

Supplementary Information

New insights from Thailand into the maternal genetic history of Mainland Southeast Asia

The supplementary information consists of Supplementary Text, ten Supplementary Figures and eight Supplementary Tables:

Supplementary Text Description of Approximate Bayesian Computation (ABC) analyses and additional details on mtDNA haplogroups.

Supplementary Figure S1 Relative frequency of shared haplotypes within and among 73 Thai/Lao populations. Population abbreviations are in Supplementary Table S1.

Supplementary Figure S2 Discriminant Analysis of Principal Components (DAPC) results based on linguistic groups (a) and geographic groups (b).

Supplementary Figure S3 MDS plots, based on the Φ_{st} distance matrix for 73 populations, of dimension 1 vs. 2 (a), dimension 1 vs. 3 (b) and dimension 2 vs. 3 (c). Red, black and dark blue symbols indicate AA, TK and ST populations, respectively. The stress value is 0.0711. Population abbreviations are shown in Supplementary Table S1.

Supplementary Figure S4 Pairwise Φ_{st} values with P-values obtained by 1000 permutations. Population abbreviations are in Supplementary Table S1.

Supplementary Figure S5 MDS plot based on the Φ_{st} distance matrix for 134 populations. The stress value is 0.0961. Symbols represent linguistic families: $\blacktriangle = \text{Sino-Tibetan}$, $\blacksquare = \text{Tai-Kadai}$, $\bullet = \text{Austroasiatic}$, $\spadesuit = \text{Austronesian}$, $\blacksquare = \text{Dravidian}$ and Indo-European. Black and red indicate Thai/Lao and ISEA populations, respectively while pink and green represent Indian and Chinese populations. Yellow indicates populations from Myanmar, Cambodia and Vietnam. Population abbreviations are shown in Supplementary Table S1.

Supplementary Figure S6 Networks of major haplogroups.

Supplementary Figure S7 Bayesian skyline plots (BSP) for each individual population. The 95% highest posterior density limits are indicated by the blue shading. Population abbreviations are in Supplementary Table S1.

Supplementary Figure S8 Evolution of the ABC-RF prior error rate with respect to the number of trees in the forest for the ABC analysis of the origins of Central Thai groups (A) and of the relationships between populations from different MSEA language families (B).

Supplementary Figure S9 Linear Discriminate Analysis (LDA) plot for the fit between the observed data and the simulated data generated by each model for the origin of Central Thai groups (a) and the relationships of populations from different language families (b).

Supplementary Figure S10 Posterior distributions of the parameters estimated for the demic diffusion (A) and continuous migration (B) models. The X axis covers the range of the (uniform) prior distributions. Abbreviations are in Supplementary Table S5.

Supplementary Table S1 Details for the populations used in the comparative analyses.

Supplementary Table S2 Haplogroup information in all studied samples.

Supplementary Table S3 Haplogroup frequencies (in percentage) observed in 22 populations. Bold letter indicates new haplogroups which were not found in our previous study of Thai/Lao populations.

Supplementary Table S4 Random forests confusion matrix and classification error for the ABC analysis of Central Thai origins.

Supplementary Table S5 Votes assigned to each model by the Random Forest procedure and posterior probability of the selected model in the ABC analysis of Central Thai origins.

Supplementary Table S6 Parameters estimation for the demic diffusion and continuous migration model in the ABC analysis of Central Thai origins. NcAA is the current effective population size of Austroasiatic populations, NcCT is the current central Thai effective population size, NcDAI is the current effective population size for Southern Chinese populations, M1 is the migration rate to Austroasiatic groups from Central Thai populations and M2 is the migration rate to Central Thai populations from Austroasiatic groups.

Supplementary Table S7 Random forests confusion matrix and classification error for the ABC analysis of the relationships between populations from different MSEA language families.

Supplementary Table S8 Votes assigned to each model by the Random Forest procedure and posterior probability of the selected model in the ABC analysis of the relationships between populations from different MSEA language families.

Supplementary Text

Description of Approximate Bayesian Computation (ABC) analyses

An ABC approach was utilized to test different demographic scenarios concerning the relationships of SEA language families and the origin of central Thai (CT) populations. Employing an ABC methodology allowed us to simulate the evolution of complete mitochondrial sequences, by means of coalescent theory, under different competing models and to select the model that was best able to recreate the variation observed in our populations. The simulations were generated considering prior distributions associated with different model parameters. For the maternal origin of CT populations, we considered the same three demographic scenarios tested in our previous

study¹ for the origins of North/Northeastern Thai and Laos populations: demic diffusion; an endogenous origin (with cultural diffusion of the TK language); and continuous migration (Figure 2). The demic diffusion model postulates a first split of AA-speaking Mon (MO) and Khmer (KH) from the TK-speaking populations (Xishuangbanna Dai and CT) ~3 kya² followed by a later split of CT from Xishuangbanna Dai ~1.2 kya (Figure 2a).¹¹³ The endogenous scenario involves instead an early split of the Xishuangbanna Dai from CT and AA groups, with a later division of CT and AA ~0.8 kya (Figure 2b).⁴ The continuous migration model incorporates the same demographic history as the demic diffusion model, but includes additional gene-flow between AA and CT after first contact (0.8 kya) (Figure 2c).

For all of the models in the CT origin test, we assumed constant population sizes that were allowed to vary among groups, a fixed mutation rate from Fu *et al.*⁵ (4.80 x 10^{-7}), and fixed separation times based on historical records. We assigned a uniform prior on the effective population size of the three groups over the interval 1,000-100,000 and on the migration rate for the admixture model between 0.01-0.20. The mtDNA genomes from CT groups (n = 210) were generated in the present study, while Mon (MO) sequences consisted of 49 new sequences generated in the present study plus an additional 153 MO and KH sequences reported previously. The Xishuangbanna Dai sequences were obtained from a previous study.

For testing the genetic relationships of populations from the different SEA language families by analyzing five tree-like demographic histories (Figure 3), we assume expanding population sizes, a fixed mutation rate⁵ (4.80 x 10⁻⁷), and fixed separation times based on historical records. We assigned a uniform prior distribution on both the current and ancestral effective population sizes over the range 1,000-100,000 and 1,000-50,000, respectively. We combined our Thai/Lao data with selected published mtDNA genomic data as follows: 1,219 TK sequences (present study and previous studies^{1,6}), 876 AN sequences⁷⁻¹¹, 627 AA sequences (present study and previous studies^{1,12}) and 440 ST sequences (present study and previous studies^{1,13}) (Supplementary Table

S1). Due to the uneven sample sizes of these four groups, we simulated 440 sequences for each of the model populations as 440 sequences represents the smallest sample size; thus, the final dataset consists of 1,760 sequences.

Because of the computational cost of simulating a large number of complete mitochondrial sequences, we utilized a novel approach based on a machine learning tool called "Random Forests". This new method can greatly reduce the number of simulations required to select the corrected model from a set of competing ones. ABC- Random Forests uses a machine-learning algorithm (based on a reference table of simulations) to predict the most suitable model at each possible value of a set of covariates (i.e. all summary statistics used to summarize the data), and a classification algorithm which allows one to overcome the difficulties in the choice of the summary statistics, while also gaining a larger discriminative power among the competing models (see details in Pudlo *et al.*¹⁷).

To generate the simulated datasets, we used the software package ABCtoolbox¹⁹ running 10,000 simulations for each model. We computed a set of summary statistics using arlsumstat²⁰ describing both within-population (number of haplotypes, haplotype diversity, total and private number of segregating sites, average number of pairwise differences for each population and Tajima's D)²¹⁻²³, and between-population diversity (Φ_{st} and mean number of pairwise differences between populations)²⁴⁻²⁵. We randomly resampled 440 sequences from AA, AN and TK groups before computing the summary statistics for the observed data, so as to make them comparable with the simulated data.

The ABC model choice was conducted with the R package *abcrf* ¹⁷. We built the reference table using the function *abcrf* and employing a forest of 500 trees, as this number was suggested to provide the best trade-off between computational efficiency and statistical precision ¹⁷. However, we checked that this number was sufficient by plotting the estimated values of the prior error rate as a function of the number of trees in the forest using the function *err.abcrf* (Supplementary Figure

S8). We also made sure that our set of models were able to reconstruct the variability found in the observed mitochondrial sequences and that, inside each set, we were able to discriminate between different scenarios. In order to achieve these goals we plotted the reference table on the LDA axes using the function *plot.abcrf* and calculated the classification error using as PODs each dataset of our reference table. We carried out the actual model comparisons and obtained the posterior probabilities of the winning models using the function *predict*. As both the demic diffusion and continuous migration models resulted difficult to distinguish (Supplementary Table S3) and received a similar amount of votes in our model selection procedure (Supplementary Table S4), we estimated the parameters involved in these scenarios. We ran additional simulations (200,000) for both models and applied the script available at:

http://code.google.com/p/popabc/source/browse/#svn%2Ftrunk%2Fscripts.

