

Supplementary Table 1: Molecular diversity indices for populations in West Timor based on Y chromosome STRs ($n = 14$)

Population	N	h	S	Diversity Indices				
				Haplotype diversity	SD	MNPD	SD	Nucleotide diversity
Umaklaran	41	29	13	0.97	0.013	8.38	3.96	0.60
Fatuketi	35	26	13	0.98	0.011	6.72	3.25	0.48
Tialai	24	18	13	0.96	0.025	8.35	4.01	0.60
Raimanawe	50	32	13	0.96	0.017	7.53	3.58	0.54
Kamanasa	62	52	14	0.99	0.005	7.99	3.76	0.57
Kletek Rainan	17	15	13	0.99	0.025	8.80	4.27	0.63
Kletek Suai	15	11	13	0.95	0.040	7.70	3.80	0.55
Kletek Wefatuk	15	14	13	0.99	0.028	7.60	3.76	0.54
Kateri	50	29	13	0.96	0.015	6.66	3.20	0.48
Laran	50	45	13	1.00	0.005	7.45	3.54	0.53
Kakaniuk	47	26	13	0.95	0.017	7.57	3.60	0.54
Umanen Lawalu	49	41	14	0.99	0.007	8.29	3.91	0.59
Besikama	42	32	13	0.98	0.012	8.52	4.02	0.61

Abbreviations: N, number of sequences; h, number of haplotypes; S, number of polymorphic sites; MNPD, mean number of pairwise differences; SD, standard deviation.

Supplementary Table 2: Y chromosome haplogroup frequencies (percentages) in West Timor

	TIM_BS n=42	TIM_FT n=35	TIM_KM n=62	TIM_KT n=50	TIM_KK n=47	TIM_KR n=17	TIM_KS n=15	TIM_KW n=15	TIM_LR n=50	TIM_RM n=50	TIM_TI n=24	TIM_UM n=41	TIM_UL n=49	TIMOR n=497
C-RPS4Y	2.4		3.2		8.5	11.8		6.7	6.0	4.0		12.2	4.1	4.4
C-M38	9.5	37.1	22.6	46.0	25.5	23.5	20.0	53.3	32.0	24.0	4.2	17.1	30.6	26.6
C-P355	14.3	28.6	11.3	12.0	2.1	5.9	6.7		8.0	24.0	20.8	4.9	14.3	12.5
C-M208			1.6								4.2			0.4
C-P343		2.9			2.1									0.4
D-M116												2.4		0.2
E-P1								2.0						0.2
F-P14*				2.0	2.1						20.8	2.4	2.0	1.8
J-M172			1.6					6.7						0.4
K-P397	9.5	2.9	19.4		4.3	5.9		6.7	14.0	8.0	16.7	19.5	10.2	9.9
K-P79	2.4													0.2
K-P336						5.9						2.4		0.2
M-P34	9.5		12.9	2.0		17.6	13.3	6.7	2.0	2.0		17.1	6.1	6.2
O-P201	4.8		14.5	2.0	4.3				2.0	2.0			2.0	3.4
O-JST002611	2.4					5.9								0.4
O-M134		5.7			2.1				2.0	2.0				1.0
O-M119	11.9	2.9		2.0	4.3				2.0		4.2		2.0	2.4
O-P203		2.9							4.0			7.3		1.2
O-M110	9.5	8.6	1.6	18.0	12.8		6.7	6.7	10.0	6.0	8.3	4.9	6.1	8.0
O-M95				2.0			6.7		2.0	2.0				0.8
P-P295	4.8	8.6	4.8	14.0	25.5	5.9	6.7	6.7	10.0	18.0	16.7	2.4	10.2	10.9
Q-P36													2.0	0.2
S-M254	14.3		4.8			11.8	40.0	6.7	2.0	8.0	4.2	7.3	6.1	6.0
S-P377	4.8		1.6		6.4	11.8			2.0				4.1	2.2

Populations: TIM_BS: Besikama; TIM_FT: Fatuketi; TIM_KM: Kamanasa; TIM_KT: Kateri; TIM_KK: Kakaniuk; TIM_KR: Kletek Rainan; TIM_KS: Kletek Suai; TIM_KW: Kletek Wefatuk; TIM_LR: Laran; TIM_RM: Raimanawe; TIM_TI: Tialai; TIM_UM: Umaklaran; TIM_UL: Umanen Lawalu.

