi Sody, 1936), 2 larger sister species (C. brunnea Jentink, 1888; and C. orientalis Jentink, 1890), and a medium-sized, long-tailed species (C. abscondita Esselstyn et al., 2014). Subsequent to the description of C. abscondita (Esselstyn et al. 2014), additional collections in Central and East Java revealed that specimens of an undescribed small shrew species from Mt. Gede in West Java were incorrectly assigned by Esselstyn et al. (2013, 2014) to C. maxi (Demos et al. 2016). With the re-identification of these specimens, C. maxi is again thought to be restricted to East Java and the Lesser Sunda Islands, with all other Javan Crocidura considered endemic to Java (Kitchener et al. 1994; Demos et al. 2016). As part of their broad phylogeographic analyses, Demos et al. (2016) inferred low levels of unidirectional gene flow from the new species described herein to the syntopic Mt. Gede population of C. monticola.

In this study, we distinguish the new species previously identified as *C. maxi* from other Javan and Sumatran *Crocidura*

species on the basis of multilocus sequence data, morphometric analyses, and qualitative morphological characters.

MATERIALS AND METHODS

Specimens of Crocidura were collected during small mammal surveys of the montane forests and secondary habitats of Java that included 5 montane regions across the length of the island (Fig. 1). These specimens are deposited at Field Museum of Natural History, Chicago (FMNH), Louisiana State University Museum of Natural Science, Baton Rouge (LSUMZ), and Museum Zoologicum Bogoriense, Bogor (MZB; Appendix 1). We included additional comparative material from Ambrose Monell Cryo Collection, American Museum of Natural History (AMCC); American Museum of Natural History (AMNH); University of Kansas Natural History Museum (KU); National University of Taiwan (NTU); National Museum of Natural History, Smithsonian Institution, Washington, DC (USNM); and Western Australia Museum (WAM; Appendix 1). Shrew specimens were captured using a combination of Victor snap traps and pitfall traps with and without drift fences. Specimens were measured and weighed, and either prepared as skins and skeletons or fixed in formalin and later preserved in 70% ethanol before being deposited at the aforementioned museums. Field methods followed guidelines approved by the American Society of Mammalogists (Sikes et al. 2016).

The new species described here is most similar in size and morphology to *C. maxi*, but is phylogenetically closest to *C. monticola*. On the basis of external characters and measurements, specimens of the new species were identified in previous publications as *C. maxi* (Esselstyn et al. 2013, 2014). Therefore, most comparisons of the new species are with *C. maxi* and *C. monticola*. Additional comparisons for select measurements and characters are also made to the other 3 Javan *Crocidura* (*C. abscondita*, *C. brunnea*, and *C. orientalis*).

External measurements (in millimeters) were transcribed from field notes and include total length (Total), tail length (Tail), head and body length (HB), hindfoot length with claw (HF), ear length (Ear), and body mass (Mass, in grams).



Fig. 1.—Map of collecting localities for specimens of *Crocidura* from Java examined in this study. Elevation indicated by gray shading as defined on map. Inset shows Java's position (black) in Southeast Asia.

Craniodental measurements used are identical to those from Esselstyn et al. (2014): condylo–incisive length (CIL), breadth of braincase (BBC), interorbital width (IOW), rostral length (RL), rostral width (RW), postpalatal depth (PPD), postpalatal length (PPL), distance from occipital condyle to glenoid fossa (CGF), upper toothrow length (UTL), distance from alveolar of P⁴ to M³ (P⁴–M³), and labial width at M² (M²–M²). Principal component (PC) analysis of log-transformed craniodental variables based on a variance–covariance matrix was used to assess morphometric variation and visualize the degree of morphometric distinction among putative species. We also calculated standard summary statistics from univariate measurements. All statistical analyses were performed using R (R Core Team 2014) and PAST (Hammer et al. 2001).

Esselstyn et al. (2013, 2014) identified 2 small species of shrews co-occurring on Mt. Gede, West Java. They referred these specimens to C. maxi and C. monticola. Their analyses used DNA sequences from 9 unlinked loci to infer a sister relationship between the 2 species, but after additional specimen collections and DNA sequencing, it is now clear those previous studies lacked specimens of true C. maxi (Demos et al. 2016). Demos et al. (2016) added homologous sequences for true C. maxi from Mt. Ijen, East Java (near the type locality of East Besoeki [Besuki], East Java), additional populations of C. monticola from Mts. Ijen, Salak (West Java), and Slamet (Central Java), and for several Sumatran species of Crocidura. These more recent results indicate that the specimens previously referred to C. maxi by Esselstyn et al. (2013, 2014) represent an undescribed species. We are confident in our identification of C. maxi from Mt. Ijen on the basis of their 1) close phylogenetic relationship to C. maxi specimens identified by Kitchener et al. (1994) from the Indonesian islands of Alor, Aru, and Bali; 2) morphological similarity as described by Kitchener et al. (1994); and 3) the close proximity of Mt. Ijen to the type locality for C. maxi.