When estimating model parameters we reduced the dimensionality of the space of statistics via Partial Least Square (PLS) transformation. ¹⁹ Under this approach, we defined a set of orthogonal linear- combinations of summary statistics best explaining the variance in the model parameter space. After the inspection of the Root Mean Square Error Plots, we selected 10 PLS to calculate the posterior probabilities of model parameters.

References

- Kutanan W, Kampuansai J, Srikummool M, et al. Complete mitochondrial genomes of Thai and Lao populations indicate an ancient origin of Austroasiatic groups and demic diffusion in the spread of Tai–Kadai languages. Hum Genet 2017; 136: 85–98.
- 2. Sun H, Zhou C, Huang X, *et al.* Autosomal STRs provide genetic evidence for the hypothesis that Tai people originate from Southern China. *PLoS ONE* 2013; **8**: e60822.
- 3. Pittayaporn P. Layers of Chinese loanwords in proto-southwestern Tai as evidence for the dating of the spread of southwestern Tai. *Manusya J Humanit* 2014; **20**: 47–68.

- 4. Baker C, Phongpaichit P (eds). *A history of Thailand*, 2nd edn. Cambridge University Press: Cambridge, UK, 2009.
- 5. Fu Q, Mittnik A, Johnson PL, *et al.* A revised timescale for human evolution based on ancient mitochondrial genomes. *Curr Biol* 2013; **23**: 553–559.
- 6. Diroma MA, Calabrese C, Simone D, *et al*. Extraction and annotation of human mitochondrial genomes from 1000 Genomes Whole Exome Sequencing data. *BMC Genom* 2014; **15**: S2.
- 7. Gunnarsdottir ED, Li M, Bauchet M, Finstermeier K, Stoneking M. High-throughput sequencing of complete human mtDNA genomes from the Philippines. *Genome Res* 2011a; **21**: 1–11.
- 8. Gunnarsdóttir ED, Nandineni MR, Li M, *et al.* Larger mitochondrial DNA than Y-chromosome differences between matrilocal and patrilocal groups from Sumatra. *Nat Commun* 2011b; **2**: 228.
- 9. Jinam TA, Hong LC, Phipps ME, *et al.* Evolutionary history of continental southeast Asians: "early train" hypothesis based on genetic analysis of mitochondrial and autosomal DNA data. *Mol Biol Evol* 2012; **29**: 3513–3527.
- 10. Ko AMS, Chen CY, Fu Q, et al. Early Austronesians: into and out of Taiwan. Am J Hum Genet 2014; 94: 426–436.
- 11. Delfin FS, Ko AMS, Li M, *et al.* Complete mtDNA genomes of Filipino ethnolinguistic groups:

 A melting pot of recent and ancient lineages in the Asia-Pacific region. *Eur J Hum Genet*2014; **22**: 228–237.
- 12. Zhang X, Qi X, Yang Z, *et al.* Analysis of mitochondrial genome diversity identifies new and ancient maternal lineages in Cambodian aborigines. *Nat Commun* 2013; **4**: 2599.

- 13. Zhao M, Kong QP, Wang HW, *et al.* Mitochondrial genome evidence reveals successful Late Paleolithic settlement on the Tibetan Plateau. *Proc Natl Acad Sci USA* 2009; **106**: 21230–21235.
- 14. Zheng HX, Yan S, Qin ZD, *et al*. Major population expansion of East Asians began before Neolithic time: evidence of mtDNA genomes. *PLoS ONE* 2011; **6**: e25835.
- 15. Summerer M, Horst J, Erhart G, *et al.* Large-scale mitochondrial DNA analysis in Southeast Asia reveals evolutionary effects of cultural isolation in the multi-ethnic population of Myanmar. *BMC Evol Biol* 2014; **14**: 17.
- 16. Li YC, Wang HW, Tian JY, et al. Ancient inland human dispersals from Myanmar into interior East Asia since the Late Pleistocene. Sci Rep 2015; 5: 9473.
- 17. Pudlo P, Marin JM, Estoup A, Cornuet JM, Gautier M, Robert CP. Reliable ABC model choice via random forests. *Bioinformatics* 2016; **32**: 859–866.
- 18. Breiman L. Random forests. *Machine learning* 2001; **45**: 5–32.
- 19. Wegmann D, Leuenberger C, Neuenschwander S, Excoffier L. ABCtoolbox: a versatile toolkit for approximate Bayesian computations. *BMC Bioinformatics* 2010; **11**: 116.
- 20. Excoffier L, Lischer HEL. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour* 2010; 10: 564–567.
- 21. Tajima F. Evolutionary relationship of DNA sequences in finite populations. *Genetics* 1983;105: 437–460.
- 22. Tajima F. Measurement of DNA polymorphism. In: Takahata N, Clark AG (eds). Mechanisms of Molecular Evolution. Introduction to Molecular Paleopopulation Biology. Sinauer Associates Inc: Maryland, USA, 1993, pp 37–59.

- 23. Tajima F. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 1989; **123**: 585–595.
- 24. Weir BS, Cockerham CC. Estimating F-statistics for the analysis of population structure. *Evolution* 1984; **38**: 1358–1370.
- 25. Michalakis Y, Excoffier L. A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. *Genetics* 1996; **142**: 1061–1064.

Additional details on mtDNA haplogroups

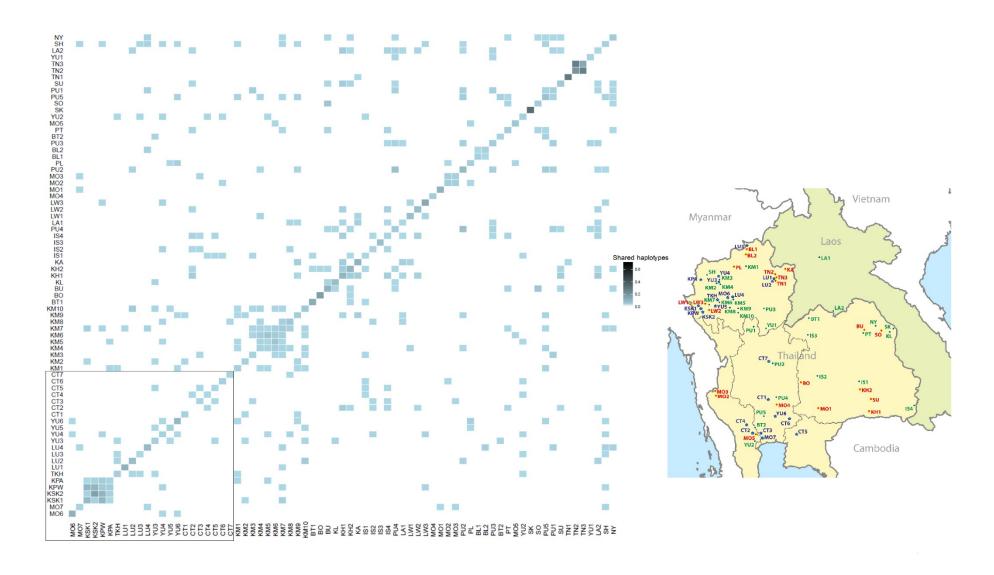
There are many lineages with ages older than 30 kya found in our Thai/Lao samples, e.g. B4, B5, D, F1, F3, M7, M*, M12, M13, M17, M21, M71, M73, M74, M91, R9, R22, N10 and U. Many of them are major lineages and distributed in our Thai/Lao samples as well as in other SEA populations, and have been previously discussed. Here, we focus on some uncommon ancient lineages, i.e. M*, M17, M21, M71, M73, M91 and U. Nineteen sequences were classified as superhaplogroup M* (i.e., they could not be classified into other M sublineages) and date to ~54.27 kya; most of them occur in the Mon (52.63%) and Karen (KPA) (15.79%). M17 bifurcated to M17a and M17c ~40.90 kya, and 61.11% of the M17 lineages are found in Central Thai (CT) groups. M17a is proposed to be an early mtDNA lineage that originated in MSEA and migrated to ISEA, 2-3 while M17c was previously found in the Philippine populations.⁴ We here date the variation within these lineages to ~29.02 kya (M17a) and ~32.18 kya (M17c) (Table 3). M21 bifurcates ~42.73 kya to (M21b and M21a, with ages for the variation within each clade of 34.54 kya and 3.93 kya, respectively. M21b was found in AA-speaking and CT groups whereas M21a is new lineage in Thai/Lao populations, found in the Karen and MO7. M21a is most common among the Semang and M21b is found in both the Semang and Senoi from Malaysia.⁵ Two major sublineages of M71 are M71(151T) and M71a. Although M71 is rare (~0.02%) in our study, its frequency is higher than reported previously in MSEA^{6,7-8} and ISEA.⁴ The estimated divergence time of M71 is \sim 31.22 kya, slightly lower than a previous estimate of \sim 39.40 kya. The variation within M71(151T) and M71a are ~23.56 kya and ~24.00 kya, respectively. About 50% of M71a is from CT individuals, with the remainder found in other TK groups and in the Blang, an AA group. M73 was mostly contributed by the MO (44.44%) and CT (44.44%). It was also reported previously at low frequency in MSEA^{6,7-8} and ISEA.⁴ We dated this lineage to ~36.21 kya, consistent with a previous estimate of ~37.80 kya. Notably, M17, M21, M71 and M73 are ancient maternal lineages of SEA found in both MSEA and ISEA, reflecting linkages between the early lineages in SEA.9

