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Integrative taxonomy and biogeography of Asian yellow house bats (Vespertilionidae: *Scotophilus*) in the Indomalayan Region

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Running title: Integrative taxonomy and biogeography of Asian *Scotophilus*

Abstract

Yellow house bats (*Scotophilus*) have been known for centuries as a widespread genus of vesper bats in the Indomalayan Region. Despite this, their taxonomic status and phylogeographical patterns remain unclear due to differing criteria employed by early taxonomists and inconsistencies between morphological and molecular assessments. To address these issues, we undertook a comparative phylogeographic analysis of Asian *Scotophilus* spp. using integrated genetic and morphological analyses of samples collected across the region. These demonstrate that yellow house bats in Asia can be classified into just two widespread species, namely the smaller *S. kuhlii* (e.g., FA ≤ 53.1 mm, GLS ≤ 20.18 mm) and the larger *S. heathii* (e.g., FA ≥ 53.4 mm, GLS ≥ 20.85 mm), which occur in sympatry in different parts of the Indomalayan Region. Although these two sympatric species share similar eco-ethological preferences, they differ considerably in their geographic distributions and intraspecific variation in mtDNA sequences and morphological traits. These disparities were likely misinterpreted as indicating potential cryptic diversity in previous studies, whereas we suggest they are related to interspecific differences in sex-biased gene flow and phenotypic plasticity to adapt to varying environments. Our study highlights the importance of using multiple datasets to resolve taxonomic uncertainties and reconstruct demographic and phylogeographic histories of sympatric species.

Keywords: integrative taxonomy, comparative phylogeography, multiple datasets, sympatric species, *Scotophilus*.

1. Introduction

Species constitute one of the fundamental units of study in many fields of biology. However, the multiplicity of species concepts and criteria used to delineate species boundaries have led to considerable taxonomic confusion and numerous controversies (Aldhebiani, 2018; de Queiroz, 2005, 2007). A typical example of such confusion concerns the systematics of the genus *Scotophilus* Leach, 1821 (family Vespertilionidae), which comprises yellow house bats that are widely distributed in the Old World tropics.

Until the third edition of Mammal Species of the World (Wilson & Reeder, 2005), most mammalogists adopted the Biological Species Concept (Mayr, 1942). As such, they usually regarded morphological and ecological variation and/or geographical isolation as indicative of reproductive isolation among sister species and considered that the systematics of mammals were relatively complete and accurate (Baker & Bradley, 2006). Following taxonomic reviews in the 20th century, Simmons (2005) recognised 12 valid species in the genus *Scotophilus*, most of which were polytypic and widely distributed in the Old World tropics. Since this time, exploration of poorly studied regions and development of analytical approaches that integrate morphological, molecular and acoustic data have altered taxonomic opinions regarding the utility of different species concepts and associated criteria for delineating species boundaries, including within *Scotophilus* spp. (Baker & Bradley, 2006; Demos, Webala, Bartonjo, & Patterson, 2018; Francis et al., 2010; Trujillo, Patton, Schlitter, & Bickham, 2009; Vallo & Van Cakenberghe, 2017). For instance, recent integrative studies have revealed that certain traditionally accepted and polytypic species of *Scotophilus* in Africa actually comprise several distinct species which were previously unrecognized or subsumed as subspecies due to their morphological similarities. As a consequence, the number of valid African *Scotophilus* species has increased from eight (Simmons, 2005) to 18 and will likely continue to grow as further cryptic forms of widespread taxa are found (Demos et al., 2018; Trujillo et al., 2009; Vallo, Reeder, Vodzak, & Benda, 2019; Vallo & Van Cakenberghe, 2017).

Prior to 1940, Asian *Scotophilus* included 17 distinct taxa (species and subspecies) (Figure 1; Table S1). Between 1940 and 2000, several studies addressed the taxonomic status of *Scotophilus* spp. in Asia on the basis of their morphology (Table 1). These led to consensus among early bat taxonomists that two sizes of yellow house bats, small and large, co-occur in many parts of the Indomalayan Region (Corbet & Hill, 1992; Hill & Thonglongya, 1972; Shamel, 1942; Siddiqi, 1960; Tate, 1942). However, these studies frequently employed differing criteria to interpret morphological variation in their specimen material and consequently applied different taxonomic approaches to the same geographical populations. A typical example of this is apparent in the differences in the systematics of the two yellow house bats present in the Sunda Islands (e.g. Java, Bali, Belitung and Borneo).

Within the Sunda Islands, Tate (1942) included all specimens of yellow house bats collected on Java and nearby islands in three subspecies of *S. temminckii* Horsfield, 1824 (=*S. kuhlii* Leach, 1821; see Hill & Thonglongya, 1972): *S. t. temminckii*, *S. t. collinus* Sody, 1936 and *S. t. solutatus* Sody, 1936. In contrast, Shamel (1942) classified the small and large yellow house bats on Java into two distinct species, *Pachyotus* (=*Scotophilus*) *temminckii* and *P. solutatus*, respectively. The taxonomic situation became more complex when Siddiqi (1960) also classified Javanese yellow house bats into two species, the smaller *S. t. temminckii* and the larger *S. heathii* Horsfield, 1831. However, specimens of the larger species and females of the smaller taxon recognized by Siddiqui (1960) were morphologically comparable to those of *P. solutatus* (*sensu* Shamel, 1940). Likewise, Corbet & Hill (1992) allocated Asian *Scotophilus* into just two polytypic species, namely (1) *S. kuhlii*, including subspecies/synonyms occurring widely in the Indomalayan Region: *castaneus* Horsfield, 1851, *collinus*, *consobrinus* Allen, 1906, *fulvus* Gray, 1843, *gairdneri* Kloss, 1917, *panayensis* Sody, 1928, *solutatus*, *swinhoei* Blyth, 1860, *temminckii*, and *wroughtoni* Thomas, 1897; and (2) *S. heathii*, including the synonyms *belangeri* Geoffroy I., 1834, *flaveolus* Horsfield, 1851, *insularis* Allen, 1906, *luteus* Blyth, 1851 and *watkinsi* Sanborn 1952 which were restricted to mainland Asia, plus *celebensis* Sody, 1928 endemic to Sulawesi. This view ignored the treatments of Shamel (1942) and Siddiqi (1960) in considering all yellow house bats on Java and nearby islands as representatives of *S. kuhlii* sensu lato (s.l.) and created extensive overlap in the morphology of bats of this taxon (i.e. FA: 45–59 mm) and those of *S. heathii* s.l. (i.e. FA ≥55 mm) (Figure 1; Table 1).

Contrary to earlier treatments (Corbet & Hill, 1992; Shamel, 1942; Siddiqi, 1960; Tate, 1942), Kitchener, Packer, & Maryanto (1997) argued that smaller *Scotophilus* with a FA of less than 54 mm on the Greater (Java and Borneo) and Lesser Sunda Islands were readily identifiable as two distinct species, namely the smaller *S. collinus* (i.e. mean FA values for males and females: 49.1 and 50.9 respectively) and the larger *S. kuhlii* (i.e. mean FA values for males and females are 51.8 and 52.5, respectively). Both of these species were further divided into two morphological forms, but only those of the larger species, *S. kuhlii* s.l. were regarded as two subspecies, namely *S. k. temminckii* inhabiting West and East Java and its relatively smaller sister *S. k. solutatus*, occurring on East Java and Bali. On East Java, two subspecies of *S. kuhlii* were recognised in adjacent mountain ranges separated by the Bondoyudo River plains, and the authors even suggested that they might be distinct species.

In light of the above, the taxonomic status of several subspecies or races of two polytypic species, *S. kuhlii* s.l. and *S. heathii* s.l. (*sensu* Corbet & Hill, 1992) is likely inaccurate. Many of these taxa were originally described as distinct species or subspecies that were individually distinguished by certain morphological variation (pelage colour or body size) and/or geographical hiatus (Figure 1; Table S1). Nonetheless, Simmons (2005) and subsequent authors (Hutson, Kingston, Francis, & Suyanto, 2008; Moratelli et al. 2019; Sinaga & Maryanto, 2008; Srinivasulu & Srinivasulu, 2019a, 2019b) recognised only four distinct species of Asian *Scotophilus*: (1) *S. kuhlii,* found widely in the Indomalayan Region; (2) *S. collinus,* occurring sporadically in the Greater and Lesser Sunda Islands; (3) *S. heathii,* found in mainland Asia, except for the southern Indochinese subregion; and (4) *S. celebensis*, endemic to Sulawesi (Figure 1). Among these, the separation of *S. celebensis* from *S. heathii* was regarded as provisional (Simmons, 2005) because it was based solely on the disjunct distributions of the two taxa (Corbet & Hill, 1992; Tate, 1942).

Recent genetic studies have improved our understanding of the taxonomy and biogeography of Asian yellow house bats (Francis et al., 2010; Hisheh, How, Suyanto, & Schmitt, 2004; Trujillo et al., 2009; Yu, Chen, Li, & Wu, 2012). In relation to morphological taxonomy, the separation of the two differently sized species, *S. kuhlii* and *S. heathii*, has been highly supported by genetic analyses (Francis et al., 2010; Trujillo et al., 2009). Within *S. kuhlii* s.l., all recent studies have found little genetic differences between geographically distant populations in the Lesser Sunda Islands (Hisheh et al., 2004), from Vietnam, Peninsular Malaysia and the Philippines (Trujillo et al., 2009), from northern and southern Indochina (Laos and Vietnam) (Francis et al., 2010) and from Hainan Island and mainland China (Yu et al., 2012). These data indicate that gene flow across the studied populations of *S. kuhlii* has not been limited by geographical distance or sea-barriers (Hisheh et al., 2004; Yu et al., 2012). While comparable morphological data are still lacking due to inadequate or disparate sampling, it is very likely that *S. kuhlii* represents a monotypic species. For this reason, research is needed to confirm the taxonomic validity of species or subspecies that were subsumed into or distinguished from *S. kuhlii* on the basis of morphology alone.

Compared to *S. kuhlii* s.l., *S. heathii* s.l. has similar eco-ethological preferences. However, the latter species may have a greater dispersal ability due to its higher wing loading and aspect ratio e.g. 15 vs 11.6 and 8.0 vs 6.96, respectively (Francis, 2008; Luo et al., 2019; Norberg & Rayner, 1987; Zhu, Chmura, & Zhang, 2012). If so, this would suggest that *S. heathii* s.l. could also maintain a strong gene flow between its geographically distant populations. This inference is supported by Trujillo et al. (2009) who analysed genetic divergences in mtDNA (*Cytb*) and nuDNA (zinc finger Y – *zfy* gene) sequences between *S. heathii* from Yunnan (China) and northern and southern Vietnam and suggested that these represent the same species. Conversely, Francis et al. (2010) found that *S. heathii* bats from northern Indochina (northern Vietnam and Laos) and southern Indochina (southern Vietnam) constituted two highly divergent clusters of *COI* gene sequences. Because comparable *COI* divergence exist between many closely related species in the Vespertilioninae, Francis et al. (2010) suggested that Indochinese *S. heathii* may represent a complex of different species. However, since mitochondrial data are maternally inherited, they should not be solely used to draw taxonomic conclusions (Dool et al., 2016; Hassanin, An, Ropiquet, Nguyen, & Couloux, 2013; Nesi, Nakouné, Cruaud, & Hassanin, 2011; Tu et al., 2017; Tu, Hassanin, Furey, Son, & Csorba, 2018). For a more integrative approach, the hypothesis of Francis et al. (2010) requires further testing with biparental genetic markers, geographically denser sampling and additional morphological data.

This paper presents a phylogeographic study of Asian *Scotophilus* based on new specimens obtained from different areas in the Indomalayan Region. To this end, we integrate genetic and morphological analyses to (1) address taxonomic uncertainties (e.g. misidentifications or potential cryptic diversity) regarding Asian yellow house bats; (2) evaluate patterns in the demographic and evolutionary histories of species recognized by this study; and (3) consider the roles of ecological factors in shaping the current distributions, population genetic structures and morphological variation of the species. Our overall aim was to improve understanding of the taxonomy and biogeography of Asian yellow house bats.

2. Materials and Methods

2.1. Taxonomic sampling

In this study, we examined 106 bats of *S. kuhlii* s.l. (n=76) and *S. heathii* s.l. (n=30) collected from different parts in the Indomalayan Region (Figure 1; Appendix 1). Of which, 97 voucher specimens are held in the Institute of Ecology and Biological Resources (IEBR, Hanoi, Vietnam), the Hungarian Natural History Museum (HNHM, Budapest, Hungary), the Centre for Biodiversity Conservation (CBC, Royal University of Phnom Penh, Cambodia), the University of Tokyo Hokkaido Forest (UTHF, Furano, Hokkaido, Japan), and the University of the Philippines Los Banos Museum of Natural History (UPLB-MNH, Laguna, Philippines) (See Appendix 1). Tissue samples were collected from the chest muscles of voucher specimens or from the patagium (biopsy punches; 3 mm diameter) of released individuals and preserved in 95% ethanol and stored at -20°C.

We generated 38 *Cytb* (complete cytochrome b; 1,140 bp) and 43 *COI* (fragment of cytochrome c oxidase subunit I; 705-1554 bp) sequences from 49 examined individuals encountered during field surveys undertaken by the authors between 2008 and 2019 (30 specimens were sequenced for both mitochondrial genes) (Appendix 1). These newly generated sequences were compared to the 20 *Cytb* and 41 *COI* sequences available in GenBank for Asian *Scotophilus* spp. collected from other localities in the Indomalayan Region by other authors (Figure 1; Table S2) to explore their phylogeographic and phylogenetic relationships. We further sequenced two nuclear genes including intron 9 of *TUFM* (elongation factor Tu, mitochondrial precursor) and intron 6 of *ZFYVE27* (zinc finger, FYVE domain containing 27) for six selected specimens of *S. kuhlii* (n=2) and *S. heathii* (n=4) to test any incongruence between mitochondrial and nuclear DNA phylogenies. Based on previous studies (Roehrs, Lack, & Van Den Bussche, 2010; Trujillo et al., 2009), *Murina cyclotis* Dobson, 1872 of the subfamily Murininae and *Eptesicus pachyomus* Dobson, 1871 of the subfamily Vespertilioninae were chosen as outgroups in phylogenetic analyses. Genetic sequences available for these outgroup species in GenBank are indicated in Table S2.

2.2. Genetic analyses

DNA extraction, amplification, sequencing

Total DNA was extracted from tissue samples using the QIAGEN DNAeasy Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Primer sets used for PCR amplification of *Cytb* were Mt-14724F/Cyb-15915R (Irwin, Kocher, & Wilson, 1991), Cyb-14726F/Cyb-15909R (Arai et al., 2016) and for *COI* were UTyrLA/C1L705 (Hassanin et al., 2012) or MammMt-5533F/MammMt-7159R (Arai et al., 2019) and for *TUFM* and *ZFYVE27* were TUFM-EX9U/TUFM-EX10L and ZFYVE27-EX6U/ZFYVE27-EX7L, respectively (Hassanin et al. 2013) (See Table S3 for more details).

Amplifications were done in a volume of 20 µl including 3 µl of Buffer 10X with $MgCl_2$, 2 µl of dNTP (6.6 mM), 0.12 µl of Taq DNA polymerase (2.5 U, Qiagen, Hilden, Germany) and 0.5–1 µl of the two primers at 10 µM. The standard PCR conditions were as follows: 4 min at 95ºC; 5 cycles of denaturation/annealing/extension with 45 s at 95ºC, 1 min at 60ºC and 1 min at 72ºC, followed by 30 cycles of 30 s at 95ºC, 45 s at 55ºC, and 1 min at 72ºC, followed by 10 min at 72ºC. PCR products were resolved by electrophoresis on a 1.5% agarose gel stained with ethidium bromide and visualized under UV light.

Both strands of PCR products were sequenced using Sanger sequencing on an ABI 3730 automatic sequencer at the Centre National de Séquençage (Genoscope) in Evry (France) and ABI 3730xl DNA Analyzer at the Infectious Disease Surveillance Center, Japan. The sequences were edited and assembled using CodonCode Aligner Version 3.7.1 (CodonCode Corporation) and Genetyx v11 software (Genetyx Corporation, Japan). Heterozygous positions (double peaks) of nuclear gene sequences were scored using the IUPAC ambiguity codes. Sequences generated for this study were deposited in the EMBL/DDBJ/GenBank database under the accession numbers MT820574-MT820611, MT820613-MT820624, MT820574-MT820611 (Appendix 1).

Phylogeographic analyses using mtDNA sequences

The number of haplotypes, haplotype diversity (h), and nucleotide diversity (π) for the two nominal species, *S. kuhlii* s.l. and *S. heathii* s.l., were calculated from the alignments of 82 *COI* (576 bp) and 58 *Cytb* (1140 bp) sequences (Alignments S1 and S2) using DNASP v5.10 (Librado & Rozas, 2009). Maximum parsimony haplotype networks were reconstructed using the TCS algorithm in PopArt (Leigh & Bryant, 2015). Following this, a hierarchical analysis of molecular variation (AMOVA) (Excoffier, Smouse & Quattro, 1992) was performed with 1,000 permutations in Genodive v.3.0 (Meirmans & Tienderen, 2004) to quantify the genetic variation within and among groups in the TCS network.