Supplementary Table 3: Regional Y chromosome haplogroup frequencies (percentages) in West Timor and surrounding islands

	TIM n=497	SUM n=649	FLO n=394	LEM n=92	SLW n=177	PAN n=26	ALO n=22	MAL n=30	BLI n=502	TAB n=48	PHI n=47	PNG n=48	MEL n=70	AUS n=40
C-RPS4Y	4.4	1.2	25.4	3.3	7.3		4.5		1.8			2.1		
C-M38	26.6	49.5	5.8	4.3	4.0	7.7	18.2	16.7				6.3	12.9	
C-P355	12.5	1.5	9.1	17.4	0.6	3.8	36.4	16.7						
C-M208	0.4											2.1	1.4	
C-P343	0.4		4.3	17.4		3.8								
C-M347													55.0	
C-M217										2.1	2.1			
D-M116	0.2													
E-P1	0.2													
F-P14*	1.8	0.2	1.5	5.4	2.3									
H-M69									3.4					
H-Apt					1.1									
J-M304									0.2					
J-M172	0.4				0.6									
J-M12									0.4					
K-M526*					0.6									
K-P261									1.0					
K-P397	9.9	4.5	6.1	3.3	5.1	3.8	4.5	16.7				6.3	1.4	
K-P405		0.3												
K-P60													45.0	
K-P79	0.2											4.2	8.6	
K-P315												2.1	8.6	
K-P336	0.2					22.7								
K-P378					1.1							31.9		
L-M20								2.4						

Populations: TIM: Timor; SUM: Sumba; FLO: Flores; LEM: Lembata; ALR: Alor; PTR: Pantar; SLW: Sulawesi; MAL: Maluku; BLI: Bali; TAB: Taiwanese Aboriginals; PHIL: Philippines; PNG: Papua New Guinea; MEL: Melanesia; AUS: Australia. All data, except Timor, from Karafet and colleagues¹⁴.

Supplementary Table 4: mtDNA haplogroup frequencies (percentages) in West Timor

	TIM_BS n=42	TIM_FT n=35	TIM_KM n=67	TIM_KT n=50	TIM_KK n=49	TIM_KR n=31	TIM_KS n=20	TIM_KW n=20	TIM_LR n=50	TIM_RM n=50	TIM_TI n=24	TIM_UM n=41	TIM_UL n=50	TIMOR n=529
B4a	7.1	17.1	7.5	4.0	2.0	45.2	15.0	15.0	6.0	8.0	20.8		4.0	9.6
B4a1a1a	2.4		6.0	36.0	2.0		5.0		18.0	6.0			4.0	7.4
B4b1		2.9	3.0	2.0	2.0		25.0	15.0	6.0			2.4	4.0	3.6
B4c1b3	2.4		3.0						2.0				2.0	0.9
B4c2	7.1													0.6
B5a						3.2		15.0						0.8
B5b1			6.0						2.0	2.0		4.9		1.5
D			3.0	4.0										0.8
D4		5.7												0.4
D5			1.5											0.2
D5b1c	2.4		3.0	22.0										2.6
E		2.9	7.5				5.0		4.0	8.0	8.3		2.0	3.0
E1a1a	16.7	17.1	1.5	6.0	4.1	9.7		5.0	6.0	2.0			4.0	5.5
E1b	9.5	11.4	7.5			12.9		5.0	8.0	4.0	20.8	12.2		6.4
E2	2.4					5.0								0.4
F1a			1.5					5.0	2.0				2.0	0.8
F1a3			1.5						18.0	2.0	4.2		6.0	2.8
F1a4	16.7	2.9	13.4	8.0	53.1	6.5	30.0	30.0		18.0	16.7	9.8	48.0	19.3
F3								5.0	2.0				4.0	0.8
M*	4.8	2.9	3.0	2.0					2.0	8.0	4.2	12.2		3.2
M7													2.4	0.2
M7c3	2.4			8.0		3.2				4.0		4.9		1.9
M7c3c		2.9	10.4		14.3		5.0	5.0	4.0	14.0		9.8	2.0	5.9
N*	2.4	5.7								2.0				0.8
N9a	7.1		1.5	2.0		6.5						4.0		1.7

P*	2.4	4.5		2.0								0.9
Q	2.4				3.2	5.0		2.0		2.4		0.9
Q1	9.5	25.7	14.9	4.0	18.4	6.5		14.0	16.0	25.0	29.3	10.0
R*				2.0		3.2						0.4
R9c1	2.4	2.9					2.0	4.0		9.8	4.0	2.1
Y2				2.0		5.0	4.0					0.8

* Mitochondrial paragroups: Individuals were not assigned to any derived haplogroup.