M91, dated to ~35.98 kya, is another proposed indigenous SEA haplogroup. The age estimated here is slightly lower than in a previous study of sequences from Myanmar (~39.55 kya). ¹⁰ A sublineage, M91a, dates to ~15.87 kya and is found in MO, Karen (KPA) and CT (Supplementary Table S2). Interestingly, haplogroup U is the second oldest lineage in this study with an age of ~52.60 kya, which is slightly higher than a recent estimate of 49.60 kya. ¹¹ Subhaplogroups U1 and U2, which are restricted to CT groups, are autochthonous to the Near East¹² and South Asia¹³, respectively. In summary, the CT groups contrast with other Thai/Lao groups in exhibiting several ancient haplogroups (especially basal M lineages) at low frequency.

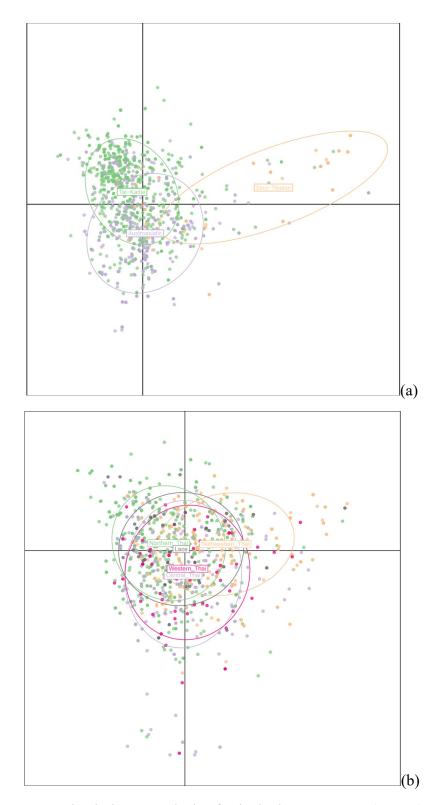
References

- Kutanan W, Kampuansai J, Srikummool M, et al. Complete mitochondrial genomes of Thai and Lao populations indicate an ancient origin of Austroasiatic groups and demic diffusion in the spread of Tai–Kadai languages. Hum Genet 2017; 136: 85–98.
- Bellwood P (eds). First Islanders: Prehistory and Human Migration in Island Southeast Asia. 1st edn.
 John Wiley & Sons: NJ, USA, 2017.
- 3. Tumonggor MK, Karafet TM, Hallmark B, *et al*. The Indonesian archipelago: An ancient genetic highway linking Asia and the Pacific. *J Hum Genet* 2013; **58**: 165–173.
- 4. Tabbada KA. Trejaut J, Loo JH, *et al.* Philippine Mitochondrial DNA Diversity: A Populated Viaduct between Taiwan and Indonesia? *Mol Biol Evol* 2010; **27**: 21-31.
- 5. Hill C, Soares P, Mormina M, *et al.* Phylogeography and ethnogenesis of aboriginal Southeast Asians. *Mol Biol Evol* 2006; **23**: 2480–2491.
- 6. Zhang X, Qi X, Yang Z, et al. Analysis of mitochondrial genome diversity identifies new and ancient maternal lineages in Cambodian aborigines. *Nat Commun* 2013; **4**: 2599.

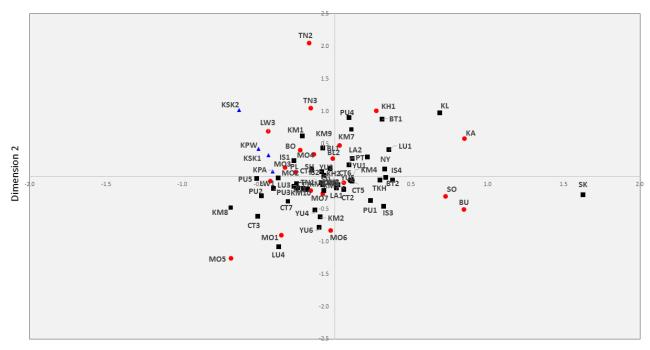
- 7. Peng MS, Quang HH, Dang KP, *et al.* Tracing the Austronesian footprint in mainland Southeast Asia: a perspective from mitochondrial DNA. *Mol Biol Evol* 2010; **27**: 2417–2430.
- 8. Bodner M, Zimmermann B, Röck A, *et al.* Southeast Asian diversity: first insights into the complex mtDNA structure of Laos. *BMC Evol Biol* 2011; **11**:49.
- 9. Jinam TA, Hong LC, Phipps ME, *et al*. Evolutionary history of continental southeast Asians: "early train" hypothesis based on genetic analysis of mitochondrial and autosomal DNA data. *Mol Biol Evol* 2012; **29**: 3513-3527.
- 10. Li YC, Wang HW, Tian JY, et al. Ancient inland human dispersals from Myanmar into interior East Asia since the Late Pleistocene. *Sci Rep* 2015; **5**: 9473.
- 11. Larruga JM, Marrero P, Abu-Amero KK, Golubenko MV, Cabrera VM. Carriers of mitochondrial DNA macrohaplogroup R colonized Eurasia and Australasia from a southeast Asia core area. *BMC Evol Biol* 2017; **17**: 115.
- 12. Derenko M, Malyarchuk B, Bahmanimehr A, *et al.* Complete Mitochondrial DNA Diversity in Iranians. *PLoS ONE* 2013; **8**: e80673.
- 13. Palanichamy MG, Sun C, Agrawal S, et al. Phylogeny of Mitochondrial DNA Macrohaplogroup N in India, Based on Complete Sequencing: Implications for the Peopling of South Asia. Am J Hum Genet 2004; 75: 966-978.



Supplementary Figure S1 Relative frequency of shared haplotypes within and among 73 Thai/Lao populations. Population abbreviations are in Supplementary Table S1.

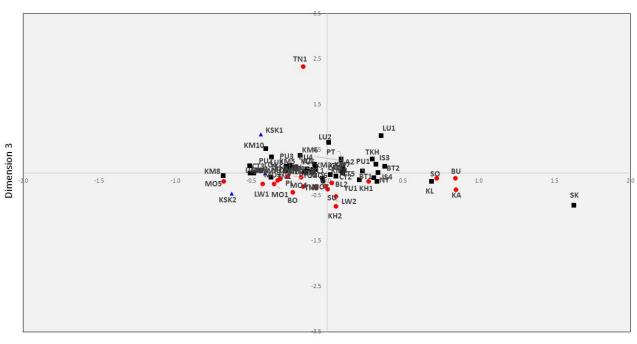


Supplementary Figure S2 Discriminant Analysis of Principal Components (DAPC) results based on linguistic groups (a) and geographic groups (b). Colors in (a) represent Tai-Kadai (green), Sino-Tibetan (orange) and Austroasiatic (light purple) populations. Colors in (b) represent Northern (green), Northeastern (orange), Central (light purple) and Western (pink) Thai populations.