Here, we reanalyze a subset of the multilocus sequence data set from Demos et al. (2016). The data include the mitochondrial gene cytochrome-b (*Cytb*) and 8 unlinked nuclear loci: apolipoprotein B (*ApoB*), brain-derived neurotrophic factor (*BDNF*), breast cancer susceptibility 1 (*BRCA1*), growth hormone receptor exon 10 (*GHR10*), mast cell growth factor (*MCGF*), prostaglandin E4 receptor (*PTGER4*), recombination activating protein 1(*RAG1*), and von Willebrand factor exon 28 (*vWF*).

We calculated interspecific uncorrected sequence divergences (*p*-distances) for *Cytb* in DnaSP v.5 (Librado and Rozas 2009). We inferred a species tree for the new species, *C. monticola*, and *C. maxi* from Java and the Lesser Sunda Islands combined with several close relatives from Sumatra (*C. neglecta* Jentink, 1888; *C.* sp. 1 [sensu Demos et al. 2016]), Borneo (*C.* cf. *neglecta*), Taiwan (*C. kurodai* Jameson and Jones, 1977), and mainland Southeast Asia (*C. fuliginosa* Blyth, 1856). For those Javan and Sumatran species for which samples from more than 1 disjunct population exist, we assigned samples from separate localities as terminal taxa, resulting in 12 tips in these analyses. We estimated the most appropriate model of sequence evolution for

each unpartitioned nuclear gene using the Bayesian information criterion and a model-specific maximum likelihood topology in jModelTest v.2.1.7 (Darriba et al. 2012). We estimated a species tree in *BEAST 2.1.1 (Drummond et al. 2012) using the 8 nuclear (nDNA) alignments. In order to facilitate convergence in the species tree algorithm, we limited the number of phased sequences to no more than 3 individuals (6 alleles) per terminal tip. The substitution, clock, and tree models were unlinked across all loci. The uncorrelated lognormal relaxed clock was applied to each locus with a Yule tree prior and the constant-root population size model. Four replicate analyses were conducted with runs of 2×10^8 generations, with parameters sampled every 10³ steps. Convergence was assessed in Tracer v.1.6 (Rambaut and Drummond 2007) with effective sample size values > 200 used as an estimate for parameter convergence. The first 10% of trees were removed as burn in and the maximum clade credibility tree was assembled using LogCombiner v.2.1.1 and TreeAnnotator v.2.0.3 (Drummond et al. 2012).

RESULTS

On the basis of molecular phylogenetic (Demos et al. 2016) and morphological evidence described below, we recognize a series of specimens of *Crocidura* from Mt. Gede, Java, previously assigned to *C. maxi* (Esselstyn et al. 2013, 2014), as a new and distinct species.

Crocidura umbra, new species

Crocidura maxi: Esselstyn et al., 2013:4977. *Crocidura maxi*: Esselstyn et al., 2014:220.

Holotype.—An adult male specimen (FMNH 212783) preserved in fluid with the skull removed and cleaned (Fig. 2a), collected on 18 November 2010 on Mt. Gede, Java. External measurements of the holotype are head and body length of 70 mm, tail length of 48 mm, hindfoot length with claw of 13 mm, ear conch height of 9 mm, and mass of 5.2 g. The specimen was fixed originally in 10% formalin and subsequently transferred to 70% ethanol. An aliquot of liver tissue was taken from the specimen in the field and preserved in home-brewed RNA*later* prior to final cryogenic storage at FMNH. The body and skull are in excellent condition with the exception of a missing left M³. The adult dentition is fully erupted and the basisphenoid–basioccipital suture is closed (Fig. 2a). The holotype will be transferred to MZB for permanent curation.

Type locality.—Indonesia, West Java, Mt. Gede, Cibodas, 6.74818°S, 106.99388°E, 1,611 m elevation.