Phylogenetic reconstruction

Phylogenetic trees of Asian *Scotophilus* spp. were reconstructed from DNA alignments (Alignments S3-S7) using Bayesian Inference (BI) and Maximum-Likelihood (ML) methods. DNA sequences were aligned with Aliview v. 1.25 (Larsson, 2014). No gaps and stop codons were found in the alignments of the mitochondrial *COI* and *Cytb* protein-coding genes. In contrast, a few gaps were included in the alignments of the nuclear genes, but their positions were not ambiguous. The indels (insertion or deletion) shared by at least two taxa in the alignments of each nuclear gene were coded as additional characters ("1": insertion; "0": deletion) and analysed as a separate partition in the Bayesian analyses. The models of nucleotide evolution were selected under jModelTest V. 2.1.7 (Posada, 2008) using the Akaike Information Criterion (AIC): GTR+G for *COI* dataset, GTR+I for *Cytb* dataset, and HKY model for *TUFM* and *ZFYVE27* datasets. The BI analyses were conducted with MrBayes v. 3.2.5 (Ronquist et al., 2012) using five separate datasets: (1) *COI* (48 sequences; 657 bp), (2) *Cytb* (39 sequences; 1140 bp), (3) *TUFM* (642 bp and 10 indels; 8 sequences), *ZFYVE27* (734 bp and 7 indel; 8 sequences) and (5) nuDNA (combining two nuclear genes; 1376 bp and 17 indels, 8 sequences) (Alignments S3-S7). The posterior probabilities (PP) were calculated using four independent Markov chains run for 10^7 Metropolis-coupled MCMC generations, with trees sampled every 1000 generations and a burn-in of 25%. The ML analyses of *COI, Cytb,* and nuDNA datasets (Alignments S3, S4, and S7) were conducted with W-IQ-TREE tool available online (http://iqtree.cibiv.univie.ac.at/) (Hoang et al., 2018; Nguyen et al., 2015; Trifinopoulos et al., 2016) with 1,000 ultrafast bootstrap replicates. Pairwise genetic distances between divergent phylogenetic lineages or TCS clusters were calculated with PAUP* v. 4b10 (Swofford, 2003) using the uncorrected p-distance.

Isolation by distance (IBD)

Geographic distances (km) between geographic haplogroups of *S. kuhlii* s.l. and *S. heathii* s.l. were generated using Geographic Distance Matrix Generator v.1.2.3 (Ersts, 2020). We tested for correlations between pairwise mtDNA distances (minimum p- distance) and geographic distances with the Mantel test (Mantel, 1967) using the generalized linear model in PAST 3.07 (Hammer, Harper, & Ryan, 2001). We also tested for correlations between pairwise genetic distance ($F_{ST}/(1-F_{ST})$) and geographic distance (ln km) for three geographic haplogroups of *S. heathii* identified in the *COI* network.

Historical demography

The dynamics of effective female population size within *S. kuhlii* s.l. and *S. heathii* s.l. were estimated from the Bayesian skyline plot (BSP) method in BEAST v.2.5.2 (Bouckaert et al., 2019) using *COI* datasets (Alignment S1). As no calibration point (fossil record or biogeographic event) was available for *Scotophilus*, we used a mutation rate of 2×10^{-8} per site per million years for the *COI* gene based on previous studies (i.e. Tu et al. 2017; Mao et al. 2010). The model of evolution of *COI* dataset was selected by jModelTest. We ran a MCMC chain of 20 million generations, sampled every 1000 generations and a burn-in of 10% with uncorrelated lognormal relaxed molecular clock. Tracer v.1.6 (available in the BEAST package) was used to assess the adequacy of chain mixing and MCMC chain convergence using ESS values of >200 and to visualize the Bayesian skyline plots.

2.3. Morphological analyses

Morphological comparison

Ninety-seven *Scotophilus* specimens listed in the Appendix 1 were included in our morphological analyses. All of the specimens examined were adults, as confirmed by the presence of fully ossified metacarpal-phalangeal joints.

External measurements were taken to the nearest 0.1 mm from alcohol-preserved specimens. These included: FA—length of forearm, Tib—tibia length, from the knee joint to the ankle. Craniodental measurements were taken to the nearest 0.01 mm using digital calipers under a stereomicroscope. These included: GSL—total length of skull, from the most anterior part of the upper incisors to the occiput; SL—greatest length of skull, the anterior rim of the alveolus of the 1st upper incisor to the most posteriorly projecting point of the occipital region; CBL—condylobasal length, from the occipital condyles to the anterior of the alveolus of the 1st upper incisor; CCL—condylo-canine length, from the exoccipital condyle to the most anterior part of the canine; ZB—greatest width of the skull across the zygomatic arches;

C^1C^1—greatest width across the upper canines between their buccal borders; M^3M^3—greatest width across the crowns of the last upper molars; CM^3—maxillary toothrow length, from the anterior of the upper canine to the posterior of the crown of the 3rd upper molar; ML—length of mandible, from the anterior rim of the alveolus of the first lower incisor to the most posterior part of the condyle; and CM_3—mandibular toothrow length, from the anterior of the lower canine to the posterior of the crown of the 3rd lower molar.

In this study, we initially assigned our study specimens to different groups based on molecular data. Specimens lacking genetic information were classified into molecular groups according to their geographic origin (Figure 1). As specimens examined within these groups lacked sexual dimorphism in all external and craniodental measurements (T-test, $p > 0.05$), the phenetic affinity of the identified taxa with both sexes combined was inferred through univariate and multivariate analyses of their morphometrics. Principal Component Analysis (PCA) of study specimens was undertaken in PAST 3.07 (Hammer et al, 2001) using 10 log-transformed craniodental measurements. Equalities of mean values of morphometrics and PC scores among different taxa were tested using a one-way analysis of variance (ANOVA), followed by a post-hoc Tukey HSD multiple comparison test for unequal sample sizes (Tukey-Kramer) (Zar, 1999).

3. RESULTS

3.1. Genetic analyses

3.1.1. Phylogeographic patterns of Asian Scotophilus *inferred from mtDNA sequences*

In the *COI* alignment (576 bp), 22 haplotypes were identified among 43 individuals of *S. kuhlii* s.l., and 18 haplotypes among 39 individuals of *S. heathii* s.l.. In the *Cytb* alignment (1,140 bp), 27 haplotypes were identified among 46 bats of *S. kuhlii* s.l. and seven among 12 bats of *S. heathii* s.l. (Figure 1; Appendix 1; Table S2). Haplotype (Hd) and nucleotide diversities (π) calculated from *COI* and *Cytb* alignments for the entire populations of each taxon were high i.e. >0.86 and >0.01, respectively (Table S4). In both species, most individuals examined, even those from the same locations, carried unique *COI* or *Cytb* haplotypes.

The *COI* and *Cytb* networks reconstructed for *S. kuhlii* s.l. and *S. heathii* s.l. have a "bush-like" shape without ancestral haplotype (Figure 1). However, mtDNA haplotypes derived from geographically distant populations of *S. kuhlii* s.l. were found to be intermixed or identical, whereas those of *S. heathii* s.l. displayed geographical patterns. For instance, the *COI* TCS network shows the separation of three clusters of haplotypes derived from individuals of *S. heathii* s.l. collected in: (1) Indian Subcontinent, including those collected from locations 1–3 in Pakistan and location 4 in India; (2) southern Indochina, including those found from locations 16–21; and (3) northern Indochina, including samples found from locations 9–12. AMOVA analyses revealed that pairwise genetic distances (Fst) among these three clusters were between 0.11–0.433 and significant (Table S5). Likewise, in the *Cytb* TCS network, the private haplotypes found in three geographic areas (Yunnan (China), northern and southern Vietnam (or Indochina) were also separated (Figure 1).

Consistent with the observed divergences in phylogeographic patterns between the two species, Mantel tests (Figure S1A) showed a lack of statistically significant correlations between pairwise mtDNA and geographic distances in *S. kuhlii* s.l. (correlation $R^2<0.3$) and the opposite in *S. heathii* s.l. ($R^2\geq0.66$) (Figure S1A). Similarly, the genetic differentiation among three geographic haplotype clusters of the latter taxon obtained from *COI* network analysis largely resulted from IBD effects (Table S5; Figure S1B).

3.1.2. Phylogeny of the Asian Scotophilus *inferred from mtDNA sequences*

The phylogenetic trees reconstructed from the alignments of mtDNA genes (*COI* and *Cytb*) are depicted in Figures 2 and S2. The genus *Scotophilus* and the two species *S. kuhlii* s.l. and *S. heathii* s.l. were monophyletic in all phylogenetic analyses with maximal support (PP/BP=1/100). Interspecific distances (uncorrected p-distance) calculated from *COI* and *Cytb* datasets between these nominal species were ≥13.9% and ≥14.5% respectively (Table 2).

Within *S. kuhlii* s.l., all *COI* and *Cytb* trees included bats from different geographic areas or subunits of the Indomalayan Region i.e. (1) India and Myanmar, (2) Indochina (Cambodia, Laos, Vietnam) and southern China, (3) Peninsular Malaysia, and (4) the Philippines intertwined with a maximum intraspecific genetic divergence (p-distance) calculated from *COI* and *Cytb* sequences of ≤2.3% (Figures 2a–b and S2; Table 2).

Within *S. heathii* s.l., our *COI* trees (Figures 2a and S2) recovered four lineages, A, B, C, and D. Haplotypes from southern Indochina appeared in lineages A (comprising those collected from locations No. 16, 17, 19, 20 and 21 in Figure 1) and B (those collected from location No. 18 in Figure 1), whereas haplotypes from northern Indochina and the Indian Subcontinental (India and Pakistan) formed two sister lineages, C and D respectively. The range of pairwise p-distances estimated from *COI* sequences between lineages A and B were 1.1–2.3%, 2.6–4.0% between C and A+B, 5.1–7.1% between C and D, and 5.2–7.6% between A+B and D. The maximum intraspecific variation within these lineages (i.e. lineage A) was ≤1.6% (Table 2). Likewise, in our *Cytb* tree (Figures 2b and S2), a single individual from Yunnan (China) occupied a basal position to a clade (PP/BP=1/93) united by two well-supported sister lineages, one containing haplotypes in northern Vietnam (=lineage C; Figure 2a) and the other containing haplotypes from southern Vietnam (=lineage A; Figure 2a) (PP/BP=1/97-100). The range of p-distances calculated from *Cytb* sequences between these three lineages was 3.7–4.2%, whereas those within populations in northern and southern Vietnam were ≤1% (Table 2).

3.1.3. Phylogeny of Indochinese Scotophilus *based on nuDNA sequence analyses*

Bayesian trees reconstructed from three nuDNA datasets i.e. *TUFM*, *ZFYVE27* and concatenation of the two nuDNA introns from selected individuals of *S. kuhlii* and *S. heathii* from the northern and southern Indochinese geographical units, as revealed by analyses of mtDNA sequences, are presented in Figures 2c, S2 and S3, respectively. Consistent with mtDNA trees, the nuDNA trees supported the monophyly of *Scotophilus* and the two nominal species, *S. kuhlii* and *S. heathii*, with maximum robustness (PP/BP=1). More specifically, in the *TUFM* and *ZFYVE27* sequence alignments, all *Scotophilus* bats examined shared a total of 8 and 3 indels respectively, whereas *S. kuhlii* and *S. heathii* were diagnosed by several indels (Figure 2c). Likewise, and inconsistent with mtDNA trees, the substructure of geographical populations in *S. heathii* were not recovered in our analyses of separated or combined nuDNA sequences (Figures 2c and S2). The genetic distances calculated from concatenation of the two nuclear introns (i.e. the p-distances) between *S. kuhlii* and *S. heathii* ranged between 1.4 and 1.6%, whereas the nuDNA sequences of selected bats of both species from different geographical units were identical (p-distances <0.1%) (Table 2).

3.2. Historical demography

Our Bayesian skyline plot (BSP) analyses indicated that populations of both *S. kuhlii* and *S. heathii* have maintained their long-term stability since the Mid-Late Pleistocene (ca. 1.5-0.5 million years ago (Mya) and experienced rapid expansion since ca. 0.3 Mya (Late Pleistocene). However, since 0.1 Mya of the late Pleistocene, the total effective population size of *S. kuhlii* was always larger than *S. heathii* (Figure S4).

3.3. Morphological analyses

Asian *Scotophilus* are relatively large vespertilionids which share similar morphological characteristics: i.e. moderately-sized ears; tragus very long and narrow, tapering slightly towards tip and curving forwards; skulls thick and heavily-built, with just one pair of large, well-developed upper incisors; dental formula I1/3, C1/1, P1/2, M3/3 (Figures 3 and 4) (Corbet & Hill, 1992; Dobson, 1875; Tate, 1942).

Both univariate and multivariate analyses of morphological characters revealed a large individual originally identified as *S. kuhlii* from Java (Indonesia) (HNHM 2869.22) as an extreme outlier, distantly related to other specimens of *S. kuhlii* sensu stricto (s.s.) but resembled our specimens of *S. heathii* s.s. (Figure 5; Table 3). As genetic data were not available and its external and craniodental characters matched those of *S. ? solutatus* s.s. (*sensu* Tate, 1942; Shamel, 1942), this Javanese specimen was treated as a separate taxon in subsequent analyses. Accordingly, *S. kuhlii* s.s. differs from *S. heathii* s.s. in its smaller body and skull size: i.e. FA: 45.7–53.1 *vs* 54.3–66.0 mm and GSL: 18.30–20.18 *vs* 20.85–25.20 mm (Table 3; Figure 5; Table S6). Aside from the presence of a well-developed occipital helmet in *S. heathii* s.s., the skull morphology of both species is similar (Figure 4; Table 3). As shown in Figure 3, species identification of Asian yellow house bats based solely on pelage colour should be cautiously interpreted due to geographical variation. Although pelage colour is useful for distinguishing taxa living in sympatry, the reliability of this trait seems doubtful for comparing specimens from different countries, and it is entirely inaccurate for museum specimens due to fading of colour during preservation.

Within *S. kuhlii* s.s., specimens from mainland Asia (India, Myanmar, Indochina, Peninsular Malaysia) and the Philippines overlapped significantly in body and skull size (Figure 5; Table 3). Likewise, within *S. heathii* s.s., separation between specimens initially allocated to different mtDNA lineages was lacking, although local differentiations were recovered in phenotypes among bats of pairwise geographic populations, even at fine scales (Figure 5; Table 3). For instance, bats of *S. heathii* s.s. in southern Indochina appeared in three different morphological subgroups according to body and skull size i.e. with a FA of 54.3 to 66.0 mm or a GSL of 20.85 to 25.20 mm, respectively (ANOVA, $p \leq 0.05$; Table S7): (1) smaller bats in south-central Vietnam (location No. 21 and adjacent area, Figure 1); (2) intermediate bats in north-eastern Cambodia (location No. 18, Figure 1); and (3) larger bats in the central highlands of Vietnam (location No. 19, Figure 1) (Figure 5). Similarly, in the PCA of craniodental characters (Figure 5), *S. heathii* s.s. bats in the Indian Subcontinent and southern Indochina were separated by PC2 which is significantly correlated with the greatest width across the upper canines (C^1C^1) (Table S6).

4. Discussion

4.1. Cryptic diversity or inadequate taxonomy?

Early taxonomists differed considerably in how they delineated species boundaries between taxa allocated to *S. kuhlii* s.l. and *S. heathii* s.l. (*sensu* Corbet & Hill, 1992) in the Indomalayan Region, especially those found on the Sunda Islands (Table 1). According to our integrated analyses, *S. kuhlii* s.s. and *S. heathii* s.s. are genetically and morphologically distinct species. The former species is monotypic whereas the latter one is likely polytypic. In particular, FA —a standard measurement with low variation among bat researchers— appears to be reliable for differentiating the two species, as indicated by our PCAs on craniodental traits (Figures 5 and S5). Pairwise comparisons of FA data in different studies (Figure 6) show that apart from some larger individuals in Java and nearby islands (Belitung and Borneo), all other bats assigned to *S. kuhlii* s.l. in the Indomalayan Region are morphologically comparable to our *S. kuhlii* s.s. (i.e. FA ≤53.1 mm). This includes *S. collinus* (*sensu* Kitchener et al., 1997) which we regarded as a member of *S. kuhlii* in agreement with previous authors (e.g. Corbet & Hill, 1992; Shamel, 1942; Tate, 1942). Regarding the larger specimens of *S. kuhlii* s.l. (i.e. FA ≥53.4 mm) from Java, Belitung and Borneo, some were identified as *S. k. temminckii* in past studies (Kitchener et al., 1997; Siddiqi, 1960; Sody, 1928, 1936), whereas others were identified as *S. k. collinus* and *S. k. solutatus* (Sody, 1936; Tate, 1942). These controversial specimens and those of *S. heathii* s.l. in mainland Asia and Java (Shamel, 1942; Siddiqi, 1960; Tate, 1942), *S. ? celebensis* in Sulawesi (Sody, 1928;

Tate, 1942), and *P. solutatus* (=*S. solutatus*) in Java (Indonesia) (Shamel, 1942) are comparable to our specimens of *S. ? solutatus* s.s. and *S. heathii* s.s. (Figure 6; Tables 1 and 3). The new evidence from our study suggests that the current taxonomy of Asian *Scotophilus* spp. (i.e. Moratelli et al. 2019; Simmons, 2005) is inaccurate.