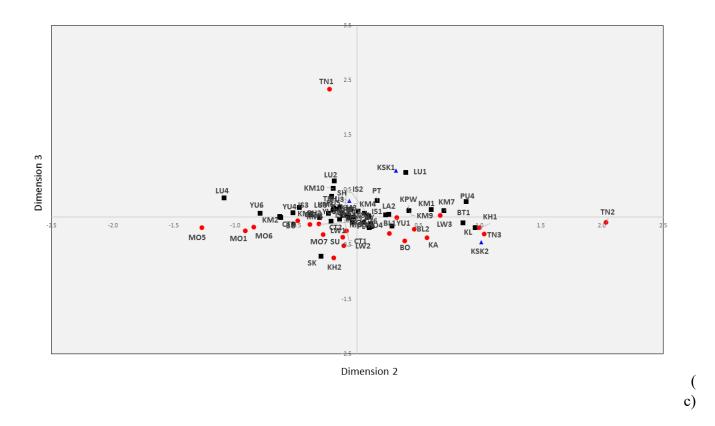
Dimension 1

(a)

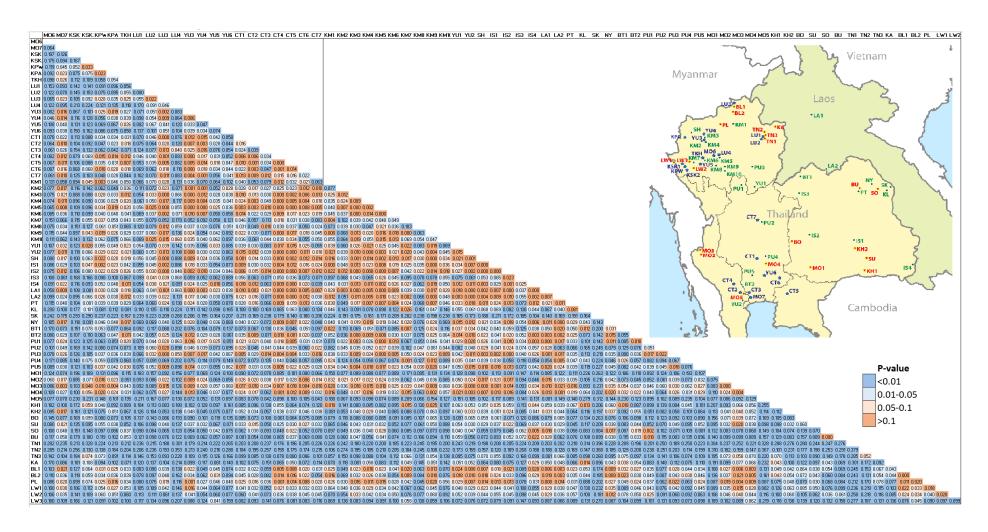


Dimension 1

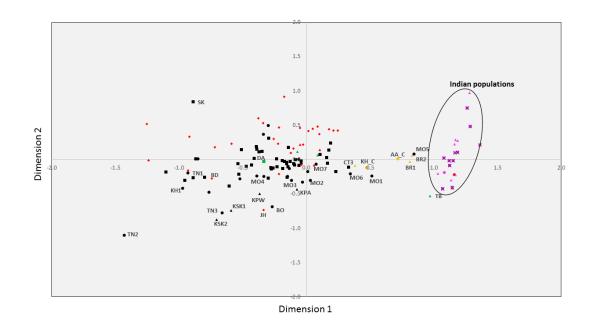
(b)

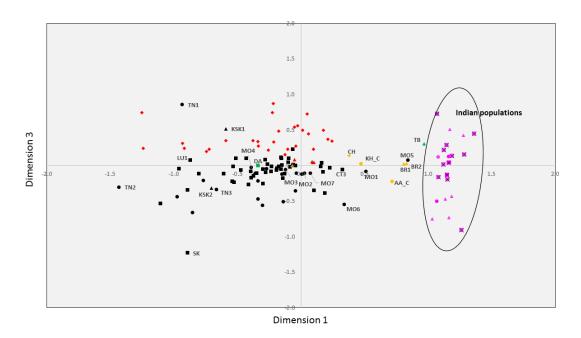


Supplementary Figure S3 The MDS plot based on the Φ_{st} distance matrix for 73 populations: dimension 1 vs. 2 (a), dimension 1 vs. 3 (b) and dimension 2 vs. 3 (c). Red, black and dark blue symbols indicate AA, TK and ST populations, respectively. The stress value is 0.0711. Population abbreviations are shown in Supplementary Table S1.

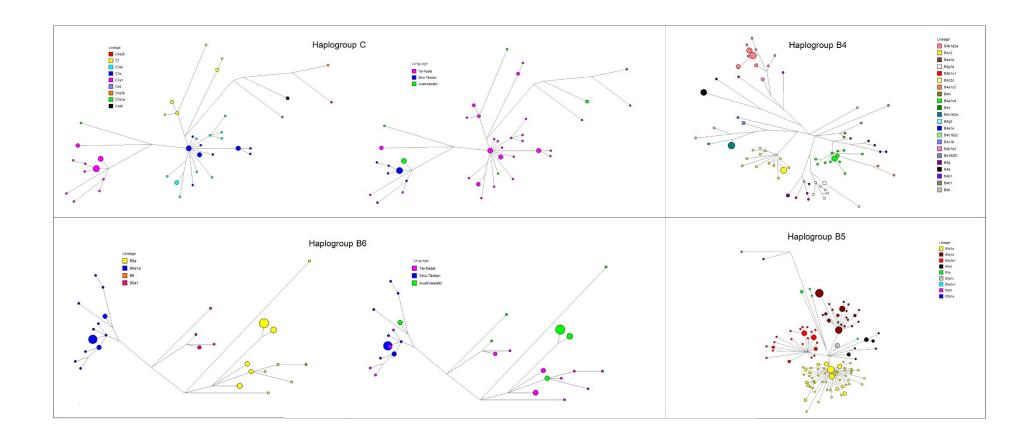


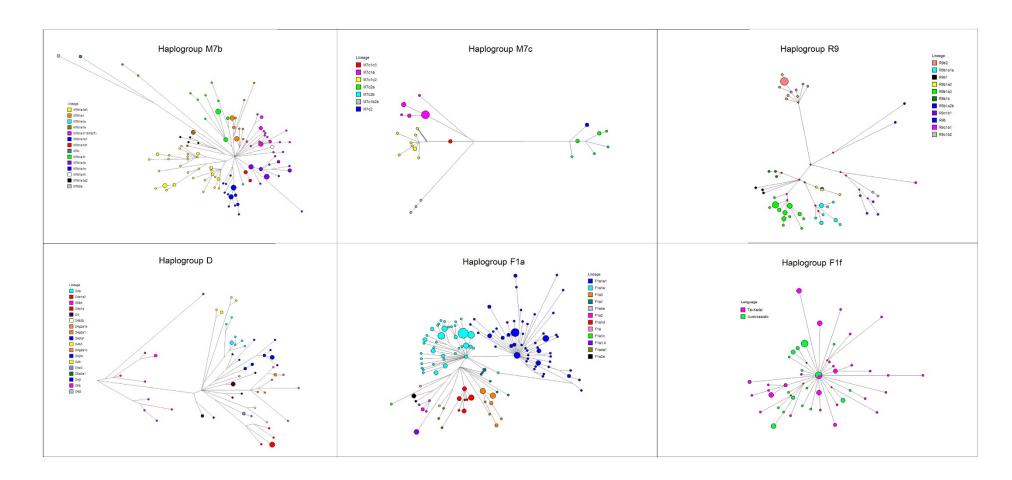
Supplementary Figure S4 Pairwise Φ_{st} with P-values executed by 1,000 permutations. Population abbreviations are in Supplementary Table S1.



