European Journal of Taxonomy 274: 1–38
http://dx.doi.org/10.5852/ejt.2017.274

ISSN 2118-9773
www.europeanjournaloftaxonomy.eu
2017 · Tu V.T. *et al.*

DNA Library of Life, research article

urn:lsid:zoobank.org:pub:DEFAD552-9C2E-497B-83CA-1E04E3353EA4

Comparative phylogeography of bamboo bats of the genus *Tylonycteris* (Chiroptera, Vespertilionidae) in Southeast Asia

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Abstract. In Southeast Asia, bats of the genus *Tylonycteris* Peters, 1872 have traditionally been classified into two wide-ranging species, *T. pachypus* (Temminck, 1840) and *T. robustula* Thomas, 1915. Our comparative phylogeographic analyses based on two mitochondrial and seven nuclear genes, combined with our multivariate morphological analyses, show that these species actually represent cryptic species complexes that share a similar biogeographic history in three major regions, i.e., Sundaland, southern

Indochina, and northern Indochina. Our molecular dating estimates suggest that Pleistocene climatic oscillations and sea level changes have repeatedly isolated ancestral populations of *Tylonycteris* spp. in distant bamboo forest refugia. The analyses indicate, however, that populations of the *T. pachypus* complex were less affected by forest fragmentation in mainland Southeast Asia than those of the *T. robustula* complex. Accordingly, we propose several taxonomic changes within the genus *Tylonycteris*: the species *T. fulvida* and *T. malayana* are revalidated, and a new species, *T. tonkinensis* Tu, Csorba, Ruedi & Hassanin sp. nov., endemic to northern Indochina, is described.

Keywords. Vespertilioninae, *Tylonycteris*, DNA phylogeny, taxonomy, biogeography.

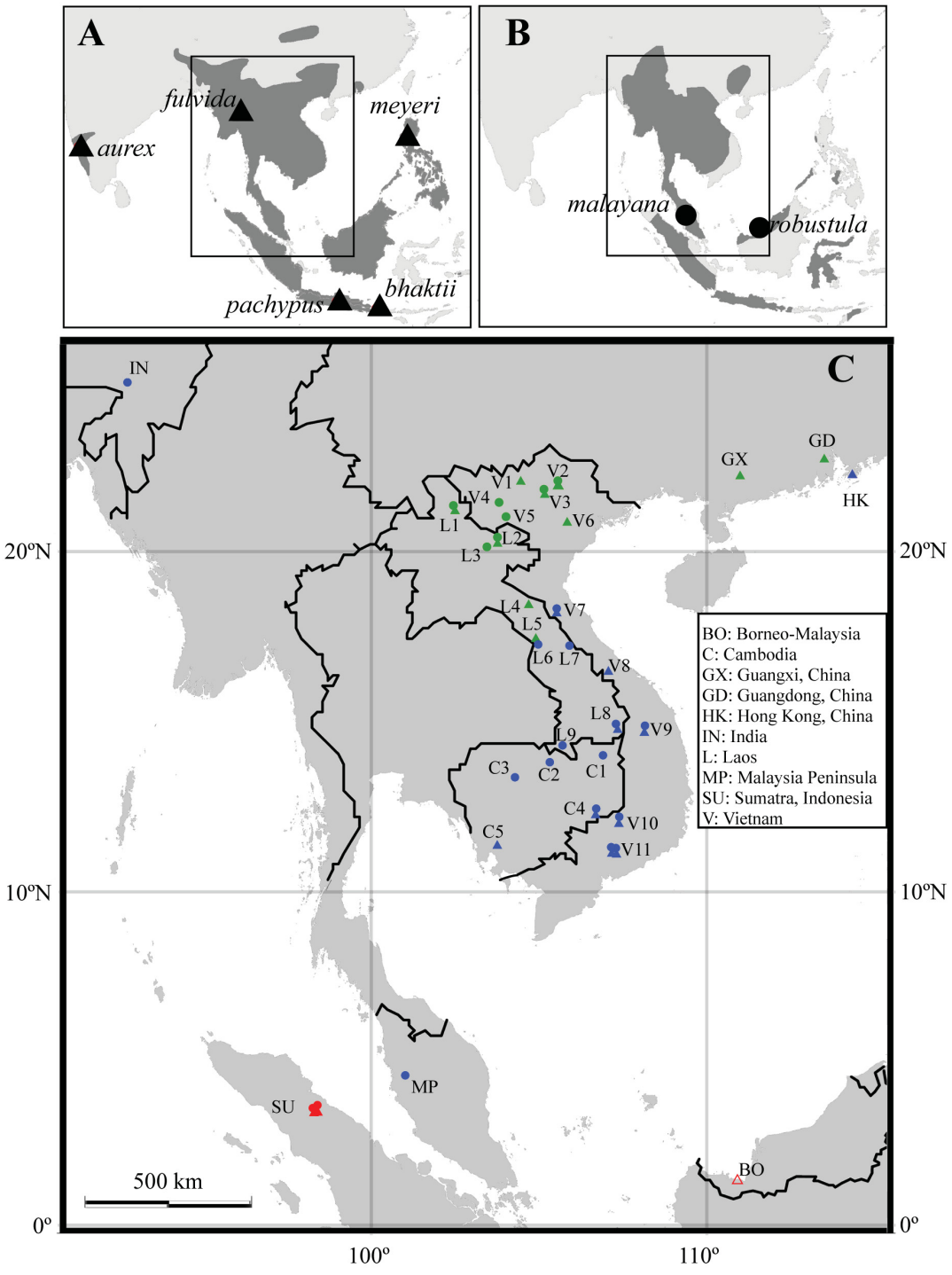
Tu V.T., Csorba G., Ruedi M., Furey N.M., Son N.T., Thong V.D., Bonillo C. & Hassanin A. 2017. Comparative phylogeography of bamboo bats of the genus *Tylonycteris* (Chiroptera, Vespertilionidae) in Southeast Asia. *European Journal of Taxonomy* 274: 1–38. http://dx.doi.org/10.5852/ejt.2017.274

Introduction

Bamboo bats of the genus *Tylonycteris* Peters, 1872 (Chiroptera, Vespertilionidae) are small-sized bats (weight: 3–10 g; forearm length: 22–32 mm) characterized by a dorsoventrally flattened skull and well-developed, fleshy pads at the base of the thumb and on the sole of the foot (Tate 1942). These morphological features are thought to be adaptations for roosting in small cavities with smooth surfaces such as the internodes of bamboo stalks or narrow crevices in trees and rocks (Feng *et al.* 2008; Medway & Marshall 1970; Thewissen & Etnier 1995). Classically, the genus was regarded as containing only two species, *T. pachypus* (Temminck, 1840) and *T. robustula* Thomas, 1915, with several further taxa included as subspecies (Fig. 1) (Tate 1942; Simmon 2005). More recently, Feng *et al.* (2008) described a third species, *T. pygmaea* Feng, Li & Wang, 2008, which is smaller than its congeners. Whereas *T. pygmaea* is considered to be endemic to Yunnan Province in southern China, the two other species have much more extensive geographic ranges that greatly overlap in Southeast Asia (Fig. 1). The range of *T. pachypus* is even more extended in the north and west, with apparently two isolated populations recorded in the Chinese provinces of Sichuan and Chongqing, and in southern India around the Western Ghats (Fig. 1) (Bates *et al.* 2008a, 2008b).

Bats of the genus *Tylonycteris* are usually associated with bamboo groves in both intact and disturbed habitats at elevations ranging from lowland up to 1500 m (Bates *et al.* 2008a, 2008b; Medway & Marshall 1970). All species can often be found roosting within bamboo internodes in colonies of up to 40 individuals, entering through narrow vertical slits created by long-horned beetles (Medway & Marshall 1970; Zhang *et al.* 2007). Females are gregarious, whereas males tend to be more solitary (Medway & Marshall 1972). At least occasionally, different species of *Tylonycteris* can occupy the same bamboo chamber (Feng *et al.* 2008; Medway & Marshall 1970). Zhang *et al.* (2005) determined that the diet of *Tylonycteris* bats is mainly composed of insects of the order Hymenoptera, which were regarded as significant pests of bamboo. Interestingly, the highest richness in bamboo species in the Asia-Pacific Region has been recorded in southern China (Bystriakova *et al.* 2003a; Yuming *et al.* 2004), the only region where the three species of *Tylonycteris* occur in sympatry. Taken together, these aspects indicate that *Tylonycteris* species are strongly dependent on bamboo vegetation.

DNA barcodes corresponding to the 5' fragment of the mitochondrial DNA (mtDNA) gene of cytochrome c oxidase subunit I (*CO1*) have been shown to be useful in species identification. Those published by Francis *et al.* (2010) for 9 and 15 samples of *T. pachypus* and *T. robustula*, respectively, have revealed high levels of intraspecific genetic variation in both taxa, suggesting the possible existence of cryptic species. More recently, Huang *et al.* (2014) showed that bats assigned to *T. pachypus fulvida* (Blyth, 1859) collected from South China and nearby regions differed karyotypically and morphologically from



mtDNA haplogroups

- ***T. pachypus*:** ▲- Sumatra (Tp1), △- Borneo, ▲- South Indo-Burma (Tp2), ▲- North Indo-Burma (Tp3)
- ***T. robustula*:** ● - Sumatra (Tr1), ● - South + West Indo-Burma (Tr2), ● - North Indo-Burma (Tr3)

Fig. 1. A–B. Maps of Asia showing the distribution range (shaded) and type localities of described subspecies of *T. pachypus* (Temminck, 1840) and *T. robustula* Thomas, 1915 (Bates *et al*. 2008a, 2008b). **C**. Localities of *Tylonycteris* specimens included in this study. Triangles and circles refer to *T. pachypus* and *T. robustula*, respectively; the colours indicate the mtDNA haplogroups found in the Bayesian analyses of *COI* and *Cytb* sequences (see Fig. 2 for details).

T. p. pachypus found in Peninsular Malaysia and Indonesia, suggesting that *T. pachypus* might be a species complex.

We present here a comprehensive comparative phylogeographic investigation of *Tylonycteris* spp. based on molecular and morphological data. Two mitochondrial markers and seven nuclear markers were sequenced to address the following questions: (1) how many mtDNA haplogroups exist in mainland Southeast Asia; (2) are these haplogroups corroborated by nuclear DNA markers; and (3) do these genetically defined groups correspond to distinct morphotypes? The ultimate goal of this study was to improve our understanding of the biogeography and taxonomy of the genus *Tylonycteris*.

Material and methods

Data sampling for genetic analyses

For this study, 63 tissue samples were collected during field expeditions within Indochina and from historical specimens housed in museum collections. Vouchers were deposited in the following institutions: the Institute of Ecology and Biological Resources (IEBR, Hanoi, Vietnam), the Muséum national d'Histoire naturelle (MNHN, Paris, France), the Muséum d'histoire naturelle de Genève (MHNG, Geneva, Switzerland), the Hungarian Natural History Museum (HNHM, Budapest, Hungary), the Centre for Biodiversity Conservation (CBC, Royal University of Phnom Penh, Cambodia), Rijksmuseum van Natuurlijke Histoire (RMNH, Leiden, Netherlands; now Naturalis Biodiversity Center) and the Zoological Museum Amsterdam (ZMA, Amsterdam, Netherlands). In addition, we included DNA sequences of *Tylonycteris* available in the GenBank nucleotide database. The origins of all samples are represented in Fig. 1 and detailed in Appendices 1 and 2.

Bats were captured using mist nets (Ecotone, Poland) and four-bank harp traps. They were measured, photographed and initially identified following morphological criteria provided by Bates & Harrison (1997), Borisenko & Kruskop (2003) and Francis (2008). Tissue samples for genetic study were collected from chest muscles of voucher specimens or from the patagium (biopsy punches; 3 mm diameter) of released bats. Tissue samples were preserved in 95% ethanol and stored at -20°C.

DNA extraction, amplification, sequencing

Total DNA was extracted from muscle or patagium samples using the QIAGEN DNAeasy Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Two mitochondrial genes were sequenced for this study: the 5' fragment of the *COI* gene and the complete cytochrome b (*Cytb*) gene. The primers used for PCR amplification of mitochondrial genes were UTyrLA and C1L705 for *COI* (Hassanin *et al.* 2012), and Thr-CH (Hassanin *et al.* 2014) and the newly designed Glu-CH (5'-AAY CAC CGT TGT AYT TCA ACT A-3') for *Cytb*. From DNA extracted from five old museum vouchers, it was not possible to amplify long PCR fragments (> 300 nt). Therefore, two further primer sets were used to amplify and sequence two overlapping fragments of *COI*: UTyrLA and Tyl-L122 (5'-AGY ART GCT CCT GGY TGA CC-3'), and Tyl-U86 (5'-GGT GCC TGA GCT GGC ATA GT-3') and Tyl-L266 (5'-TCG GGG RAA TGC TAT ATC AG-3').

The following seven nuclear markers were also sequenced for three outgroup species and 14 samples of *Tylonycteris* representing the divergent mtDNA haplotypes of Indochina: intron 2 of CHPF2 (chondroitin polymerizing factor 2), intron 6 of HDAC1 (histone deacetylase 1), intron 10 of HDAC2 (histone deacetylase 2), intron 2 of PABPN1 (poly(A) binding protein, nuclear 1), intron 6 of RIOK3 (RIO kinase 3), intron 9 of TUFM (elongation factor Tu, mitochondrial precursor), and intron 6 of ZFYVE27 (zinc finger, FYVE domain containing 27). The primers used for PCR amplifications of nuclear introns are detailed in Hassanin *et al.* (2013). For Sumatran specimens, which were collected more than one hundred years ago, it was not possible to obtain successful PCR amplification of nuclear markers.

Amplifications were done in 20 µl using 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 0.5–1.0 µl of the two primers at 10 µM. The standard PCR conditions were as follows: 4 min at 95°C; 5 cycles of denaturation/annealing/extension for 45 s at 95°C, 1 min at 60°C and 1 min at 72°C, followed by 30 cycles of 30 s at 95°C, 45 s at 55°C, and 1 min at 72°C, followed by 10 min at 72°C. 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). The sequences were edited and assembled using Codoncode Alignment v. 3.7.1 (CodonCode Corporation) and Sequencher v. 5.0 (Gene Codes Corporation). Heterozygous positions (double peaks) were scored using the IUPAC ambiguity codes. Sequences generated for this study were deposited in the GenBank database (accession numbers KX496340–KX496537; Appendix 1).

Phylogeographic analyses based on mitochondrial sequences

The 63 *COI* and 19 *Cytb* sequences newly generated in this study were compared to the 38 *COI* and four *Cytb* sequences available for *Tylonycteris* in GenBank. DNA sequences were aligned with Se-Al v. 2.0a11 (Rambaut 2002). Phylogenetic analyses of bats of the genus *Tylonycteris* were initially performed using two separate datasets: (1) *COI* (101 taxa and 728 nt), and (2) *Cytb* (23 taxa and 1140 nt). Three newly sequenced species, representing three different genera of the subfamily Vespertilioninae (*Pipistrellus* cf. *javanicus* Gray, 1838, *Eptesicus* sp. and *Hypsugo pulveratus* (Peters, 1870)), were chosen as outgroups on the basis of previous studies (Jones *et al.* 2002; Roehrs *et al.* 2010).

The Bayesian approach was used to reconstruct phylogenetic relationships. The best-fitting model of sequence evolution was selected under jModelTest (Posada 2008) using the Akaike Information Criterion (AIC). Bayesian analyses were then conducted with MrBayes v. 3.2.1 (Ronquist *et al.* 2012) using the selected HKY + G and GTR + I + G models, respectively for *COI* and *Cytb* datasets. The posterior probabilities (PP) were calculated using four independent Markov chains run for 10 000 000 Metropolis-coupled MCMC generations, with trees sampled every 1000 generations and a burn-in of 25%. Pairwise genetic distances between groups were calculated with PAUP* v. 4b10 (Swofford 2003) using the Kimura 2-parameter (K2P) correction.

Phylogenetic analyses based on eight independent markers

The phylogeny of *Tylonycteris* was further investigated using a reduced sample of 14 specimens (plus three outgroups) sequenced for multiple loci, including seven nuclear introns and a combination of two mtDNA markers (Appendix 1). These analyses were performed to test possible discordance between the phylogenetic signals extracted from independent markers. We did not obtain high quality sequences for three PCR products: *CHPF2* for two specimens of *T. pachypus* (T5009 and VN11-1138) and *PABPNI* for *Eptesicus* sp. (VN11-0076) (Appendix 1). These poor-quality sequences were not included in the alignments and coded as missing data (Ns) in the multilocus analyses. Accordingly, 10 datasets were analyzed: supermatrix (combining all the nine genes; 7572 nt), nuDNA (combining all the seven nuclear introns; 5604 nt), mtDNA (*COI* + *Cytb*; 1868 nt), *CHPF2* (858 nt), *HDAC1* (1128 nt), *HDAC2* (639 nt), *PABPNI* (677 nt), *RIOK3* (915 nt), *TUFM* (655 nt) and *ZFYVE27* (732 nt). DNA alignments were done with Se-Al v. 2.0a11 (Rambaut 2002). A few gaps were included in the alignments of the nuclear introns, but their positions were not found to be ambiguous. The models of nucleotide evolution were selected under jModeltest (Posada 2008): the GTR + I + G model for *mtDNA* markers, the HYK + G model for *CHPF2*, *PABPNI* and *ZFYVE27*, and the HKY model for *HDAC1*, *HDAC2*, *RIOK3* and *TUFM*.