Populations: TIM_BS: Besikama; TIM_FT: Fatuketi; TIM_KM: Kamanasa; TIM_KT: Kateri; TIM_KK: Kakaniuk; TIM_KR: Kletek Rainan; TIM_KS: Kletek Suai; TIM_KW: Kletek Wefatuk; TIM_LR: Laran; TIM_RM: Raimanawe; TIM_TI: Tialai; TIM_UM: Umaklaran; TIM_UL: Umanen Lawalu.

Supplementary Table 5: Regional mtDNA haplogroup frequencies (percentages) in West Timor and surrounding islands

	TIM Present Study	SUM Tumong gor et al, 2013 ⁶	FLO Tumong gor et al, 2013 ⁶	LEM Tumong gor et al, 2013 ⁶	ALR Tumong gor et al, 2013 ⁶	PTR Tumong gor et al, 2013 ⁶	SLW Tumongg or et al, 2013 ⁶	MAL Hill et al, 2007 ⁵⁸	BLI Tumong gor et al, 2013 ⁶	TAB Tabbada et al, 2010 ⁴¹	PHIL Tabbada et al, 2010 ⁴¹	PNG Friedlaen der et al, 2007 ⁴²	MEL Friedlaen der et al, 2007 ⁴²	AUS Hudjasov et al, 2007 ⁴⁵	
		n=529	n=639	n=453	n=92	n=23	n=27	n=188	n=43	n=457	n=640	n=423	n=231	n=607	n=38
B		0.3	0.2						0.4						
B4a	9.6	5.8	4.9	13	13		8	9.3	2.4	8.7	11.8	19.0	16.0		
B4a1a1a	7.4	0.6			4.3				14	0.4					
B4b1	3.6	5.6	2.9	3.3	4.3		4.3	4.7		6	7.6		0.3		
B4c1b3	0.9	4	0.3			14.8	10.6		11.8	4.9	5				
B4c2	0.6	0.2	1.2						7.4						
B5a	0.8	3.1	4	1.1					7	18.4	5.9	0.7			
B5b1	1.5	5.6	2.6	1.1	4.3		1.1		3.7		7.8				
D	0.8	2	3.8				0.5	4.7							
D4	0.4														
D5	0.2	1.4	1.8	1.1			1.6	2.3		4.8					
D5b1c	2.6	4.2	1.5	3.3											
E	3.0	1.4	1.8				2.7		1.5	2.3					
E1a1a	5.5	3	2.4	8.7	8.7	3.7	18.1	4.7	2.4	6.9	11.1	0.4	1.2		
E1b	6.4	4.7	1.1	1.1	4.3	3.7	6.9		0.7		1.4	0.4	9.1		
E2	0.4	3.3	3.1				5.9	4.7	1.1	2.8	3.3	0.4	0.2		
F1a	0.8	3.8	0.2				0.5		1.8	2.2					
F1a1		1.3	2.4	2.2			0.5	2.3	1.8	3.9	0.2				
F1a3	2.8	5.5	5.3	2.2			2.1	7	2.6		3.1				
F1a4	19.3	4.7	3.8	14.1	13		2.7	4.7	1.5		4.3				
F1ac			2				1.1		0.2						
F3	0.8	0.6								1.9					
M*	3.2	8.3	14.7	2.2	8.7	14.8	6.9	2.3	10.7						
M7	0.2							2.3	1.1						
M7b		1.4	0.7					2.3		0.2					

* Mitochondrial paragroups: Individuals were not assigned to any derived haplogroup.

Populations: TIM: Timor; SUM: Sumba; FLO: Flores; LEM: Lembata; ALR: Alor; PTR: Pantar; SLW: Sulawesi; MAL: Maluku; BLI: Bali; TAB: Taiwanese Aboriginals; PHIL: Philippines; PNG: Papua New Guinea; MEL: Melanesia; AUS: Australia.