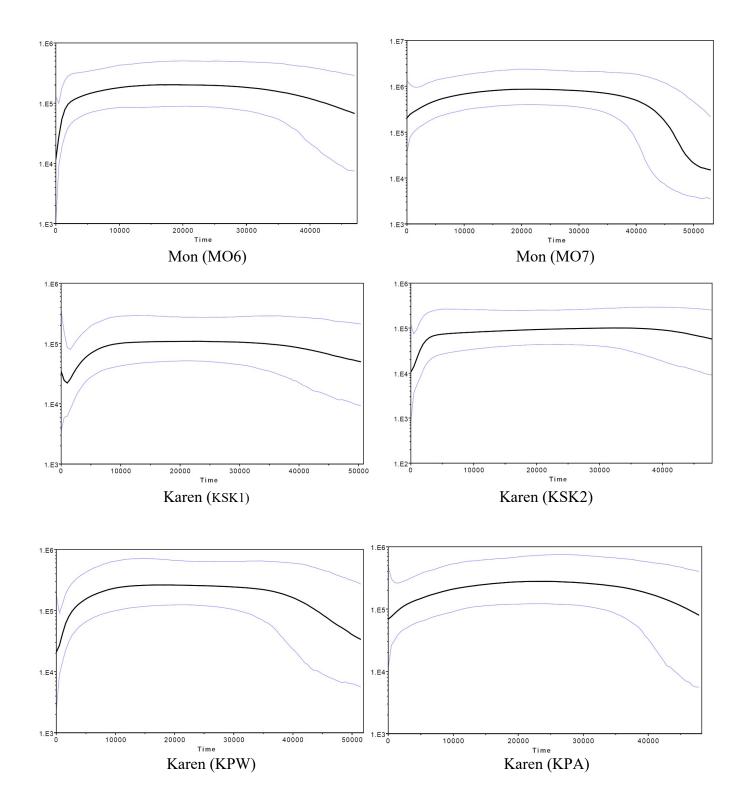


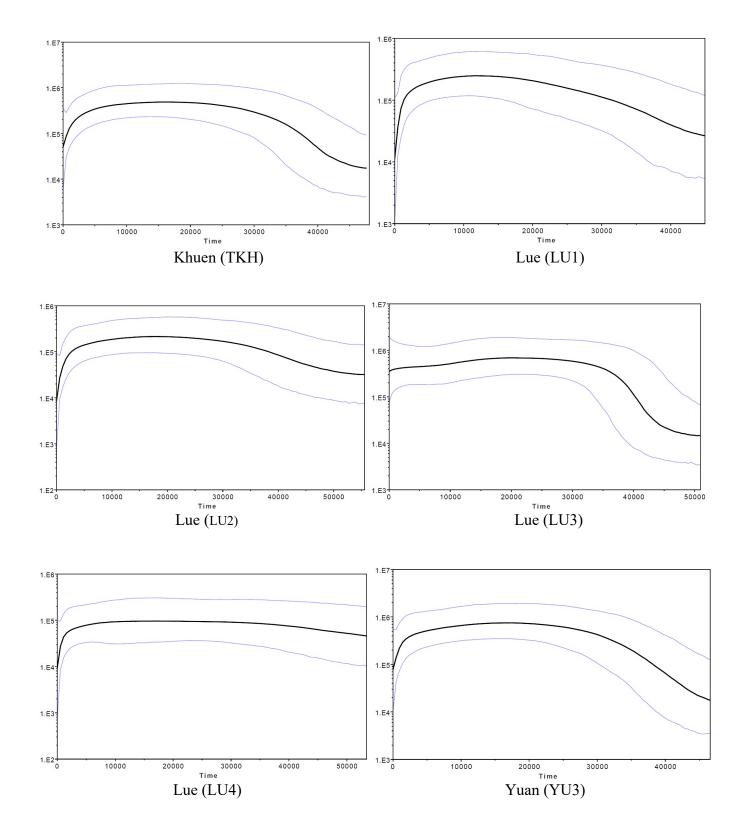
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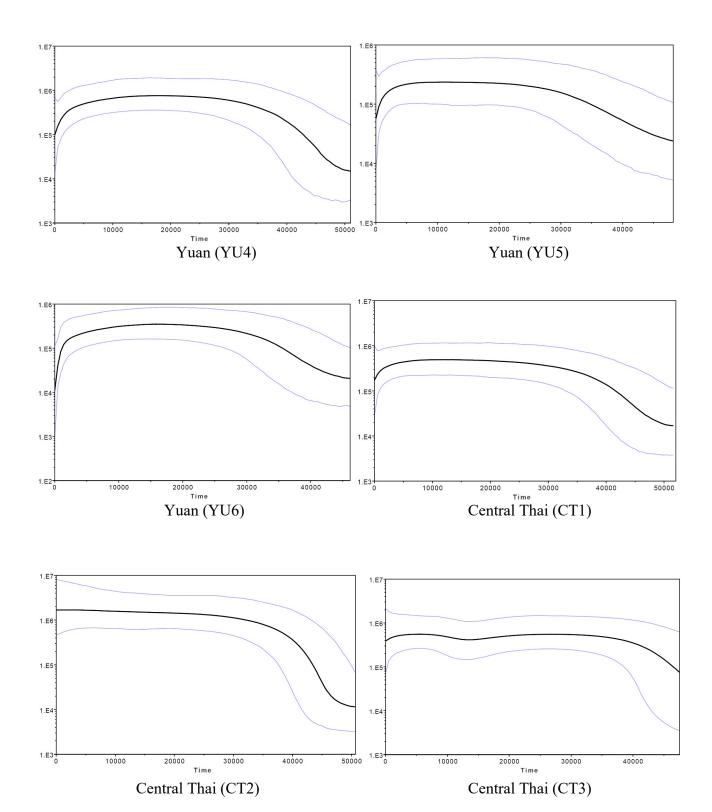


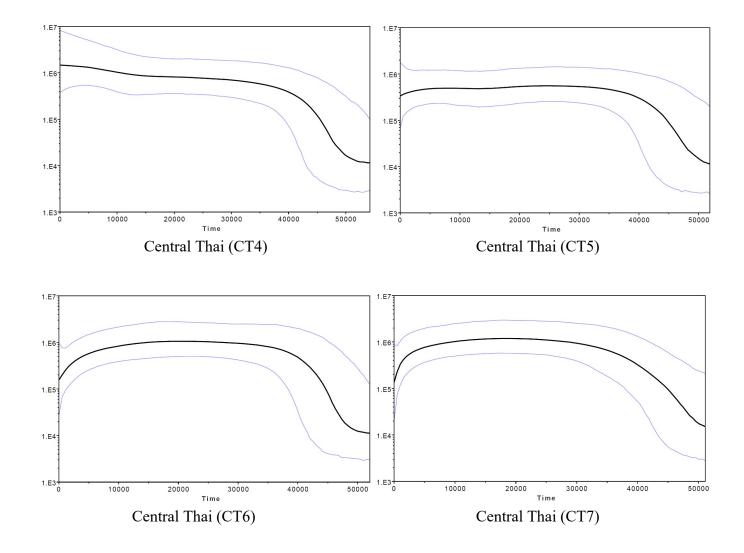


Supplementary Figure S6 Networks of major haplogroups.



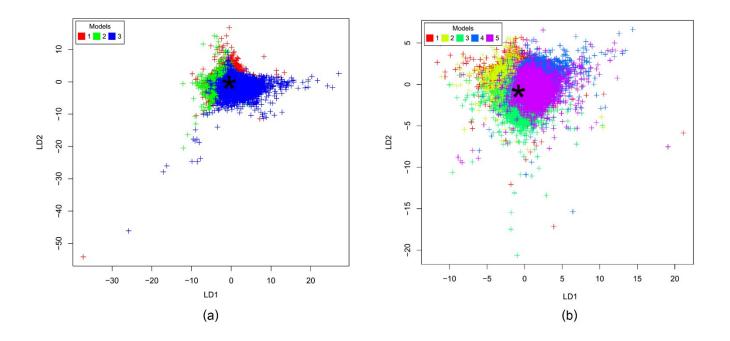






Supplementary Figure S7 Bayesian skyline plots (BSP) for each individual population. The 95% highest posterior density limits are indicated by the blue lines.

Supplementary Figure S8 Evolution of the ABC-RF prior error rate with respect to the number of trees in the forest for the ABC analysis on Central Thai origin (A) and on the relationships between populations from different MSEA language families (B)



Supplementary Figure S9 Linear Discriminate Analysis (LDA) plot for the fit between the observed data and the simulated data generated by each model for the origin of Central Thai groups (a) the relationships between populations from different MSEA language families (b).

Supplementary Figure S10 Posterior distributions of the parameters estimated for the demic diffusion

(A) and continuous migration (B) models. The X axis covers the range of the (uniform) prior distributions.

Abbreviations are in Supplementary Table S5.

Supplementary Table S1 Details for populations in the comparative analyses.