A partitioned Bayesian approach was used to account for the combination of markers with contrasted molecular properties. The mtDNA dataset was run using a GTR + I + G model for each of the three codon

positions of the two mitochondrial genes; the concatenation of seven nuclear introns and the supermatrix were run using the selected model for each gene partition. The indels shared by at least two taxa and without ambiguity in the position of the gaps were coded as additional characters ("1": insertion; "0": deletion) and analyzed as a separate partition in the Bayesian analyses. The posterior probabilities (PP) were calculated using four independent Markov chains run for 10 000 000 Metropolis-coupled MCMC generations, with tree sampling every 1000 generations, and a burn-in of 25%.

Bootstrap percentages (BP) were computed by PAUP* v. 4b10 (Swofford 2003) after 1000 replicates, using the GTR + I + G model selected by jModelTest for the supermatrix dataset. The results obtained from the separate Bayesian analyses of the eight independent molecular markers (mtDNA and the seven nuclear introns) were also analyzed for congruence using the SuperTRI method (Ropiquet *et al.* 2009). The lists of bipartitions obtained from the eight Bayesian analyses were transformed into a weighted binary matrix for supertree construction using SuperTRI v. 57 (available from http://www.normalesup.org/~bli/Programs/programs.html). Each binary character corresponds to a node, which was weighted according to its frequency of occurrence in one of the eight lists of bipartitions. In that way, the SuperTRI method takes into account both principal and secondary signals, because all phylogenetic hypotheses found during the eight separate analyses are represented in the weighted binary matrix used for supertree construction. The reliability of the nodes was assessed using three different measures. The first value is the Supertree Bootstrap Percentage (SBP), which was calculated under PAUP* v. 4b10 after 1000 BP replicates of the weighted binary matrix reconstructed with SuperTRI (941 characters; heuristic search). The second value is the ''Mean Posterior Probability'' (MPP) calculated using the lists of bipartitions obtained from Bayesian analyses of the eight datasets. The third value is the index of reproducibility (Rep), which is the ratio of the number of datasets supporting the node of interest to the total number of datasets. The MPP and Rep values were directly calculated on SuperTRI v. 57. All SuperTRI values were reported on the Bayesian tree obtained from the supermatrix analysis.

Molecular Dating

Divergence times were estimated with the Bayesian approach implemented in BEAST v. 2.1.3 (Drummond *et al.* 2012) using either a *COI* alignment of 33 taxa and 291 nt or a *Cytb* alignment of 19 taxa and 1140 nt. As no calibration point (fossil record or biogeographic event) is available for *Tylonycteris*, we employed a range of nucleotide substitution rates used for mammals (Arbogast & Slowinski 1998; Mao *et al.* 2010). We used a first mutation rate (R1) of 0.015 per site per lineage per Myr with a lower boundary of 0.01 and an upper boundary of 0.02, a second mutation rate (R2) of 0.02 ± 0.005 per site per lineage per Myr and a third mutation rate (R3) of 0.025 ± 0.005 per site per lineage per Myr. We tested R1 and R2 for the *CO1* dataset, and R2 and R3 for *Cytb* dataset. We applied a GTR + I + G model of evolution (based on jModelTest) and a relaxed-clock model with an uncorrelated log normal distribution for substitution rate. Node ages were estimated using a Yule speciation prior and 10^8 generations, with tree sampling every 1000 generations and a burn-in of 25 %. Adequacy of chain mixing and MCMC chain convergence were assessed using the ESS values in Tracer v. 1.6 (available in the BEAST package). The chronograms were generated with TreeAnnotator v. 1.8.2 (also available in the BEAST package) and visualized with FigTree v. 1.4.1 (Rambaut 2009).

Morphological analysis

For the morphological analysis, 62 adult specimens of *Tylonycteris* spp. were examined (Appendix 1). Besides mass (expressed in grams), the following external measurements were taken from living bats or museum specimens to the nearest 0.1 mm: forearm length (FA) – from the elbow to the wrist with both joints folded; head and body length (HB) –from the tip of the face/chin to the anus; tail length (Tail) – from the anus to the tip of the tail; ear length (Ear) – from the base of the ear, where it attaches to the head, to the tip of the pinna. Craniodental measurements were taken to the nearest 0.01 mm with the use of digital calipers under a stereo microscope and included: GLS – greatest length of skull, from

the anterior rim of the alveolus of the 1st upper incisor to the most posteriorly projecting point of the occipital region; CCL – condylo-canine length, from the exoccipital condyle to the most anterior part of the canine; UCI – from the anterior rim of the alveolus of the first upper incisor to the posterior rim of the alveolus of the canine; CC – greatest width across the upper canines from their labial borders; M^3M^3 – greatest width across the crowns of the last upper molars from their labial borders; IC – least width of the interorbital constriction; MB – greatest distance across the mastoid region; BW – greatest width of the braincase; CM^3 – maxillary toothrow length, from the anterior of the upper canine to the posterior of the crown of the 3rd molar; ML – mandible length, from the anterior rim of the alveolus of the 1st 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.

Data analyses

Bats were classified into six geographic groups on the basis of our molecular results. Two distinct principle component analyses (PCAs) were done on the craniodental characters with PAST (Hammer *et al.* 2001) and irrespective of sexes: (1) the log-transformed raw measurements to assess an overall size factor (usually PC1; Barlow *et al.* 1997; Lindenfors *et al.* 2007) and (2) log-transformed standardized data (raw score/geometric mean) to assess shape factors (Jungers *et al.* 1995). The statistically significant difference in mean values of craniodental measurements and PC mean scores between different groups were then tested by one-way analysis of variance (ANOVA, $p \leq 0.05$) followed by Turkey HSD post-hoc test for unequal sample sizes (Zar 1999).

Results

Phylogeographic analyses based on mitochondrial sequences

Results derived from the analysis of COI sequences

We sequenced a *COI* fragment of 728 nt for 55 individuals of *Tylonycteris* and three outgroup taxa collected across Indochina and adjacent areas. Five additional *COI* sequences of 291 nt were also obtained from old specimens collected from Sumatra (Appendix 1). Our sequences were aligned with all other *COI* sequences available in the GenBank database, producing a final alignment containing 101 specimens. The Bayesian analyses reconstructed from the two *COI* alignments of either 728 nt (Fig. 2) or 291 nt (Appendix 3) showed very similar topologies. Both analyses support the monophyly of the genus *Tylonycteris* (PP = 1). As noted by Huang *et al.* (2014), one specimen labelled as *T. robustula* in GenBank (accession number HM914921) appears within the clade containing all sequences of *T. pachypus*. Examination of the voucher, ZMMU S-186637 held at the Zoological Museum of Moscow State University, indicates that its cranium clearly fits the diagnosis of *T. pachypus* (S. Kruskop, in litt.) and that it should be relabelled accordingly. Otherwise, our results strongly support the monophyly of both species of *Tylonycteris*. In the *COI* trees (Fig. 2; Appendix 3), the *T. pachypus* clade is further subdivided into three divergent and highly supported geographic subclades (PP = 1) named Tp1 (Sumatra), Tp2 (southern Indochina) and Tp3 (northern Indochina). A similar phylogeographic pattern is also detected in *T. robustula*, with three strongly supported geographic subclades named Tr1 (Sumatra), Tr2 (southern Indochina + northwestern India + Peninsular Malaysia) and Tr3 (northern Indochina). Within Indochina, the area of separation between the northern and southern Indochinese subclades corresponds to the region located between Vu Quang (Ha Tinh Province, Vietnam) and Ban Houana (Khammouane Province, Laos) for *T. pachypus*, whereas it corresponds to the region situated between Nam Et (Houaphan Province, Laos) and Vu Quang (Vietnam) for *T. robustula* (Fig. 1).

Results derived from the analysis of *Cytb* sequences

The Bayesian analysis of the newly generated *Cytb* sequences (n = 19) and those of *Tylonycteris* downloaded from GenBank (n = 4) produced a topology (Fig. 2) similar to those obtained with the *COI* gene tree, with two exceptions: (1) *T. robustula* is paraphyletic because one specimen collected

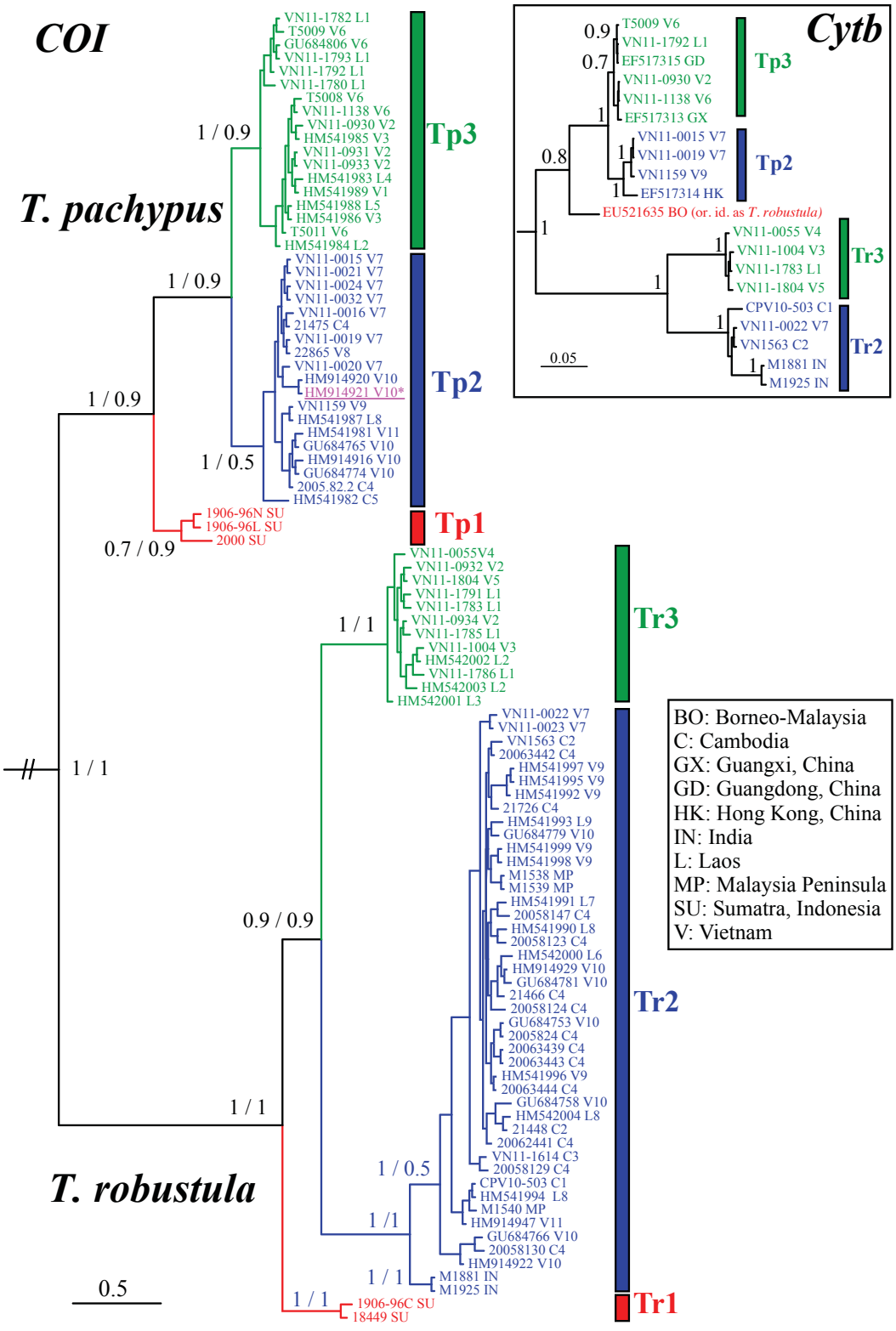


Fig. 2. Bayesian tree obtained from the analyses of *COI* and *Cytb* genes. The values on the nodes indicate posterior probabilities (PP). In the *COI* tree, PPs were calculated from two DNA alignments of *CO1* sequences (728 nt or 291 nt; see text for details). The sequence HM914921 was obtained from a specimen originally identified as *T. robustula*. In the *Cytb* tree, the sequence EU521635 was obtained from a specimen originally identified as *T. robustula*.

in Borneo, originally identified as *T. robustula* (GenBank accession number EU521635; Anrawali *et al.* unpubl.), appears as sister to the clade containing all *T. pachypus* specimens (PP = 0.8); and (2) two sequences of *T. pachypus* from Guangdong and Guangxi (China) are nested within the northern Indochinese subclade (Tp3) of *T. pachypus* (PP = 0.7) as expected, but a sequence of *T. pachypus* from Hong Kong appears as sister to the southern Indochina subclade (Tp2) (PP = 1) (Fig. 2). These results are consistent with the phylogeographic pattern reported in Huang *et al.* (2014).

Genetic distances

The comparisons of interspecific genetic variations, as estimated by K2P distances, indicate that the specimens from Sumatra differ from those of mainland Southeast Asia by 6.0–6.1% and 5.7–7.5% in partial *COI* sequences (calculated from an alignment of only 291 nt) for *T. pachypus* and *T. robustula*, respectively (Appendix 5). The K2P distances for *Cytb* sequences (404 nt) between the specimen of *T. robustula* from Borneo and those representing the Tp2 and Tp3 subclades from Southeast Asia's mainland (Indochina and southern China including Guangdong, Guangxi, and Hong Kong) range from 5.7 to 6.4%. This is significantly smaller than the distance of 12.4–13.8% measured for other specimens assigned to *T. robustula* (Appendix 5). Within Indochina, the mean K2P distances inferred from the alignments of *COI* (728 nt) and *Cytb* (1140 nt) sequences are 2.8% (2.2–3.5%) and 2.8% (2.6–3.0%) between Tp2 and Tp3 of *T. pachypus*, and 6.5% (5.5–7.4%) and 9.5% (8.6–10.4%) between Tr2 and Tr3 of *T. robustula*, respectively. The maximal intraspecific distances in *Cytb* sequences between the specimen from Hong Kong and those from Tr2 and Tr3 are 2.0% and 3.2% (3.1–3.2%), respectively (data not shown in Appendix 5).

Supermatrix and SuperTRI analyses of eight independent DNA markers

The Bayesian trees reconstructed from the concatenation of the seven nuDNA introns (5604 nt and 54 indels) or based on the supermatrix of 7526 characters (mtDNA + nuDNA; 7472 nt and 54 indels) are shown in Fig. 3 and Appendix 4, respectively. In the tree of Fig. 3, we report the bootstrap proportions obtained from the ML analyses (Appendix 6), as well as the results of the SuperTRI analyses (SBP, MPP, and Rep, Appendix 7). All these analyses support with maximal values of robustness the monophyly of the genus *Tylonycteris* and that of the two species, *T. pachypus* and *T. robustula* (Fig. 3). Both species can be diagnosed by several indels in the nuclear dataset: 10 for *T. pachypus* and three for *T. robustula* (Fig. 3). For instance, all specimens of *T. pachypus* share a large insertion of ca 250 nt in *RIOK3* and a deletion of 47 nt in *HDAC2*, while a deletion of two bases (CA) in *CHPF2* was found in all specimens of *T. robustula* (Fig. 3).

Within *T. robustula*, the two geographic mtDNA haplogroups corresponding to Tr2 and Tr3 in Indochina were recovered with high support values ($PP_{nuDNA} / PP_{supermatrix} = 1$; BP = 100; SBP > 95; MPP > 0.54; Rep > 0.5; Fig. 3). Other relationships within *T. robustula* are not robust.

Within *T. pachypus*, no substructure was consistent. The Tp2 subclade was recovered in the supermatrix analyses with modest support and SuperTRI analyses revealed that this node was only supported by the mtDNA dataset. The Tp3 subclade was not found to be monophyletic in supermatrix and superTRI analyses, but the weak support values indicate a lack of resolution rather than real discordance between the datasets.

Interspecific divergences, as estimated from K2P distances, were calculated using the concatenation of the seven nuclear genes (5604 nt). The distances between *T. pachypus* and *T. robustula* range from 1.6 to 1.8%. The divergences between specimens of northern and southern Indochina range from 0.4 to 0.6% for *T. robustula* and from 0 to 0.2% for *T. pachypus*. The maximum intraspecific variation within each geographical population is 0.2% for both species (Appendix 5).

Molecular divergence time estimates

Our molecular dating estimations based on two different mtDNA alignments (*COI* and *Cytb*) and three different rates of substitution (Table 1) suggest that the common ancestor of extant *Tylonycteris* spp. was already present during the Pliocene epoch (6.56–3.84 Mya), and that the diversification of major geographic clades within both *T. pachypus* and *T. robustula* occurred during the Early Pleistocene.