Paratypes.—Three males (FMNH 212779, 212788, and 213409), 2 females (FMNH 212793 and 213410), and 1 sex unknown (FMNH 213408), all collected in 2010 from the type locality, are designated as paratypes. All 6 paratypes are adults preserved as fluid specimens with skulls removed and cleaned.

Distribution.—Crocidura umbra is known only from 1 small area on Mt. Gede-Pangrango, West Java, with samples collected at 1,611 and 1,950 m elevation. Recent surveys on 4



Fig. 2.—Scanning electron micrographs of the cranium and mandibles of a) *Crocidura umbra* new species (FMNH 212783 [holotype]) and b) *C. monticola* (FMNH 212775). From top to bottom are dorsal, ventral, and lateral views of the cranium and lateral and occlusal views of the mandible.

other mountains in West, Central, and East Java did not find the new species (Esselstyn et al. 2013; Demos et al. 2016).

Etymology.—Latin noun for both "ghost," a reference to the discovery happening on Mt. Gede, the best-surveyed area in Indonesia, and "shadow," a reference to MZB being situated in the shadow of Mt. Gede. We recommend "Javan ghost shrew" as an English common name.

Nomenclatural statement.—A life number was obtained for the new species *Crocidura umbra*: urn:lsid:zoobank. org:pub:75EDA49A-EC74-4453-BD07-E1193AEBE055.

Diagnosis.—Among members of *Crocidura* from Southeast Asia, *C. umbra* is a small (5.0–7.2 g; Table 1), brown shrew with a relatively thick, medium-length tail that is approximately three-fourths of head and body length. The pelage

is gray at the base with dark brown tips on the dorsum and slightly lighter, medium brown tips on the venter. The tail is moderately bristled along the proximal half of its length. The skull is small (17.2–18.3 mm CIL; Table 1) and the maxilla broad relative to the breadth of the braincase. The anterior terminus of the premaxilla is notably vertical in lateral profile above the 1st upper incisors. In comparison to the other 5 species of *Crocidura* known from Java Island, the new species is smaller in all external and cranial measurements than *C. abscondita, C. brunnea*, and *C. orientalis*; larger in breadth of braincase, rostral width, and postpalatal length (nonoverlapping size ranges) than *C. monticola*; and distinctly darker in ventral pelage, but similar in external and skull proportions to allopatric *C. maxi*.

Table 1.—External and craniodental descriptive statistics (mean ± 1 SD, range) for Crocidura umbra n. sp., C. monticola, and C. maxi. The
"sympatric" and "allopatric" columns refer to populations of C. monticola in sympatry (Mt. Gede) or allopatry (all other sites) with C. umbra. The
values in bold are for variables with nonoverlapping ranges with C. umbra. Exceptions to sample sizes (n) are provided as footnotes. All measure-
ments are in millimeters except mass (g). BBC = breadth of braincase; CIL = condylo-incisive length; HB = head and body length; HF = hindfoot
length with claw; P ⁴ –M ³ = distance from alveolar of P ⁴ to M ³ ; CGF = distance from occipital condyle to glenoid fossa; IOW = interorbital width;
M^2-M^2 = labial width at M^2 ; Mass = body mass; PPD = postpalatal depth; PPL = postpalatal length; RL = rostral length; RW = rostral width;
Tail = tail length; Total = total length; UTL = upper toothrow length.