Citation	Population	Language	Country	Sample size	Code
Present study	Mon	Austroasiatic	Thailand	24	MO6
Present study	Mon	Austroasiatic	Thailand	25	MO7
Present study	Karen	Sino-Tibetan	Thailand	25	KSK1
Present study	Karen	Sino-Tibetan	Thailand	13	KSK2
Present study	Karen	Sino-Tibetan	Thailand	24	KPW
Present study	Karen	Sino-Tibetan	Thailand	25	KPA
Present study	Khuen	Tai-Kadai	Thailand	25	TKH
Present study	Lue	Tai-Kadai	Thailand	25	LU1
Present study	Lue	Tai-Kadai	Thailand	23	LU2
Present study	Lue	Tai-Kadai	Thailand	25	LU3
Present study	Lue	Tai-Kadai	Thailand	16	LU4
Present study	Yuan	Tai-Kadai	Thailand	25	YU3
Present study	Yuan	Tai-Kadai	Thailand	25	YU4
Present study	Yuan	Tai-Kadai	Thailand	26	YU5
Present study	Yuan	Tai-Kadai	Thailand	25	YU6
Present study	CentralThai	Tai-Kadai	Thailand	30	CT1
Present study	CentralThai	Tai-Kadai	Thailand	30	CT2
Present study	CentralThai	Tai-Kadai	Thailand	30	CT3
Present study	CentralThai	Tai-Kadai	Thailand	30	CT4
Present study	CentralThai	Tai-Kadai	Thailand	30	CT5
Present study	CentralThai	Tai-Kadai	Thailand	29	CT6
Present study	CentralThai	Tai-Kadai	Thailand	31	CT7
Kutanan <i>et al</i> . ¹	KhonMueang	Tai-Kadai	Thailand	25	KM1
Kutanan <i>et al</i> . ¹	KhonMueang	Tai-Kadai	Thailand	25	KM2
Kutanan et al. ¹	KhonMueang	Tai-Kadai	Thailand	24	KM3
Kutanan et al. ¹	KhonMueang	Tai-Kadai	Thailand	25	KM4
Kutanan et al.1	KhonMueang	Tai-Kadai	Thailand	23	KM5
Kutanan et al.1	KhonMueang	Tai-Kadai	Thailand	25	KM6

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Kutanan et al. ¹	KhonMueang	Tai-Kadai	Thailand	25	KM7
Kutanan et al. ¹	KhonMueang	Tai-Kadai	Thailand	25	KM8
Kutanan <i>et al</i> . ¹	KhonMueang	Tai-Kadai	Thailand	24	KM9
Kutanan et al. ¹	KhonMueang	Tai-Kadai	Thailand	25	KM10
Kutanan et al. ¹	Yuan	Tai-Kadai	Thailand	17	YU1
Kutanan et al. ¹	Yuan	Tai-Kadai	Thailand	25	YU2
Kutanan et al. ¹	Shan	Tai-Kadai	Thailand	25	SH
Kutanan et al. ¹	LaoIsan	Tai-Kadai	Thailand	25	IS1
Kutanan et al. ¹	LaoIsan	Tai-Kadai	Thailand	25	IS2
Kutanan et al. ¹	LaoIsan	Tai-Kadai	Thailand	25	IS3
Kutanan et al. ¹	LaoIsan	Tai-Kadai	Thailand	25	IS4
Kutanan et al. ¹	Lao	Tai-Kadai	Laos	25	LA1
Kutanan et al. ¹	Lao	Tai-Kadai	Laos	24	LA2
Kutanan et al. ¹	Phutai	Tai-Kadai	Thailand	25	PT
Kutanan <i>et al</i> . ¹	Kalueng	Tai-Kadai	Thailand	25	KL
Kutanan et al. ¹	Seak	Tai-Kadai	Thailand	26	SK
Kutanan et al. ¹	Nyaw	Tai-Kadai	Thailand	25	NY
Kutanan et al. ¹	BlackTai	Tai-Kadai	Thailand	25	BT1
Kutanan et al. ¹	BlackTai	Tai-Kadai	Thailand	25	BT2
Kutanan et al. ¹	Phuan	Tai-Kadai	Thailand	25	PU1
Kutanan <i>et al</i> . ¹	Phuan	Tai-Kadai	Thailand	25	PU2
Kutanan et al. ¹	Phuan	Tai-Kadai	Thailand	25	PU3
Kutanan et al. ¹	Phuan	Tai-Kadai	Thailand	25	PU4
Kutanan <i>et al</i> . ¹	Phuan	Tai-Kadai	Thailand	25	PU5
Kutanan <i>et al</i> . ¹	Mon	Austroasiatic	Thailand	25	MO1
Kutanan <i>et al</i> . ¹	Mon	Austroasiatic	Thailand	23	MO2
Kutanan <i>et al</i> . ¹	Mon	Austroasiatic	Thailand	15	MO3
Kutanan <i>et al</i> . ¹	Mon	Austroasiatic	Thailand	25	MO4
Kutanan <i>et al</i> . ¹	Mon	Austroasiatic	Thailand	22	MO5
Kutanan <i>et al</i> . ¹	Khmer	Austroasiatic	Thailand	19	KH1
Kutanan <i>et al</i> . ¹	Khmer	Austroasiatic	Thailand	25	KH2
Kutanan <i>et al</i> . ¹	Nyahkur	Austroasiatic	Thailand	23	ВО
Kutanan <i>et al</i> . ¹	Suay	Austroasiatic	Thailand	25	SU
Kutanan <i>et al</i> . ¹	So	Austroasiatic	Thailand	25	SO
Kutanan <i>et al</i> . ¹	Bru	Austroasiatic	Thailand	24	BU
Kutanan <i>et al</i> . ¹	Htin	Austroasiatic	Thailand	25	TN1
Kutanan et al. ¹	Htin	Austroasiatic	Thailand	25	TN2
Kutanan <i>et al</i> . ¹	Htin	Austroasiatic	Thailand	25	TN3
Kutanan et al. ¹	Khamu	Austroasiatic	Thailand	25	KA
Kutanan <i>et al</i> . ¹	Blang	Austroasiatic	Thailand	25	BL1
Kutanan <i>et al</i> . ¹	Blang	Austroasiatic	Thailand	25	BL2
Kutanan <i>et al</i> . ¹	Paluang	Austroasiatic	Thailand	25	PL
Kutanan et al. ¹	Lawa	Austroasiatic	Thailand	22	LW1
Kutanan et al. ¹	Lawa	Austroasiatic	Thailand	24	LW2
Kutanan et al. ¹	Lawa	Austroasiatic	Thailand	24	LW3
Zhang et al. ²	Khmer	Austroasiatic	Cambodia	18	KH_C
Zhang et al. ²	Austroasiatic	Austroasiatic	Cambodia	65	AA_C
Summerer et al. ³	Barma	Sino-Tibetan	Myanmar	20	BR1
Li et al. (2015) ⁴	Barma	Sino-Tibetan	Myanmar	73	BR2
(= • • •)			- /		

Peng et al. (2010) ⁵	Cham	Austronesian	Vietnam	16	СН
Diroma <i>et al.</i> (2014) ⁶	Dai	Tai-Kadai	China	56	DA
Zheng <i>et al.</i> $(2011)^7$	Han	Sino-Tibetan	China	55	HN S
Zheng <i>et al.</i> $(2011)^7$	Han	Sino-Tibetan	China	89	HN_N
-			Tibet and southern		ТВ
Zhao et al. (2009) ⁸	Tibetan	Sino-Tibetan	China	23	
Jinam <i>et al</i> . (2012) ⁹	Temuan	Austronesian	West Malysia	18	TM
Jinam <i>et al</i> . (2012) ⁹	Seletar	Austronesian	West Malysia	21	SE
Jinam <i>et al</i> . (2012) ⁹	Jehai	Austronesian	West Malysia	24	JH
Jinam <i>et al</i> . (2012) ⁹	Bidayuh	Austronesian	Indonesia	23	BD
Gunnarsdóttir <i>et al</i> . (2011b) 10	Semende	Austronesian	Indonesia	35	SMD
Gunnarsdóttir <i>et al</i> . (2011b) 10	Besemah	Austronesian	Indonesia	36	BS
Gunnarsdottir et al. (2011a) 11	Mamanwa	Austronesian	The Philippines	32	MM
Gunnarsdottir <i>et al.</i> (2011a) ¹¹	Manobo	Austronesian	The Philippines	40	MAN
Gunnarsdottir <i>et al.</i> (2011a) ¹¹	Surigaonon	Austronesian	The Philippines	25	SR
Delfin <i>et al.</i> (2014) 12	Abaknon	Austronesian	The Philippines	30	AB
Delfin et al. (2014) 12	Aeta Bataan	Austronesian	The Philippines	21	AEB
Delfin et al. (2014) 12	Bagalot	Austronesian	The Philippines	30	BAG
Delfin <i>et al.</i> (2014) ¹²	Ibaloi	Austronesian	The Philippines	26	IB
Delfin et al. (2014) 12	Ifugao	Austronesian	The Philippines	29	IF
Delfin <i>et al.</i> (2014) 12	Ivatan	Austronesian	The Philippines	29	IV
Delfin <i>et al.</i> (2014) 12	Kalangoya	Austronesian	The Philippines	26	KAG
Delfin et al. (2014) 12	Kankanaey	Austronesian	The Philippines	30	KAN
Delfin et al. (2014) 12	Maranao	Austronesian	The Philippines	18	MR
Ko et al. (2014) 13	Saisiat	Austronesian	Taiwan	24	SAI
Ko <i>et al</i> . (2014) 13	Atayal	Austronesian	Taiwan	50	ATA
Ko <i>et al</i> . (2014) 13	Tsou	Austronesian	Taiwan	48	TSO
Ko <i>et al</i> . (2014) 13	Bunun	Austronesian	Taiwan	51	BUN
Ko <i>et al</i> . (2014) 13	Puyuma	Austronesian	Taiwan	39	PUY
Ko <i>et al</i> . (2014) ¹³	Rukai	Austronesian	Taiwan	25	RUK
Ko et al. (2014) 13	Paiwan	Austronesian	Taiwan	50	PAI
Ko et al. (2014) 13	Ami	Austronesian	Taiwan	19	AMI
Ko et al. (2014) 13	Tao	Austronesian	Taiwan	25	TAO
Ko et al. (2014) 13	Hakka	Sino-Tibetan	Taiwan	23	HAK
Ko et al. (2014) 13	Minnan	Sino-Tibetan	Taiwan	25	MIN
Ko et al. (2014) 13	Makatao	Austronesian	Taiwan	50	MAK
Chandrasekar et al. (2009) 14	Malpaharia	Austroasiatic	East India	15	ML
Chandrasekar et al. (2009) 14	Munda	Austroasiatic	East India	31	MUN
`		1			AD
` /					DR
`		•			DB
` ,					GL JK
					KMR
`		•			KU
`		•			KD
` ,		1			KR
` ,		•			KK
Chandrasekar et al. (2009) 14	Munda Andh Dirang Monpa Dongri Bhill Gallong Jenu Kuruba Kamar Kathakur Kathodi Katkari Korku	Austroasiatic Indo-European Sino-Tibetan Indo-European Sino-Tibetan Dravidian Indo-European Indo-European Indo-European Indo-European Austroasiatic	East India Central India Northeast India West India Northeast India South India Central India West India West India West India West India Central India Central India	31 19 30 43 39 79 53 19 15 21 15	I