Kitchener et al. (1997) assigned yellow house bats with forearm lengths of ≤ 54 mm from the Sunda Islands to either *S. collinus* s.s. or *S. kuhlii* s.s. depending on body size i.e. the mean FA values for males / females of each taxon were 49.1 / 50.9 and 51.8 / 52.5, respectively. Their *a priori* species identifications were then checked by multiple regressions and discriminant function analyses (DFAs) of morphological characters. However, certain issues render the classification of Kitchener et al. (1997) unconvincing. For instance, our pairwise comparison of FA values among the taxa recognised by different authors (Figure 6) reveals that their assumption of the upper limit in forearm length for *S. kuhlii* s.l. was unjustified and that their study materials may have contained misidentified individuals of a larger form (or *S. heathii* s.s / *S. ? solutatus*). Our study and previous taxonomic works (i.e. Hill & Thonglongya, 1972; Shamel, 1942; Tate, 1942) have also confirmed the existence of individual variation in size and pelage among bats of the same *Scotophilus* species found in either sympatry or allopatry. As a consequence, the initial species identification of specimens by Kitchener et al. (1997) was uncertain particularly when considering the extensive overlap in all morphometrics given for their recognised taxa with pooled geographic populations i.e. the range of FA values for males / females of each taxon were 44.6–51.9 / 45.2–52.8 and 50.5–54.0 / 50.3–53.7, respectively. In addition to potential misidentification of specimens assigned *a priori*, the sample sizes of these taxa were heavily skewed in the two DFAs of Kitchener et al. (1997) which set apart two putative taxa (i.e. the datasets for males and females of *S. collinus* / *S. kuhlii* were 82 / 10 and 124 / 8, respectively). As such imbalanced datasets tend to produce unsatisfactory classifiers, the classification of *a priori* taxa in these DFAs should be considered doubtful, even if statistically significant (López, Fernández, García, Palade, & Herrera, 2013). Consistent with this, separation between the two *a priori* taxa was not recovered in their DFA of skull characters of both sexes combined (as there is no sexually dimorphic effects on size) using another simulated dataset for *S. collinus / S. kuhlii* (i.e. 178 / 19 respectively). In particular, Hisheh et al. (2004) considered that *S. kuhlii* s.l. bats throughout the Lesser Sunda Islands can be treated as a panmictic unit. According to Kitchener et al. (1997), the study area of Hisheh et al. (2004) encompasses two sister species which overlap in size, *S. k. solutatus* and *S. collinus*, i.e. FA values (in mm) of their males /

females are 50.3–52.4 / 50.5–52.9 and 44.6–51.9 / 46.4–52.8, respectively. This evidence suggests that the classification of different cryptic species within *S. kuhlii* s.l. in the Sunda Islands by Kitchener et al. (1997) reflected only artificial groups of a single species which resemble our *S. kuhlii* s.s.. The latter taxonomic inference is supported by the high overlap in FA values between different putative species determined by Kitchener et al. (1997) and our *S. kuhlii* s.s. (Figure 6). Moreover, from a biogeographical viewpoint, geographical distance and/or marine straits between the Lesser Sunda Islands (=study areas of Hisheh et al. (2004)) and other parts in the Indomalayan Region (our study) (Figure 1) cannot be considered as a reliable barrier to long-range dispersal and associated gene flow within this species (Hisheh et al., 2004; Yu et al., 2012; this study).

Based on *COI* distances of >2%, Francis et al. (2010) suggested that two cryptic species may be recognized in *S. heathii*, one from northern Indochina (haplotype C-h7 in Figure 2a) and the other from southern Indochina (haplotypes A-h11, h14, h15, and h17 in Figure 2a). Our phylogeographic analyses of *COI* sequences from a wider taxonomic sampling (including specimens from Cambodia and Vietnam) do not support this view. For instance, our haplotypes from Indochina fall into three lineages (i.e. A, B and C) (Figures 2a and S2). However, the pairwise p-distances calculated from *COI* sequences between these lineages ranged between 1.1–4.0% and overlapped with their intraspecific variation (i.e. lineages A: 0–1.6%) (Table 2). In particular, the interspecific divergences between our Indochinese lineages (A, B and C) were not supported by either nuDNA or morphological analyses (Figures 2c, S2 and 5; Table 2). This is consistent with the results of Trujillo et al. (2009), in which *S. heathii* bats from Yunnan (southern China), northern Vietnam and southern Vietnam have identical zfy gene sequences, whereas they belong to three divergent *Cytb* haplogroups (p-distances: 3.7-4.2%) (Figures 2b and S2; Table 2). Indeed, the absence of structured signals in nuclear datasets may be a consequence of low mutation rates or incomplete lineage sorting of these loci (i.e. Hassanin et al. 2013). However, the mito-nuclear discordance of *S. heathii* may be best explained by female philopatry and male biased dispersers (Arnold & Wilkinson, 2015; Rivers, Butlin, & Altringham, 2005; Tu et al., 2017). As such, the hypothesis of potential cryptic diversity within Indochinese *S. heathii* s.l. can be ruled out. Similarly, the relatively higher genetic divergence of *COI* sequences among two morphologically overlapping populations of *S. heathii* s.l. in spatially distant subregions (Indochina lineages A, B, and C and the Indian Subcontinent lineage D (5.1–7.6% p-distances) (Figures 1-3 and S2; Table 2)) might also be attributable to their potential sex-biased gene flow followed by IBD effects (Figure S1). Thus, while further analyses of samples from the intervening zones (Nabhan & Sarkar, 2012; Zwickl & Hillis, 2002) and/or nuclear markers are needed, the possibility that allopatric speciation events have occurred without morphological changes in the above populations of *S. heathii* s.s. (Bickford et al., 2007; Tu et al., 2017, 2018) would seem very unlikely.

All larger forms of yellow house bats on Java and nearby islands (i.e. FA values of ≥53.4 mm and GSL ≥20.5 mm) were previously referred to as *S. kuhlii* (Corbet & Hill, 1992; Simmons, 2005). However, their taxonomic identity was an issue of debate for many years (Figure 6; Table 1). Having examined one of these controversial specimens from Java (HNHM 2869.22), our results suggest that it resembles *S. heathii* s.s. more than *S. kuhlii* s.s. (Figures 4–6; Table 3). This specimen was collected by the Novara Expedition (1857–1859) and its identity was determined by the British zoologist, Oldfield Thomas (1858–1929). It should be noted that before Hill & Thonglongya (1972) confirmed that *S. kuhlii* should replace *S. temminckii* as the correct name for smaller Asian yellow house bats, *S. kuhlii* was used for larger bats by several taxonomists (Allen, 1906; Osgood, 1932; Shamel, 1942; Thomas, 1897) instead of *S. heathii* which other authors used (Siddiqi, 1960; Tate, 1942) (Table 1). This suggests that the previous allocation of our examined specimen (and *S. ? solutatus* s.s.) and the larger *Scotophilus* from Java and nearby islands (i.e. Borneo) as *S. kuhlii* s.l. (*sensu* Corbet & Hill, 1992; Simmons, 2005; Kitchener et al., 1997; Tate, 1942) was misguided. In addition, while research on Asian bats has intensified in recent years, most survey effort has focused on habitats such as tropical forests which support high bat diversity (Kingston, 2010), whereas bats that live commensally with humans are comparatively neglected (Jung & Threlfall, 2016). *Scotophilus* spp. are one of the most common bats in urban-rural habitats but roost in high shelters (e.g. roofs of houses) and forage in open spaces above the effective range of ground-based live-traps (i.e. mist-nets and harp traps) (Bates & Harrison, 1997; Francis, 2008; Hisheh et al., 2004). As such, they are likely under-surveyed in many regions of Southeast Asia (Figure 1). Consequently, the disjunct distribution of large yellow house bats between mainland Asia (or *S. heathii* s.s.) and Sulawesi (*S. celebensis* s.s.) (Figure 1) determined by previous authors (e.g. Corbet & Hill, 1992; Simmons, 2005; Tate, 1942) may be due to erroneous naming of voucher specimens and gaps in survey coverage. Alternatively, the separation of *S. celebensis* from *S. heathii* by Simmons (2005) could be regarded as unsubstantiated. Thus, until additional analyses further elucidate their phylogenetic relationships, all large yellow house bats (i.e. FA ≥53.4 mm and GSL ≥20.5 mm

(Figure 6; Tables 1 and 3) occurring in mainland Asia (*S. heathii* s.s.), Java and nearby islands (*S. ? solutatus*), and Sulawesi (*S. celebensis* s.s.) should be regarded as representatives of a single species: *S. heathii*.

4.2. Comparative phylogeography of Asian Scotophilus spp.

The absence of star-like TCS haplotype networks (Figure 1) and results of BSP analyses (Figure S4) in our study indicate that both *S. kuhlii* and *S. heathii* have maintained a constant or expanding effective population size through time since the Mid-Pleistocene. The distribution and lack of bottlenecks in the evolutionary history of Asian yellow house bats in the Indomalayan Region thus differs strikingly from other co-distributed bat species i.e. bamboo bats (*Tylonycteris* spp.) (Tu et al., 2017), woolly bats (*Kerivoula* spp.) (Khan et al., 2010; Tu et al., 2018), horseshoe bats (*Rhinolophus* spp.) (Mao et al., 2010). This may be attributable to eco-ethological differences (Avise, 2000; Hassanin et al., 2016; Moussy et al., 2013). For example, Asian yellow house bats are strong dispersers and aerial-hawking, open space foragers (Bates & Harrison, 1997; Francis, 2008; Norberg & Rayner, 1987), whereas *Tylonycteris*, *Kerivoula* and *Rhinolophus* spp. have a much weaker dispersal ability because they are mostly forest-dwelling taxa (Khan et al., 2010; Mao et al., 2010; Tu et al., 2017; 2018). Thus, unlike forest-dependent species, the dispersal and associated gene flow among geographic populations of Asian *Scotophilus* might be less restricted by physical and ecological barriers (Hisheh et al., 2004; Yu et al., 2012). In addition, while the population structure and dynamics of species with low dispersal capacities were strongly influenced by the past compression and expansion of forests during glacial and interglacial periods in the Pleistocene (Khan et al., 2010; Mao et al., 2010; Tu et al., 2017; 2018) and by current patterns of deforestation (Kingston, 2010), Asian *Scotophilus* spp. may have been less affected by such changes.

Our comparative phylogeographic analyses show that geographically distant populations of *S. kuhlii* in the Indomalayan Region possess very low genetic and morphological variation, whereas those of *S. heathii* in mainland Asia display divergent mtDNA sequences and phenotypes (see section 4.1 for more detail). As discussed above, the incongruences in phylogeographic patterns of mtDNA genetic diversity between the two sympatric species may be attributable to sex-biased gene flows (Avise, 2000; Slatkin, 1987). More specifically, the phylogenetic signals in our mtDNA and nuDNA datasets for *S. heathii* may be consistent with female philopatry and male-biased dispersal whereas those for *S. kuhlii* do not exhibit sex-biased dispersal (Arnold & Wilkinson, 2015; Rivers et al., 2005; Tu et al., 2017). As both species may have maintained gene flows among their populations, the geographic variation observed in external and craniodental traits might reflect their phenotypic plasticity to adapt to selective forces imposed by environmental variability, as well as interspecific competition for common resources in areas of sympatry (Chevin, Lande, & Mace, 2010; Ghalambor, McKay, Carroll, & Reznick, 2007; Lande, 2014; Spaeth, 2009; Tienderen, 1997; Zamudio, Bell, & Mason, 2016).

The low morphological variation of *S. kuhlii* specimens throughout the Indomalayan Region suggests that the species has maintained a generalist phenotype suited to a variety of environments. By contrast, the morphological variability of *S. heathii* indicates that its geographic populations, even those at small scales, may have evolved plastic phenotypes that suit the particular habitats they inhabit (Chevin et al., 2010; Lande, 2014; Spaeth, 2009; Tienderen, 1997; Zamudio et al., 2016). As such, the generalist populations of *S.kuhlii* may have experienced fewer adverse effects from climate and associated habitat changes than *S. heathii*. They may also have had more advantages than *S. heathii* when colonizing and adapting to new habitats (Bonte et al., 2012; Ghalambor et al., 2007; Hollander, Verzijden, Svensson, & Brönmark, 2014; Kelly, Panhuis, & Stoehr, 2012; Lande, 2014; Moussy et al., 2013). These inferences suggest that *S. heathii* bats may be more philopatric to their native areas compared to *S. kuhlii*. If so, this would explain how *S. kuhlii* can occupy a wider geographic range in the Indomalayan Region relative to *S. heathii* (Figure 1), even though *S. heathii* would be expected to have greater dispersal ability based on its wing morphology.

It should be noted that bats of *S. kuhlii* and *S. heathii* found either in sympatry or allopatry do not overlap in most morphological traits (i.e. FA; Figures 3-6). As discussed above, it is very likely that *S. kuhlii* and *S. heathii* have co-existed for a long time. Both species are also known to share similar eco-ethological preferences and feeding guilds (Bates & Harrison, 1997; Francis, 2008; Norberg & Rayner, 1987). Because prey density usually decreases from cluttered to open habitats, strong interspecific competition likely occurred in areas of sympatry during their evolutionary history (Grether et al., 2013; Müller et al., 2012; Roeleke, Johannsen, & Voigt, 2018). To reduce niche overlap, co-existing related species typically evolve mechanisms for resource partitioning (Chevin et al., 2010; Lande, 2014; Tienderen, 1997) that result in detectable differences in morphology or echolocation call parameters (Kingston et al., 2001; Zhang et al., 2007). This would be consistent with the morphological differences between the two species.

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Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article and/or its supplementary materials.

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Figures

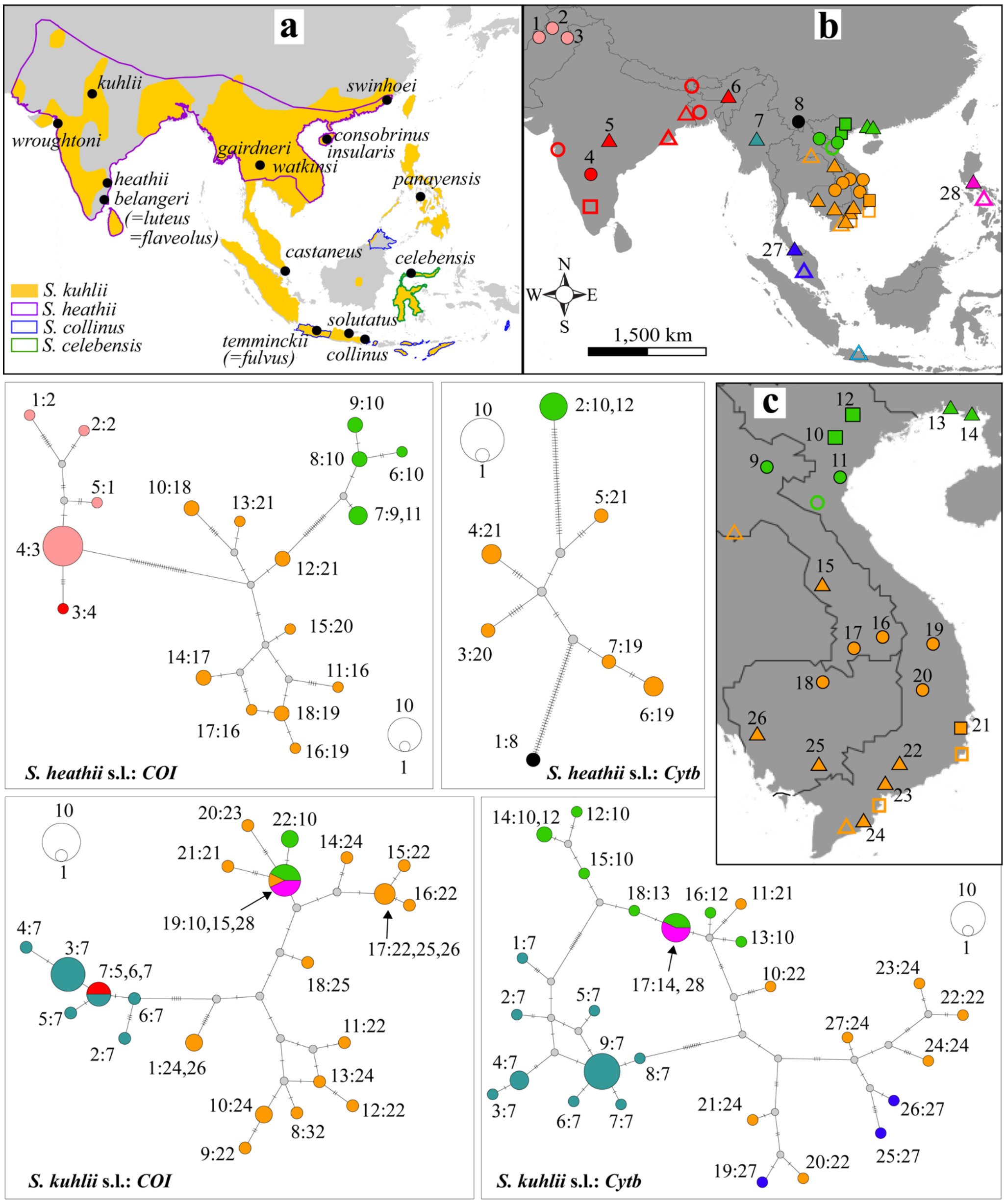


Figure 1. Phylogeographic patterns of Asian *Scotophilus* spp. based on the mitochondrial (*COI* and *Cytb*) markers.

a: IUCN distribution range of four recently recognized Asian *Scotophilus* spp. Black dots refer to type localities of formerly described species or subspecies of *Scotophilus* in Asia (See Table S1). Note that the type locality of *S. kuhlii* in India is uncertain. b–c: Locations for which specimens of *S. kuhlii, S. heathii* or both species were collected are shown as triangles, circles and squares, respectively. Localities for specimens included in genetic analyses are filled and numbered whereas those of individuals in morphological analyses only are empty. MtDNA haplotypes of examined specimens in the TCS networks and their corresponding locations are indicated by the same colour and by numbers before and after colons, respectively (See Appendix 1 and Table S2 for more details).