Variable	$C. \ umbra \ (n = 15)$	C. monticola $(n = 31)$	<i>C. monticola</i> sympatric (<i>n</i> =13)	<i>C. monticola</i> allopatric $(n = 18)$	<i>C. maxi</i> (<i>n</i> =7)
CIL	17.81 ± 0.28	16.47 ± 0.50	16.05 ± 0.25	16.78 ± 0.39	18.38 ± 0.61
	17.17-18.29	15.53-17.55	15.53-16.32	16.18-17.55	17.59-19.45
BBC	8.27 ± 0.19	7.44 ± 0.21	7.33 ± 0.16	7.52 ± 0.20	8.10 ± 0.24
	8.06-8.67	7.08-8.04	7.08-7.65	7.30-8.04	7.75-8.39
IOW	4.05 ± 0.09	3.56 ± 0.14	3.49 ± 0.08	3.61 ± 0.16	3.84 ± 0.14
	3.88-4.21	3.30-3.90	3.30-3.63	3.40-3.90	3.63-4.05
RL	6.97 ± 0.13	6.40 ± 0.28	6.17 ± 0.18	6.57 ± 0.22	6.96 ± 0.19
	6.81-7.24	5.75-7.01	5.75-6.41	6.26-7.01	6.67-7.21
RW	2.49 ± 0.12	2.03 ± 0.08	2.07 ± 0.07	2.00 ± 0.07	2.42 ± 0.09
	2.26-2.81	1.92-2.19	1.95-2.19	1.92-2.13	2.24-2.53
PPD	3.35 ± 0.12	3.02 ± 0.13	3.01 ± 0.10	3.03 ± 0.16	3.29 ± 0.17
	3.14-3.59	2.71-3.30	2.74-3.11	2.71-3.30	3.11-3.53
PPL	8.17 ± 0.16	7.48 ± 0.19	7.36 ± 0.11	7.56 ± 0.20	8.50 ± 0.22
	7.95-8.61	7.12-7.87	7.15-7.48	7.12-7.87	8.12-8.72
CGF	7.46 ± 0.18	6.78 ± 0.15	6.69 ± 0.09	6.84 ± 0.16	7.54 ± 0.23
	7.10-7.72	6.58-7.16	6.58-6.83	6.61-7.16	7.24-7.82
UTL	7.63 ± 0.12	7.07 ± 0.26	6.88 ± 0.19	7.21 ± 0.22	7.71 ± 0.16
	7.45-7.82	6.56-7.63	6.56-7.17	6.88-7.63	7.48-7.91
P^4-M^3	4.41 ± 0.14	4.01 ± 0.15	3.89 ± 0.11	4.09 ± 0.12	4.18 ± 0.10
	4.22-4.71	3.71-4.41	3.71-4.03	3.94-4.41	4.06-4.32
M^2-M^2	5.09 ± 0.11	4.68 ± 0.16	4.54 ± 0.09	4.78 ± 0.12	5.26 ± 13
	4.92-5.24	4.42-4.98	4.42-4.68	4.64-4.98	5.05-5.45
Total	$120.5^{a} \pm 3.98$	103.7 ± 7.20	97.9 ± 4.42	107.9 ± 5.74	115.3 ± 5.24
	115-127	90-117	90-106	100-117	110-123
HB	$67.9^{a} \pm 2.02$	60.8 ± 4.49	59.9 ± 4.54	61.4 ± 4.48	68.3 ± 6.68
	63-71	53-70	54-70	53-69	57-75
Tail	$52.6^{a} \pm 3.72$	42.9 ± 5.80	38.0 ± 2.12	46.5 ± 4.9	47.0 ± 3.46
	47-58	35-59	35-41	41–59	44-53
HF	$12.1^{a} \pm 0.66$	11.2 ± 0.70	10.9 ± 0.76	11.4 ± 0.61	12.3 ± 0.52
	11-13	10-12	10-12	10-12	12-13
Mass	$6.11^{a} \pm 0.65$	$4.2^{b} \pm 0.68$	3.9 ± 0.52	$4.5^{\circ} \pm 0.69$	5.8 ± 0.88
	5.0-7.2	3.4-6.3	3.4–5.1	3.4–6.3	4.3-6.9

^a n = 14.

 $^{\circ} n = 15.$

Description.-Dorsal pelage is dark brown and comprised of bicolored hairs with medium to dark gray bases and chocolate brown tips; ventral pelage slightly paler than dorsum. Pelage length is 4-5 mm at mid-dorsum. Hairs on the pinnae are pale tan at the base of the ear and brown at the margin. Hairs on the dorsal surface of the hindfeet are dark brown from the base to the tips. The dorsal surface of the hindfeet have scattered white hairs distally. The lips are unpigmented and the rhinarium is gray-brown. The mystacial vibrissae are mostly 10-12 mm in length, but the longest extend to 16 mm. Vibrissae on the chin are sparse and short. The palmar integument is pale brown and the plantar integument is darker brown. Dark brown scales are apparent on the distal and lateral margins of the hindfeet; digits I-IV are medium brown and digit V is darker brown on the plantar surface. On both the fore and hindfeet, the thenar and hypothenar pads are dark brown, the 1st and 4th interdigital pads are medium brown, and the 2nd and 3rd interdigital

Downloaded from https://academic.oup.com/jmammal/article-abstract/98/1/183/2658451 by Louisiana State University user on 06 August 2018 pads are light brown. Claws on the fore and hindfeet are well developed. The tail is medium length (mean Tail/HB \pm *SD*, 78 \pm 6%), with moderately dense, 3–4 mm long bristles along the proximal half. Applied hairs on the tail are dark brown dorsally, but lighter brown ventrally on the proximal one-third and transitioning to medium brown distally.