Chandrasekar et al. (2009) 14	Lachungpa	Sino-Tibetan	Northeast India	25	LAH
Chandrasekar et al. (2009) 14	Lepcha	Sino-Tibetan	Northeast India	20	LP
Chandrasekar et al. (2009) 14	Mathakur	Indo-European	West India	11	MT
Chandrasekar et al. (2009) 14	Madia	Dravidian	East India	20	MAA
Chandrasekar et al. (2009) 14	Nihal	Indo-European	Central India	28	NI
		Dravidian/Indo-			DD
Chandrasekar et al. (2009) 14	Pauri Bhuiya	European	Central India	32	PB
Chandrasekar et al. (2009) 14	Shertukpen	Sino-Tibetan	Northeast India	15	ST
	Sonowal				COL
Chandrasekar et al. (2009) 14	Kachari	Indo-European	Northeast India	19	SOK
Chandrasekar et al. (2009) 14	Toto	Sino-Tibetan	Northeast India	28	TO
Chandrasekar et al. (2009) 14	Wanchoo	Sino-Tibetan	Northeast India	22	WA

Notes: 1) All populations were used in genetic distance calculations while those used for the model selection ABC analyses are highlighted in yellow.

2) The compared mtDNA sequences datasets from most previous studies were retrieved from the NCBI Genbank database with the following accession numbers; Kutanan *et al.*¹ (KX456435–KX457668), Zhang *et al.*² (KC887456 to KC887497), Summerer *et al.*³ (JX289092-JX289135), Li *et al.*⁴ (KP345975-KP346066), Peng *et al.*⁵ (GQ301863–GQ301886), Zhao *et al.*⁸ (GU014563-GU014569), Jinam *et al.*⁹ (AP012346–AP012431), Gunnarsdóttir *et al.*¹⁰ (HM596644 -HM596715), Gunnarsdóttir *et al.*¹¹ (GU733718–GU733826), Delfin *et al.*¹² (KC993902–KC994161), Ko *et al.* (2014)¹³ (KF540506-KF541055) and Chandrasekar *et al.*¹⁴ (FJ383814- FJ383174). The sequences of Zheng *et al.*⁷ were retrieved from http://www.phylotree.org/mtDNA_seqs.htm and the sequences from Diroma *et al.*⁶ were obtained via personal communication with the authors.

Reference

- Kutanan W, Kampuansai J, Srikummool M, et al. Complete mitochondrial genomes of Thai and Lao populations indicate an ancient origin of Austroasiatic groups and demic diffusion in the spread of Tai–Kadai languages. Hum Genet 2017; 136: 85–98.
- 2. Zhang X, Qi X, Yang Z, *et al.* Analysis of mitochondrial genome diversity identifies new and ancient maternal lineages in Cambodian aborigines. *Nat Commun* 2013; **4**: 2599.
- 3. Summerer M, Horst J, Erhart G, *et al.* Large-scale mitochondrial DNA analysis in Southeast Asia reveals evolutionary effects of cultural isolation in the multi-ethnic population of Myanmar. *BMC Evol Biol* 2014; **14**: 17.

- 4. Li YC, Wang HW, Tian JY, *et al*. Ancient inland human dispersals from Myanmar into interior East Asia since the Late Pleistocene. *Sci Rep* 2015; **5**: 9473.
- 5. Peng MS, Quang HH, Dang KP, *et al.* Tracing the Austronesian footprint in mainland Southeast Asia: a perspective from mitochondrial DNA. *Mol Biol Evol* 2010; **27**: 2417–2430.
- 6. Diroma MA, Calabrese C, Simone D, *et al.* Extraction and annotation of human mitochondrial genomes from 1000 Genomes Whole Exome Sequencing data. *BMC Genom* 2014; **15**: S2.
- 7. Zheng HX, Yan S, Qin ZD, *et al.* Major population expansion of East Asians began before Neolithic time: evidence of mtDNA genomes. *PLoS ONE* 2011; **6**: e25835.
- 8. Zhao M, Kong QP, Wang HW, *et al.* Mitochondrial genome evidence reveals successful Late

 Paleolithic settlement on the Tibetan Plateau. *Proc Natl Acad Sci USA* 2009; **106**: 21230-21235.
- 9. Jinam TA, Hong LC, Phipps ME, *et al.* Evolutionary history of continental southeast Asians: "early train" hypothesis based on genetic analysis of mitochondrial and autosomal DNA data. *Mol Biol Evol* 2012; **29**: 3513-3527.
- 10. Gunnarsdóttir ED, Nandineni MR, Li M, et al. Larger mitochondrial DNA than Y-chromosome differences between matrilocal and patrilocal groups from Sumatra. Nat Commun 2011b; 2: 228.
- 11. Gunnarsdottir ED, Li M, Bauchet M, Finstermeier K, Stoneking M. High-throughput sequencing of complete human mtDNA genomes from the Philippines. *Genome Res* 2011a; **21**: 1–11.
- 12. Delfin FS, Ko AMS, Li M, *et al.* Complete mtDNA genomes of Filipino ethnolinguistic groups: A melting pot of recent and ancient lineages in the Asia-Pacific region. *Eur J Hum Genet* 2014; **22**: 228-237.
- 13. Ko AMS, Chen CY, Fu Q, et al. Early Austronesians: into and out of Taiwan. Am J Hum Genet 2014; 94: 426–436.

14. Chandrasekar A, Kumar S, Sreenath J, *et al.* Updating phylogeny of mitochondrial DNA macrohaplogroup M in India: dispersal of modern human in South Asian corridor. *PLoS ONE* 2009; **4**: e7447.

Supplementary Table S2 Haplogroup information in all studied samples. (an excel file)

Supplementary Table S3 Haplogroup frequencies (in percentage) observed in 22 populations. Bold letter indicates new haplogroups which were not found in our previous study of Thai/Lao populations.