Morphological comparisons

Most craniodental measurements of available *T. pachypus* specimens are significantly smaller than those of *T. robustula* (ANOVA, $p \leq 0.05$); whereas those of the three molecular groups within each taxon are overlapping (Table 2). This is reflected in univariate variation of GLS measurements (Fig. 4A) or on the multivariate component of PC*1 based on raw data. This component accounted for 86.9% of total variance and was correlated positively with all characters (Table 3), confirming that it represents an overall size factor (Fig. 4B). The range of PC*1 scores of *T. pachypus* (between -2.0 and -0.58) is significantly smaller than that of *T. robustula* (between -0.38 and 1.33) (ANOVA, $p \leq 0.05$).

The PCA of log-transformed standardized data, which better reflects shape factors, reveals that the two first PCs, PC1 and PC2, accounted for 37.2 and 19.3% of total variance, respectively (Table 3), with a

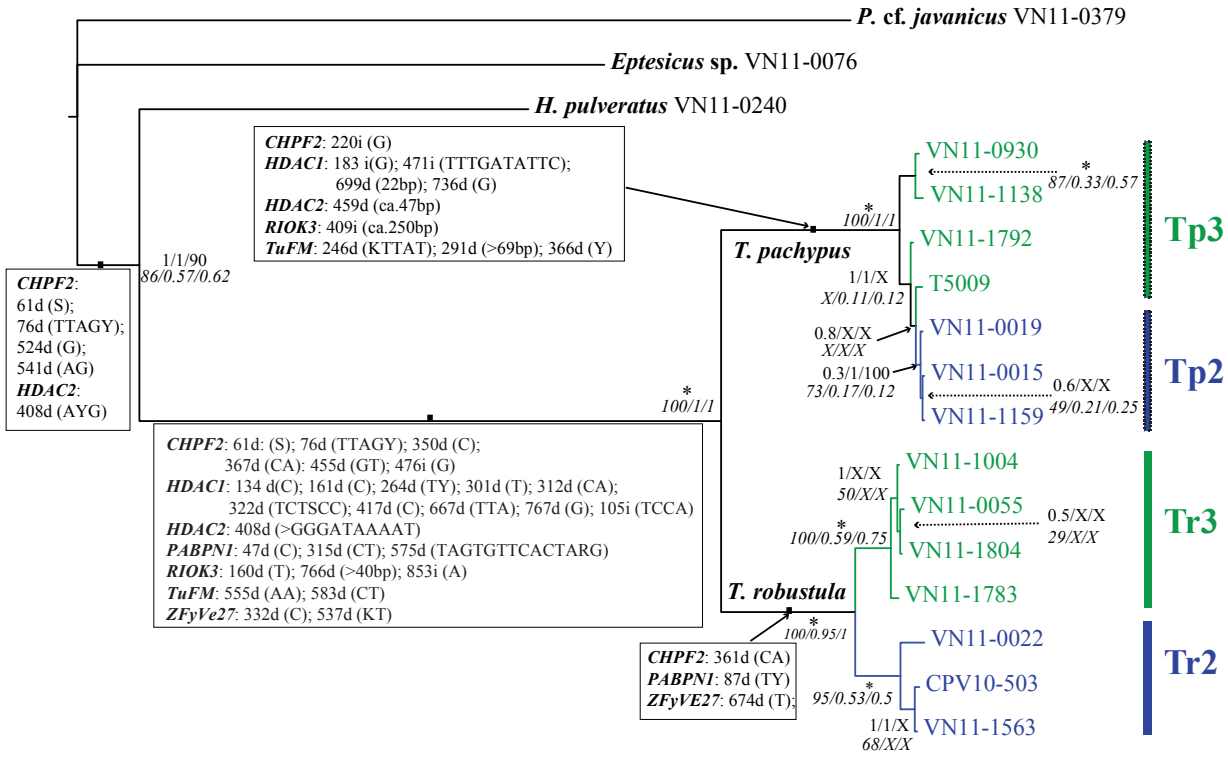


Fig. 3. Bayesian tree reconstructed from the analysis of the seven concatenated nuDNA introns. The seven independent markers are *CHPF2* (858 nt), *HDAC1* (1128 nt), *HDAC2* (639 nt), *PABPNI* (677 nt), *RIOK3* (915 nt), *TUFM* (655 nt) and *ZFYVE27* (732 nt). The values indicated on the branches are the Bayesian posterior probabilities (PP_{nuDNA} and $PP_{supermatrix}$), maximum likelihood bootstrap (BP), Supertree Bootstrap percentage (SBP), Mean posterior probability (MPP) and Reproducibility index (Rep). An asterisk indicates that the node was supported by maximal values of robustness ($PP_{nuDNA} = PP_{supermatrix} = 1$; BP = 100). The letter "X" indicates that the node was not found in the analysis. 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.

Table 1. Bayesian mean node ages in million years ago (Mya) estimated with three different rates of substitution, 0.015 ± 0.005 (R1), 0.02 ± 0.005 (R2) and 0.025 ± 0.005 (R3) per lineage per Myr, and using two different DNA alignements: subunit I of cytochrome c oxidase (*COI*) or cytochrome b (*Cytb*).

Taxa	*COI* R1	*COI* R2	*Cytb* R2	*Cytb* R3	Epochs
Tylonycteris	5.27	3.84	6.56	5.27	Pliocene
T. robustula	3.07	2.22	NA	NA	E Pleist.
Indochina	2.36	1.70	2.97	2.38	E Pleist.
Northern Indochina	0.90	0.65	0.26	0.21	M Pleist.
Southern Indochina	1.38	1.00	0.48	0.39	E/M Pleist.
Sumatra	0.27	0.20	NA	NA	M Pleist.
T. pachypus	2.70	1.96	2.44	1.96	E Pleist.
Indochina	1.35	0.99	0.98	0.79	E/M Pleist.
Northern Indochina	0.45	0.33	0.25	0.20	M/L Pleist.
Southern Indochina + HK	NA	NA	0.54	0.43	M Pleist.
Southern Indochina	0.71	0.52	0.12	0.10	M/L Pleist.
Sumatra	0.73	0.53	NA	NA	M Pleist.

Abbreviations: HK = Hong Kong; NA = not applicable; Pleist. = Pleistocene; E = Early; M = Middle; L = Late.

significant difference in shape components between *Tylonycteris* taxa (ANOVA, $p \leq 0.05$). In the plot of PC1 against PC2 (Fig. 4C), we found an overlap between *T. pachypus* and *T. robustula*. However, this multivariate projection also shows that, within *T. pachypus*, bats of Tp1 form a cluster clearly separated from bats of Tp2 and Tp3. Within *T. robustula*, morphological overlap between haplogroups is more extensive, but most Tr2 individuals tend to separate from Tr1 and Tr3 ones.

Systematics of the genus *Tylonycteris*

In earlier taxonomic accounts (e.g., Tate 1942 or Medway 1973), the three distinct species *T. fulvida* (sometimes incorrectly referred to as *rubidus*) (type locality [t.l.]: Schwe Gyen, Myanmar), *T. meyeri* Peters, 1872 (t.l.: South Luzon) and *T. aurex* Thomas, 1951 (t.l.: Astoli, south of Mumbai, India) were treated as subspecies of *T. pachypus* (t.l.: Java), while *T. malayana* Chasen, 1940 (t.l.: Peninsular Malaysia) was considered as a subspecies of *T. robustula* (t.l.: Borneo). Most subsequent authors followed these taxonomic recommendations (Corbet & Hill 1992; Simmons 2005; Bates *et al.* 2008a, 2008b; Francis 2008). Previous karyological and genetic data (Francis *et al.* 2010; Huang *et al.* 2011) and our new phylogenetic analyses, however, support a clear division between populations of *Tylonycteris* spp. from mainland Southeast Asia vs those of the Sundaic islands, such as Sumatra and Borneo. We propose therefore to restrict the species names *T. pachypus* and *T. robustula* to populations from the Sundaland, where the type specimens were collected (Fig. 1), and to revalidate the following two species names previously used for populations occurring in mainland Southeast Asia (Tate 1942; Medway 1973): the first is *T. fulvida*, which was originally described as *Scotophilus fulvidus* from Sittang River (Myanmar) and should be applied to continental bats of the *T. pachypus* complex; the second is *T. malayana*, which was originally described from Perak (Peninsular Malaysia) and should be applied to populations of the *T. robustula* complex found in Peninsular Malaysia, southern Indochina, and northern India. In contrast, all bats of the *T. robustula* species complex endemic to northern Indochina should be included in the new species described below. Representatives from other isolated populations, notably from the Philippines and the Western Ghats in India would need further scrutiny to assess their taxonomic rank, but certainly represent further distinct taxa.

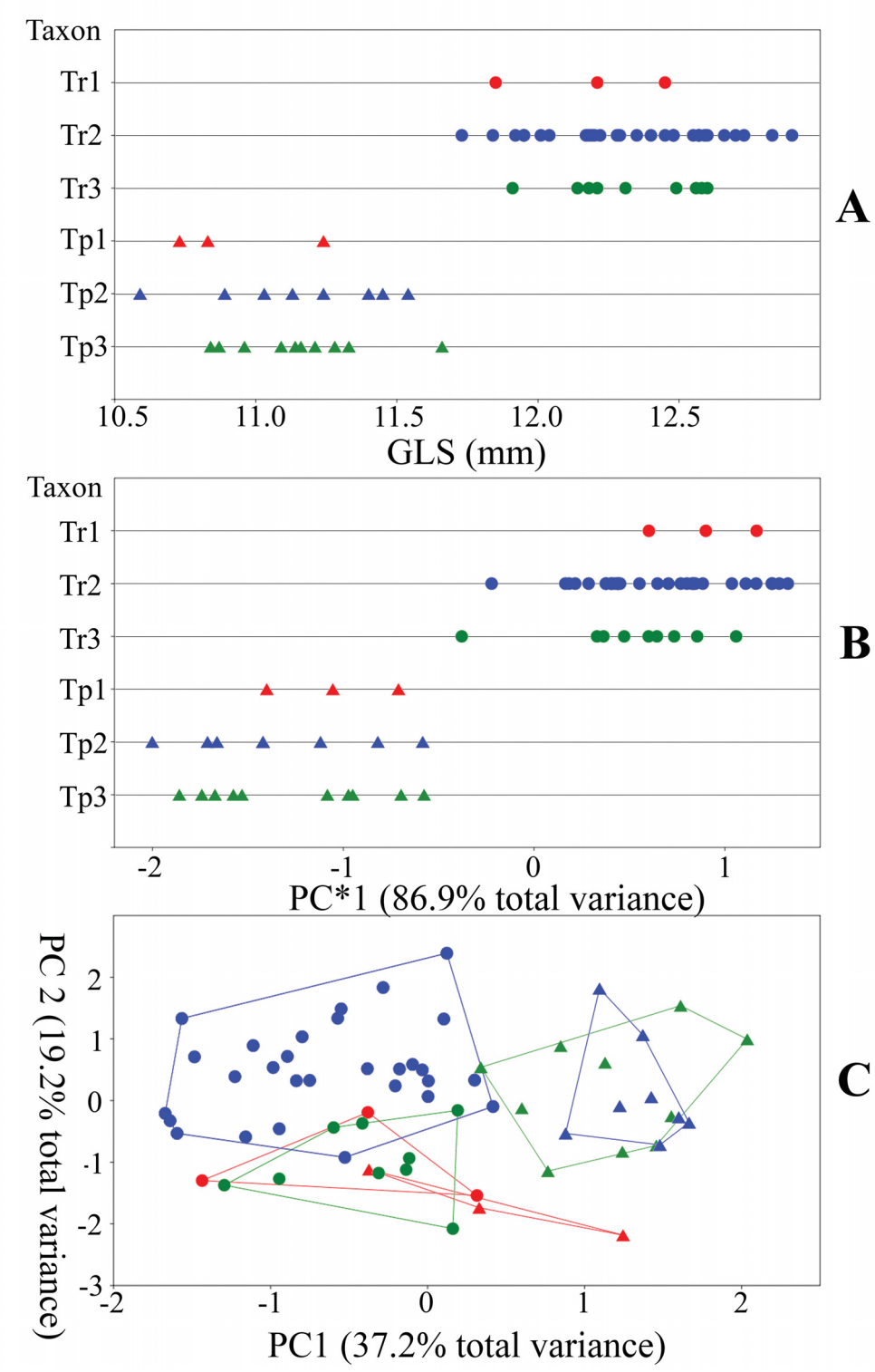


Fig. 4. Scatter plots obtained from morphological analyses of *Tylonycteris* spp. **A**. Range of GLS measurements of specimens within each group of *Tylonycteris* spp. **B**. Range of PC*1 scores of specimens of *Tylonycteris* spp. obtained from PCA of log-transformed raw data of craniodental measurements. **C**. Plot of PC 1 against PC 2 obtained from PCA on log-transformed standardized data. Triangles and circles refer to *T. pachypus* s. lat. and *T. robustula* s. lat., respectively. Colour patterns indicate the mtDNA haplogroups: green for Tp3 and Tr3 (northern Indochina); blue for Tp2 and Tr2 (other regions of the Southeast Asian mainland); red for Tp1 and Tr1 (Sundaland).

Table 2. Selected external and craniodental measurements (in mm) of *Tylonycteris* spp. Values are given as mean, standard deviation (SD), (n) and min–max. Acronyms and definitions for measurements are given in Material and methods.

	T. pachypus			*T. robustula*		
Character	**Tp1** (n = 3)	**Tp2** (n = 8)	**Tp3** (n = 10)	**Tr1** (n = 3)	**Tr2** (n = 29)	**Tr3** (n = 9)
FA	–	24.9, 0.4 (6) 24.4–26.4	25.7, 0.6 (7) 24.1–25.3	–	26.4, 0.5 (6) 25.5–27.5	26.7, 1.1 (5) 25.1–27.8
GLS	10.93, 0.27 10.73–11.24	11.16, 0.32 10.59–11.54	11.15, 0.24 10.84–11.66	12.17, 0.30 11.85–12.45	12.34, 0.31 11.73–12.90	12.33, 0.24 11.91–12.60
CCL	10.19, 0.21 10.00–10.41	10.26, 0.28 9.72–10.69	10.25, 0.18 9.99–10.54	11.36, 0.26 11.08–11.59	11.23, 0.28 10.66–11.82	11.29, 0.24 10.82–11.62
CC	3.57, 0.13 3.43–3.68	3.51, 0.13 3.27–3.67	3.52, 0.11 3.29–3.69	4.12, 0.11 4.00–4.21	4.09, 0.16 3.81–4.46	3.92, 0.16 3.60–4.12
UCI	1.81, 0.10 1.70–1.88	1.66, 0.04 1.62–1.76	1.66, 0.08 1.52–1.79	2.04, 0.06 1.98–2.10	1.98, 0.10 1.77–2.17	1.98, 0.08 1.86–2.10
M^3M^3	4.74, 0.08 4.65–4.80	4.86, 0.20 4.62–5.15	4.91, 0.21 4.48–5.24	5.67, 0.17 5.53–5.86	5.54, 0.20 5.13–5.90	5.47, 0.14 5.28–5.76
IC	3.46, 0.08 3.37–3.51	3.28, 0.10 3.16–3.42	3.33, 0.21 3.02–3.72	3.97, 0.09 3.90–4.08	3.76, 0.14 3.52–4.01	3.90, 0.19 3.48–4.11
MB	7.08, 0.07 7.00–7.14	7.02, 0.25 6.59–7.38	6.85, 0.16 6.52–7.04	7.70, 0.33 7.32–7.89	7.43, 0.18 7.14–7.80	7.34, 0.17 7.04–7.54
BW	6.49, 0.14 6.33–6.58	6.29, 0.15 6.00–6.51	6.40, 0.26 6.14–6.83	7.10, 0.27 6.80–7.29	6.78, 0.21 6.40–7.36	6.88, 0.34 6.54–7.50
CM^3	3.50, 0.15 3.37–3.66	3.48, 0.16 3.27–3.73	3.54, 0.14 3.33–3.79	4.05, 0.10 3.98–4.17	4.06, 0.12 3.74–4.31	3.96, 0.10 3.81–4.12
ML	7.77, 0.19 7.56–7.93	7.79, 0.35 7.38–8.32	7.81, 0.24 7.41–8.13	8.56, 0.16 8.41–8.73	8.71, 0.23 8.25–9.19	8.59, 0.25 8.08–8.99
CM_3	3.78, 0.13 3.65–3.90	3.74, 0.21 3.51–4.09	3.76, 0.17 3.51–4.03	4.26, 0.14 4.18–4.43	4.32, 0.13 4.08–4.53	4.16, 0.15 3.92–4.47

Phylum Chordata Haeckel, 1874
Class Mammalia Linnaeus, 1758
Order Chiroptera Blumenbach, 1779
Family Vespertilionidae Gray, 1821
Subfamily Vespertilioninae Gray, 1821

Tylonycteris tonkinensis Tu, Csorba, Ruedi & Hassanin sp. nov.
urn:lsid:zoobank.org:act:C59B0774-79D6-4A84-9489-CF04BE35FC49
Fig. 5B

Tylonycteris robustula Thomas, 1915 (partim): 227.

Tylonycteris robustula – Osgood 1932: 236. — Tate 1942: 268. — Hendrichsen *et al.* 2001: 90. — Kruskop 2013: 221. — Thomas *et al.* 2013: 229.