Supplementary Table 6: Standardized genetic distances in West Timor (G'_{ST})

	Number of Subpopulations	G'_{ST}			
		mtDNA HVS I	mtDNA SNP	Y STR	Y SNP
All Indonesian populations	70	0.86	0.57	0.97	0.79
Timor	13	0.69	0.42	0.72	0.24

**Supplementary Table 7: Mantel tests for genetic, linguistic and geographic associations
(Based on seven populations in Timor that are primarily monolingual)**

Correlation	<i>r</i>	<i>P</i>
	(correlation coefficient)	
Full correlation		
mtDNA HVS1 Slatkin and geography	0.12	0.27
mtDNA HVS1 Slatkin and language	0.13	0.37
mtDNA SNP Slatkin and geography	0.12	0.31
mtDNA SNP Slatkin and language	0.15	0.31
YSTR Slatkin and geography	0.36	0.097
YSTR Slatkin and language	0.24	0.19
YSNP Slatkin and geography	0.43	0.08
YSNP Slatkin and language	-0.31	0.90
Geography and language	0.02	0.43
Partial correlation		
mtDNA HVS1 Slatkin and geography (language*)	0.12	0.29
mtDNA HVS1 Slatkin and language (geography *)	0.13	0.37
mtDNA SNP Slatkin and geography (language*)	0.12	0.31
mtDNA SNP Slatkin and language (geography*)	0.15	0.34
YSTR Slatkin and geography (language*)	0.36	0.12
YSTR Slatkin and language (geography*)	0.25	0.19
YSNP Slatkin and geography (language*)	0.46	0.057
YSNP Slatkin and language (geography*)	-0.35	0.93
Geography and language (HVS1 Slatkin*)	0.0051	0.46
Geography and language (mtDNA SNP Slatkin*)	0.0027	0.46
Geography and language (YSTR Slatkin*)	-0.071	0.57
Geography and language (YSNP Slatkin*)	0.18	0.26

* Indicates constrained parameter.

Supplementary Table 8: Asian admixture rates in Indonesia and surrounding islands (Cox and colleagues⁹; Timor data is from the present study)

Region	Location	Population	Asian Admixture			
			All	A	X	Difference
ISEA	Taiwan	Aboriginal	1.00	1.00	1.00	0
	Philippines	Filipino	1.00	1.00	1.00	0
Indonesia	Sumatra	Toba	1.00	1.00	1.00	0
	Nias	Nias	1.00	1.00	1.00	0
	Mentawai	Mentawai	1.00	1.00	1.00	0
	Java	Dieng	0.98	0.96	1.00	0.04
	Java	Javanese	1.00	1.00	1.00	0
	Borneo	Borneo	1.00	0.99	1.00	0.01
	Bali	Bali	0.99	0.95	1.00	0.04
	Sulawesi	Sulawesi	0.97	0.91	0.99	0.08
	Flores/Lembata	Flores/Lembata	0.66	0.62	0.69	0.08
	Sumba	Sumba	0.81	0.74	0.86	0.12
Melanesia	Alor	Alor	0.51	0.49	0.54	0.04
	Timor	Timor	0.64	0.58	0.70	0.12
	PNG	Coastal	0.14	0.13	0.16	0.03
	Bismarck Archipelago	New Britain	0	0	0	0
	Bougainville	Nasioi	0	0	0	0
	Vanuatu	Maewo	0.06	0.02	0.14	0.12

Supplementary Table 9: Asian admixture rates in West Timor populations

Region	Location	Population	Asian Admixture			
			All	A	X	Difference
Indonesia	Timor	Besikama	0.65	0.59	0.71	0.12
		Fatuketi	0.64	0.59	0.69	0.10
		Kakaniuk	0.67	0.58	0.74	0.16
		Kamanasa	0.62	0.54	0.69	0.15
		Kateri	0.61	0.56	0.66	0.10
		Kletek Rainan	0.63	0.59	0.67	0.08
		Kletek Suai	0.63	0.58	0.67	0.09
		Kletek Wefatuk	0.72	0.66	0.77	0.11
		Laran	0.62	0.57	0.67	0.10
		Reinawe	0.63	0.57	0.68	0.11
		Tialai	0.62	0.59	0.65	0.06
		Umaklaran	0.63	0.57	0.68	0.11
		Umanen Lawalu	0.68	0.58	0.78	0.20
Mean			0.64	0.58	0.70	0.11

Supplementary Table 10: Tentative estimates of the Time of the Most Recent Common Ancestor (TMRCA) of selected mitochondrial DNA haplogroups inferred from the ρ statistic within Indonesia and major surrounding islands