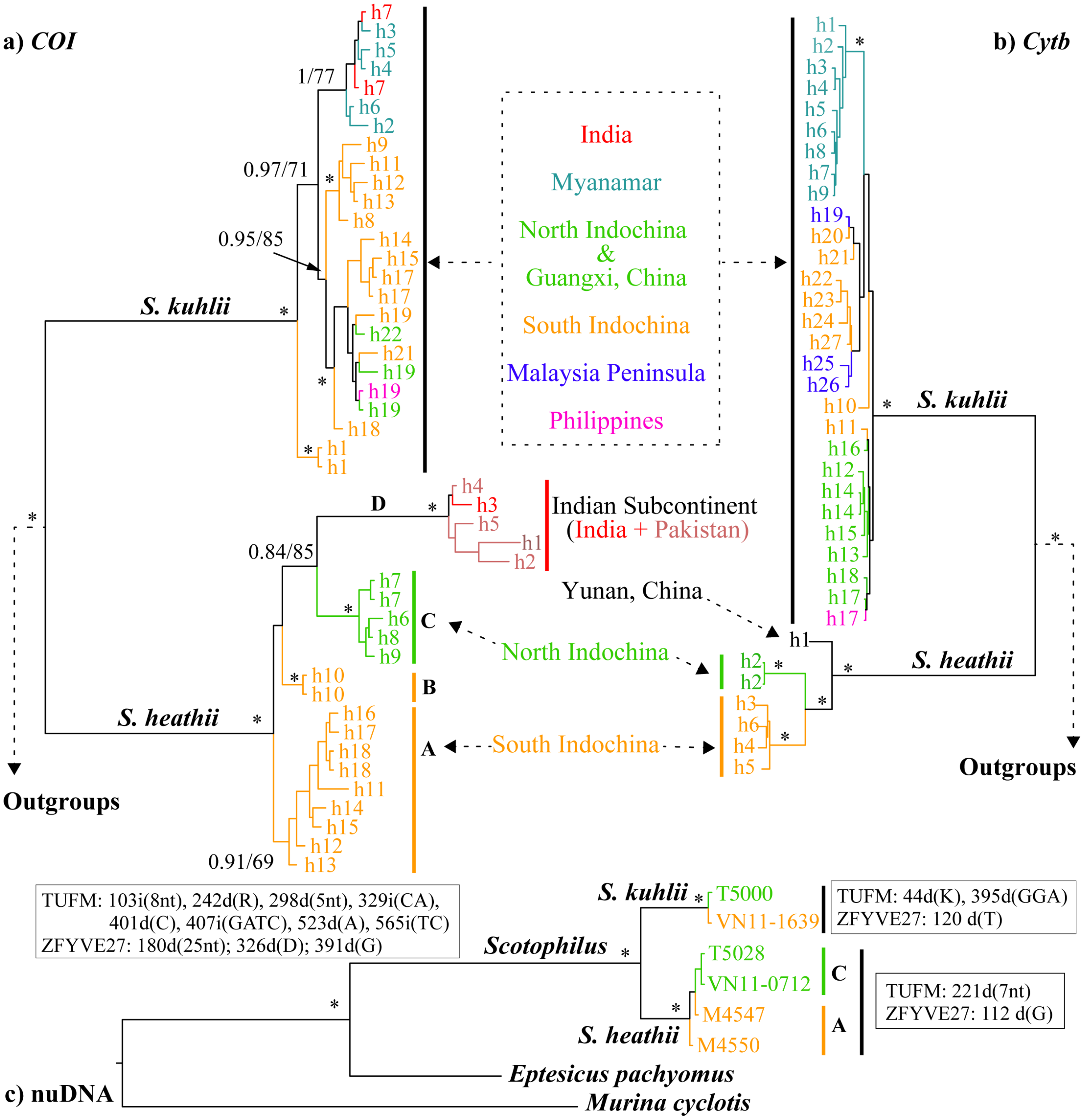


Figure 2. Phylogenetic trees of Asian *Scotophilus* spp. and outgroups. Values on nodes indicate Bayesian posterior probabilities (PP)/Maximum-Likelihood bootstrap percentage (BP) (PP<0.7 and BP <70% are not shown). The asterisks (*) indicate that the node was supported by PP≥0.9/BP≥90. The colours of mtDNA haplotypes match those in Figure 1. The position and nature of all diagnostic indels (i: insertion; d: deletion) shared by at least two taxa in the alignments of nuclear genes are indicated in boxes.

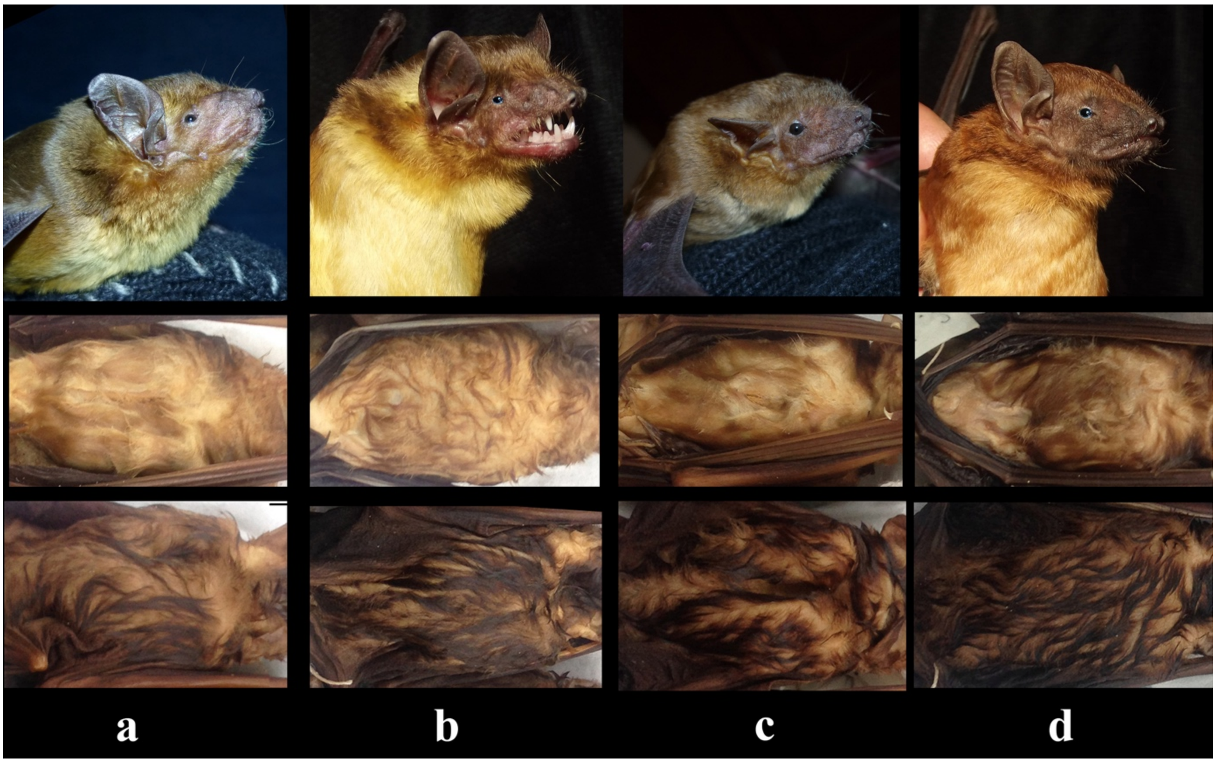


Figure 3. Live and wet specimens (not to scale) of *Scotophilus heathii* and *S. kuhlii* collected in sympatry in two different regions of Vietnam.

Northern Vietnam (Location 10): a – *S. heathii* / c – *S. kuhlii*. South-Central Vietnam (Location 21): b – *S. heathii* / d – *S. kuhlii*

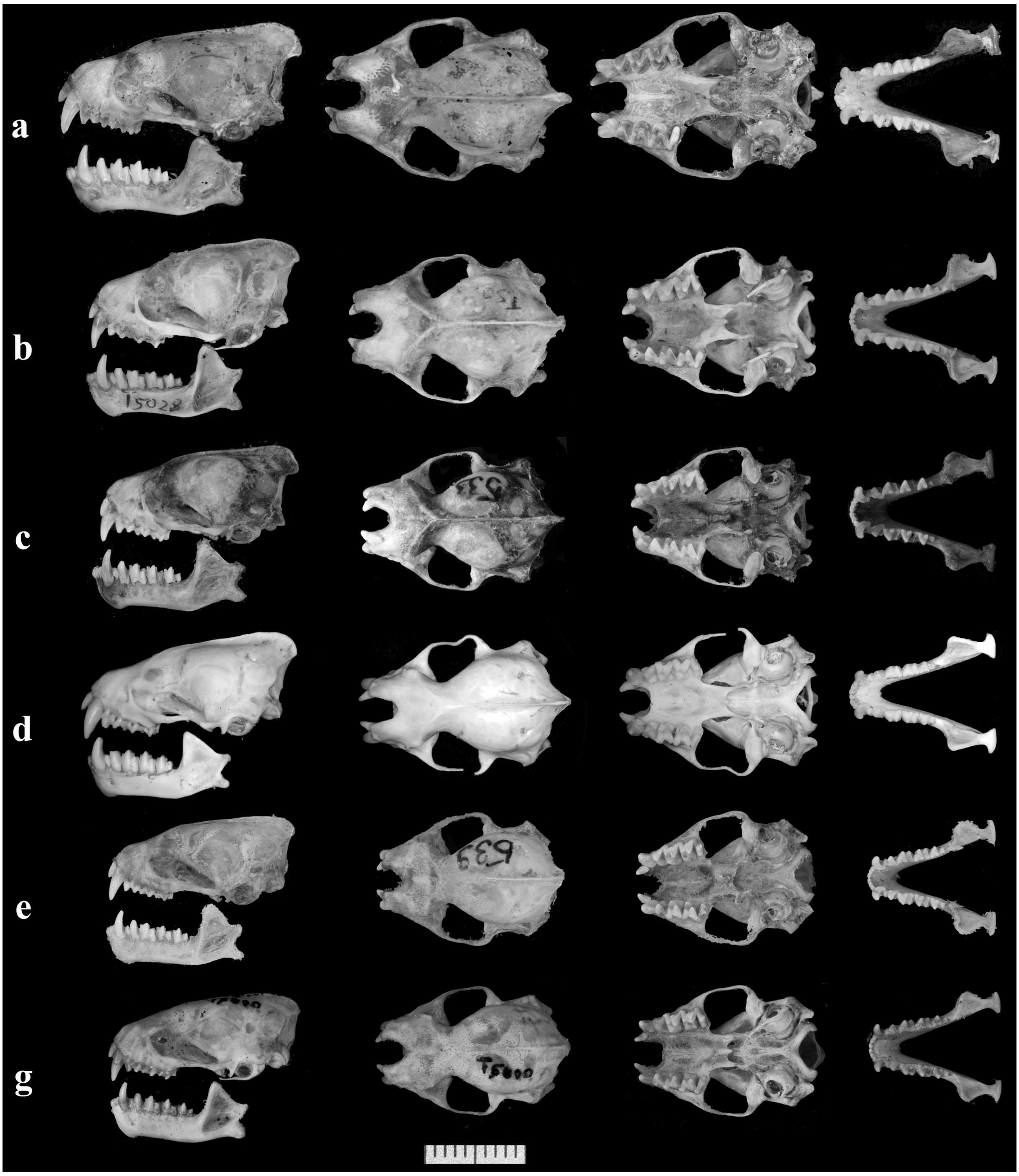


Figure 4. Skull profiles of selected Asian *Scotophilus* spp.

S. heathii s.s.: a – Highland Central Vietnam (loc. 19; IEBR-M-4550), b – Northern Vietnam (loc. 10; IEBR. T5028) and c – South Central Vietnam (loc. 21; IEBR. VN17-533); *S. ? solutatus* s.s.: d – Java, Indonesia (HNHM 2869.22); and *S. kuhlii* s.s.: e – South Central Vietnam (loc. 21; IEBR. VN17-539) and g – Northern Vietnam (loc. 10; IEBR. T5000). Scale =10 mm.

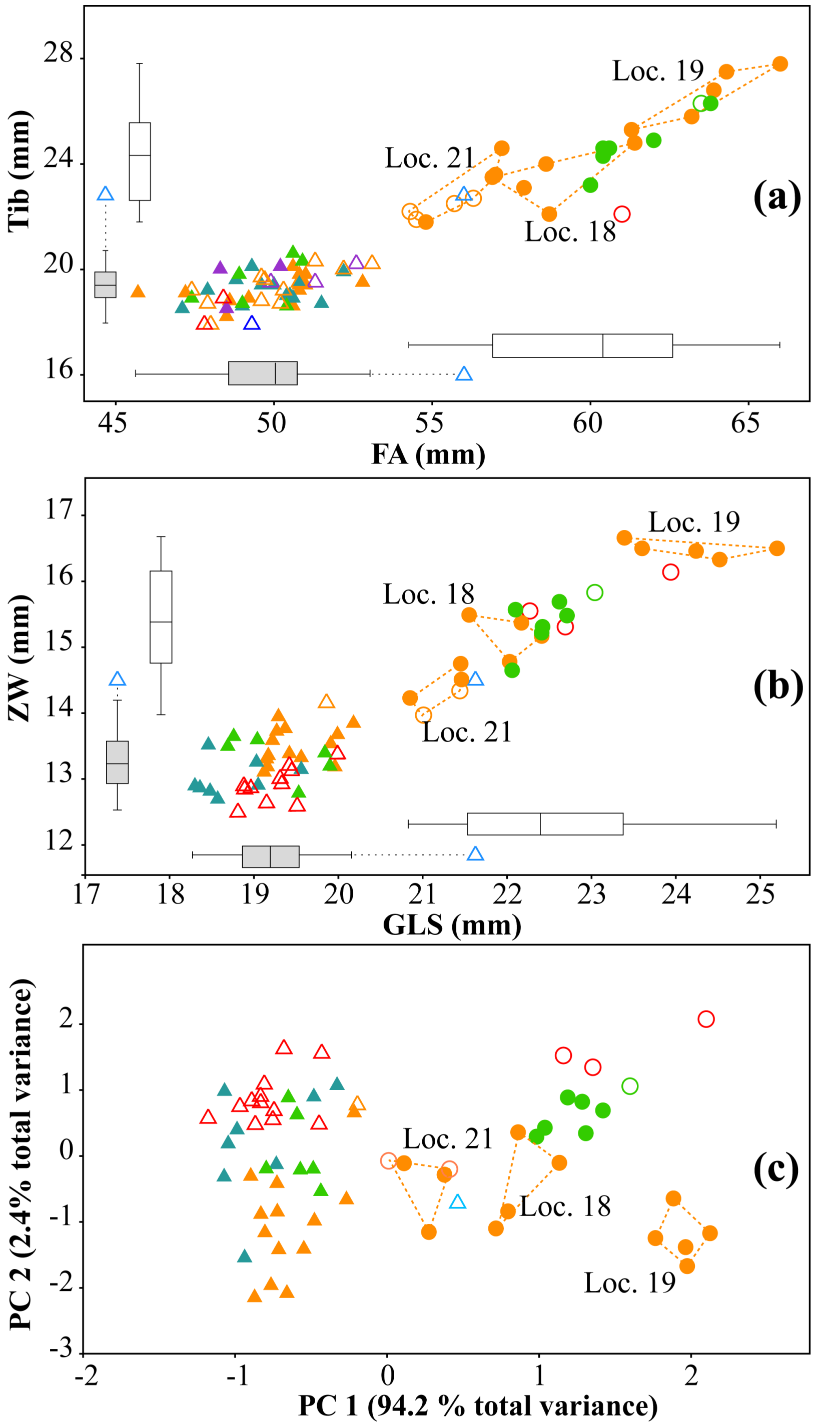


Figure 5. Scatter plots from morphological analyses of Asian *Scotophilus* spp. a and b: Variation in external (FA vs. Tib) and skull traits (GSL vs. ZB) among specimens of *Scotophilus* spp., respectively. Boxplots (with an interquartile range) to find outliers in the datasets were embedded; c: Plot of PC 1 against PC 2 from PCA on log-transformed craniodental measurements. The legends of symbols follow Figure 1. Bats of *S. heathii* found in three spatially isolated locations (18, 19, and 21) in Southern Indochina (Figure 1) appear as three relatively separated subpopulations.