Etymology

The specific epithet refers to the current restricted occurrence of the new species in north-eastern Laos and northern Vietnam (Fig. 1). The Vietnamese portion of this region was previously called “Tonkin”

Table 3. Factor loadings of craniodental characters for the PCs obtained from principle component analyses (PCAs) of specimens of *Tylonycteris*. Acronyms and definitions for measurements are given in Material and methods. The first PCA is based on raw data (PC*1), while the second PCA is based on the log-transformed, standardized data (PC1 and PC2). Values in bold indicate the most significant loadings.

	Raw data	Standardized data	
Characters	**PC*1**	**PC1**	**PC2**
GLS	**0.25**	0.14	0.04
CCL	**0.23**	0.22	-0.02
CC	**0.36**	-0.18	0.25
UCI	**0.41**	**-0.62**	**-0.38**
M^3M^3	**0.32**	-0.02	0.30
IC	**0.34**	-0.15	**-0.62**
MB	0.17	**0.47**	-0.10
BW	0.19	**0.43**	-0.28
CM^3	**0.35**	-0.22	0.29
ML	**0.27**	0.09	0.16
CM_3	**0.34**	-0.17	**0.35**
Eigenvalue	0.0082	0.0006	0.0003
% variance	86.9	37.2	19.3

during the Nguyễn dynasty and French colonial era (from the 19th to the mid-20th centuries) to separate it from the country's centre (Annam) and southern regions (Cochinchina). The proposed English name is "Tonkin's greater bamboo bat" and the proposed Vietnamese name is 'Dơi ống tre Bắc Bộ'.

Type material

Holotype

VIETNAM: ♂, Copia Nature Reserve, Co Ma commune, Thuan Chau District, Son La Province, 21°21.727′ N, 103°30.562′ E, 1286 m a.s.l., 9 May 2011, Vuong Tan Tu leg. (IEBR-VN11-0055; field number Tu.090511.3; tissue code VN11-0055). Body in alcohol, skull removed. Mass: 4 g. Measurements (in mm): FA: 27.0; HB: 41.0; Tail: 33.0; Ear: 11.0; GLS: 12.60; CCL: 11.59; UCI: 1.98; CC: 4.06; M^3M^3: 5.46; IC: 3.97; MB: 7.42; BW: 7.07; CM^3: 3.96; ML: 8.64; CM_3: 4.22. Accession numbers of mitochondrial and nuclear sequences: KX496422–KX496429.

Paratypes

LAOS: 3 adult ♂♂ (MHNG 1926.059, MNHN 2006-90, MNHN 2006-93), 1 adult ♀ (MHNG 1926.057), Hat Hin, Nam Sing River, Phongsaly Province, 21°40.14′ N, 102°13.26′ E, 2004, Manuel Ruedi leg., body in alcohol, skull removed.

VIETNAM: 1 ♂ (IEBR-VN11-1804), Hang Kia, Pa Co Nature Reserve, Hoa Binh Province, 20°44.910′ N, 104°54.900′ E, 1080 m a.s.l., 2012, Vuong Tan Tu leg., body in alcohol, skull removed.

Accession numbers of DNA sequences for paratypes are given in Appendix 1.

Referred material

Specimens identified as *T. robustula* collected from Na Don, Phuong Vien, Cho Don (Bac Kan Province) and Na Hang Nature Reserve (Tuyen Quang Province) (Appendix 1) are also referred to *T. tonkinensis* sp. nov.

Description

A member of the *T. robustula* species complex comprising representatives of the Tr3 haplogroup found in northern Indochina. Externally, individuals are small, with a forearm length of 25.1–27.8 mm (Table 2). The head is dorsoventrally very flattened. Pelage coloration is relatively variable, more or less golden red at the base of the dorsal fur, to dark brown near the tips of the dorsal hairs, and lighter golden brown on the underparts (Fig. 5B). The ears have a triangular shape, with broadly rounded tips. The tragus is short and blunt. The wing membranes are dark brown. The base of thumbs and soles of hind feet have fleshy pads (Fig. 5B).

The skull is small (GLS: 11.91–12.60 mm), lightly built and very flat (Fig. 5B). The rostrum is short. The sagittal crest is absent. The lambdoid crests are well developed. The dental formula is I2/3 C1/1, P1/2, M3/3 = 32. The first upper incisor (I^2) is bicuspidate, with small cusps on cingulum. I^3 is unicuspidate, about half the height and crown area of I^2. The upper canine has a posterior supplementary cusp. A diastema between I^3 and the upper canine is clearly visible. The protocones of M^1 and M^2 are well-developed. M^2 appreciably exceeds M^1 in width, and its width clearly exceeds its length. M^3 is relatively smaller and without a metastyle. The three lower incisors are tricuspidate. The first (PM_2) and second (PM_4) premolars are approximately equal in height and crown area (Fig. 5B).

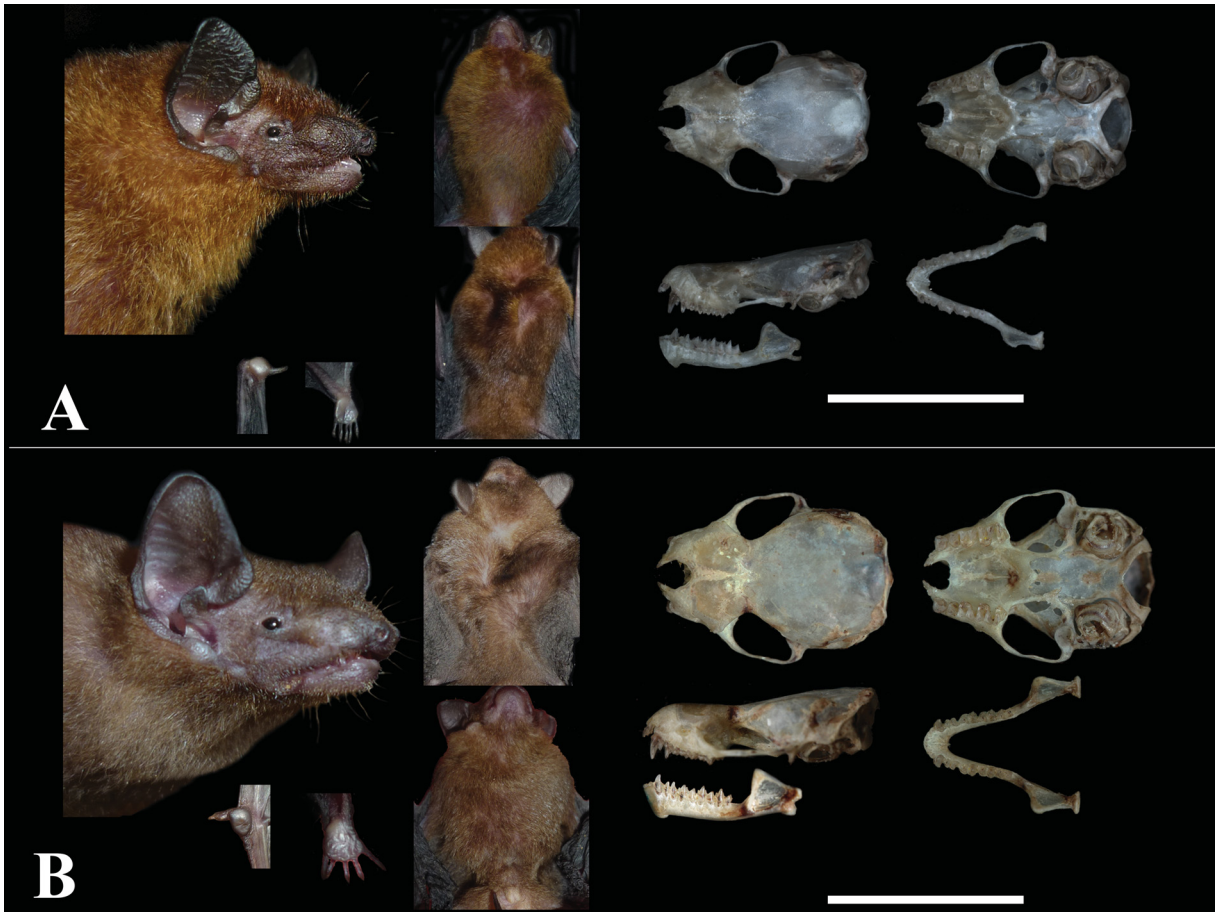


Fig. 5. Morphological characteristics of the two nominal species of the genus *Tylonycteris* Peters, 1872. **A**. *T. pachypus* (Temminck, 1840) (corrected taxon name is *T. fulvida* (Blyth, 1859)), IEBR-VN11-0015. **B**. *T. robustula* Thomas, 1915 (corrected taxon name is *T. tonkinensis* Tu, Csorba, Ruedi & Hassanin sp. nov.), holotype, IEBR-VN11-0055. Head profiles, ventral and dorsal views, fleshy pads at the base of the thumb and on the sole of the foot, and different views of the skull (dorsal, ventral and lateral). Scale = 10 mm.

Remarks

In northern Indochina, *T. tonkinensis* sp. nov. can be distinguished from *T. fulvida* and *T. pygmaea* by its significantly larger body and skull size (Table 2; Figs 4–5; see Feng *et al*. 2008 for comparisons with *T. pygmaea*), by K2P distances of at least 12% for *Cytb* and *COI* sequences and by K2P distances of at least 1.5% for the concatenation of the seven nuclear genes (5604 nt) (Appendix 5). Within the *T. robustula* complex, *T. tonkinensis* sp. nov. is morphologically overlapping with *T. robustula*, found in Sumatra, and *T. malayana* (= Tr2 haplogroup), collected from the Southeast Asian mainland, but differs from the first taxon by K2P distances of at least 5.2% in *COI* sequences (Appendix 5) and from the latter by K2P distances of at least 5.5%, 8.6% and 0.4% calculated from *COI* sequences (657 nt), *Cytb* sequences (1140 nt) and the concatenation of seven nuclear DNA sequences (5604 nt), respectively (Appendix 5).

Ecology and habitat

Like other species of *Tylonycteris*, *T. tonkinensis* sp. nov. is associated with woody bamboo groves. The new species is usually found in sympatry with the smaller species *T. fulvida* (Fig. 1). In northwestern Vietnam, bats of the new species were captured in mist-nests set near bamboo groves in forest edges adjacent to rural-residential areas at relatively high elevations, e.g., at 1010 m a.s.l in Hang Kia, Pa Co Nature Reserve (Hoa Binh Province) or at 1286 m a.s.l in its type locality. In Laos, the known localities are found at lower elevations, between 500 and 800 m a.s.l.

Distribution

Currently, the new species is known to occur in north-eastern Laos and northern Vietnam only (Fig. 1).

Discussion

Cryptic species diversity in the genus *Tylonycteris*

Previous studies have detected high levels of genetic and karyological variation among specimens identified as either *T. pachypus* or *T. robustula* collected from different geographic locations in mainland Southeast Asia (Francis *et al.* 2010; Huang *et al.* 2014). Our *COI* analyses further revealed the existence of three divergent geographic haplogroups for both *T. pachypus* and *T. robustula*, for each corresponding to Sumatra, northern Indochina and southern Indochina (but also northwestern India and Peninsular Malaysia for *T. robustula*). Our *Cytb* dataset confirmed the existence of these geographic haplogroups in mainland Southeast Asia. In addition, a specimen of *T. pachypus* collected from Borneo and originally described as *T. robustula* was found to be highly divergent from the two other Indochinese haplogroups (Fig. 2). In the absence of *Cytb* data for Sumatran specimens, it is impossible to know whether they share the same mitochondrial lineage as those from Borneo.

Taken together, our mtDNA analyses show that all haplotypes sequenced for insular *Tylonycteris* are very divergent from those identified in mainland Southeast Asia. However, genetic inferences based on the maternally inherited mitochondrial genes are prone to be discordant with the true evolutionary history of the taxa, owing to various evolutionary processes, such as mtDNA introgression, incomplete lineage sorting or female philopatry (Avise 2000; Ballard & Whitlock 2004; Hassanin & Ropiquet 2007; Nesi *et al.* 2011; Rivers *et al.* 2005). Here, the geographic pattern of mtDNA diversity observed for the two species of *Tylonycteris* could be the consequence of female philopatry, i.e., the behavior of remaining in, or returning to the natal territory. Indeed, bat species with philopatric females generally display high geographic structure when relationships are examined with maternally inherited markers, such as the mitochondrial DNA. This pattern can disappear with biparentally inherited markers when adult males are able to disperse over long distances, allowing gene flow between otherwise isolated populations (Castella *et al.* 2001; Hassanin *et al.* 2015; Hulva *et al.* 2010; Pereira *et al.* 2009; Rivers

et al. 2005). Behavioral and population genetic studies in southern China have shown that *T. pachypus* bats are philopatric to their natal area and that philopatry is especially pronounced in females (Hua *et al.* 2011, 2013). Although no data are available for *T. robustula*, female philopatry can also be predicted for this species, because it shares similar morphological, behavioural and ecological traits with *T. pachypus* (Medway 1972; Medway & Marshall 1970, 1972; Zhang *et al.* 2007). The social organization of *T. pachypus* and *T. robustula*, combined with their fragmented habitats, is therefore expected to result in limited gene flow between populations, especially among matrilines from distant geographic localities. For both species, this prediction is corroborated by the analyses of mtDNA markers, with the identification of three divergent geographically non-overlapping haplogroups. For *T. robustula*, this phylogeographic pattern is also supported by the nuclear sequence data, as the two Indochinese clades were recovered monophyletic with all the three introns containing enough nucleotide variation at the intra-specific level, i.e., *CHPF2*, *HDAC1* and *TUFM* (Appendix 8). By contrast, our nuclear analyses (Fig. 3; Appendices 4, 8) do not support the reciprocal monophyly of the two Indochinese clades of *T. pachypus*, suggesting that gene flow was maintained by male dispersal or, alternatively, that their separation was too recent to be detected with our nuclear genes.

Nucleotide distances estimated from mtDNA genes between northern and southern Indochinese populations of *T. robustula* were more than twice those of *T. pachypus* (6.5% vs 2.8% in *COI*; 9.5% vs 2.8% in *Cytb*), indeed supporting a more recent divergence for the latter taxon, if we assume equal evolutionary rates. Similarly, the nuclear distances between the two Indochinese clades of *T. robustula* were between 0.41 and 0.56%, which is more than twice those calculated between Indochinese individuals of *T. pachypus* (0–0.2%; Appendix 5) and in the range of interspecific distances found in other groups of Laurasiatheria, such as fruit bats of the tribes Myonycterini (Nesi *et al*. 2013) and Scotonycterini (Hassanin *et al*. 2015), or cattle and buffalo of the tribe Bovini (Hassanin *et al*. 2013). Although none of the nuclear markers could be sequenced for Sumatran and Bornean *Tylonycteris*, their high mtDNA divergence from Indochinese populations (> 5.7 % in both *COI* and *Cytb* sequences; Appendix 5) suggests they might represent distinct lineages based on nuclear markers as well. In agreement with this view, our multivariate morphological analyses revealed that Indochinese bats of the *T. pachypus* complex constitute a distinct group separated from those collected on Sumatra. For the *T. robustula* complex, morphological overlap between haplogroups is more extensive, but pairwise comparisons of their PC mean scores support the distinctness of adjacent geographical taxa, such as Tr2 and Tr3 on the Southeast Asian mainland.

The close morphological similarity among taxa of *Tylonycteris* suggests that they have evolved under the influences of similar and specialized habitats, i.e., woody bamboo vegetation. Molecular evidence indicates, however, that *T. pachypus* should be split into at least two distinct species, *T. pachypus* on the Sunda islands (Sumatra and/or Borneo) and *T. fulvida* in mainland Southeast Asia, and that *T. robustula* should be divided into at least three species, with *T. robustula* on Sumatra, *T. malayana* in southern and western mainland Southeast Asia, and *T. tonkinensis* sp. nov. in northern Indochina.

The evolution of *Tylonyteris* spp. in Southeast Asia during the Pleistocene

Given that both species complexes, here named *T. pachypus* s. lat. and *T. robustula* s. lat., are usually found in sympatry across most of their geographic ranges in Southeast Asia, they are expected to share a common phylogeographical history. Our estimates of divergence times based on mtDNA sequences suggest that the genus *Tylonycteris* diversified during the Pliocene epoch (*Cytb*: 5.92 ± 0.65 Mya; *COI*: 4.56 ± 0.72 Mya) (Table 1). During the Miocene and until the early Pliocene, Southeast Asia was generally covered by large tracks of rain forests as a consequence of warm and humid climatic conditions (Meijaard & Groves 2006; Morley 2000). Thus, ancestors of both *Tylonycteris* species complexes were presumably widely distributed across Southeast Asia during the Pliocene.