The skull is small for a *Crocidura* species, with a broad, short rostrum and broad, vertically compressed braincase. In lateral profile, the top of the skull is relatively flat from the height of the braincase and the anterior portion of the rostrum. The anterior portion of the premaxilla is nearly vertical in profile down to the insertion of I¹. The zygomatic plate is narrow with the anterior border aligned with the middle of M¹ and the posterior border aligned with the middle of M². The interorbital constriction is relatively broad (mean approximately 22% of the condyle–incisive length). The foramen magnum is broad and oval-shaped. I¹ is falciform with a rounded tip and emerges

 $^{^{\}rm b} n = 28.$

from the premaxilla at a right angle from the plane of the palate. I^1 is large relative to I^2 and I^3 , which are half the length of I^1 . M^1 and M^2 are square-shaped in occlusal view and M^3 is relatively small in comparison.

Comparisons.—*Crocidura umbra* is readily distinguished from larger *Crocidura* species known from West and Central Java (*C. brunnea*, *C. orientalis*, and *C. abscondita*) on the basis of overall body size, tail length, hindfoot length, and mass (Esselstyn et al. 2014). The skull of *C. umbra* averages smaller than *C. brunnea*, *C. orientalis*, and *C. abscondita* in all measurements (Esselstyn et al. 2014). Although *C. umbra* is only moderately smaller than the syntopic *C. abscondita* in body size (mean HB \pm *SD*: *C. umbra*, 67.9 \pm 2.0; *C. abscondita*, 73.5 \pm 3.2), it is readily distinguished by relative and absolute tail lengths (mean Tail/HB \pm *SD*: *C. abscondita*, 120 \pm 6%; *C. umbra*, 78 \pm 6%).

Although C. umbra, presently only known from Mt. Gede in West Java, and the widespread Javan C. monticola overlap to some extent in their ranges of external measures, the new species is larger on average in all external dimensions than C. monticola. On Mt. Gede, where C. umbra and C. monticola are syntopic, the population of C. monticola is smaller, and there is no overlap between the 2 species in any craniodental measurements and in total length and tail length (Table 1). C. umbra is similar to C. maxi in external dimensions and mass and would not be distinguishable on the basis of external dimensions. However, the overall pelage of C. umbra has a brown hue compared to the gray color of C. maxi (as observed from Mt. Ijen specimens). The ventral pelage of C. maxi is especially paler than in C. umbra and at mid-venter, hairs are almost white at the tips; the new species has a darker, brown-tipped ventral pelage. In addition, our current understanding of their geographic ranges is that they are entirely allopatric, with C. maxi restricted to East Java. C. maxi is also phylogenetically distant from C. umbra in the species tree (see "Results") and past inferences (Esselstyn et al. 2013; Demos et al. 2016). Given the significant and readily diagnosable differences in body size between C. umbra and the larger Javan species of C. abscondita, C. brunnea, and C. orientalis, along with the distant phylogenetic relationship with C. maxi, we restrict the remaining morphological comparisons to those of the new species with the other small shrew present in West Java, C. monticola.

The pelage of *C. umbra* is chocolate brown on the dorsum compared to the more gray-brown tint of the pelage of *C. monticola*. The pelage of *C. umbra* is less strongly bicolored than that of *C. monticola*. The ears are somewhat paler gray-brown in *C. umbra* compared to *C. monticola*, the lip pigmentation slightly lighter, and the ventral integument of the fore and hindfeet are lighter. The tail of *C. umbra* is bristled along the proximal half of its length and these bristles are noticeably denser than in *C. monticola*. However, this character is known to vary geographically in some species (Ruedi 1995). The tail integument varies along the length in *C. umbra* where there is a lighter brown integument on the ventral surface of the proximal portion of the tail compared to the uniformly dark brown integument in *C. monticola*. The tail is also noticeably thicker and longer on

average in *C. umbra* than in the *C. monticola* (52.6 ± 3.72 versus 42.9 ± 5.80 in *C. monticola*). Pelage length on the mid-dorsum of *C. umbra* is 4–5 mm compared to 2–3.5 mm in *C. monticola*. The longest facial vibrissae are proportionally longer in *C. umbra* compared to *C. monticola*. As described above, all of the mean external measurements of *C. monticola* are smaller in the Mt. Gede population relative to other mountain populations of *C. monticola* and to *C. umbra*. The ranges of measurements are discrete for total length (*C. umbra* = 115–127, *C. monticola* = 90–106) and tail length (range for *C. umbra* = 47–58, for *C. monticola* = 35–41) on Mt. Gede.