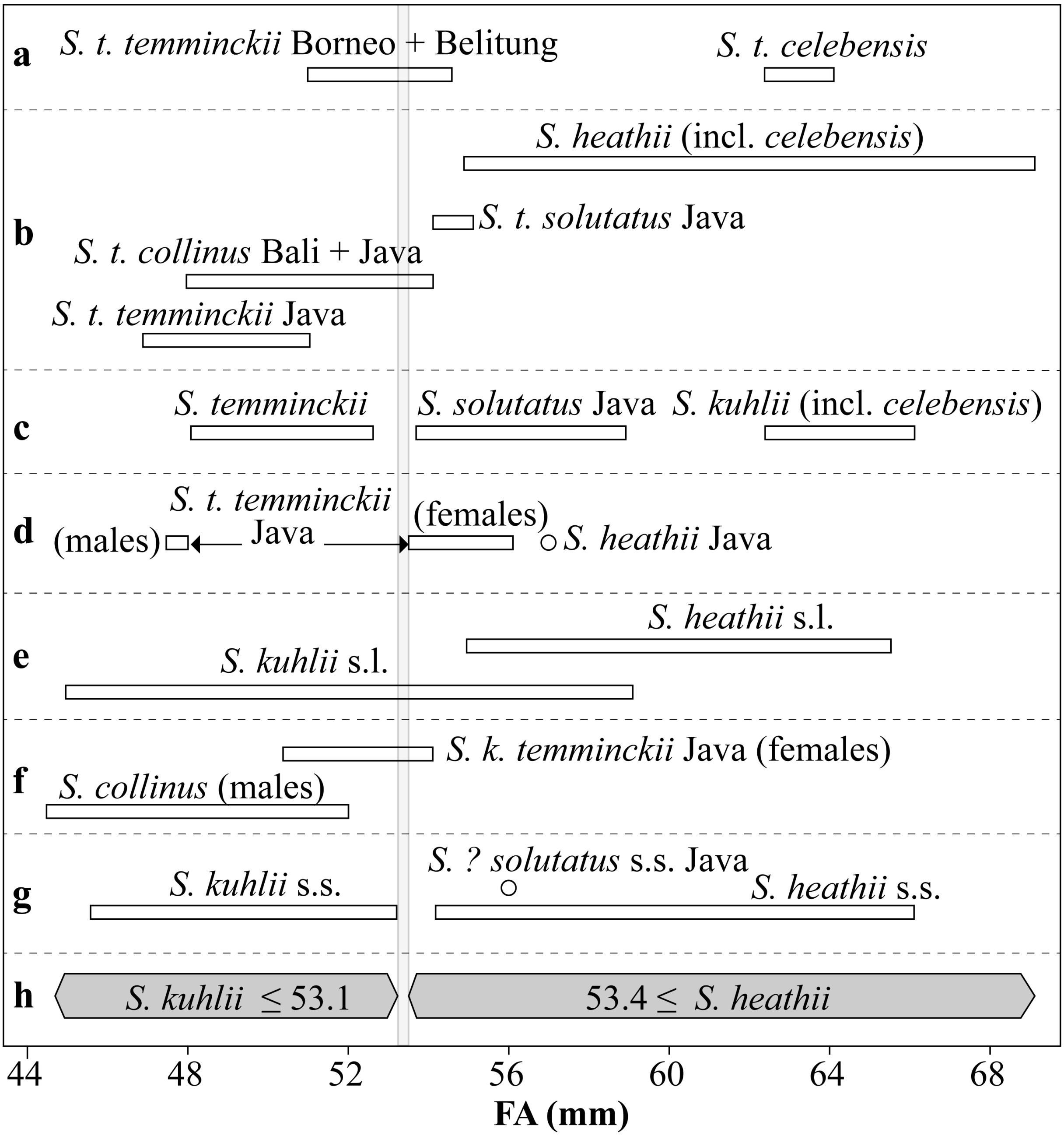


Figure 6. Pairwise comparison of FA ranges (min–max) for Asian *Scotophilus* spp. recognized in present and previous studies.

a – Sody, 1928; b – Tate, 1942; c – Shamel, 1942; d – Siddiqi, 1960; e – Corbet & Hill, 1992; f – Kitchener et al. (1997); g – this study; and h – desired ranges for S. *kuhiii* and *S. heathii*.

Tables

Table 1: Synopsis of taxonomic studies on Asian *Scotophilus* between 1940–2000(†).

Author	Reference materials	Taxonomic treatment
[1]	India, Hainan, Taiwan (Formosa), Malacca, Java, Bali, Luzon	*S. kuhlii* (forearm length (FA, in mm): 41, immature holotype) *S. temminckii* (=*fulvus*) (FA ≤ 51) includes *consobrinus*, *castaneus*, *wroughtoni*, *panayensis, collinus*, and *gairdneri* as similar sized races/ synonyms and a larger sized Javanese one, *solutatus* (FA: 55-55).
	India: Kashmir, Myanmar (Burma), Hainan, Sri Lanka (Ceylon)	*S. heathii* (FA ≥ 54) with *belangeri* (=*luteus*=*flaveolus*), *insularis*, and *celebensis* as races/synonyms
[2]	Java, Singapore, Thailand, French Indochina	*Pachyotis temminckii* (FA: 48.2-52.5) includes *temminckii*=*castaneus*, *consobrinus*, *wroughtoni*, *panayensis, collinus*, and *gairdneri* as synonyms/ races
	West Java, Depok, Pelabuhan	*P. solutatus* (FA: 53.8-58.9)
	Thailand (Siam), French Indochina, Ceylon	*Pachyotis kuhlii* (FA: 60-66) includes *insularis*, and *celebensis* as races
[3]	Thailand	*S. s. solutatus*: Java, and *S. solutatus watkinsi* ssp. nov. (FA: 55.5-60.5). Type locality: Pak Nam Pho, Nakhon Sawan, Thailand.
[4]	Collection in the British Museum (N.H.)	*S. t. temminckii* (FA of males / females: 47.6-47.9 / 53.4-56.0): Java
		S. t. castaneus (FA: 47.6-52.4): Malay Peninsula; Upper Burma; South China; India: Calcutta
		S. t. wroughtoni (FA: 45.2-52.4): Ceylon and India
		S. h. heathii (56.2-63.8): Ceylon, India, Pakistan, Burma, Siam and Java
[5]	Collection in the British Museum (N.H.)	*S. kuhlii* replaced *S. temminckii* as generic name of smaller sized species. Its subspecies/ races include *temminckii*, *castaneus*, *collinus*, *consobrinus*, *gairdneri*, *panayensis*, *solutalus* and *wroughtoni.*
		S. h. heathii (Indian and Burma) and *S. h.* (?) *watkinsi* (FA: 61.2-61.4)
[6]	Synthesis from previous studies	*S. kuhlii* (FA: 45-59) comprises *castaneus*, *consobrinus*, *fulvus*, *gairdneri*, *panayensis*, *solutanus*, *swinhoei*, *temminckii*, and *wroughtoni* as synonyms/races found throughout the Indomalayan Region. The largest specimens (or *S. k. solutanus*) occur apprarently in Java.
		S. heathii (FA: 55-65.5) includes *belangeri*, *flaveolus*, *insularis*, *luteus*, *watkinsi* (from Afghanistan to Vietnam) and probably *celebensis* (Sulawesi) as subspecies/synonyms
[7]	*S. kuhlii* s. l. (FA< 54) collected from Greater (Java), Lesser Sunda and Borneo islands	- *S. k. temminckii* (FA of males: 52.2-53.7): Java and *S. k. solutatus* (FA of males: 50.3-52.4): East Java and Bali; - *S. collinus*: Sundaic form (FA of males: 45.2-51.4): Sabah, Java and Bali; Nusa Tenggara form (FA of males: 44.6-51.9): Lombok to Timor

(†) Prior to the mid-20th century, Asian *Scotophilus* included 17 taxa (species and subspecies) described as new to science (detailed in Figure 1; Table S1). Authors: 1 – Tate, 1942; 2 – Shamel, 1942; 3 – Sanborn, 1952; 4 – Siddiqui, 1960; 5 – Hill & Thonglongya, 1972; 6 – Corbet & Hill, 1992; 7 – Kitchener et al., 1997

Table 2: Range (min–max) of uncorrected p-distances (%) between Asian *Scotophilus* spp. and selected outgroups, based on *COI* (*Cytb*) (below the diagonal) and nuDNA (above the diagonal) datasets.

Taxon		**1**	**2**	**3**	**4**			
					A	**B**	**C**	**D**
1. *M. cyclotis*			9.5	11.3-11.7	11.3-11.3	NA	11.6-11.6	NA
2. *E. pachyomus*		20.1 (19.6)		6.8-7.0	6.8-6.9	NA	6.8-6.9	NA
3. *S. kuhlii*		21.1 -22.7 (19.7-20.3)	20.9-21.8 (21.1-21.7)	**0.0** **2.3(2.2)**	1.4-1.6	NA	1.5-1.7	NA
4. *S. heathii*	**A**	21.5-22.1 (22.1-22.5)	20.5-21.2 (21.1-21.3)	13.9-15.2 (14.5-15.9)	**0.0** **1.6 (1.0)**	NA	0.0-0.1	NA
	B	21.8-21.8 (NA)	21.3-21.3 (NA)	14.3-15.4 (NA)	1.1-2.3 (NA)	**NA** **0.0 (NA)**	NA	NA
	C	21.8-22.1 (21.8-21.8)	21.0 - 21.2 (20.6-20.6)	14.3 - 15.7 (15.2-16.0)	2.6-4.0 (3.7-3.9)	NA (3.8-4.2)	**0.0** **0.6 (0-0)**	NA
	D	21.9-23.4 (NA)	20.7 -21.3 (NA)	16.0-18.3 (NA)	5.2-7.6 (NA)	5.5-6.9 (NA)	5.1-7.1 (NA)	**NA** **2.0 (NA)**
	E	NA (21.4)	NA (21.1)	NA (14.0-14.9)	NA (4.0-4.0)	NA (NA)	NA (3.8-4.2)	NA (NA)

Taxon: A – D and E are corresponding lineages A – D and Yunnan, China of *S. heathii* in Figure 2. Values in diagonal in bold show the maximum intraspecific distances within each taxon calculated from the respective datasets; NA – not available.

Table 3: External and craniodental measurements (in mm) of Asian *Scotophilus* spp. Values are given as mean ± SD, n; min–max. Acronyms and definitions for measurements are given in the Materials and Methods section.

Character	***S. kuhlii* s.l.**							***S. heathii* s.l.**		
	North Indochina	**South Indochina**	**Myanmar**	**Indian Subcontinental**	**Malaysia Peninsula**	**Philippines**	**Indonesia (Java)***	**North Indochina**	**South Indochina**	**Indian Subcontinental**
FA	49.5 ± 1.3; 6 47.4 – 50.9	49.9 ± 1.7; 26 45.7 – 53.1	49.8 ± 1.4; 14 47.1 – 52.2	48.1 ± 0.4; 2 47.8 – 48.4	49.3; 1	50.1 ± 1.6; 6 48.3 – 52.6	56.0; 1	61.5 ± 1.6; 7 60.0 – 63.8	58.9 ± 3.7; 17 54.3 – 66.0	61.0; 1
Tib	19.6 ± 0.9; 6 18.7 – 20.7	19.4 ± 0.6; 26 18.0 – 20.4	19.3 ± 0.5; 14 18.6 – 20.2	18.5 ± 0.8; 2 18.0 – 19.0	18.0; 1	19.7 ± 0.6; 6 18.6 – 20.3	22.9; 1	24.9 ± 1.1; 7 23.2 – 26.3	24.1 ± 2.0; 17 21.8 – 27.8	22.1; 1
GSL	19.29 ± 0.54; 6 18.69 – 19.90	19.54 ± 0.37; 14 19.15 – 20.18	18.73 ± 0.44; 8 18.30 – 19.56	19.24 ± 0.34; 12 18.81 – 19.99	–	–	21.68; 1	22.48 ± 0.35; 7 22.06 – 23.04	22.52 ± 1.41; 14 20.85 – 25.20	22.97 ± 0.87; 3 22.27 – 23.94
SL	18.45 ± 0.52; 6 17.66 – 19.11	18.70 ± 0.27; 14 18.19 – 19.11	18.25 ± 0.51; 8 17.64 – 18.98	18.17 ± 0.33; 12 17.68 – 18.80	–	–	20.43; 1	21.37 ± 0.35; 7 20.80 – 21.90	21.43 ± 1.34; 14 19.78 – 23.50	21.83 ± 0.96; 3 20.97 – 22.87
CBL	17.59 ± 0.25; 6 17.30 – 17.90	17.49 ± 0.30; 14 16.95 – 17.90	17.30 ± 0.58; 8 16.48 – 18.05	17.10 ± 0.33; 12 16.40 – 17.61	–	–	18.76; 1	19.76 ± 0.33; 7 19.31 – 20.26	19.74 ± 1.26; 14 17.97 – 21.39	20.00 ± 0.76; 3 19.32 – 20.82
CCL	17.55 ± 0.19; 6 17.24 – 17.81	17.71 ± 0.30; 14 17.27 – 18.21	17.34 ± 0.53; 8 16.80 – 18.11	17.44 ± 0.28; 12 16.93 – 17.83	–	–	19.26; 1	20.01 ± 0.42; 7 19.46 – 20.48	19.94 ± 1.30; 14 18.14 – 21.61	20.47 ± 0.85; 3 19.72 – 21.40
ZB	13.39 ± 0.32; 6 12.82 – 13.68	13.61 ± 0.30; 14 13.22 – 14.19	13.05 ± 0.27; 8 12.73 – 13.55	12.96 ± 0.26; 12 12.53 – 13.41	–	–	14.54; 1	15.39 ± 0.39; 7 14.65 – 15.83	15.36 ± 0.97; 14 13.97 – 16.66	15.67 ± 0.43; 3 15.31 – 16.14
C^1C^1	6.26 ± 0.10; 6 6.10 – 6.35	6.14 ± 0.24; 14 5.75 – 6.58	6.23 ± 0.27; 8 5.95 – 6.66	6.39 ± 0.17; 12 6.11 – 6.68	–	–	6.69; 1	7.39 ± 0.22; 7 7.04 – 7.77	7.08 ± 0.33; 14 6.63 – 7.63	7.89 ± 0.43; 3 7.56 – 8.38
M^3M^3	8.72 ± 0.22; 6 8.36 – 9.03	8.45 ± 0.26; 14 7.95 – 8.89	8.32 ± 0.21; 8 8.00 – 8.63	8.39 ± 0.14; 12 8.11 – 8.63	–	–	8.95; 1	9.72 ± 0.28; 7 9.29 – 10.12	9.51 ± 0.49; 14 8.79 – 10.36	9.81 ± 0.31; 3 9.63 – 10.16
CM^3	6.62 ± 0.09; 6 6.49 – 6.77	6.52 ± 0.19; 14 6.35 – 6.99	6.52 ± 0.14; 8 6.34 – 6.75	6.58 ± 0.16; 12 6.30 – 6.84	–	–	7.26; 1	7.55 ± 0.13; 7 7.32 – 7.68	7.26 ± 0.44; 14 6.72 – 7.94	7.74 ± 0.23; 3 7.59 – 8.00
ML	13.69 ± 0.13; 6 13.49 – 13.82	13.75 ± 0.22; 14 13.41 – 14.13	13.72 ± 0.26; 8 13.32 – 14.10	13.41 ± 0.24; 12 13.09 – 13.90	–	–	14.89; 1	15.86 ± 0.32; 7 15.34 – 16.24	15.51 ± 1.05; 15 14.14 – 17.00	15.81 ± 0.62; 3 15.25 – 16.47
CM_3	7.53 ± 0.17; 6 7.30 – 7.80	7.42 ± 0.21; 14 7.09 – 7.88	7.36 ± 0.17; 8 7.12 – 7.67	7.36 ± 0.11; 12 7.20 – 7.56	–	–	7.92; 1	8.65 ± 0.20; 7 8.37 – 8.89	8.28 ± 0.47; 15 7.63 – 8.96	8.79 ± 0.33; 3 8.57 – 9.17

* – This specimen resembles *S. ?. solutatus* (Shamel, 1942; Tate, 1942)

Appendix 1. Asian *Scotophilus* specimens were collected and/or examined directly by the authors in this study.

See Material and Methods for acronyms of museums and genetic markers. (†) – tissue samples only. (‡) –Localities for specimens included in molecular analyses are indicated by numbers (after colon) as shown in Figure 1. Country codes include: IN – India, KH – Cambodia; LA – Laos; and VN – Vietnam. **(§)** – Numbers (in parentheses) after Genbank accession numbers for *Cytb* and *COI* sequences of *Scotophilus* specimens are respective haplotypes shown in Figure 1 and 2.