Our molecular dating estimates indicate that the basal geographic splits within the two species complexes, i.e., between mainland Southeast Asia and Sumatra, took place approximately at the same time during the Early Pleistocene (between 2.70 and 1.96 Mya for *T. pachypus*, between 3.07 and 2.22 Mya for *T. robustula*; Table 1). The Pleistocene epoch is characterized by the onset of repeated cycles of cold glacial and warm interglacial periods as the results of the glaciations/deglaciations of the Northern Hemisphere, which implied contraction and expansion of rain forests in Asia (An *et al.* 2001; Meijaard & Groves 2006; Morley 2000). As bats of the genus *Tylonycteris* are highly dependent on woody bamboo vegetation for roosting, foraging and mating (Kunz 1982; Medway 1972; Medway & Marshall 1970, 1972), their Pleistocene biogeographic history was firmly constrained by the distribution of such bamboo habitats. The current distribution of woody bamboo species in Asia (Bystriakova *et al.* 2003b; Fig. 6) indicates that eight disjunctive biogeographic regions have higher species richness (> 5 species) for some bamboo genera: southern India (*Ochlandra* Thwaites), northern Myanmar (*Cephalostachyum* Munro), southern China (*Dendrocalamus* Nees and *Bambusa* Schreb.), Hainan Island (*Bambusa*), northwestern Thailand (*Dendrocalamus* and *Gigantochloa* Kurz ex Munro), Peninsular Malaysia (*Gigantochloa*), Sumatra and Borneo (*Gigantochloa* and *Schizostachyum* Nees). All these regions may therefore have acted as distinct bamboo forest refugia during the glacial periods of the Pleistocene (Fig. 6). Evidence for a number of these postulated glacial refugia has been reported in previous studies for many organisms, including bats (Flanders *et al.* 2011; Khan *et al.* 2010; Lin *et al.* 2014; Mao *et al.* 2013). Accordingly, we propose that the contraction of woody bamboo forests into different glacial refugia had fragmented the distribution of the Pliocene ancestors of both *T. pachypus* s. lat. and *T. robustula* s. lat. In addition, we can assume that Pleistocene glacial periods resulted in higher interspecific competition between co-distributed species of *Tylonycteris*, because the supply of most suitable resources was more limited in glacial bamboo forest refugia (Medway & Marshall 1970). As noted in previous studies, *T. pachypus* s. lat. has a more manoeuvrable flight in cluttered habitats and forages on smaller insects than *T. robustula* s. lat. (Zhang *et al.* 2005, 2007). Moreover, Medway & Marshall (1970) found that the smaller *T. pachypus* s. lat. can roost in the internodes with small entrance holes, which the larger *T. robustula* s. lat. is unable to enter. These differences suggest, therefore, that the smaller *T. pachypus* s. lat. have greater advantages than the larger *T. robustula* s. lat. in interspecific competition when natural resources are limited. Hence, isolated populations of *T. robustula* s. lat. may have been more exposed to bottlenecks and therefore more vulnerable to local extinction than those of co-distributed *T. pachypus* s. lat.

During interglacial periods of the Early Pleistocene, warmer and humid conditions resulted in the expansion of woody bamboo forests, which in turn may have favored the restoration of connectivity between isolated populations of both complexes. However, the isolated populations may have been connected or not, depending on their dispersal capacity and the distances between refugia. In *T. robustula* s. lat., these processes may have taken longer, because of its lower population abundance (Lande & Barrowclough 1987; Shaffer 1981), and may have been prevented in cases of extinction of transitional populations (Huang *et al.* 2014 and references therein). This scenario is supported by the fact that *T. pachypus* s. lat. is usually found to be more abundant than *T. robustula* s. lat. in bamboo forests (Zhang *et al.* 2004; Medway & Marshall 1972) and by the wider geographic range of *T. pachypus* s. lat. (Bates *et al.* 2008a, 2008b; Fig. 1). Knowing this, the body size differences between the two species complexes may be the key factor explaining why the basal divergence of northern Indochinese populations occurred earlier in *T. robustula* s. lat. (i.e., *T. tonkinensis* sp. nov) than in *T. pachypus* s. lat., i.e., 2.97–1.70 vs 1.35–0.79 Mya (Table 1). During Pleistocene interglacials, exchanges of *Tylonycteris* spp. between the continent and the islands of Sundaland were probably prevented because of the long distances between the glacial forest refugia, as well as the higher sea levels (Fig. 1). Our molecular dating estimates corroborate this scenario, as continental populations of *Tylonycteris* spp. from Indochina and Peninsular Malaysia diverged from insular populations (Sumatra and Borneo) in the Early Pleistocene (Table 1).

Implications for conservation

Previous studies considered *T. pachypus* and *T. robustula* to be common species and thus classified them as *Least Concern* in the IUCN Red List (Bates *et al.* 2008a, 2008b). Since our study reveals that both species in fact represent several species with more restricted distributions, the IUCN status of the different taxa should be reassessed urgently, including that of the new species, *T. tonkinensis* sp. nov. In addition, our study suggests that several biogeographic regions have acted as Pleistocene glacial refugia. This information is very important for developing more effective conservation strategies, particularly

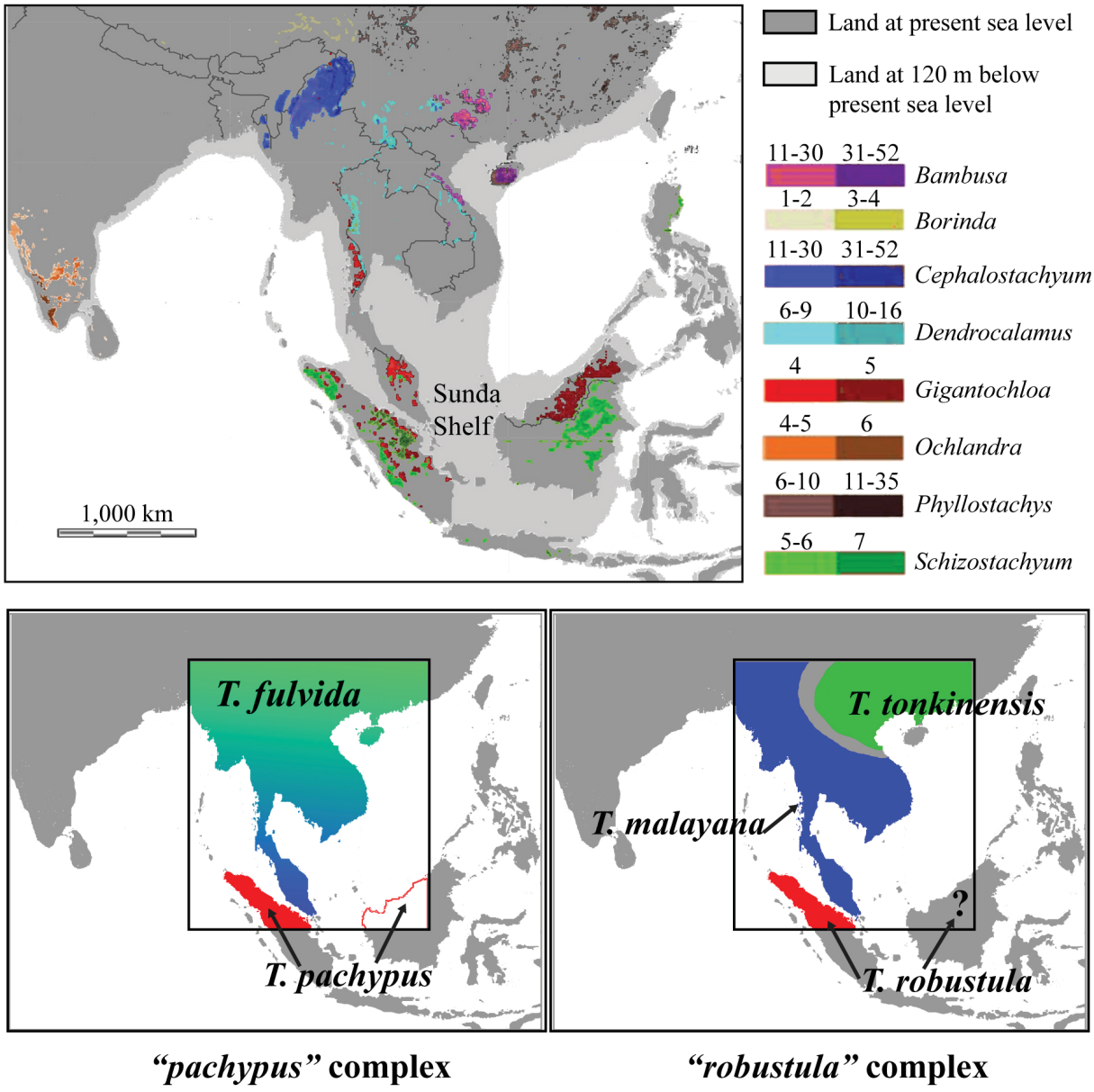


Fig. 6. Distribution and species richness for the eight genera of woody bamboo forests currently found in Asia (adapted and modified from Bystriakova *et al.* 2003b) and putative geographic range areas of the species of *Tylonycteris*. During glacial periods of the Pleistocene, the highlighted biogeographic regions may have acted as bamboo refugia for *Tylonycteris* spp. Moreover, sea level falls (at around 120 m below present) exposed land bridges, such as the entire Sunda Shelf (adapted from Voris 2000), connecting the islands to the continent.

given the high current rates of deforestation affecting most natural habitats in Southeast Asia (Kingston 2010; Sodhi *et al.* 2010; Tordoff *et al.* 2012).

Acknowledgements

In Vietnam, we would like to thank P.D. Tien (IEBR), D.Q. Thang and N.X. Nghia (Ngoc Linh NR) and N.T. Son (Vu Quang NP) for their support during the field surveys. We are grateful to L.X. Canh, T.H. Thai, N.V. Sinh and other colleagues of the IEBR, Hanoi for administrative assistance. The field research was done under the permissions of the People's Committees of numerous provinces and the Vietnamese Ministry of Agriculture and Rural Development (Vietnam Administration of Forestry). In Cambodia, we would like to acknowledge T.D. Vong, V. Hul and S. In for their support during the field surveys, and C. Garnero-Morena, G. Diffloth, B. Kilian, A. Beschaouch and N. Hayashi-Denis for their assistance. In Laos, field surveys were realized with the help of R. Kirsch, A. Cibois and J. Fuchs. The field trips were organized by UNESCO under the permissions of the National Authority for Preah Vihear, the President, His Excellency Mr. Chuch Phoeurn and the Director of the Preah Vihear temple, Prof. Hang Soth. We thank collection managers and curators who provided samples from museum specimens: W. van Bohemen and S. van der Mije at the Naturalis Biodiversity Center (Leiden, The Netherlands); and J.-M. Pons and A.M. Ohler at the MNHN (Paris, France). This research was supported by the "ATM Barcode" funded by the MNHN, the "Bibliothèque du Vivant" network funded by the CNRS, the MNHN, the INRA and the CEA (Genoscope), the "Société des Amis du Muséum et du Jardin des Plantes", Rufford Foundation (UK), the SYNTHESYS Project, which is financed by European Community Research Infrastructure Action under the FP7 "Capacities" Program, the Hungarian Scientific Research Fund (OTKA) K112440, the Vietnamese Ministry of Education and Training, the French government (through Campus France Agency and "CROUS de Paris"), and the Leg Prevost of MNHN.

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Manuscript received: 2 March 2016
Manuscript accepted: 1 August 2016
Published on: 9 February 2017
Guest editors: Line Le Gall, Frédéric Delsuc, Stéphane Hourdez, Guillaume Lecointre and Jean-Yves Rasplus
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Appendix 1. Taxonomic sampling, geographic origin, genetic markers used (see Material and methods for definitions of acronyms; NA = not available) and specimens that were morphologically examined (ME). Vouchers, if available, are housed in IEBR, MHNG, HNHM, MNHN, CBC, RMNH or ZMA (see Material and methods for explanation of museum acronyms). Abbreviations: NBCA = National Biodiversity Conservation Area; NP = National Park; NR = Nature Reserve; PF = Protected Forest. Question marks (?) refer to examined bats without genetic information.

Museum	Original name	Corrected name	Tissue code	Catalogue no. / Field no.	Sex	Locality	Province	Country	Code on Fig.1	*Cytb*	*COI*	HDAC2	CHPF2	HDAC1	RIOK3	ZFyVE27	TuFM	PABPNI	Haplo-group	ME
IEBR	*Eptesicus* sp.	*Eptesicus* sp.	VN11-0076	Tu.110511.15	–	Copia NR, Coma	Son La	Vietnam	–	KX496340	KX496341	KX496342	KX496343	KX496344	KX496345	KX496346	KX496347	NA	–	–
IEBR	*Hypsugo pulveratus*	*Hypsugo pulveratus*	VN11-0240	Tu.22061140	–	Copia NR, Coma	Son La	Vietnam	–	KX496348	KX496349	KX496350	KX496351	KX496352	KX496353	KX496354	KX496355	KX496356	–	–
IEBR	*Pipistrellus* cf. *javanicus*	*Pipistrellus* cf. *javanicus*	VN11-0379	Tu.300711.7	–	Nui chua NP	Ninh Thuan	Vietnam	–	KX496357	KX496358	KX496359	KX496360	KX496361	KX496362	KX496363	KX496364	KX496365	–	–
MNHN	*T. pachypus*	*T. pachypus*	1906-96N	1906-96N	–	Pane Bandar Barbu 1200m	Sumatra	Indonesia	SU	NA	KX496366	NA	NA	NA	NA	NA	NA	NA	Tp1	–
ZMA	*T. pachypus*	*T. pachypus*	2000	2000	–	Deli	Sumatra	Indonesia	SU	NA	KX496536	NA	NA	NA	NA	NA	NA	NA	Tp1	–
MNHN	*T. pachypus*	*T. pachypus*	1906-96L	1906-96L	–	Pane Bandar Barbu 1200m	Sumatra	Indonesia	SU	NA	KX496537	NA	NA	NA	NA	NA	NA	NA	Tp1	–
MHNG	*T. pachypus*	*T. pachypus*	–	MHNG1481.035	♂	Binjai, Medan	Sumatra	Indonesia	SU	NA	NA	NA	NA	NA	NA	NA	NA	NA	Tp1	x
MHNG	*T. pachypus*	*T. pachypus*	–	MHNG1481.042	♀	Binjai, Medan	Sumatra	Indonesia	SU	NA	NA	NA	NA	NA	NA	NA	NA	NA	?Tp1	x
MHNG	*T. pachypus*	*T. pachypus*	–	MHNG1481.045	♂	Binjai, Medan	Sumatra	Indonesia	SU	NA	NA	NA	NA	NA	NA	NA	NA	NA	?Tp1	x
IEBR	*T. pachypus*	*T. fulvida*	VN11-0015	Tu.220411.7	♂	Vu Quang NP	Ha Tinh	Vietnam	V7	KX496473	KX496474	KX496475	KX496476	KX496477	KX496478	KX496479	KX496480	KX496481	Tp2	x
IEBR	*T. pachypus*	*T. fulvida*	VN11-0016	Tu.220411.8	♀	Vu Quang NP	Ha Tinh	Vietnam	V7	NA	KX496482	NA	NA	NA	NA	NA	NA	NA	Tp2	x
IEBR	*T. pachypus*	*T. fulvida*	VN11-0019	Tu.220411.11	♀	Vu Quang NP	Ha Tinh	Vietnam	V7	KX496483	KX496484	KX496485	KX496486	KX496487	KX496488	KX496489	KX496490	KX496491	Tp2	–
IEBR	*T. pachypus*	*T. fulvida*	VN11-0020	Tu.220411.12	♀	Vu Quang NP	Ha Tinh	Vietnam	V7	NA	KX496492	NA	NA	NA	NA	NA	NA	NA	Tp2	x
IEBR	*T. pachypus*	*T. fulvida*	VN11-0021	Tu.220411.13	♂	Vu Quang NP	Ha Tinh	Vietnam	V7	NA	KX496493	NA	NA	NA	NA	NA	NA	NA	Tp2	–
IEBR	*T. pachypus*	*T. fulvida*	VN11-0024	Tu.230411.3	♀	Vu Quang NP	Ha Tinh	Vietnam	V7	NA	KX496494	NA	NA	NA	NA	NA	NA	NA	Tp2	x
IEBR	*T. pachypus*	*T. fulvida*	VN11-0032	Tu.230411.11	♂	Vu Quang NP	Ha Tinh	Vietnam	V7	NA	KX496495	NA	NA	NA	NA	NA	NA	NA	Tp3	x
HNHM	*T. pachypus*	*T. fulvida*	22865	22865	–	Bac Huong Hoa NR	Quang Tri	Vietnam	V8	NA	KX496462	NA	NA	NA	NA	NA	NA	NA	Tp2	–
IEBR	*T. pachypus*	*T. fulvida*	VN11-1159	–	–	Ngoc Linh mountain	Kon Tum	Vietnam	V9	KX496515	KX496516	KX496517	KX496518	KX496519	KX496520	KX496521	KX496522	KX496523	Tp2	–
IEBR	*T. pachypus*	*T. fulvida*	–	2013116-B34	♀	Vinh Cuu NR	Dong Nai	Vietnam	V11	NA	NA	NA	NA	NA	NA	NA	NA	NA	?Tp2	x
HNHM	*T. pachypus*	*T. fulvida*	2005.82.2	2005.82.2./ 21505/ SBCA2	♂	Seima NBCA	Mondolkiri	Cambodia	C4	NA	KX496383	NA	NA	NA	NA	NA	NA	NA	Tp2	x
HNHM	*T. pachypus*	*T. fulvida*	21475	2005.81.31./ 21475/CSOCA31	♂	Seima NBCA	Mondolkiri	Cambodia	C4	NA	KX496392	NA	NA	NA	NA	NA	NA	NA	Tp2	x
IEBR	*T. pachypus*	*T. fulvida*	VN11-0930	T.200708.1	♂	Na Don, Phuong Vien, Cho Don	Bac Kan	Vietnam	V2	KX496496	KX496497	KX496498	KX496499	KX496500	KX496501	KX496502	KX496503	KX496504	Tp3	x
IEBR	*T. pachypus*	*T. fulvida*	VN11-0931	T.200708.3	♂	Na Don, Phuong Vien, Cho Don	Bac Kan	Vietnam	V2	NA	KX496505	NA	NA	NA	NA	NA	NA	NA	Tp3	x
IEBR	*T. pachypus*	*T. fulvida*	VN11-0933	T.200708.2	♂	Na Don, Phuong Vien, Cho Don	Bac Kan	Vietnam	V2	NA	KX496506	NA	NA	NA	NA	NA	NA	NA	Tp3	–
IEBR	*T. pachypus*	*T. fulvida*	VN11-1138	VN11-1138	♀	Uy No, Dong Anh	Hanoi	Vietnam	V2	KX496507	KX496508	KX496509	NA	KX496510	KX496511	KX496512	KX496513	KX496514	Tp3	x