The skull is larger overall in C. umbra compared to C. monticola, the zygomatic plate somewhat broader, and the braincase less rounded. When viewed in lateral profile, no transition from the interorbital region to the braincase is apparent in C. umbra, where a continuous plane connects the peak of the braincase with the terminus of the rostrum. In contrast, this plane in C. monticola is interrupted by an upward deflection in the proximal portion of the braincase (Fig. 2b). The rostrum of C. umbra is notably broader than in C. monticola (Fig. 2; *C. umbra*, $RW = 2.49 \pm 0.12$; *C. monticola*, $RW = 2.03 \pm 0.08$) and the range of values are entirely nonoverlapping between the taxa (Table 1). C. umbra is distinctly larger in cranial variables for breadth of braincase and postpalatal length compared to C. monticola, and these variables also have nonoverlapping ranges (Table 1). Viewed in lateral profile, the nasal branch of the premaxilla terminates frontally in a nearly vertical plane in C. umbra; a more gently sloping plane is seen in C. monticola (Fig. 2).

The dentition of *C. umbra* is larger in all dimensions than in *C. monticola*, although similar in proportions. I¹ of *C. umbra* is more procumbent, larger, more robust, and emerges from the premaxilla in amore horizontal plane relative to those of *C. monticola*, which emerges at a downward angle when viewed in lateral profile (Fig. 2).

A PC analysis was performed on 11 log-transformed craniodental variables for specimens of C. umbra, C. monticola, and C. maxi (Table 2; Fig. 2). This analysis readily distinguishes C. umbra from all populations of C. monticola along the 1st axis (reflects size; Table 2), and to a lesser extent, the 2nd axis (Fig. 3). In addition, the Mt. Gede population of C. monticola is separated from C. monticola individuals on 3 other mountains located in West, Central, and East Java along the 2nd PC. C. monticola is syntopic with C. umbra only on Mt. Gede and these 2 taxa are separated completely on PC1 and partially on PC2. The ranges of 8 of 11 craniodental measurements and 4 external measurements overlap between C. umbra and the combined populations of C. monticola from Mts. Ijen, Salak, Slamet, and Gede. However, when measurements from C. umbra are compared to only the Mt. Gede population of C. monticola, they show complete separation in ranges for all 11 craniodental variables, and total length and tail length in external dimensions (Table 1). These skull and body size differences of Mt. Gede C. monticola from other populations of C. monticola are reflected in the discrete multivariate space that these individuals occupy (Fig. 3). Neither of the first 2 PCs distinguishes *C. umbra* from *C. maxi* (Fig. 3). However, these 2 species differ in pelage color (see above) and are unambiguously distinguishable on the basis of their genetic distances and

Table 2.—Variable loadings from principal components analysis performed on 11 craniodental measurements of *Crocidura umbra*, *C. monticola*, and *C. maxi* from Java. PC = principal component. BBC = breadth of braincase; CIL = condylo–incisive length; P⁴– M^3 = distance from alveolar of P⁴ to M³; CGF = distance from occipital condyle to glenoid fossa; IOW = interorbital width; M²–M² = labial width at M²; PPD = postpalatal depth; PPL = postpalatal length; RL = rostral length; RW = rostral width; UTL = upper toothrow length.

Variable	Corre	lations
	PC1	PC2
CIL	0.256	0.269
BBC	0.272	-0.076
IOW	0.327	-0.050
RL	0.257	0.387
RW	0.508	-0.734
PPD	0.293	0.027
PPL	0.274	0.114
CGF	0.272	0.077
UTL	0.240	0.324
$P^{4}-M^{3}$	0.245	0.244
$M^2 - M^2$	0.276	0.217
Cumulative percent variance	82.08	88.97
Eigenvalue	0.0068	0.0006

phylogenetic relationships (see below). Moreover, available evidence suggests they are allopatric.

Molecular genetic analyses.—Uncorrected mitochondrial *Cytb* genetic distances between *C. umbra* and *C. monticola* ranged from 3.7% to 3.8% (Table 3). Genetic distances between other species and population pairs ranged from 10.1% to 15%. The genetic distances of *C. umbra* to populations of *C. monticola* indicate a relatively recent history of evolutionary independence.