Locations	mtDNA Haplogroup E1a1a		mtDNA Haplogroup F1a4		mtDNA Haplogroup P		mtDNA Haplogroup Q	
	Years BP ± SD	ρ ± SD	Years BP ± SD	ρ ± SD	Years BP ± SD	ρ ± SD	Years BP ± SD	ρ ± SD
Indonesia (Lesser Sunda Islands)	6,700 ± 3,100	0.351 ± 0.16	5,150 ± 1,600	0.268 ± 0.08				
Timor	4,050 ± 2,900	0.211 ± 0.15	3,050 ± 1,200	0.158 ± 0.06	59,600 ± 16,050	3.109 ± 0.84	26,000 ± 9,250	1.356 ± 0.48
Philippines	3,850 ± 2,500	0.200 ± 0.13	950 ± 600	0.048 ± 0.03				
Taiwan	4,000 ± 1,800	0.210 ± 0.09						
Aborigines								
New Guinea					53,700 ± 11,850	2.8 ± 0.62	38,900 ± 8,350	2.03 ± 0.43

Note: Dates before present (BP) are rounded to the nearest 50 years.

Supplementary Table 11: Time to the Most Recent Common Ancestor (TMRCA) of selected Y chromosome haplogroups in individuals from Indonesia, Philippines (Aeta), Oceania and Australia using Batwing[†]

Y Chromosome Haplogroup	TMRCA (95% Confidence Interval)
	Years BP
C-RPS4Y	41,650 (17,850 – 92,100)
C-M38	32,950 (14,350 – 70,850)
C-P343	16,000 (6,500 – 36,550)
C-P355	13,600 (5,600 – 30,200)
C-M347	15,900 (5,950 – 39,550)
M-P256	30,600 (13,100 – 66,600)
S-M230	22,750 (9,750 – 49,300)

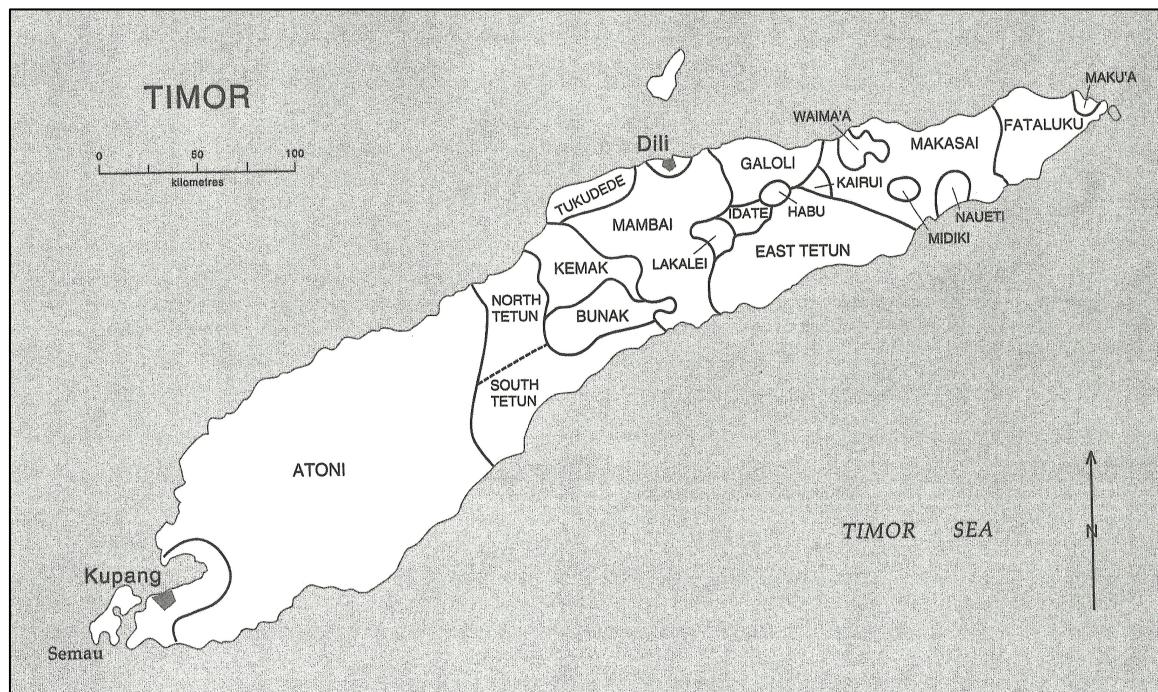
Note: Dates before present (BP) are rounded to the nearest 50 years.