Taxon	**Museum/Sample code**	**Location(‡)**	**Genbank Accession No.**			
			***Cytb*(§)**	***COI*(§)**	***TUFM***	***ZFYVE27***
S. heathii (♂)	HNHM 65.23.1.	Bharatpur, Rajasthan, IN	–	–	–	–
S. heathii (♀)	HNHM 92.120.1. (11951)	Elephanta caves, Mumbai, Maharashtra, IN	–	–	–	–
S. heathii (♂)	HNHM 93.36.1. (12175)	Coimbatore, Tamil Nadu, IN	–	–	–	–
S. heathii (♂)	HNHM 93.37.1. (12207)	Sevoke, IN	–	–	–	–
S. heathii (♂)	HNHM 92.119.1. (11526)	N.Salt Lake, Nalbani, BD	-	-	–	–
S. heathii (♂)	IEBR.Tu.04.09.09.1 (T5028)	Xom Hau, Dong Anh, Hanoi, VN: 10	–	MT821518 (6)	–	–
S. heathii (♀)	IEBR.Tu.10.08.09.1 (T5029)	Ho Tay, Tay Ho, Ha Noi, VN: 10	–	MT821519 (8)	–	–
S. heathii (♀)	IEBR.VN15-47 (VN6149)	Xom Hau, Dong Anh, Hanoi, VN: 10	MT820603 (2)	MT821506 (9)	–	–
S. heathii (♂)	IEBR.VN15-49 (VN6151)	Xom Hau, Dong Anh, Hanoi, VN: 10	MT820604 (2)	MT821505 (9)	–	–
S. heathii (♀)	IEBR.VN15-50 (VN6152)	Xom Hau, Dong Anh, Hanoi, VN: 10	MT820605 (2)	MT821504 (8)	–	–
S. heathii (♀)	IEBR.VN11-0712	Ngoc Lac, Thanh Hoa, VN: 11	–	MT821517 (7)	–	–
S. heathii (♀)	IEBR.PH24 (VN7297)	Pu Huong, Nghe An, VN	–	–	–	–
S. heathii (♂)	CBC01250 (VN11-1648)	Preah Vihear protected forest, KH: 18	–	MT821520 (10)	–	–
S. heathii (♀)	CBC01251	Preah Vihear protected forest, KH: 18	–	–	–	–
S. heathii (♀)	CBC01252 (VN11-1649)	Preah Vihear protected forest, KH: 18	–	MT821521(10)	–	–
S. heathii (♀)	CBC01260	Preah Vihear protected forest, KH: 18	–	–	–	–
S. heathii (♂)	HNHM 2014.11.26 (23702)	Preah Vihear protected forest, KH	–	–	–	–

Taxon	**Museum/Sample code**	**Location**(‡)	**Genbank Accession No.**			
			Cytb(§)	***COI***(§)	***TUFM***	***ZFYVE27***
S. heathii (♀)	IEBR.M4547	Ba To, Quang Ngai, VN: 19	–	–	–	–
S. heathii (♀)	IEBR.M4548	Ba To, Quang Ngai, VN: 19	–	–	–	–
S. heathii (♂)	IEBR.M4550 (VN7293)	Ba To, Quang Ngai, VN: 19	–	–	–	–
S. heathii (♂)	IEBR.M4553 (VN1736/VN7294)	Ba To, Quang Ngai, VN: 19	MT820609 (6)	MT821522 (18)	MT820615	MT820621
S. heathii (♀)	IEBR.M4554 (VN7295)	Ba To, Quang Ngai, VN: 19	MT820611 (7)	MT821507 (16)		
S. heathii (♂)	IEBR.M4555 (VN1737/VN7296)	Ba To, Quang Ngai, VN: 19	MT820610 (6)	MT821523 (18)	MT820616	MT820622
S. heathii (♀)	IEBR.VN17-532 (VN7299)	Loi Hai, Ninh Thuan, VN: 21	MT820606 (3)	MT821509 (12)	–	–
S. heathii (♂)	IEBR.VN17-533 (VN7300)	Loi Hai, Ninh Thuan, VN: 21	MT820607 (3)	MT821510 (12)	–	–
S. heathii (♀)	IEBR.VN17-536 (VN7302)	Loi Hai, Ninh Thuan, VN: 21	MT820608 (5)	MT821508 (13)	–	–
S. heathii (♀)	IEBR.VN17-537	Lien Huong, Binh Thuan, VN	–	–	–	–
S. heathii (♂)	IEBR.VN17-538 (VN7303)	Lien Huong, Binh Thuan, VN	–	–	–	–
S. heathii (?)	IEBR.Tu.18.5.17.2	Lien Huong, Binh Thuan, VN	–	–	–	–
S. heathii (♂)	IEBR.Tu.18.5.17.3	Lien Huong, Binh Thuan, VN	–	–	–	–
S. kuhlii (♂)	HNHM 92.121.1. (11548)	Calcutta, West Bengal, IN	–	–	–	–
S. kuhlii (♂)	HNHM 92.123.1. (11600)	Calcutta, West Bengal, IN	–	–	–	–
S. kuhlii (♂)	HNHM 92.123.2. (11601)	Calcutta, West Bengal, IN	–	–	–	–
S. kuhlii (♂)	HNHM 92.123.3. (11602)	Calcutta, West Bengal, IN	–	–	–	–
S. kuhlii (♂)	HNHM 92.123.4. (11603)	Calcutta, West Bengal, IN	–	–	–	–
S. kuhlii (♂)	HNHM 92.123.5. (11604)	Calcutta, West Bengal, IN	–	–	–	–
S. kuhlii (♂)	HNHM 92.123.6. (11605)	Calcutta, West Bengal, IN	–	–	–	–
S. kuhlii (♂)	HNHM 92.155.1. (11606)	Calcutta, West Bengal, IN	–	–	–	–
S. kuhlii (♂)	HNHM 92.123.7. (11608)	Calcutta, West Bengal, IN	–	–	–	–
S. kuhlii (♂)	HNHM 92.123.8. (11609)	Calcutta, West Bengal, IN	–	–	–	–

Taxon	**Museum/Sample code**	**Location(‡)**	**Genbank Accession No.**			
			***Cytb*(§)**	***COI*(§)**	***TUFM***	***ZFYVE27***
S. kuhlii (♂)	HNHM 93.34.1. (12100)	Ganespur, West Bengal, IN	–	–	–	–
S. kuhlii (♀)	HNHM 93.35.1. (12176)	Mettupalayam, Tamil Nadu, IN	–	–	–	–
S. kuhlii (♂)	HNHM 92.122.1. (11565)	Ruined Hindu temple, Konarka, Orissa, IN	–	–	–	–
S. kuhlii (♂)	MM3297(†)	Nay Pyi Daw, Myanmar: 7	MT820592 (9)	–	–	–
S. kuhlii (♂)	MM3298(†)	Nay Pyi Daw, Myanmar: 7	MT820576 (3)	–	–	–
S. kuhlii (♀)	MM3300(†)	Nay Pyi Daw, Myanmar: 7	MT820593 (9)	–	–	–
S. kuhlii (♀)	MM3301(†)	Nay Pyi Daw, Myanmar: 7	MT820577 (4)	–	–	–
S. kuhlii (♀)	MM3302(†)	Nay Pyi Daw, Myanmar: 7	MT820583 (8)	–	–	–
S. kuhlii (♀)	MM3303(†)	Nay Pyi Daw, Myanmar: 7	MT820594 (9)	–	–	–
S. kuhlii (♂)	MM3304(†)	Nay Pyi Daw, Myanmar: 7	MT820578 (1)	–	–	–
S. kuhlii (♀)	MM3305(†)	Nay Pyi Daw, Myanmar: 7	MT820579 (1)	–	–	–
S. kuhlii (♀)	UTHF.MM3168B1	Nay Pyi Daw, Myanmar: 7	MT820584 (9)	MT821492 (3)	–	–
S. kuhlii (♂)	UTHF.MM3169B2	Nay Pyi Daw, Myanmar: 7	MT820581 (6)	MT821503 (5)	–	–
S. kuhlii (♂)	UTHF.MM3170B3	Nay Pyi Daw, Myanmar: 7	MT820575 (2)	MT821491 (6)	–	–
S. kuhlii (♂)	UTHF.MM3171B4	Nay Pyi Daw, Myanmar: 7	MT820585 (9)	MT821502 (7)	–	–
S. kuhlii (♀)	UTHF.MM3172B5	Nay Pyi Daw, Myanmar: 7	MT820586 (9)	MT821499 (3)	–	–
S. kuhlii (♀)	UTHF.MM3173B6	Nay Pyi Daw, Myanmar: 7	Unassigned (9)	MT821498 (3)	–	–
S. kuhlii (♀)	UTHF.MM3174B7	Nay Pyi Daw, Myanmar: 7	MT820587 (9)	MT821500 (4)	–	–
S. kuhlii (♀)	UTHF.MM3175B8	Nay Pyi Daw, Myanmar: 7	MT820574 (1)	MT821490 (2)	–	–
S. kuhlii (♀)	UTHF.MM3176B9	Nay Pyi Daw, Myanmar: 7	MT820588 (9)	MT821497 (3)	–	–
S. kuhlii (♀)	UTHF.MM3182B13	Nay Pyi Daw, Myanmar: 7	MT820589 (9)	MT821496 (3)	–	–
S. kuhlii (♀)	UTHF.MM3183B14	Nay Pyi Daw, Myanmar: 7	MT820590 (9)	MT821495 (3)	–	–
S. kuhlii (♀)	UTHF.MM3184B15	Nay Pyi Daw, Myanmar: 7	MT820591 (9)	MT821501 (7)	–	–

Taxon	**Museum/Sample code**	**Location(‡)**	**Genbank Accession No.**			
			***Cytb*(§)**	***COI*(§)**	***TUFM***	***ZFYVE27***
S. kuhlii (♀)	UTHF.MM3185B16	Nay Pyi Daw, Myanmar: 7	MT820582 (7)	MT821494 (3)	–	–
S. kuhlii (♀)	UTHF.MM3186B17	Nay Pyi Daw, Myanmar: 7	MT820580 (5)	MT821493 (3)	–	–
S. kuhlii (♂)	IEBR.Tu.04.05.10.1 (T5000)	Xom Hau, Dong Anh, Hanoi, VN: 10	–	MT821515 (16)	MT820617	MT820623
S. kuhlii (♂)	IEBR.Tu.04.09.09.3 (T5001)	Xom Hau, Dong Anh, Hanoi, VN: 10	–	MT821516 (16)	–	–
S. kuhlii (♂)	IEBR.VN15-43 (VN6145)	Xom Hau, Dong Anh, Hanoi, VN: 10	MT820599 (15)	MT821486 (16)	–	–
S. kuhlii (♂)	VN15-44 (VN6146)(†)	Xom Hau, Dong Anh, Hanoi, VN: 10	MT820597 (13)	MT821488 (15)	–	–
S. kuhlii (♂)	IEBR.VN15-45 (VN6147)	Xom Hau, Dong Anh, Hanoi, VN: 10	MT820596 (12)	MT821489 (15)	–	–
S. kuhlii (♂)	IEBR.VN15-51 (VN6153)	Xom Hau, Dong Anh, Hanoi, VN: 10	MT820598 (14)	MT821487 (16)	–	–
S. kuhlii (♂)	IEBR.VN17-46 (VN7397)	Xom Hau, Dong Anh, Hanoi, VN: 10	–	Unassigned (16)	–	–
S. kuhlii (♂)	HNHM 98.46.1. (16574)	Vientiane, LA	–	–	–	–
S. kuhlii (♀)	IEBR.VN17-355 (VN7298)	Loi Hai, Ninh Thuan, VN: 21	–	–	–	–
S. kuhlii (♀)	IEBR.VN17-534 (VN7301)	Loi Hai, Ninh Thuan, VN: 21	MT820595 (11)	MT821482 (14)		
S. kuhlii (♂)	IEBR.VN17-539 (VN7304)	Lien Huong, Binh Thuan, VN	–	–	–	–
S. kuhlii (?)	IEBR.Tu.18.5.17.1	Lien Huong, Binh Thuan, VN	–	–	–	–
S. kuhlii (♀)	IEBR.VN19-04	Vinh Loi, Bac Lieu, VN	–	–	–	–
S. kuhlii (♀)	IEBR.VN19-05	Vinh Loi, Bac Lieu, VN	–	–	–	–
S. kuhlii (♀)	IEBR.VN19-06	Vinh Loi, Bac Lieu, VN	–	–	–	–
S. kuhlii (♀)	IEBR.VN19-07	Vinh Loi, Bac Lieu, VN	–	–	–	–
S. kuhlii (♀)	IEBR.VN19-08	Vinh Loi, Bac Lieu, VN	–	–	–	–
S. kuhlii (♀)	IEBR.VN19-09	Vinh Loi, Bac Lieu, VN	–	–	–	–
S. kuhlii (♀)	IEBR.VN19-10	Vinh Loi, Bac Lieu, VN	–	–	–	–
S. kuhlii (♀)	IEBR.VN19-11	Vinh Loi, Bac Lieu, VN	–	–	–	–
S. kuhlii (♀)	IEBR.VN19-12	Vinh Loi, Bac Lieu, VN	–	–	–	–

Taxon	**Museum/Sample code**	**Location(‡)**	**Genbank Accession No.**			
			***Cytb*(§)**	***COI*(§)**	***TUFM***	***ZFYVE27***
S. kuhlii (♀)	IEBR.VN19-13	Vinh Loi, Bac Lieu, VN	–	–	–	–
S. kuhlii (♂)	IEBR.VN19-14	Vinh Loi, Bac Lieu, VN	–	–	–	–
S. kuhlii (♀)	IEBR.VN19-15	Vinh Loi, Bac Lieu, VN	–	–	–	–
S. kuhlii (♂)	CBC01861	Bang Chureng, S'ang, Kandal, KH: 25	–	–	–	–
S. kuhlii (♀)	CBC01862	Bang Chureng, S'ang, Kandal, KH: 25	–	–	–	–
S. kuhlii (♀)	CBC01863	Bang Chureng, S'ang, Kandal, KH: 25	–	–	–	–
S. kuhlii (♀)	CBC01864	Bang Chureng, S'ang, Kandal, KH: 25	–	–	–	–
S. kuhlii (♂)	CBC01865	Bang Chureng, S'ang, Kandal, KH: 25	–	–	–	–
S. kuhlii (♂)	CBC01866 (VN11-1642)	Bang Chureng, S'ang, Kandal, KH: 25	–	MT821512 (12)	–	–
S. kuhlii (♂)	CBC01867 (VN11-1640)	Bang Chureng, S'ang, Kandal, KH: 25	–	MT821513 (11)	–	–
S. kuhlii (♂)	CBC01870 (VN11-1641)	Prey Toch, Moung Russey, Battambang, KH: 26	–	MT821514 (11)	–	–
S. kuhlii (♂)	CBC01871 (VN11-1639)	Prey Toch, Moung Russey, Battambang, KH: 26	–	MT821511 (1)	MT820617	MT820624
S. kuhlii (♂)	HNHM 98.14.27. (15672)	Selangor, Malaysia	–	–	–	–
S. kuhlii (♂)	UPLB-MNH paa1548 (MT1706)	Quezon city, Luzon Island, Philippines: 28	MT820600 (17)	MT821484 (16)	–	–
S. kuhlii (♂)	UPLB-MNH paa1550 (MT1707)	Quezon city, Luzon Island, Philippines: 28	MT820601 (17)	MT821483 (16)	–	–
S. kuhlii (♀)	UPLB-MNH paa1552 (MT1709)	Quezon city, Luzon Island, Philippines: 28	MT820602 (17)	MT821485 (16)	–	–
S. kuhlii (♂)	UPLB-MNH jdva1064 (3873)	Sibuyan Island, Philippines	–	–	–	–
S. kuhlii (♀)	UPLB-MNH jdva1065 (3874)	Sibuyan Island, Philippines	–	–	–	–
S. kuhlii (♀)	UPLB-MNH jdva1066 (3875)	Sibuyan Island, Philippines	–	–	–	–
S. ? solutatus	HNHM 2869.22 (ori. ID: *S. kuhlii*)	Java, Indonesia	–	–	–	–

SUPPORTING INFORMATION

Integrative taxonomy and biogeography of Asian yellow house bats (Vespertilionidae: *Scotophilus*) in the Indomalayan Region

Vuong Tan Tu, Tamás Görföl, Gábor Csorba, Satoru Arai, Fuka Kikuchi, Dai Fukui, Daisuke Koyabu, Neil M. Furey, Saw Bawm, Kyaw San Lin, Phillip Alviola, Chu Thi Hang, Nguyen Truong Son, Tran Anh Tuan, and Alexandre Hassanin

List of Alignments

Alignment S1. Alignment of *COI* sequences included in the TCS analysis.

Alignment S2. Alignment of *Cytb* sequences included in the TCS analysis.

Alignment S3. Alignment of *COI* sequences included in the phylogenetic analyses.

Alignment S4. Alignment of *Cytb* sequences included in the phylogenetic analyses.

Alignment S5. Alignment of *TuFM* sequences included in the phylogenetic analyses.

Alignment S6. Alignment of *ZFYVE27* sequences included in the phylogenetic analyses.

Alignment S7. Alignment of nuDNA sequences (combining *TuFM* and *ZFYVE27* genes) included in the phylogenetic analyses.

List of Tables

Table S1. List of species and subspecies of the Asian *Scotophilus* described as new to science prior to the mid-20th century.

Table S2. Genetic sequences of Asian *Scotophilus* spp. and outgroups in GenBank included in molecular analyses in the present study.

Table S3. Primers used to amplify and sequence DNA in the present study.

Table S4. mtDNA divergence within Asian *Scotophilus* spp.

Table S5. COI genetic variation in three clusters of geographic populations of *S. heathii* s.l..

Table S6. Factor loading for two first PCs obtained from PCA of cranial characters.

Table S7. Pairwise comparisons (ANOVA-tests) of morphometrics among geographical populations of *S. heathii* s.l. in Indochina.

List of Figures

* References cited in the Supporting Information

Table S1. List of species and subspecies of Asian *Scotophilus* described as new to science prior to the mid-20th century.