Museum	Original name	Corrected name	Tissue code	Catalogue no. / Field no.	Sex	Locality	Province	Country	Code on Fig.1	*Cytb*	*COI*	HDAC2	CHPF2	HDAC1	RIOK3	ZFyVE27	TuFM	PABPN1	Haplo-group	ME
HNHM	*T. pachypus*	*T. fulvida*	–	22834/BL15	♂	Na Hang	Tuyen Quang	Vietnam	V3	NA	NA	NA	NA	NA	NA	NA	NA	NA	?Tp3	x
IEBR	*T. pachypus*	*T. fulvida*	T5008	Tu.100310.2	♂	Mai Lam, Dong Anh	Hanoi	Vietnam	V6	NA	KX496463	NA	NA	NA	NA	NA	NA	NA	Tp3	x
IEBR	*T. pachypus*	*T. fulvida*	T5009	Tu.270709.1	♂	Mai Lam, Dong Anh	Hanoi	Vietnam	V6	KX496464	KX496465	KX496466	NA	KX496467	KX496468	KX496469	KX496470	KX496471	Tp3	x
IEBR	*T. pachypus*	*T. fulvida*	T5011	Tu.100310.1	♀	Mai Lam, Dong Anh	Hanoi	Vietnam	V6	NA	KX496472	NA	NA	NA	NA	NA	NA	NA	Tp3	x
MHNG	*T. pachypus*	*T. fulvida*	VN11-1780	MHNG1926.054	♀	Naten	Phongsaly	Laos	L1	NA	KX496524	NA	NA	NA	NA	NA	NA	NA	Tp3	x
MHNG	*T. pachypus*	*T. fulvida*	VN11-1782	MHNG1926.056	♀	Hat Hin, Nam Sing River	Phongsaly	Laos	L1	NA	KX496525	NA	NA	NA	NA	NA	NA	NA	Tp3	x
MHNG	*T. pachypus*	*T. fulvida*	VN11-1792	MHNG1926.055	♂	Hat Hin, Nam Sing River	Phongsaly	Laos	L1	KX496526	KX496527	KX496528	KX496529	KX496530	KX496531	KX496532	KX496533	KX496534	Tp3	x
MNHN	*T. pachypus*	*T. fulvida*	VN11-1793	CG-2006-89	♀	Hat Hin, Nam Sing River	Phongsaly	Laos	L1	NA	KX496535	NA	NA	NA	NA	NA	NA	NA	Tp3	–
ZMA	*T. robustula*	*T. robustula*	–	18449	♀	Berastagi, Sumatra	Sumatra	Indonesia	SU	NA	KX496367	NA	NA	NA	NA	NA	NA	NA	Tr1	x
MNHN	*T. robustula*	*T. robustula*	1906-96C	1906-96C	–	Pane Bandar Barbu 1200m	Sumatra	Indonesia	SU	NA	KX496368	NA	NA	NA	NA	NA	NA	NA	Tr1	–
MHNG	*T. robustula*	*T. robustula*	–	MHNG1481.025	♂	Binjai, Medan	Sumatra	Indonesia	SU	NA	NA	NA	NA	NA	NA	NA	NA	NA	?Tr1	x
MHNG	*T. robustula*	*T. robustula*	–	MHNG1481.038	♀	Binjai, Medan	Sumatra	Indonesia	SU	NA	NA	NA	NA	NA	NA	NA	NA	NA	?Tr1	x
IEBR	*T. robustula*	*T. malayana*	VN11-0022	Tu.230411.1	♂	Vu Quang NP	Ha Tinh	Vietnam	V7	KX496401	KX496402	KX496403	KX496404	KX496405	KX496406	KX496407	KX496408	KX496409	Tr2	x
IEBR	*T. robustula*	*T. malayana*	VN11-0023	Tu.230411.2	♀	Vu Quang NP	Ha Tinh	Vietnam	V7	NA	KX496410	NA	NA	NA	NA	NA	NA	NA	Tr2	x
IEBR	*T. robustula*	*T. malayana*	–	2013I8-B15	♂	Vinh Cuu NR	Dong Nai	Vietnam	V11	NA	NA	NA	NA	NA	NA	NA	NA	NA	?Tr2	x
HNHM	*T. robustula*	*T. malayana*	–	23066/PL42	♂	Vinh Cuu NR	Dong Nai	Vietnam	V11	NA	NA	NA	NA	NA	NA	NA	NA	NA	?Tr2	x
HNHM	*T. robustula*	*T. malayana*	–	23067/PL55	♂	Vinh Cuu NR	Dong Nai	Vietnam	V11	NA	NA	NA	NA	NA	NA	NA	NA	NA	?Tr2	x
HNHM	*T. robustula*	*T. malayana*	–	23080/PL135	♂	Vinh Cuu NR	Dong Nai	Vietnam	V11	NA	NA	NA	NA	NA	NA	NA	NA	NA	?Tr2	x
IEBR	*T. robustula*	*T. malayana*	–	NTS321	♀	Cat Tien NP	Dong Nai	Vietnam	V11	NA	NA	NA	NA	NA	NA	NA	NA	NA	?Tr2	x
IEBR	*T. robustula*	*T. malayana*	–	VC-10	♀	Vinh Cuu NR	Dong Nai	Vietnam	V11	NA	NA	NA	NA	NA	NA	NA	NA	NA	?Tr2	x
HNHM	*T. robustula*	*T. malayana*	–	2005.82.52./ 21555	♀	–	Khammoun	Laos	L7	NA	NA	NA	NA	NA	NA	NA	NA	NA	?Tr2	x
MNHN	*T. robustula*	*T. malayana*	CPV10-503	CPV10-503	♀	Virachey NP, Taveng Village	Rattanakiri	Cambodia	C1	KX496369	KX496370	KX496371	KX496372	KX496373	KX496374	KX496375	KX496376	KX496377	Tr2	x
HNHM	*T. robustula*	*T. malayana*	21448	2005.81.4./ 21448/ CSOCA04	♂	Preah Vihear PF	Preah Vihear	Cambodia	C2	NA	KX496390	NA	NA	NA	NA	NA	NA	NA	Tr2	x
CBC	*T. robustula*	*T. malayana*	VN11-1563	CBC01275	♂	Baray Mts, Preah Vihear PF	Preah Vihear	Cambodia	C2	KX496411	KX496412	KX496413	KX496414	KX496415	KX496416	KX496417	KX496418	KX496419	Tr2	x
CBC	*T. robustula*	*T. malayana*	VN11-1614	CBC00910	♀	Kbal Spean, Koulen NP	Siem Reap	Cambodia	C3	NA	KX496420	NA	NA	NA	NA	NA	NA	NA	Tr2	x
CBC	*T. robustula*	*T. malayana*	–	CBC00908	♀	Kbal Spean, Koulen NP	Siem Reap	Cambodia	C3	NA	NA	NA	NA	NA	NA	NA	NA	NA	?Tr2	x
HNHM	*T. robustula*	*T. malayana*	21466	2005.81.22./ 21466/CSOCA22	♂	Seima NBCA	Mondolkiri	Cambodia	C4	NA	KX496391	NA	NA	NA	NA	NA	NA	NA	Tr2	x
HNHM	*T. robustula*	*T. malayana*	21726	2006.34.36.	–	Seima NBCA	Mondolkiri	Cambodia	C4	NA	KX496393	NA	NA	NA	NA	NA	NA	NA	Tr2	–
HNHM	*T. robustula*	*T. malayana*	2005.81.23	2005.81.23./ 21467/CSOCA23	♂	Seima NBCA	Mondolkiri	Cambodia	C4	NA	KX496378	NA	NA	NA	NA	NA	NA	NA	Tr2	x
HNHM	*T. robustula*	*T. malayana*	2005.81.24	2005.81.24./ 21468/CSOCA24	♂	Seima NBCA	Mondolkiri	Cambodia	C4	NA	KX496379	NA	NA	NA	NA	NA	NA	NA	Tr2	x

Museum	Original name	Corrected name	Tissue code	Catalogue no. / Field no.	Sex	Locality	Province	Country	Code on Fig.1	*Cytb*	*COI*	HDAC2	CHPF2	HDAC1	RIOK3	ZFyVE27	TuFM	PABPNI	Haplo-group	ME
HNHM	*T. robustula*	*T. malayana*	2005.81.29	2005.81.29./ 21473/CSOCA29	♀	Seima NBCA	Mondolkiri	Cambodia	C4	NA	KX496380	NA	NA	NA	NA	NA	NA	NA	Tr2	x
HNHM	*T. robustula*	*T. malayana*	2005.81.30	2005.81.30./ 214747/CSOCA30	♂	Seima NBCA	Mondolkiri	Cambodia	C4	NA	KX496381	NA	NA	NA	NA	NA	NA	NA	Tr2	x
HNHM	*T. robustula*	*T. malayana*	2005.81.47	2005.81.47./ 21491/CSOCA47	♂	Seima NBCA	Mondolkiri	Cambodia	C4	NA	KX496382	NA	NA	NA	NA	NA	NA	NA	Tr2	x
HNHM	*T. robustula*	*T. malayana*	2005.82.4	2005.82.4./ 21507/ SBCA4	♀	Seima NBCA	Mondolkiri	Cambodia	C4	NA	KX496384	NA	NA	NA	NA	NA	NA	NA	Tr2	x
HNHM	*T. robustula*	*T. malayana*	2006.34.39	2006.34.39	–	Seima NBCA	Mondolkiri	Cambodia	C4	NA	KX496385	NA	NA	NA	NA	NA	NA	NA	Tr2	–
HNHM	*T. robustula*	*T. malayana*	2006.34.41	2006.34.41	–	Seima NBCA	Mondolkiri	Cambodia	C4	NA	KX496386	NA	NA	NA	NA	NA	NA	NA	Tr2	–
HNHM	*T. robustula*	*T. malayana*	2006.34.42	2006.34.42	–	Seima NBCA	Mondolkiri	Cambodia	C4	NA	KX496387	NA	NA	NA	NA	NA	NA	NA	Tr2	–
HNHM	*T. robustula*	*T. malayana*	2006.34.43	2006.34.43	–	Seima NBCA	Mondolkiri	Cambodia	C4	NA	KX496388	NA	NA	NA	NA	NA	NA	NA	Tr2	–
HNHM	*T. robustula*	*T. malayana*	2006.34.44	2006.34.44	–	Seima NBCA	Mondolkiri	Cambodia	C4	NA	KX496389	NA	NA	NA	NA	NA	NA	NA	Tr2	–
HNHM	*T. robustula*	*T. malayana*	–	2005.82.6./ 21509/ SBCA01	♀	Seima NBCA	Mondolkiri	Cambodia	C4	NA	NA	NA	NA	NA	NA	NA	NA	NA	?Tr2	x
HNHM	*T. robustula*	*T. malayana*	–	2005.82.7./ 21510/ SBCA02	♀	Seima NBCA	Mondolkiri	Cambodia	C4	NA	NA	NA	NA	NA	NA	NA	NA	NA	?Tr2	x
CBC	*T. robustula*	*T. malayana*	–	CBC00478	♂	Seima NBCA	Mondolkiri	Cambodia	C4	NA	NA	NA	NA	NA	NA	NA	NA	NA	?Tr2	x
MHNG	*T. robustula*	*T. malayana*	M1538	MHNG1970.041	♀	Ulu Kenas Recreational Forest	Perak	Malaysia	MP	NA	KX496394	NA	NA	NA	NA	NA	NA	NA	Tr2	x
MHNG	*T. robustula*	*T. malayana*	M1539	MHNG1970.042	♀	Ulu Kenas Recreational Forest	Perak	Malaysia	MP	NA	KX496395	NA	NA	NA	NA	NA	NA	NA	Tr2	x
MHNG	*T. robustula*	*T. malayana*	M1540	MHNG1970.043	♂	Ulu Kenas Recreational Forest	Perak	Malaysia	MP	NA	KX496396	NA	NA	NA	NA	NA	NA	NA	Tr2	x
MHNG	*T. robustula*	*T. malayana*	M1881	MHNG1991.095	♂	Northeastern India	India	India	IN	KX496397	KX496398	NA	NA	NA	NA	NA	NA	NA	Tr2	x
MHNG	*T. robustula*	*T. malayana*	M1925	MHNG1991.096	♀	Northeastern India	India	India	IN	KX496399	KX496400	NA	NA	NA	NA	NA	NA	NA	Tr2	x
IEBR	*T. robustula*	*T. tonkinensis*	VN11-0932	T.260607.1	♀	Na Don, Phuong Vien, Cho Don	Bac Kan	Vietnam	V2	NA	KX496430	NA	NA	NA	NA	NA	NA	NA	Tr3	x
IEBR	*T. robustula*	*T. tonkinensis*	VN11-0934	T.280607.2	♀	Na Don, Phuong Vien, Cho Don	Bac Kan	Vietnam	V2	NA	KX496431	NA	NA	NA	NA	NA	NA	NA	Tr3	x
IEBR	*T. robustula*	*T. tonkinensis*	VN11-1004	T.260811.1	♂	Na Hang NR	Tuyen Quang	Vietnam	V3	KX496432	KX496433	KX496434	KX496435	KX496436	KX496437	KX496438	KX496439	KX496440	Tr3	x
HNHM	*T. robustula*	*T. tonkinensis*	22834	22834	♂	Na Hang NR	Tuyen Quang	Vietnam	V3	NA	NA	NA	NA	NA	NA	NA	NA	NA	?Tr3	x
IEBR	*T. robustula*	*T. tonkinensis*	VN11-0055	Tu.09.05.11.3	♂	Copia NR, Coma	Son La	Vietnam	V4	KX496421	KX496422	KX496423	KX496424	KX496425	KX496426	KX496427	KX496428	KX496429	Tr3	x
IEBR	*T. robustula*	*T. tonkinensis*	VN11-1804	VN11-1804	♂	Hang Kia Pa Co NR	Hoa Binh	Vietnam	V5	KX496451	KX496452	KX496453	KX496454	KX496455	KX496456	KX496457	KX496458	KX496459	Tr3	x
MHNG	*T. robustula*	*T. tonkinensis*	VN11-1783	MHNG1926.059	♂	Hat Hin, Nam Sing River	Phongsaly	Laos	L1	KX496441	KX496442	KX496443	KX496444	KX496445	KX496446	KX496447	KX496448	KX496449	Tr3	x
MNHN	*T. robustula*	*T. tonkinensis*	VN11-1785	CG-2006-93	♂	Hat Hin, Nam Sing River	Phongsaly	Laos	L1	NA	KX496460	NA	NA	NA	NA	NA	NA	NA	Tr3	–
MNHN	*T. robustula*	*T. tonkinensis*	VN11-1786	MHNG1926.057	♀	Naten	Phongsaly	Laos	L1	NA	KX496450	NA	NA	NA	NA	NA	NA	NA	Tr3	x
MNHN	*T. robustula*	*T. tonkinensis*	VN11-1791	CG-2006-93	♂	Naten	Phongsaly	Laos	L1	NA	KX496461	NA	NA	NA	NA	NA	NA	NA	Tr3	–
MHNG	*T. robustula*	*T. tonkinensis*	–	MHNG1926.058	♀	Hat Hin, Nam Sing River	Phongsaly	Laos	L1	NA	NA	NA	NA	NA	NA	NA	NA	NA	?Tr3	x