Our species tree analysis infers *C. umbra* as sister to *C. monticola* from Mt. Ijen in East Java, although with weak support, thus rendering *C. monticola* paraphyletic (Fig. 4). Support for the node uniting all populations of *C. monticola* with *C. umbra* is modest at 0.90 (Fig. 4). The clade comprising *C. umbra* + *C. monticola* is inferred as sister to a putative undescribed species from Sumatra (*C.* sp. 1). The clade formed by these 3 species is well supported as sister to a clade that includes 3 *Crocidura* species from Southeast Asia and Sumatra, to the exclusion of *C. maxi*.

DISCUSSION

Evidence from sequences of independent loci, morphometric data, and discrete morphological characters strongly support the recognition of *Crocidura umbra* as the 5th endemic species



Fig. 3.—Bivariate plot of the first 2 principal components from an analysis of 11 log-transformed craniodental measurements from specimens of *Crocidura umbra*, *C. monticola*, and *C. maxi*.

of *Crocidura* on Java and the 6th distributed on the island. Although *C. umbra* is only relatively recently diverged from populations of *C. monticola*, they occur in sympatry on Mt. Gede, the only locality from which the new species is known. In this area, the 2 species are distinguished by several non-overlapping morphological characters, particularly skull size, total length, and tail length. *C. umbra* has been collected at 1,950 and 1,611 m elevation on Mt. Gede and *C. monticola* has been collected from 1,611 and 1,377 m. Large sample sizes of *Crocidura* obtained at the aforementioned 3 elevational sites on Mt. Gede indicate syntopy between the 2 species at mid-elevation (1,611 m), whereas at higher elevation, only *C. umbra* was found and at lower elevation, only *C. monticola* was found. This distribution pattern, along with the relatively

Table 3.—Uncorrected between group genetic distances (*p*-distances) calculated for mitochondrial cytochrome-*b* (*Cytb*) sequence data for 4 species of *Crocidura*.

Species comparison	Cytb p-distance	
<i>C. umbra</i> versus <i>C. monticola</i> (all localities)	0.038	
C. umbra versus C. monticola (Mt. Gede)	0.037	
C. umbra versus C. sp. 1 (Sumatra)	0.101	
C. umbra versus C. maxi	0.146	
C. monticola (all localities) versus C. maxi	0.150	

rapid evolution of morphological disparity between the 2 species, suggests possible ecological (e.g., Nosil 2012) speciation along an elevational gradient, with divergent selection between environments producing niche separation.

Using time-calibrated nuclear DNA mutation rates for the same 8 nuclear loci used in this study, Esselstyn et al. (2013) derived median estimated divergence times between C. umbra (referred to C. maxi) and C. monticola of 177,557 generations ago (95% highest posterior density [HPD]: 60,693-391,468) using a crocidurine origin fossil calibration (13 Ma) and 1-year generation time, and 515,467 years ago (95% HPD: 175,821-1,138,360) using external calibrations for the origin of crocidurines (34 Ma). However, these estimates of divergence date and the branch lengths in the species tree may be influenced by post-divergence gene flow. Demos et al. (2016) used IMa2 analysis (Hey and Nielson 2007; Hey 2010) to estimate a low level of gene flow from C. umbra into the C. monticola population on Mt. Gede. In any event, the consistently distinct cranial and external morphological differences between C. umbra and the Mt. Gede C. monticola and those between the Mt. Gede C. monticola and other C. monticola are surprising for occurring in the face of gene flow (between C. umbra and Gede C. monticola) and in a group known for its conservative rate of morphological evolution.



substitutions / site

Fig. 4.—Species tree inferred in *BEAST using multilocus sequence data for 7 species of *Crocidura* from Southeast Asia. Numbers above branches represent Bayesian posterior probability values.

The morphological differences between populations of C. monticola suggest the possibility that the taxon contains a complex of 2 or more allopatric species. Both an mtDNA gene tree (Demos et al. 2016) and the species tree presented here render C. monticola paraphyletic. Dividing these moderately divergent allopatric populations into species would contribute to the trend of recognizing fine-scale patterns of endemism, with small terrestrial mammal species often being endemic to single mountains or mountain ranges on the large islands of Southeast Asia (e.g., Heaney et al. 2011; Esselstyn et al. 2013; Demos et al. 2016). Given the complex tectonic, geomorphological, climatic, and ecological history of Sundaland, it is difficult to hypothesize what processes generated this microendemism (Gorog et al. 2004; Esselstyn et al. 2011, 2013, 2014; Sheldon et al. 2015). Nevertheless, the morphological differences between the Mt. Gede C. monticola and other populations of the species (Fig. 3) indicate the possibility that ecological interactions with C. umbra led to character displacement. However, samples of C. umbra from additional locations lacking C. monticola would be necessary to make a strong test of character displacement (e.g., Dayan and Simberloff 1998).