No.	Taxa	FA (in mm)	Type locality	Reference
1	*S. kuhlii*	41 (immature)	?, India	Leach, 1821
2	*Vespertilio temminckii*	47-51 (small)	Java	Horsfield, 1824
3	*Nycticejus heathii*	> *V. temminckii*	Mandas, Continental India	Horsfield, 1831
4	*Vespertilio belangeri*	55.9	Pondicharry, Coromandel, India	Geoffroy I., 1834
5	*Scotophilus fulvus*	Unknown	?, Java	Gray, 1843
6	*Nycticejus luteus*	large	Bengal, Coromandel	Blyth, 1851
7	*Nycticejus castaneus*	=*N. temminckii*	Malacca	Horsfield, 1851
8	*Nycticejus flaveolus*	Large	Continental India	Horsfield, 1851
9	*Nycticejus (?) swinhoei*	50.8	Amoy (=Xiamen), China	Blyth, 1860
10	*Scotophilus wroughtoni*	50	Kim (Surat), India	Thomas, 1897
11	*Scotophilus castaneus consobrinus*	50-52	Hainan Is. China	Allen, 1906
12	*S. kuhlii insularis*	64-67	Hainan Is. China	Allen, 1906
13	*Scotophilus gairdneri*	48	Paknampo, Central Siam	Kloss, 1917
14	*Pachyotis temminckii panayensis*	48	Panay Island, Philippines	Sody, 1928
15	*Pachyotis temminckii celebensis*	63.5-64	Toli-toli, Celebes	Sody, 1928
16	*Scotophilus temminckii collinus*	48-54	Bali	Sody, 1936
17	*Scotophilus castaneus solutatus*	50-56; holotype (55)	Tjandiroto, East Java	Sody, 1936

Table S2. Genetic sequences of Asian *Scotophilus* spp. and outgroups in GenBank included in molecular analyses in the present study. (†) –Locations of specimens included in molecular analyses are indicated by numbers (after colon) as representing in Figure 1. Country codes include: IN – India, KH – Cambodia; LA – Laos; and VN – Vietnam. (‡) – Numbers (in parentheses) after Genbank accession numbers for *Cytb* and *COI* sequences of studied *Scotophilus* specimens are respective haplotypes representing in Figures 1 and 2. Reference (§): 1 – Francis et al., 2010; 2 – Ikram et al., unpublished; 3 – Kruskop et al., unpublished; 4 –Rahman & Choudhury, unpublished; 5 – Srinivasulu et al., unpublished; 6 – Tan et al., 2020; 7 – Trujillo et al., 2009; 8 – Tu et al., 2014; 9 – Tu et al., 2017; and 10 – Tu et al., 2018.

Taxon	Location (†)	Genbank Accession No.				Ref (§)
		Cytb (‡)	*COI* (‡)	*TUFM*	*ZFYVE27*	
M. cyclotis	Ngoc Linh, Kon Tum, VN	MH137367	KF772775	MH137554	MH137584	[8,10]
E. pachyomus	Copia, Son La, VN	KX496340	KX496341	KX496347	KX496346	[9]
S. heathii	Kohat, Khyber Pakhtunkhwa, Pakistan: 1	–	MG550115 (5)	–	–	[2]
S. heathii	Swabi, Khyber Pakhtunkhwa, Pakistan: 2	–	MH712738 (1)	–	–	[2]
S. heathii	Swabi, Khyber Pakhtunkhwa, Pakistan: 2	–	MH716035 (2)	–	–	[2]
S. heathii	Fateh Jang, Attock, Punjab, Pakistan: 3	–	MG199233 (4)	–	–	[2]
S. heathii	Fateh Jang, Attock, Punjab, Pakistan: 3	–	MG199234 (4)	–	–	[2]
S. heathii	Fateh Jang, Attock, Punjab, Pakistan: 3	–	MG199235 (4)	–	–	[2]
S. heathii	Fateh Jang, Attock, Punjab, Pakistan: 3	–	MG199236 (4)	–	–	[2]
S. heathii	Fateh Jang, Attock, Punjab, Pakistan: 3	–	MG199237 (4)	–	–	[2]
S. heathii	Fateh Jang, Attock, Punjab, Pakistan: 3	–	MG199238 (4)	–	–	[2]
S. heathii	Fateh Jang, Attock, Punjab, Pakistan: 3	–	MG199240 (4)	–	–	[2]
S. heathii	Fateh Jang, Attock, Punjab, Pakistan: 3	–	MG199241 (4)	–	–	[2]

Taxon	Location [†]	Genbank Accession No.				Ref [§]
		Cytb [‡]	*COI* [‡]	*TUFM*	*ZFYVE27*	
S. heathii	Fateh Jang, Attock, Punjab, Pakistan: 3	–	MG199242 (4)	–	–	[2]
S. heathii	Fateh Jang, Attock, Punjab, Pakistan: 3	–	MG544111 (4)	–	–	[2]
S. heathii	Fateh Jang, Attock, Punjab, Pakistan: 3	–	MG550114 (4)	–	–	[2]
S. heathii	Fateh Jang, Attock, Punjab, Pakistan: 3	–	MG199239 (4)	–	–	[2]
S. heathii	Fateh Jang, Attock, Punjab, Pakistan: 3	–	MF495678 (4)	–	–	[2]
S. heathii	Fateh Jang, Attock, Punjab, Pakistan: 3	–	MG299068 (4)	–	–	[2]
S. heathii	Hampi, Bellary, Karnataka, IN: 5	–	MG821185 (3)	–	–	[5]
S. heathii	-, Yunnan, China: 8	EU750946 (1)	–	–	–	[7]
S. heathii	Nam Et national protected area, LA: 9	–	HM541921 (7)	–	–	[1]
S. heathii	Nam Et national protected area, LA: 9	–	HM541922 (7)	–	–	[1]
S. heathii	Tam Dao, Vinh Phuc, VN: 12	EU750945 (2)	–	–	–	[7]
S. heathii	Ban Paam, Attapu, LA: 16	–	HM541920 (11)	–	–	[1]
S. heathii	Ban Paam, Attapu, LA: 16	–	HM541923 (17)	–	–	[1]
S. heathii	Dong Hua Sao, Champasak, LA: 17	–	HM541924 (14)	–	–	[1]
S. heathii	Dong Hua Sao, Champasak, LA: 17	–	HM541925 (14)	–	–	[1]
S. heathii	Yok Don NP, Dak Lak, VN: 20	–	HM541926 (15)	–	–	[1]
S. heathii	Yok Don NP, Dak Lak, VN: 20	EU750944 (3)	–	–	–	[7]
S. kuhlii	Tadlapet, Adilabad, Telangana, IN: 5	–	MG821195 (7)	–	–	[5]
S. kuhlii	Cachar, Assam, IN: 6	–	KT291764 (7)	–	–	[4]
S. kuhlii	Tam Dao, Vinh Phuc, VN: 12	EU750931 (16)	–	–	–	[7]

Taxon	Location (†)	Genbank Accession No.				Ref (§)
		Cytb (‡)	*COI* (‡)	*TUFM*	*ZFYVE27*	
S. kuhlii	Tam Dao, Vinh Phuc, VN: 12	EU750913 (13)	–	–	–	[7]
S. kuhlii	-, Guangxi, China: 13	LC426467 (18)	–	–	–	[6]
S. kuhlii	-, Guangxi, China: 14	LC426465 (17)	–	–	–	[6]
S. kuhlii	-, Guangxi, China: 14	LC426466 (17)	–	–	–	[6]
S. kuhlii	-, Guangxi, China: 14	LC426468 (17)	–	–	–	[6]
S. kuhlii	Savannakhet, LA: 15	–	HM541934 (16)	–	–	[1]
S. kuhlii	Cat Tien, Lam Dong, VN: 22	–	HM541935 (17)	–	–	[1]
S. kuhlii	Cat Tien, Lam Dong, VN: 22	–	HM541936 (19)	–	–	[1]
S. kuhlii	Cat Tien, Lam Dong, VN: 22	EU750924 (24)	–	–	–	[7]
S. kuhlii	Cat Tien, Lam Dong, VN: 22	–	HM541937 (13)	–	–	[1]
S. kuhlii	Cat Tien, Lam Dong, VN: 22	EU750925 (10)	–	–	–	[7]
S. kuhlii	Cat Tien, Lam Dong, VN: 22	–	HM541938 (11)	–	–	[1]
S. kuhlii	Cat Tien, Lam Dong, VN: 22	EU750926 (20)	–	–	–	[7]
S. kuhlii	Cat Tien, Lam Dong, VN: 22	–	HM541940 (9)	–	–	[1]
S. kuhlii	Cat Tien, Lam Dong, VN: 22	–	JF444113 (10)	–	–	[3]
S. kuhlii	Cat Tien, Lam Dong, VN: 22	–	JF444114 (8)	–	–	[3]
S. kuhlii	Cat Tien, Lam Dong, VN: 22	EU750923 (19)	–	–	–	[7]
S. kuhlii	Cat Tien, Lam Dong, VN: 22	EU750927 (21)	–	–	–	[7]
S. kuhlii	Ho Chi Minh, VN: 23	–	HM541927 (20)	–	–	[1]
S. kuhlii	Soc Trang, Soc Trang, VN: 24	–	HM541929 (21)	–	–	[1]

Taxon	Location (†)	Genbank Accession No.				Ref (§)
		Cytb (‡)	*COI* (‡)	*TUFM*	*ZFYVE27*	
S. kuhlii	Soc Trang, Soc Trang, VN: 24	–	HM541932 (22)	–	–	[1]
S. kuhlii	Soc Trang, Soc Trang, VN: 24	EU750930 (27)	–	–	–	[7]
S. kuhlii	Soc Trang, Soc Trang, VN: 24	–	HM541933 (21)	–	–	[1]
S. kuhlii	Soc Trang, Soc Trang, VN: 24	EU750929 (23)	–	–	–	[7]
S. kuhlii	Soc Trang, Soc Trang, VN: 24	–	HM541930 (18)	–	–	[1]
S. kuhlii	Soc Trang, Soc Trang, VN: 24	–	HM541931 (1)	–	–	[1]
S. kuhlii	Jitra, Kedah State, Malaysia: 27	EU750920 (19)	–	–	–	[7]
S. kuhlii	Jitra, Kedah State, Malaysia: 27	EU750922 (25)	–	–	–	[7]
S. kuhlii	Jitra, Kedah State, Malaysia: 27	EU750915 (26)	–	–	–	[7]
S. kuhlii	- , Luzon Island, Philippines: 28	EU750914 (17)	–	–	–	[7]

Table S3. Primers used to amplify and sequence DNA in the present study

Gene	Primer sets (5’-3’)	Amplicon length (bp)	Sources
Cytb	Mt-L14724F: CGAGATCTGAAAAACCATCGTTG Cytb-H15915R: AACTGCAGTCATCTCCGGTTTACAAGA	~ 1190	Irwin, Kocher, & Wilson, 1991.
	Cy-14726F: GACYARTRRCATGAAAAAYCAYCGT TGT Cy- 15909R: CYYCWTYIYTGGTTTACAAGACYAG	~ 1180	Arai et al., 2016
COI	MammMt-5533F: CYCTGTSYTTRRATTTACAGTYYAA MammMt-7159R: GRGGTTCRAWWCCTYCCTYTCTT	~ 1620	Arai et al., 2019
	UTyr: ACCYCTGTCYTTAGATTTACAGTC C1L705: ACTTCDGGGTGNCCRAARAATCA	~ 750	Hassanin et al., 2013
TUFM	TUFM-EX9U: CTGACTTGGGACATGGCCTGTCG TUFM-EX10L: ACGCTGGCCTTTYTCTAAGATCAT	~ 700	Hassanin et al., 2013
ZFYVE27	ZFYVE27-EX6U: GAATGTGGAGTTCTTCCGAG ZFYVE27-EX7L: GGGTTCATCCGCCGCTGCAGA	~ 750-800	Hassanin et al., 2013

Table S4. mtDNA divergence within Asian *Scotophilus* spp.

	***S. kuhlii.* s.l.**		***S. heathii* s.l**	
	***COI* (576nt)**	***Cytb* (1140nt)**	***COI* (576nt)**	***Cytb* (1140nt)**
Number of locations	13	10	13	6
Number of sequences	43	46	39	12
Number of segregating sites	41	69	69	78
Number of haplotypes	22	27	18	7
Haplotype diversity (Hd)	0.932	0.922	0.865	0.879
Nucleotide diversity (π)	0.013	0.010	0.034	0.025

Table S5. *COI* genetic variation in three clusters of geographic populations of *S. heathii* s.l..

S5A - mtDNA divergence within three populations of *S. heathii.*

	Indian Subcontinent (Pakistan + India)	Northern Indochina	Southern Indochina
Number of locations	4	4	6
Number of sequences	18	8	13
Number of segregating sites	16	5	20
Number of haplotypes	5	4	9
Haplotype diversity (Hd)	0.405	0.821	0.95
Nucleotide diversity (π)	0.0038	0.0035	0.01

S5B - Molecular variance (AMOVA) of *COI* variation in three populations of *S. heathii* s.l..

Source of variation	d.f.	SSD	Variance component	% variation	F-statistics	P values
Within populations	36	24.024	0.334	68.7	Fis=1.000	0.001
Among populations	2	8.848	0.152	31.3	Fst=0.313	0.001

S5C - Pairwise estimates of F_{ST} (below diagonal) and closest geographic distances (Km) (above diagonal) between three geographic populations of *S. heathii* s.l..

	Indian Subcontinent (Pakistan + India)	Northern Indochina	Southern Indochina
Indian Subcontinent (Pakistan + India)		2903	3116
Northern Indochina	0.433***		589
Southern Indochina	0.343***	0.11**	

*** – p ≤0.001; ** – 0.001≤p≤ 0.05

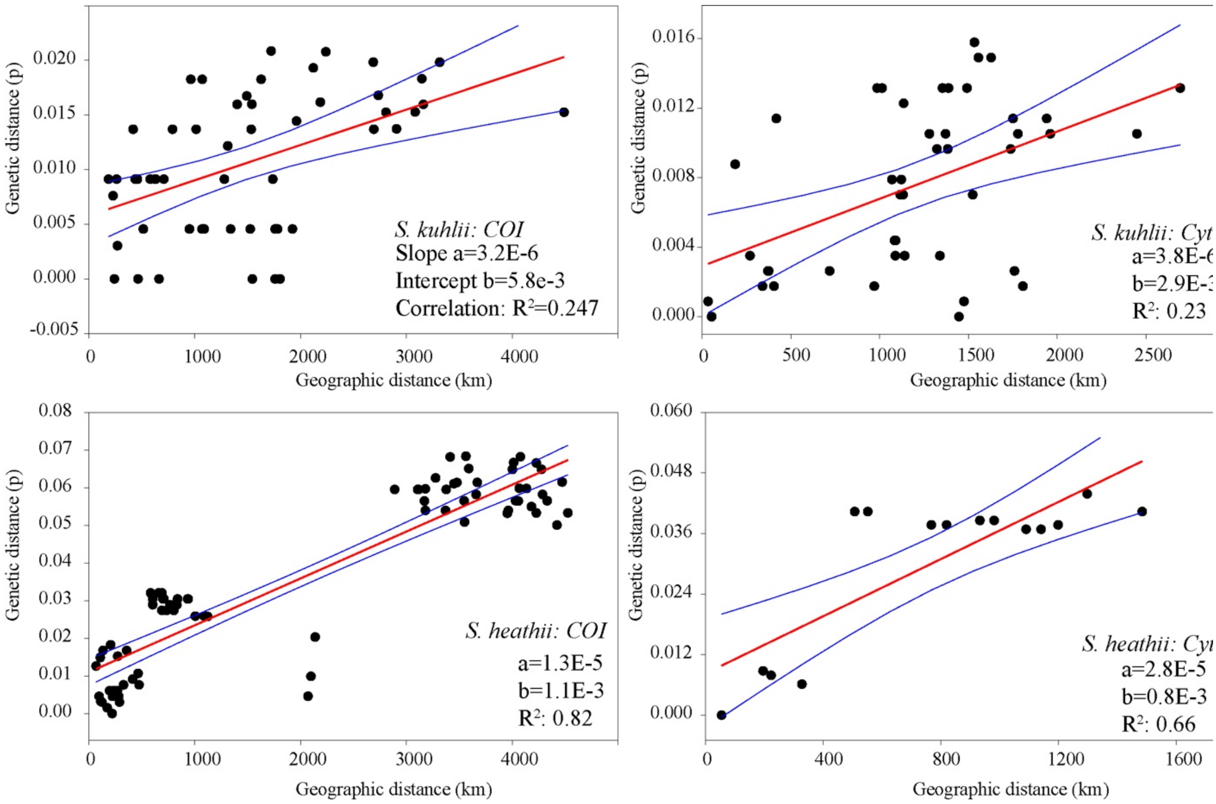
Table S6. Factor loading for two first PCs from PCA of cranial characters.