[†] <http://www.mas.ncl.ac.uk/~nijw/#batwing>

Supplementary Table 12: A list of new Y chromosome markers, position, primer information and reference SNP ID

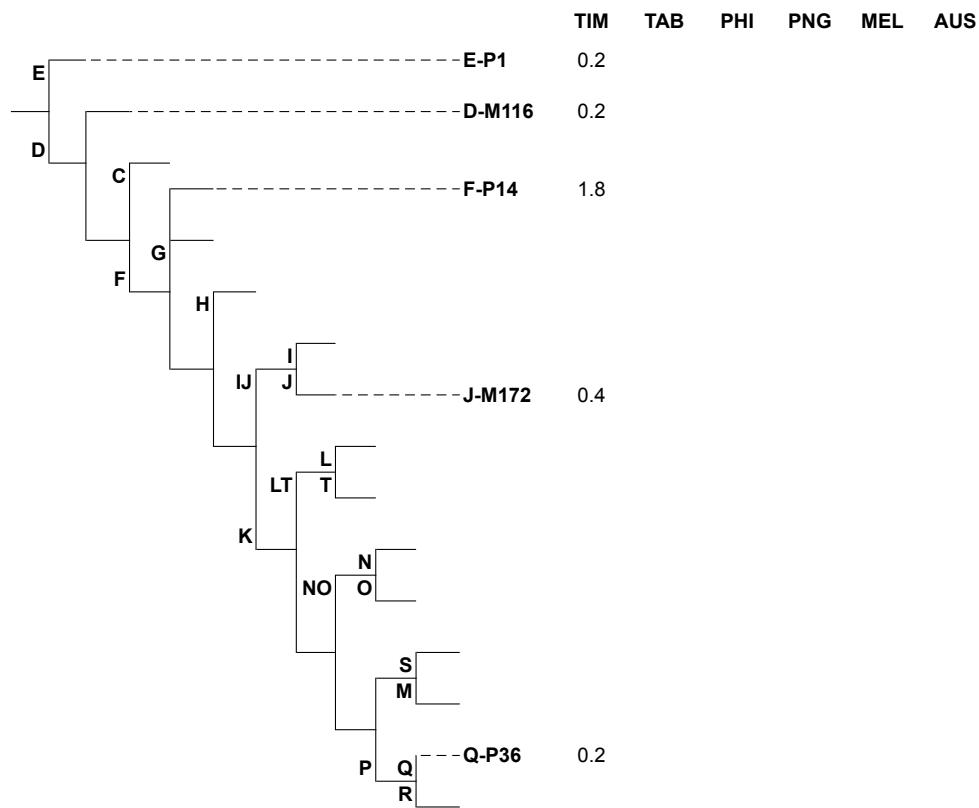
SNP	Haplogroup	Y-position (GRCh37/ hg19)	Forward Primer	Reverse Primer	PCR Size (bp)	Mutation	Site (bp)	Strand
P343	C	2,815,407	ATGAACAGAATGAATTAAATGGT	TATGTTGTAAGATGAAAAATGT	360	Cdel	85	Positive
P355	C	22,077,527	AATCTCCTGCCCTGAATGACTGTCC	TATCCTGTAATCCTTGAGCCTC	399	G→A	250	Negative
P377	S	5,847,373	CAAAAAGTGGGCAATCAGATGACT	ATTTTATTCACTTTATGGCTGAG	513	A→G	258	Positive