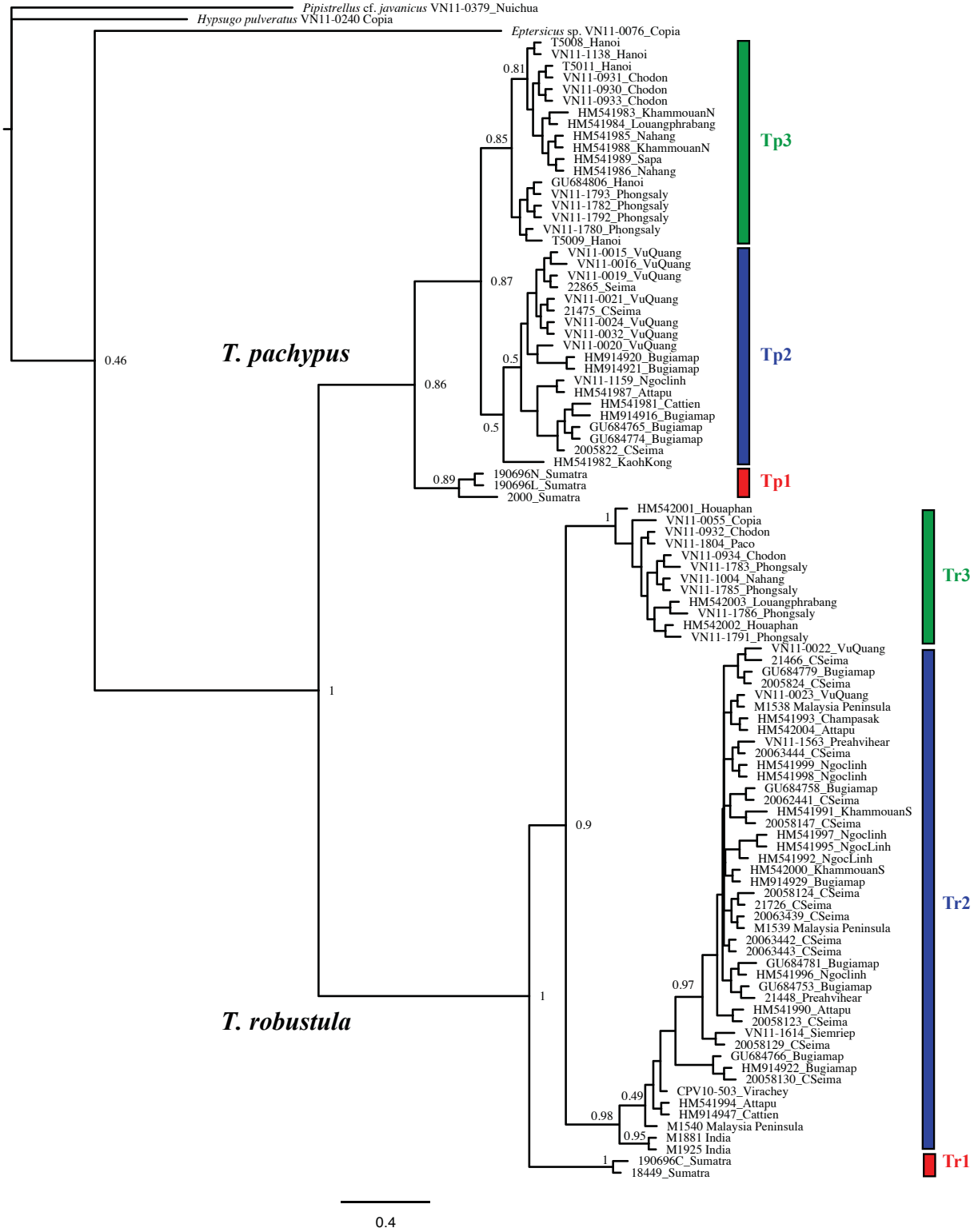
Appendix 2. GenBank accession numbers included in phylogenetic reconstructions. Abbreviations: NBCA = National Biodiversity Conservation Area; NP = National Park; NR = Nature Reserve.

Original name	Corrected name	Locality	Province	Country	Code on Fig.1	COI	Cytb	Haplo-group
T. pachypus	*T. fulvida*	Bu Gia Map NR	Binh Phuoc	Vietnam	V10	GU684765	NA	Tp2
T. pachypus	*T. fulvida*	Bu Gia Map NR	Binh Phuoc	Vietnam	V10	GU684774	NA	Tp2
T. pachypus	*T. fulvida*	Tech Centre, Thanh Xuan	Hanoi	Vietnam	V6	GU684806	NA	Tp2
T. pachypus	*T. fulvida*	Cat Tien NP	Lam Dong	Vietnam	V11	HM541981	NA	Tp2
T. pachypus	*T. fulvida*	Central Cardamom Mts, Thmar Bang	Kaoh Kong	Cambodia	C5	HM541982	NA	Tp2
T. pachypus	*T. fulvida*	Ban Keng Bit	Khammouan	Laos	L4	HM541983	NA	Tp2
T. pachypus	*T. fulvida*	Xe Kaman, Dong Amphan NBCA	Attapeu	Laos	L8	HM541987	NA	Tp2
T. pachypus	*T. fulvida*	Bu Gia Map NR	Binh Phuoc	Vietnam	V10	HM914916	NA	Tp2
T. pachypus	*T. fulvida*	Bu Gia Map NR	Binh Phuoc	Vietnam	V10	HM914920	NA	Tp2
T. pachypus	*T. fulvida*	–	Hong Kong	China	HK	NA	EF517314	Tp2
T. pachypus	*T. fulvida*	Nam Et NBCA, Nam Khan	Louangphrabang	Laos	L2	HM541984	NA	Tp3
T. pachypus	*T. fulvida*	Na Hang NR	Tuyen Quang	Vietnam	V3	HM541985	NA	Tp3
T. pachypus	*T. fulvida*	Na Hang NR	Tuyen Quang	Vietnam	V3	HM541986	NA	Tp3
T. pachypus	*T. fulvida*	1 km SW Ban Houana	Khammouan	Laos	L5	HM541988	NA	Tp3
T. pachypus	*T. fulvida*	Sa Pa Mts, Van Ban	Lao Cai	Vietnam	V1	HM541989	NA	Tp3
T. pachypus	*T. fulvida*	–	Guangxi,	China	GX	NA	EF517313	Tp3
T. pachypus	*T. fulvida*	–	Guangdong	China	GD	NA	EF517315	Tp3
T. pachypus[*]	*T. fulvida*	Bu Gia Map NR	Binh Phuoc	Vietnam	V10	HM914921	NA	–
T. robustula[+]	*T. pachypus*	–	Sarawak	Malaysia	BO	NA	EU521635	–
T. robustula	*T. malayana*	Bu Gia Map NR	Binh Phuoc	Vietnam	V10	GU684753	NA	Tr2

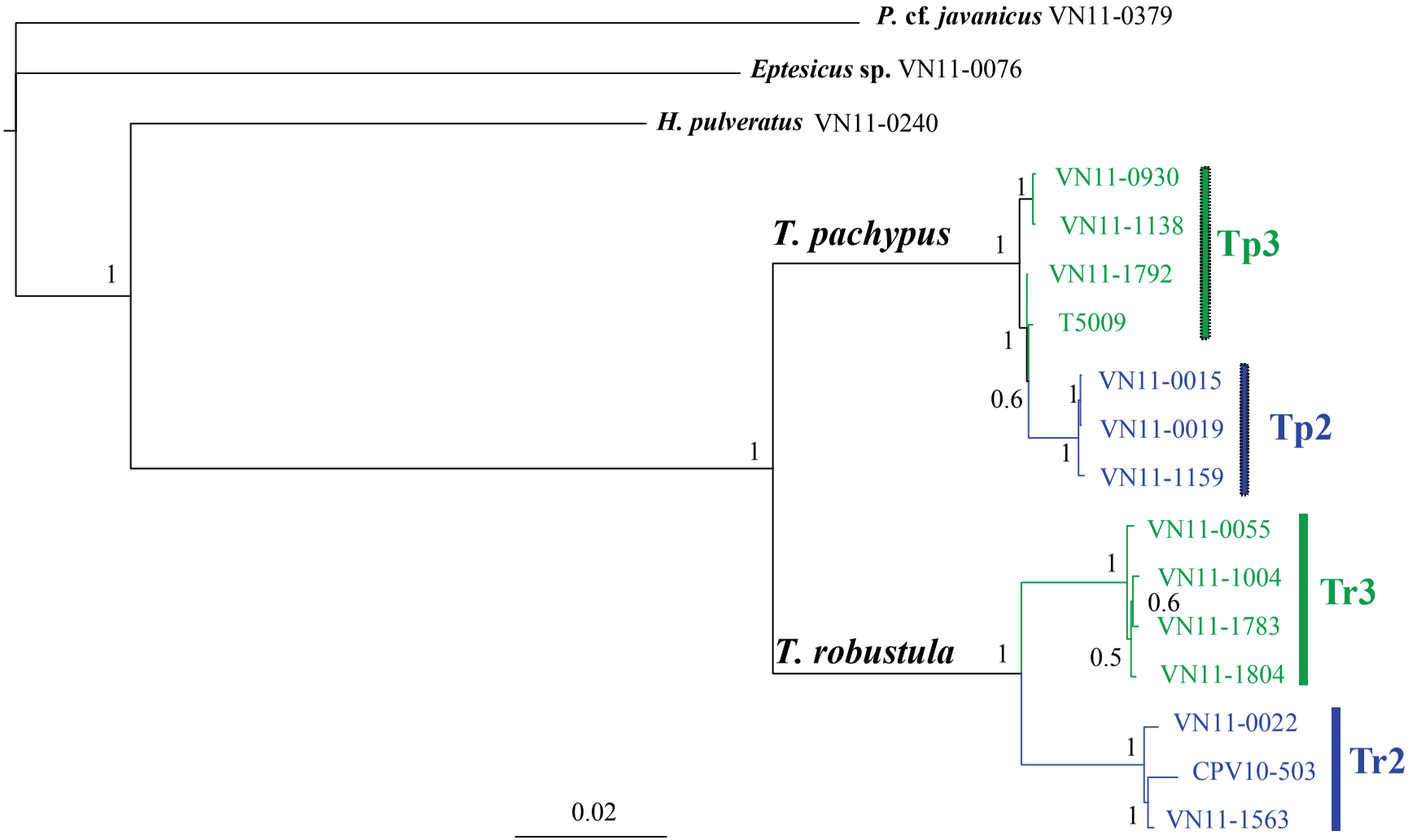
Notes: [*] Specimen originally misidentified as *T. robustula* s. lat. (see Huang *et al.* 2014).
[+] Specimen originally identified as *T. robustula*, but appears to be an unknown species of the *T. pachypus* complex.

Original name	Corrected name	Locality	Province	Country	Code on Fig.1	COI	Cytb	Haplo-group
T. robustula	*T. malayana*	Bu Gia Map NR	Binh Phuoc	Vietnam	V10	GU684758	NA	Tr2
T. robustula	*T. malayana*	Bu Gia Map NR	Binh Phuoc	Vietnam	V10	GU684766	NA	Tr2
T. robustula	*T. malayana*	Bu Gia Map NR	Binh Phuoc	Vietnam	V10	GU684779	NA	Tr2
T. robustula	*T. malayana*	Bu Gia Map NR	Binh Phuoc	Vietnam	V10	GU684781	NA	Tr2
T. robustula	*T. malayana*	Xe Kaman, Dong Amphan NBCA	Attapeu	Laos	L8	HM541990	NA	Tr2
T. robustula	*T. malayana*	Ban Xam Kang, Hin Nam No	Khammouan	Laos	L7	HM541991	NA	Tr2
T. robustula	*T. malayana*	Ngoc Linh Mountain, 5 km E of Nuoc Xa	Quang Nam	Vietnam	V9	HM541992	NA	Tr2
T. robustula	*T. malayana*	Dong Kanthung	Champasak	Laos	L9	HM541993	NA	Tr2
T. robustula	*T. malayana*	Xe Kaman, Dong Amphan NBCA	Attapeu	Laos	L8	HM541994	NA	Tr2
T. robustula	*T. malayana*	Ngoc Linh Mountain	Quang Nam	Vietnam	V9	HM541995	NA	Tr2
T. robustula	*T. malayana*	Ngoc Linh Mountain	Quang Nam	Vietnam	V9	HM541996	NA	Tr2
T. robustula	*T. malayana*	Ngoc Linh Mountain	Quang Nam	Vietnam	V9	HM541997	NA	Tr2
T. robustula	*T. malayana*	Ngoc Linh Mountain	Quang Nam	Vietnam	V9	HM541998	NA	Tr2
T. robustula	*T. malayana*	Ngoc Linh Mountain	Quang Nam	Vietnam	V9	HM541999	NA	Tr2
T. robustula	*T. malayana*	1 km SW of Ban Houana	Khammouan	Laos	L6	HM542000	NA	Tr2
T. robustula	*T. malayana*	Xe Kaman, Dong Amphan NBCA	Attapeu	Laos	L8	HM542004	NA	Tr2
T. robustula	*T. malayana*	Bu Gia Map NR	Binh Phuoc	Vietnam	V10	HM914922	NA	Tr2
T. robustula	*T. malayana*	Bu Gia Map NR	Binh Phuoc	Vietnam	V10	HM914929	NA	Tr2
T. robustula	*T. malayana*	Cat Tien NP	Lam Dong	Vietnam	V11	HM914947	NA	Tr2
T. robustula	*T. tonkinensis*	Nam Et NBCA, Ban Chak	Houaphan	Laos	L3	HM542001	NA	Tr3
T. robustula	*T. tonkinensis*	Nam Et NBCA, Nam Chong	Houaphan	Laos	L2	HM542002	NA	Tr3
T. robustula	*T. tonkinensis*	Nam Et NBCA, Nam Khan	Louangphrabang	Laos	L1	HM542003	NA	Tr3

Appendix 3. Bayesian tree of *Tylonycteris* Peters, 1872 and associated outgroups obtained from the analysis of an alignment of *COI* gene 291 nt.



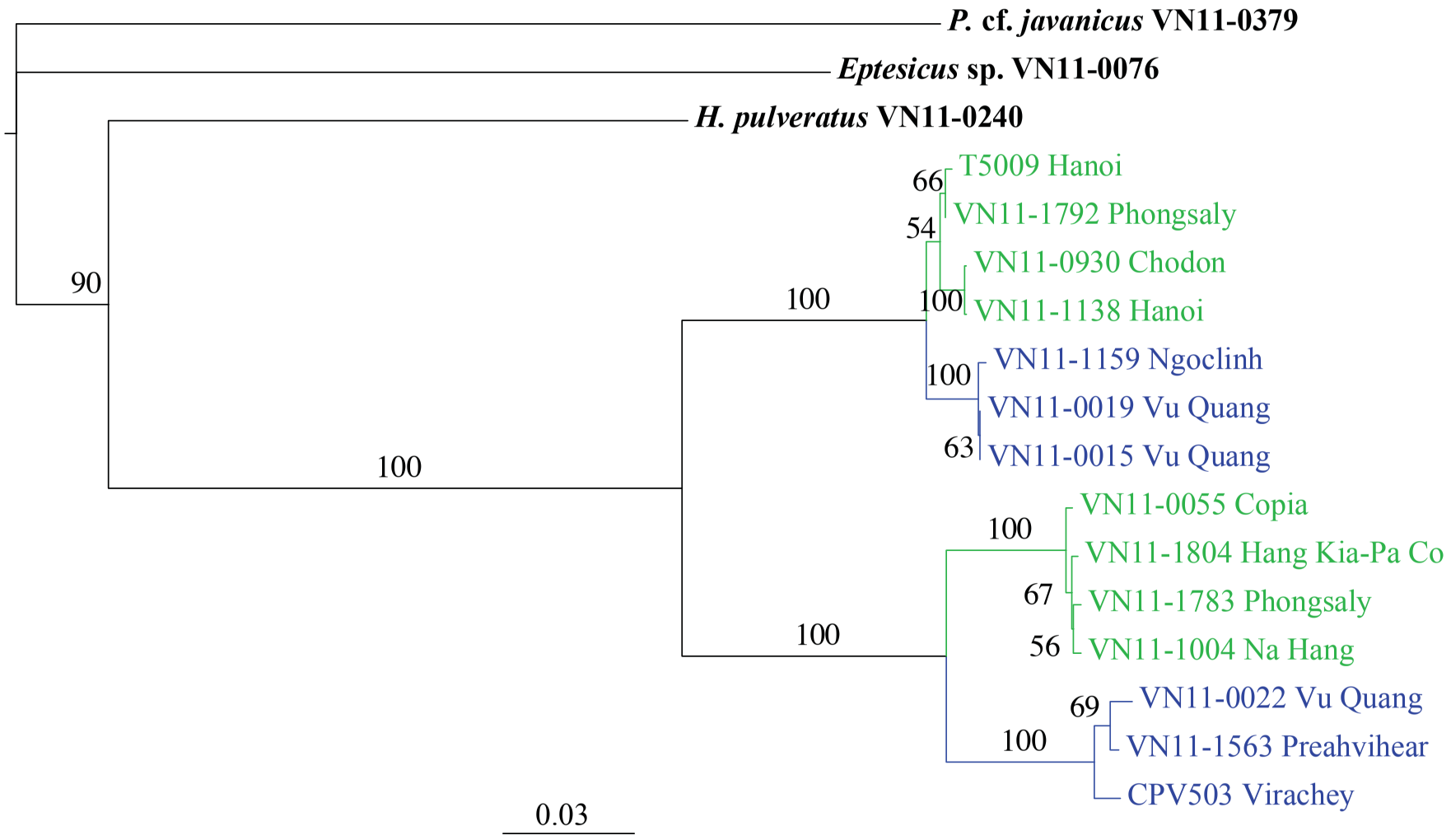
Appendix 4. Bayesian tree of *Tylonycteris* Peters, 1872 and associated outgroups obtained from the supermatrix analysis of the eight independent datasets.