	M 06	MO7	KSK 1	KSK 2	K P W	KPA	ТКН	LU1	LU2	LU3	L _{YU3}	YU4	YU5	YU6	CT1	CT2	CT 3	CT4	CT5
A+152+16362											U						_		
+ 200											4		3.85						
200											4		3.63			3.3			
A14																3			
A17			4		4. 17								23.0						
A5b1			4		1/								0						
ASDI																3.3			
B4a1a																3			
B4a1c2																			3.33
B4a1c4							4		4.35					4					
B4a1e											4								
D41.1.2												1.0			3.3	3.3			
B4b1a2a												12			3	3		2.22	
B4b1c1								1.										3.33	
B4c	1							4	21.7										
B4c1b2a									4	4									
B4c2								4								10			
B4c2c							4	4											3.33
																3.3	3.3		
B4g1a							8									3	3		
B4g2										4									
B4m	-							+		4									
B5a	-							8											
B5a1	-						4				1	4	15.2		2.2	2.2			
B5a1a		12.5						4			64	4	15.3 8	12	3.3	3.3		6.67	3.33
	12.							1								_			
B5a1b1	5						12	1	4.35				3.85			2.2			3.33
B5a1d																3.3			10
B6	<u> </u>							4								_			
B6a								Ť			12	4					3.3	3.33	

			I		Π		Ι							Τ	Ī	3		
B6a1									4									
					8.													
B6a1a			44		33	20				4						3.3		-
C4a2b																3		
C7																3.3		
C7a								4.35		12	8					3		
C/a					16			7.33		12	0							
C7 1			24	7.60	.6													
C7a1 C7a2			24	7.69	7	4			4	8			4				3.33	
									4				4				3.33	
D4															3.3			_
D4a										4			4		3			
D4b2b																		
D4e1a																	3.33	
D4e1a2																3.3		
D4g2a1										4								
D4g2a1b									4									
D4g2a1c												3.85						
D4h3											4							
D4j1a1										4								
D5b4										4							3.33	
F1a								4.35										
F1a1											4						6.67	
				46.1	29									1 2 2		2.2		
Flala			8	46.1 5	.1 7	4								3.3		3.3	3.33	
														6.6				
F1a1a1						12			8	4		3.85	4	7	10			3.33
F1a1c					-				4					-		-		
Fla1d								4.35						-				
F1a2																6.6		
F1a3																7		
F1a4a																		
F1b1+@152																	3.33	
F1c1a2	4.1	7				4												

F1d					Τ	1			Ι										
					+	4													3.33
F1d1					+		0	1											3.33
F1e							8	4							3.3				
F1e3															3				
F1f	4.1 7	12.5					4	4			6 8		7.69		3.3		6.6	6.67	10
ГП	4.1	12.3					4	+			100		7.09		3		/	0.07	10
Flg	7							4											
F2										4									
F2b1					<u> </u>			32											
F3a					1													3.33	ļ
F3a1							8				6			4	3.3				
F3b														•				3.33	
F3b (152)										8								3.33	
F4a2							4												
G2														12					
G2a1		8.33					4						7.69						
																3.3	3.3		
G2a1d2					-											3	3		
G2a1d2a													3.85						
G2b1a					4.		16			4									
G2b1a1			4	7.69	17														
G3b2														4					
															3.3				
H13a2a		4.15			+										3				
I1b	25	4.17				10													2.22
M	25	4.17				12					4								3.33
M10a1		4.17			+														
M1'20'51		4.1 /														3.3			
M12a1a	25									4		8				3			
M12a2																3.3	3.3		6.67
M12b1a2																3.3			
M12b1b												4			3.3				
M12b2																			

M13a2					4										
M13b1					•										
M13c		12.5													
M17a		12.0													3.33
M17c											3.3	3.3	10	3.33	
M17c1a1											6.6	3.3			
M17c1a1a											3.3				
M20				4. 17											
M21a		4.17	7.69	8. 33	12										
M21b															6.67
M21b2												3.3	3.3		
M24a													3.3		
M24b		4.17											2.2		
M2a1a													3.3		
M30		4.17													
M32'56															
M37e2															
M40a1	4.1 7														
M45a		4.17													
M50a1														3.33	
M51a														3.33	
M51a1a												3.3			
M55									8						
M5b									4						
M5c1															
M61								4							
M6a1a		4.17													
M71 (151T)					8										
M71a														6.67	
M71a2						4		4							6.67
M71b						4			4			3.3			

														3			
M71c																	
M72a															3.3		
M73															3	3.33	
													3.3	3.3		3.33	
M73a1													3	3			
M73b															3.3		
M74															3.3		
M74a						4							3.3				
M75										4							
															3.3	2.22	2.22
M76								_							3	3.33	3.33
M76a								8						3.3			
M79														3			
M7																3.33	
M7b																	
M7b1a1				4. 17					6		7.69		3.3		3.3		3.33
M7b1a1	4.1			1 /							7.07				3.3		
(16192T)	7				4				1			8			3		3.33
M7b1a1a	_								6						10		
M7b1a1a2	-										3.85				3.3		
M7b1a1a3						4	8.7	4	6	4			10		3.3	3.33	
	4.1					1.0											
M7b1a1b M7b1a1d1	7				4	12		8	8								6.67
M7b1a1d1					4			4									0.07
	4.1						21.7	T									
M7b1a1e1	7			1			4									3.33	
M7b1a1f		4.17		4. 17				4		8							
M7b1a1g															3.3		
M7b1a1g M7b1a1h									1						3		
MIRIO/IM									8				3.3				
M7c1a													3				<u> </u>
M7c1c2										4	3.85		3.3				

			Τ												6.6				
M7c1c3															7				
M7c2a																3.3			
M7c2b										4									
M8a2a1														28					
M91a		4.17				12											6.6 7		
M91a	4.1	4.17	+			12											/		
M9a1b1	7															2.2			
M9a5																3.3			
N10a															13. 33				
N21 (195C)											4					3.3			
N21a						4													
N22												4							
				30.7															
N8 N9a			+	7				8						8					-
N9a10								0						0					3.33
N9a6											4								3.33
R11'B6											<u> </u>							3.33	
R2+13500							4						3.85						
R21																3.3			
R22		4.17														3		3.33	
R23		,																3.33	3.33
R9b1		4.17																3.33	
					16										2.2				
R9b1a1a			16		.6 7					4					3.3				3.33
R9b1a2																		3.33	
R9b1a2b							4												
R9b1a3	4.1 7								21.7		4	4	3.85	4					
R9b1b	4.1													4					
R9b2									4.35			4			3.3				3.33
R9c1b1										4			3.85						1

U1a1c1a										3.33
U1a1c1d								3.3	3.33	
U2a1b										
U2a2								3.3		
W3a1b										

Supplementary Table S4 Random forests confusion matrix and classification error for the ABC analysis of Central Thai origins.

	Demic diffusion	Cultural diffusion	Continuous migration	Classification error
Demic diffusion	7257	214	2529	0.27
Cultural diffusion	254	9288	458	0.07
Continuous migration	4283	721	4996	0.5

Supplementary Table S5 Votes assigned to each model by the Random Forest procedure and posterior probability for the selected model in the ABC analysis of Central Thai origins.

selected mode	votes demic	votes cultural	votes continuous	Posterior	
	diffusion model	diffusion model	migration model	Probability	
1	0.515	0.081	0.404	0.604	

Supplementary Table S6 Parameters estimation for the demic diffusion and continuous migration model in the ABC analysis of Central Thai origins. NcAA is the current effective population size of Austroasiatic populations, NcCT is the current central Thai effective population size,

NcDAI is the current effective population size for Southern Chinese populations, M1 is the migration rate to Austroasiatic groups from Central Thai populations and M2 is the migration rate to Central Thai populations from Austroasiatic groups.

Demic diffusion				
	Median	Mode	95% HPD-LowB	95% HPD-UppB
NcAA	13,617	13,861	9,485	17,960
NcCT	211,902	129,884	31,972	814,367
NcDAI	31,523	20,851	6,713	84,128
Continous				
migration				
	Median	Mode	95% HPD-LowB	95% HPD-UppB
M1 (CT->AA)	0.01	0.01	0.01	0.171
M2 (AA->CT)	0.01	0.01	0.01	0.142
NcAA	14,052	14,109	9,257	18,822
NcCT	137,565	74,134	9,392	718,654
NcDAI	27,446	17,297	3,990	77,257

Supplementary Table S7 Random forests confusion matrix and classification error for the ABC analysis of the relationships between populations from different MSEA language families.

	Model 1	Model 2	Model 3	Model 4	Model 5	Classification error
Model 1	8154	1217	207	251	171	0.18
Model 2	1624	7789	481	47	59	0.22
Model 3	193	380	9035	83	309	0.1
Model 4	171	86	84	8735	924	0.13
Model 5	258	122	341	937	8342	0.17

Supplementary Table S8 Votes assigned to each model by the Random Forest procedure and posterior probability for the selected model in the ABC analysis of the relationships between populations from different MSEA language families.

Selected model	Votes model 1	Votes model 2	Votes model 3	Votes model 4	Votes model 5	Post. Prob.
1	0.509	0.311	0.037	0.112	0.031	0.656