**Supplementary Figure 1: Language groups spoken on the island of Timor
(adopted from Therik, 2004)**

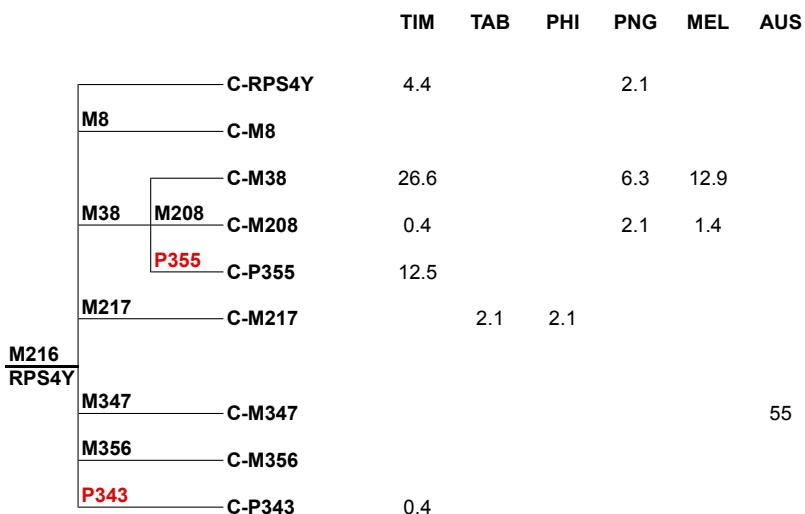


Supplementary Figure 2: Maximum Parsimony tree of Y chromosome haplogroups in West Timor

a) Simplified tree showing lineages from the D and E trunk onward.

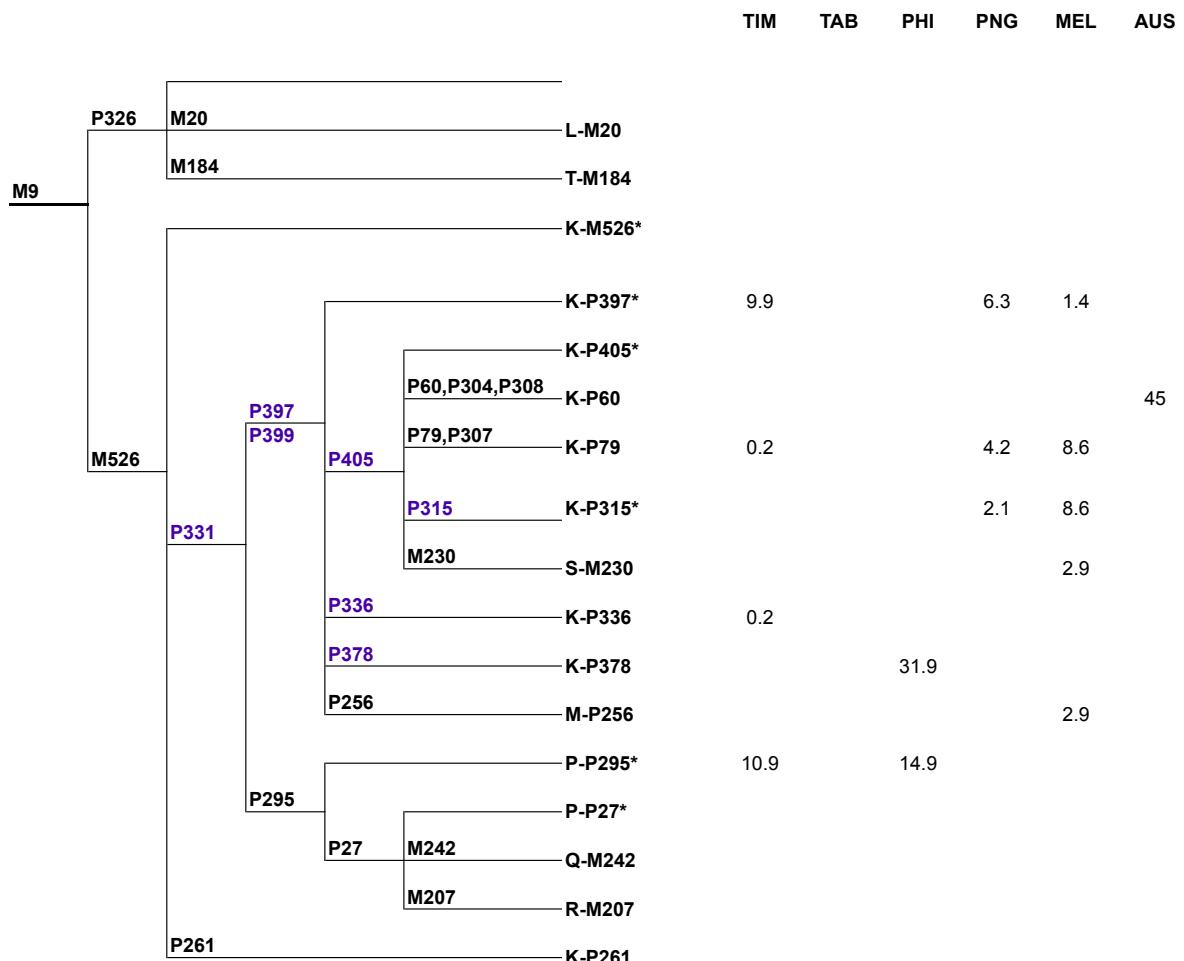


b) Detailed tree of C lineages (including newly reported haplogroups in red).