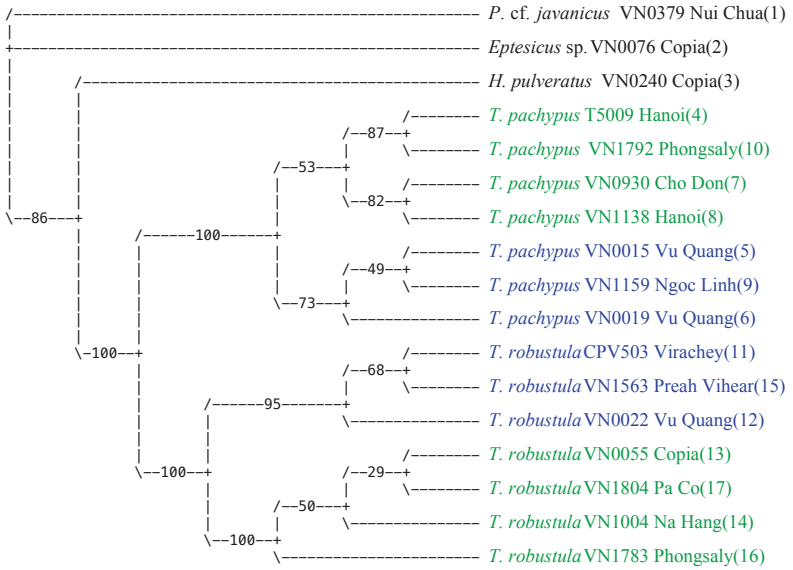
Appendix 5. Inter- and intraspecific genetic variations (% K2P; mean, minimum and maximum in percentages) calculated for *COI*, *Cytb* and nuDNA gene sequences among bats of the genus *Tylonycteris* Peters, 1872. Abbreviations: Tp = *Tylonycteris pachypus* (Temminck, 1840); Tr = *Tylonycteris robustula* Thomas, 1915; a = K2P distance calculated from alignments of 728 nt and 291nt (*) of *COI*; b = K2P distance calculated from alignments of 1140 nt of *Cytb*; c = K2P distances calculated from alignment of the concatenation of seven nuclear genes (5604 nt, nuDNA); NA = not applicable. Intraspecific distances within each geographic population are indicated in bold.

	Taxon	Gene	1	2	3	4	5	6	7	8	9	10
1	*P.* cf. *javanicus*											
2	*Eptesicus* sp.	a	17.5									
		b	18.9									
		c	5.8									
3	*H. pulveratus*	a	16.8	18.1								
		b	20.0	18.7								
		c	5.7	5.7								
4	Tp1 (Sumatra)	a	18.4*	20.2*	17.0*	**1.1* (0.0–1.7)**						
		b	NA	NA	NA	**NA**						
		c	NA	NA	NA	**NA**						
5	Tp2 (South)	a	18.3	18.7	15.9	6.1* (4.1–7.8)	**0.4 (0.0–1.5)**					
		b	19.0	19.3	18.0	NA	**1.1 (0.0–2.0)**					
		c	6.8	5.9	5.1	NA	**0.0**					
6	Tp3 (North)	a	18.4	18.7	16.3	6.0* (4.1–7.2)	2.8 (2.2–3.5)	**0.4 (0–1.1)**				
		b	18.1	19.5	18.2	NA	2.8 (2.6–3.0)	**0.5 (0.0–1.0)**				
		c	6.9	6.0	5.2	NA	0.1 (0–0.2)	**0.1 (0.0–0.2)**				
7	Tp (Borneo)	a	NA	NA	NA	NA	NA	NA				
		b	18.5	16.9	16.6	NA	6.2 (5.7–6.4)	6.1 (5.9–6.4)				
		c	NA	NA	NA	NA	NA	NA				
8	Tr1 (Sumatra)	a	20.8*	19.2*	16.4*	13.7* (11.7–14.7)	13.8* (12.4–15.9)	13.0* (12.0–14.4)	NA	**0.3***		
		b	NA	NA	NA	NA	NA	NA	NA	**NA**		
		c	NA	NA	NA	NA	NA	NA	NA	**NA**		
9	Tr2 (South)	a	18.5	19.9	17.4	14.2* (11.3–16.9)	14.3 (11.7–16.9)	13.7 (12.8–14.6)	NA	7.3* (5.2–9.4)	**1.0 (0.0–2.9)**	
		b	18.5	19.5	19.8	NA	14.4 (13.5–15.4)	13.7 (12.7–14.4)	13.2 (12.4–13.8)	NA	**2.4 (0.3–4.7)**	
		c	6.7	5.6	5.3	NA	1.8 (1.7–1.9)	1.7 (1.6–1.8)	NA	NA	**0.1 (0.0–0.2)**	
10	Tr3 (North)	a	19.8	19.3	16.6	12.9* (11.0–14.9)	13.8 (13.0–14.6)	12.6 (12.1– 13.3)	NA	5.7* (5.2–7.4)	6.5 (5.5–7.4)	**0.3 (0.0–0.7)**
		b	17.9	20.3	18.8	NA	14.1 (13.9–14.5)	13.9 (13.7–14.1)	12.3 (12.1–12.6)	NA	9.5 (8.6–10.4)	**0.6 (0.4–0.8)**
		c	6.6	5.8	5.2	NA	1.7 (1.6–1.8)	1.6 (1.5–1.8)	NA	NA	0.5 (0.4–0.6)	**0 (0.0–0.1)**

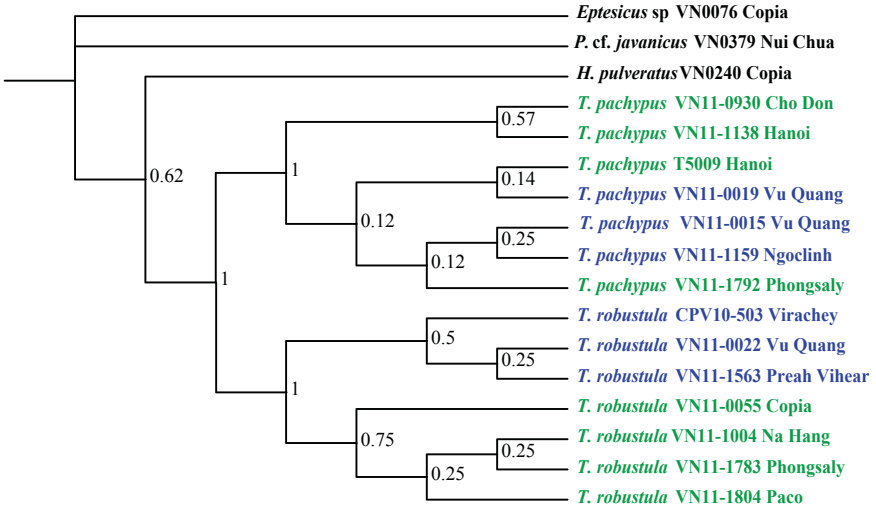
Appendix 6. Maximum likelihood tree of *Tylonycteris* Peters, 1872 and associated outgroups for the supermatrix dataset. Numbers at the nodes indicate bootstrap percentages computed by PAUP* v. 4b10 (Swofford 2003) after 1000 replicates, using the GTR + I + G model.



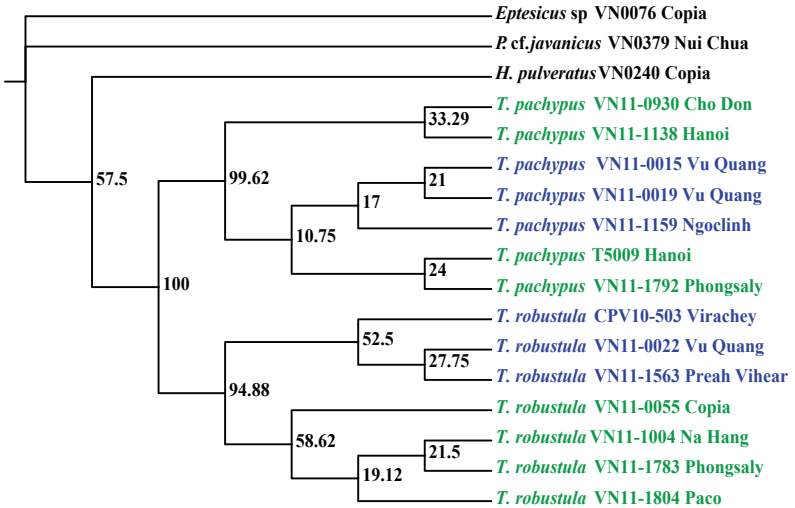
Appendix 7. 1. Supertree Bootstrap majority-rule consensus tree with Supertree Bootstrap percentages (SBP)



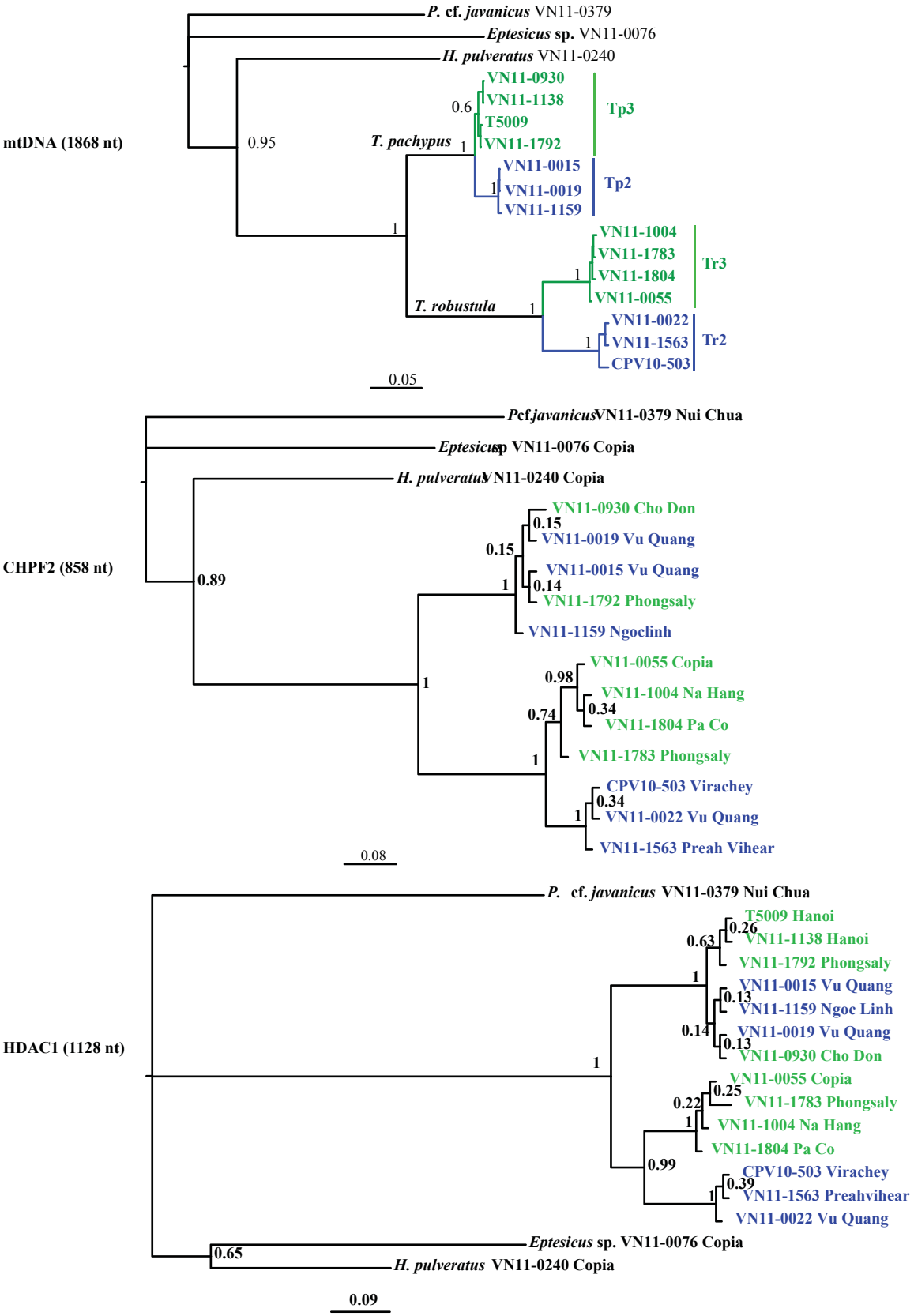
Appendix 7. 2. Mean posterior probabilities (MPP)



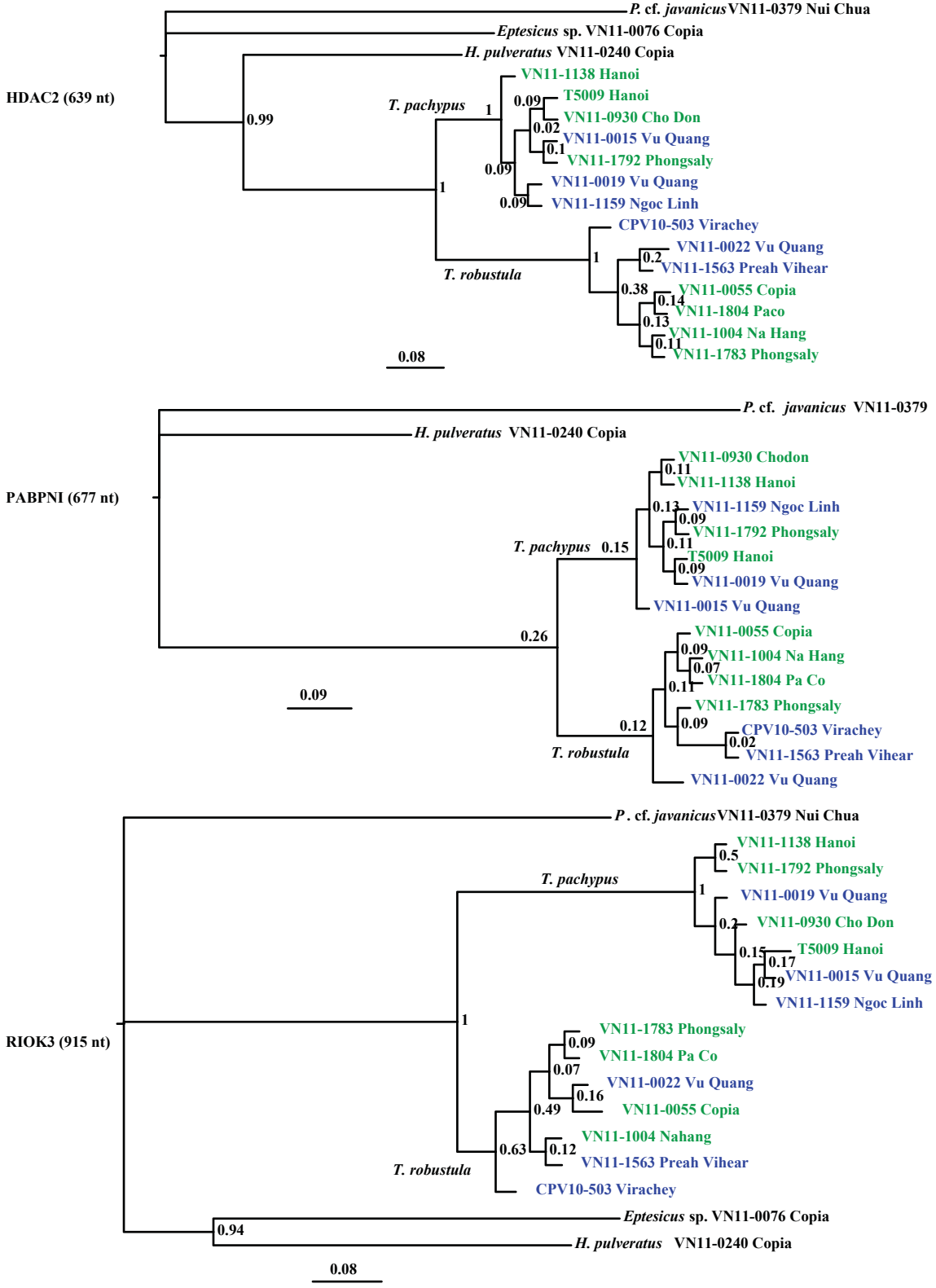
Appendix 7.3. Indices of reproducibility (Rep)



Appendix 8. Bayesian trees reconstructed from the separate analyses of the eight independent datasets (mtDNA [COI + Cytb], CHPF2, HDAC1, HDAC2, PABPN1, RIOK3, TUFM and ZFYVE27).



Appendix 8. Bayesian trees reconstructed from the separate analyses of the eight independent datasets (mtDNA [COI + Cytb], CHPF2, HDAC1, HDAC2, PABPN1, RIOK3, TUFM and ZFYVE27) (Contituned).



Appendix 8. Bayesian trees reconstructed from the separate analyses of the eight independent datasets (mtDNA [COI + Cytb], CHPF2, HDAC1, HDAC2, PABPN1, RIOK3, TUFM and ZFYVE27) (Continued).