The only species present on Java that is not readily morphologically distinguishable from *C. umbra* is the distantly related *C. maxi*. Distinguishing this cryptic pair of species in the field on the basis of external characters would be difficult and rely on color variation were it not for their allopatric distributions. Small mammal surveys to date restrict *C. maxi* to East Java and the Lesser Sunda Islands and *C. umbra* to Mt. Gede in West Java (Esselstyn et al. 2013, 2014; Demos et al. 2016). The similar proportions, size, and form of these 2 species may be the result of retention of ancestral small body size or convergent evolution.

As noted by Esselstyn et al. (2014), Mt. Gede is less than 60 km from one of the world's most populous megacities, Jakarta, and is even closer to Indonesia's largest and oldest natural history museum in Bogor. The discovery of 2 species of shrews new to science from this single mountain that is in one of the most biologically studied regions of the country is a strong indication that diversity for supposedly well-studied small mammals has been greatly underestimated in Indonesia. Continued field surveys and collection of voucher specimens will be critical to accurately inventory the vertebrate diversity of the region. This information will help guide realistic conservation assessments and provide data for more detailed evolutionary analyses, such as testing for character displacement between *C. umbra* and *C. monticola*.

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APPENDIX I

Specimens examined and/or included in genetic analyses.— Museum acronyms are given in "Materials and Methods." Specimens examined morphologically and analyzed genetically are in standard typeface, those included in only genetic analyses are indicated with an asterisk, and those examined only morphologically are italicized.

- *Crocidura fuliginosa* (*n* = 1)—Vietnam: Ha Giang, Mt. Tay Con Lin: AMCC 101526*.
- Crocidura kurodai (n = 2)—Taiwan: Nantou: NTU 980*–981*.
- *Crocidura neglecta* (*n* = 3)—Indonesia: Sumatra, Mt. Tujuh: FMNH 212877–212879.
- *Crocidura* cf. *neglecta* (n = 2)—Malaysia: Sabah, Mt. Kinabalu: KU168063*-168064*.
- Crocidura maxi (n = 16)—Indonesia: Java, East Java, Mt. Ijen: LSUMZ 37970–37982; Alor: WAM 37975*; Aru: WAM 42567*; Bali: WAM 38577*.

Crocidura monticola (*n* = 92).—Indonesia: Central Java, Mt. Slamet: LSUMZ 37984–37986, 37988–37989, 37990–37993, MZB 32148–32149, 32184; East Java, Mt. Ijen: LSUMZ 37983; West Java, Mt. Ciremai: AMNH *109356*, *109359*; Mt. Gede, Caringin, 6.82939°S, 106.92667°E, 1090 m: MZB 33646–33648; Mt. Gede, Caringin, 6.81369°S, 106.91033°E, 1140 m: MZB 33645, 33712, 33714; Mt. Gede, Cibodas, 6.74246°S, 107.00539°E, 1377 m: FMNH 212747–212757, *218663–218670*; Mt. Gede, Cibodas, 6.74818°S, 106.99388°E, 1611 m: FMNH 212747–212762, 212764–212776, 213405–213407; *218671–218686*; Mt. Gede, Kadudampit, 6.82939°S, 106.92383°E, 1055 m: MZB 33644; Mt. Salak: MZB 31720–31722.

- *Crocidura* sp. 1 (n = 15).—Indonesia: Sumatra, Mt. Singgalang: FMNH 212952, 212964–212977.
- *Crocidura umbra* (*n* = 36).—Indonesia: Java, West Java, Mt. Gede, 6.78°S, 106.98°E: USNM *481306*; Mt. Gede, Cibodas, 6.74818°S, 106.99388°E, 1611 m: FMNH 212763, 212777, 212779–212781, 212782, 212783–212793, 213408–213410, 218659, 218977; Mt. Gede, Cipanas, 6.76206°S, 106.98403°E, 1950 m: FMNH 218660–218662, 218745–218746, 218748, 218750–218755, 218757.