Characters	**PC 1**	**PC 2**
GSL	0.3500	-0.2112
SL	0.3374	-0.3527
CBL	0.2997	-0.1891
CCL	0.3054	-0.0987
ZB	0.3346	-0.2892
C^1C^1	0.3356	0.7576
M^3M^3	0.2881	0.0963
CM^3	0.2835	0.2506
ML	0.3161	-0.1212
CM_3	0.3045	0.2030
Eigenvalue	0.0106	0.0003
% variance	94.184	2.3724

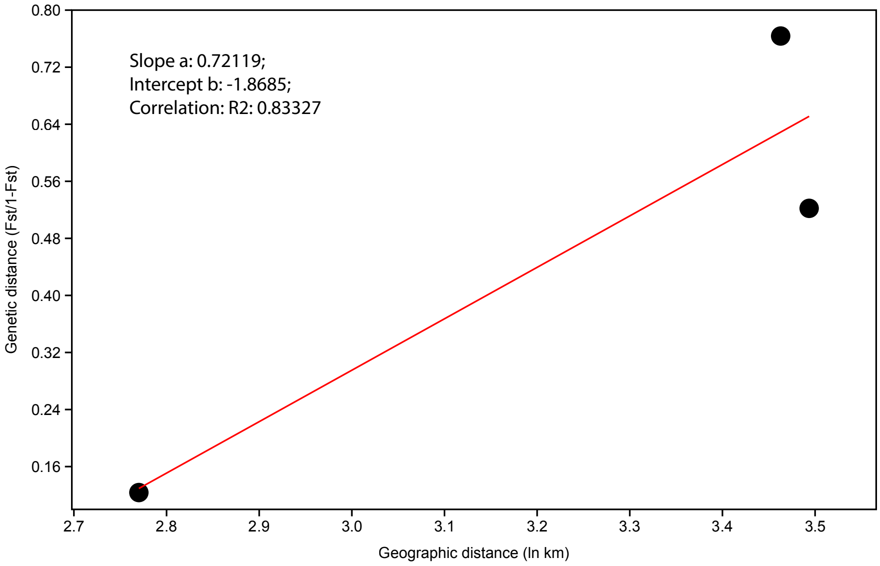
Table S7. Pairwise comparisons (ANOVA-tests) of morphometrics among geographical populations of *S. heathii* s.l. in Indochina. Acronyms and definitions for measurements are given in the Materials and Methods.

Character	**North VN**			**Highland Central VN**		**South Central VN**
	Highland Central VN (Location: 18)	**South Central (Location: 21 and nearby)**	**Cambodia (Location: 19)**	**South Central (Location: 21 and nearby)**	**Cambodia (Location: 19)**	**Cambodia (Location: 19)**
FA		***		***	***	-*
Tib				***	***	
GSL	-***	**		***	***	
SL	-***	**		***	***	
CBL	-***	***		***	***	-*
CCL	-***	***		***	***	-*
ZB	-***	**		***	***	-**
C^1C^1		***		**		
M^3M^3		**		***	**	
CM^3		***	**	***	***	-*
ML	-**	***		***	***	-*
CM_3		***		***	**	-*

Level of statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



A: Correlation between pairwise genetic divergences (minimum p-distance) and geographic distance (km) among geographic populations of Asian Scotophilus spp.



B: Correlation between pairwise genetic divergences and geographic distance among three groups of matrilines of S. heathii inferred from the COI TCS network analyses

Figure S1: Mantel tests for isolation by distance effects on pairwise genetic divergences among geographic populations of studied Scotophilus spp.

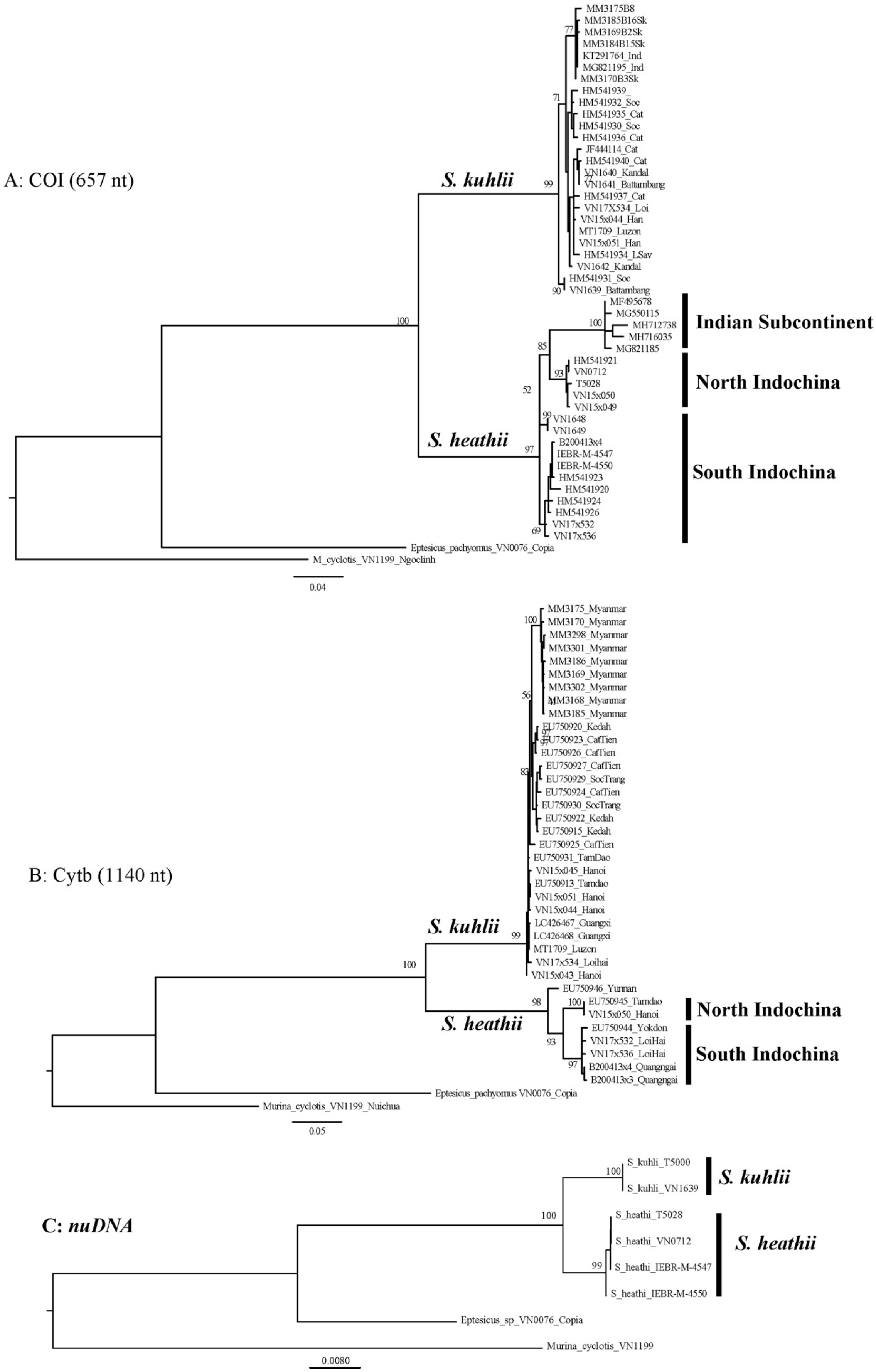


Figure S2. ML trees of Asian *Scotophilus* spp. and outgroups reconstructed from *COI*, *Cytb* and *nuDNA* sequences.

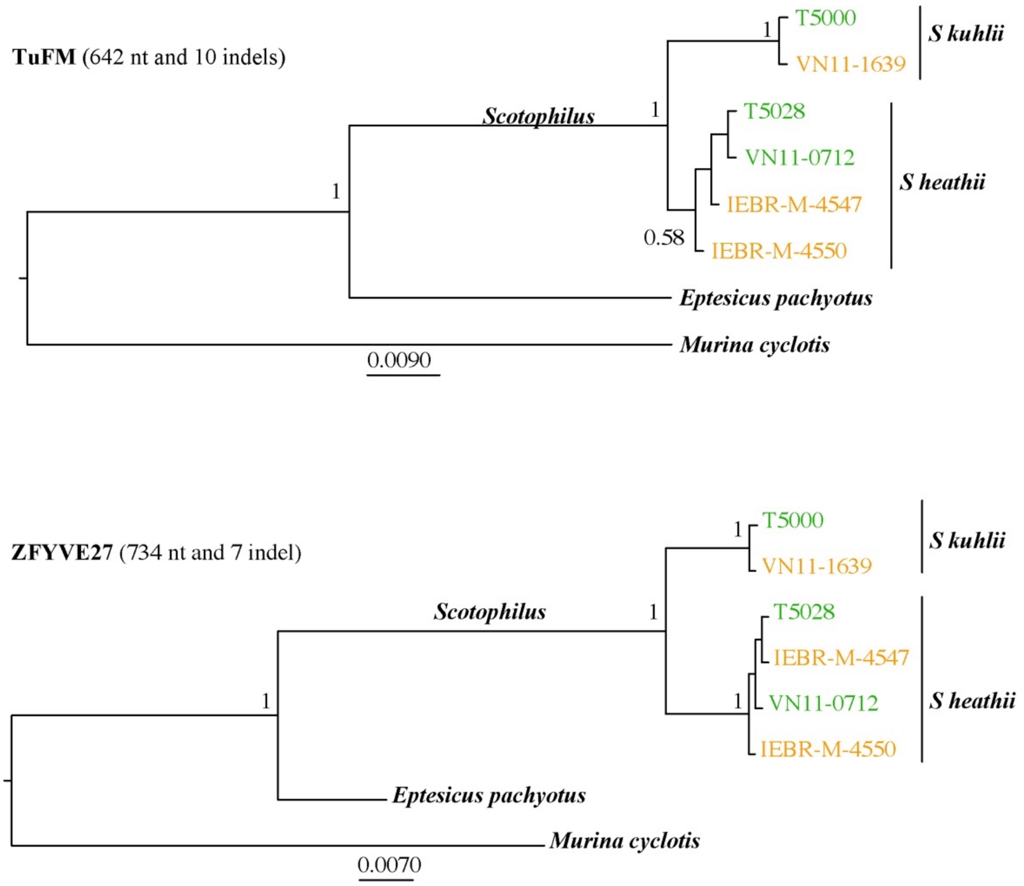
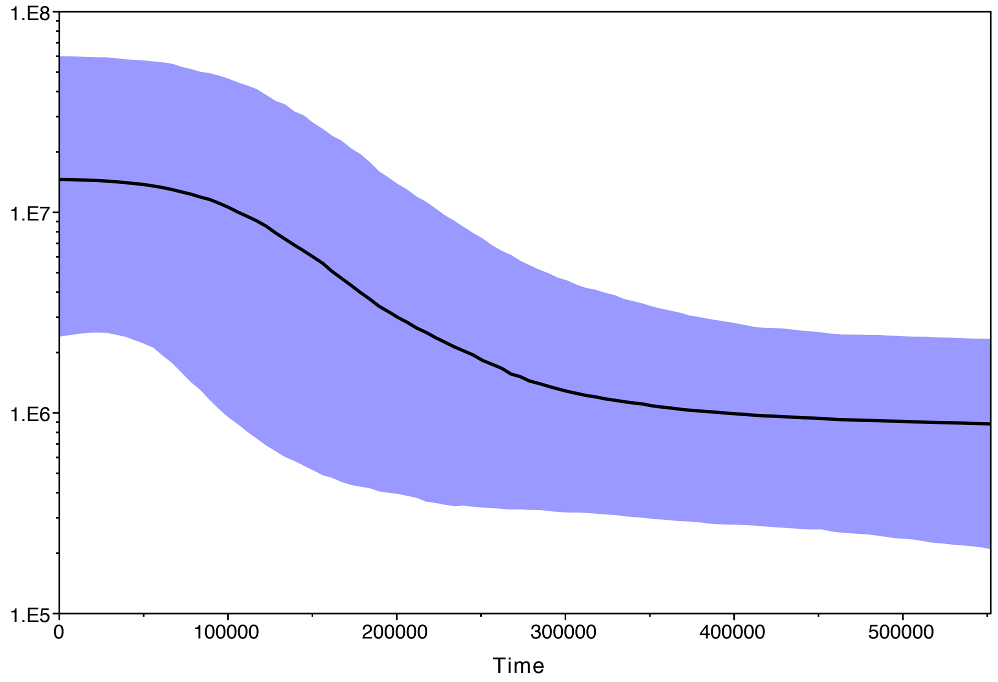


Figure S3. Bayesian trees of Asian *Scotophilus* spp. and outgroups reconstructed from *TUFM* and *ZFYVE27* sequences



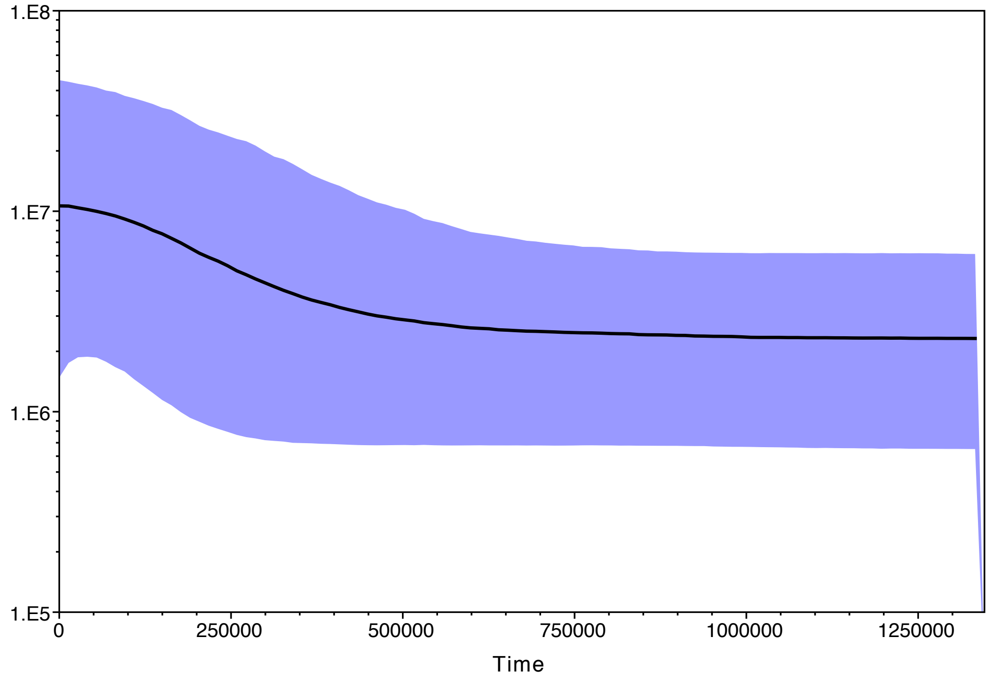


Figure S4. Bayesian skyline plot representing historical demographic trends in *S. kuhlii* (above) and *S. heathii* (below). The *X*-axis presents time as years before present. The Y-axis represents the effective female population size (*N*e). Mean estimated Ne represents as the solid line, whereas solid intervals refer the 95% highest probability density.

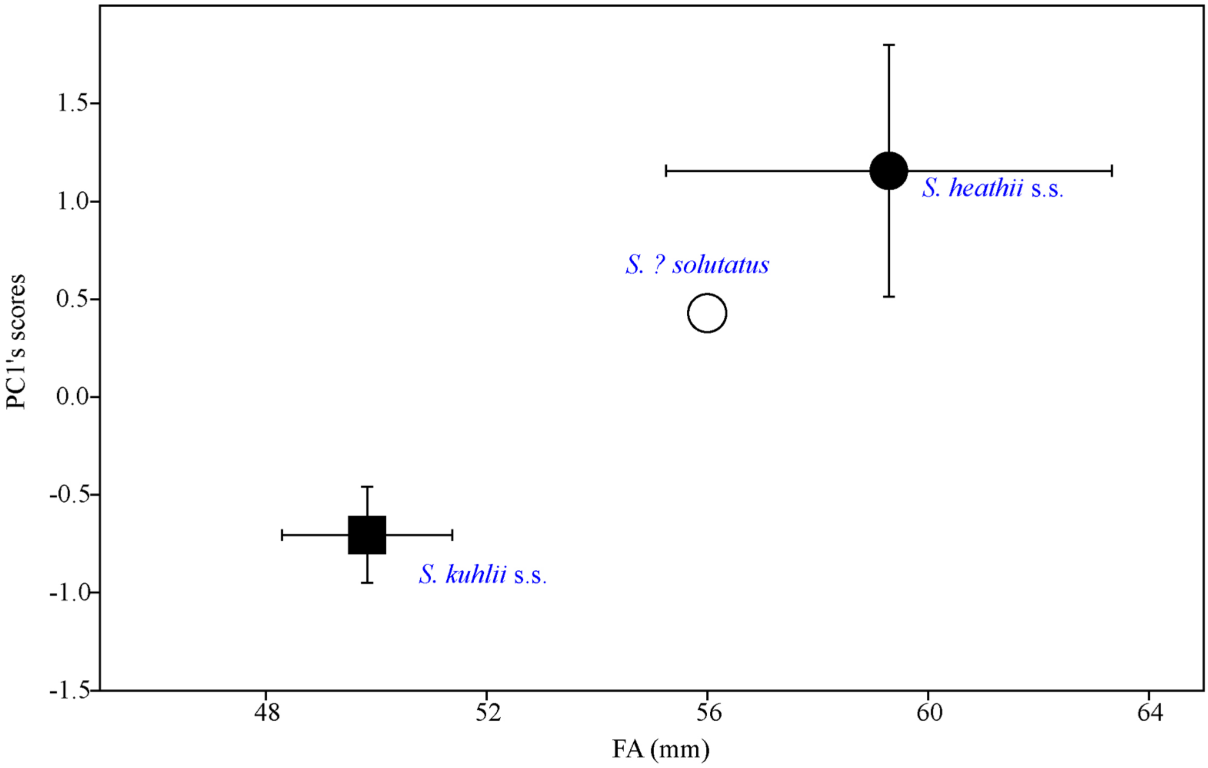


Figure S5. Correlation between mean values of PC1's scores obtained from PCA of skull characters and FA values of Asian *Scotophilus* spp.

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