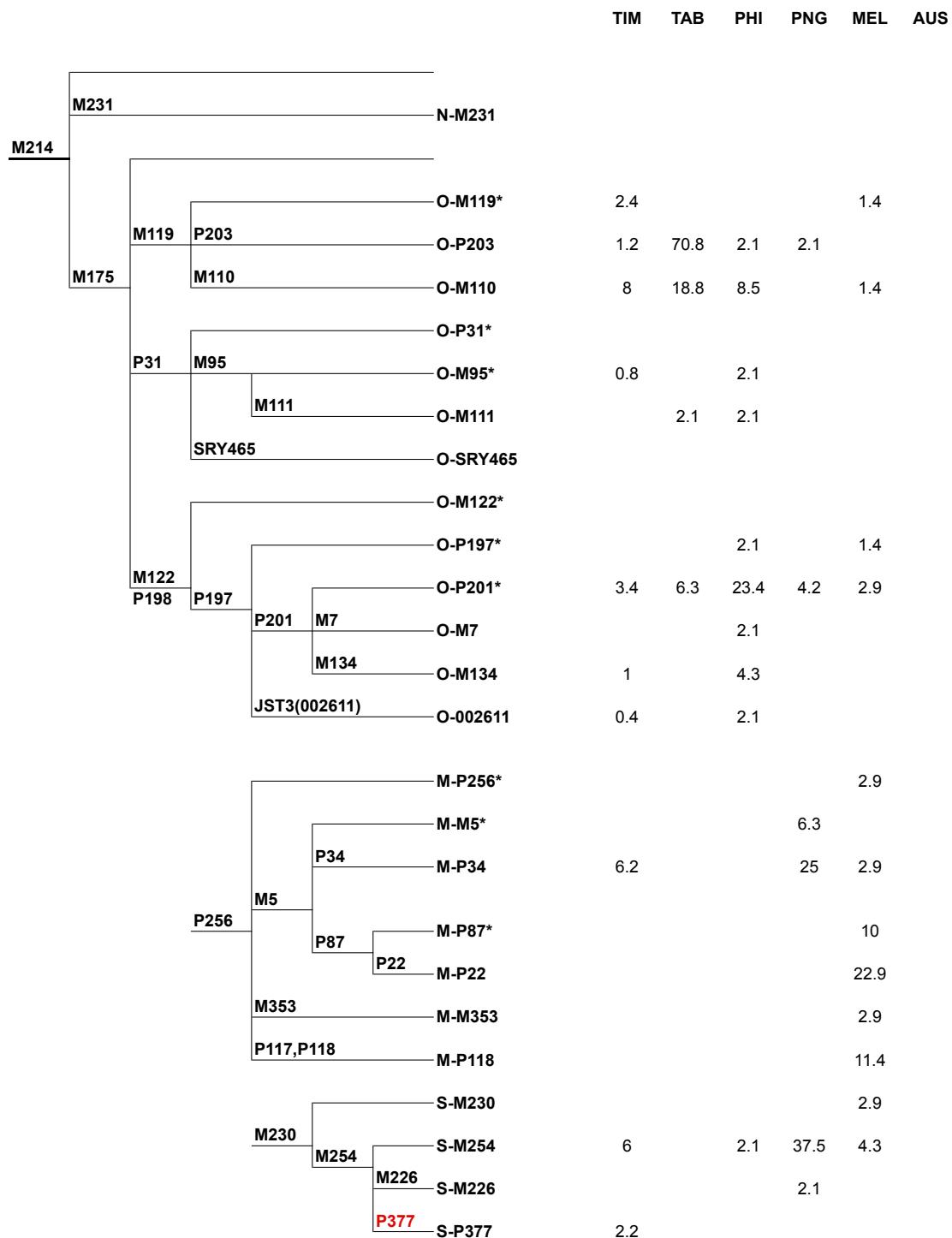
Supplementary Figure 2 *continued*

c) Lineages from the K trunk (including recently reported haplogroups by Karafet and colleagues, *in press*, in purple)

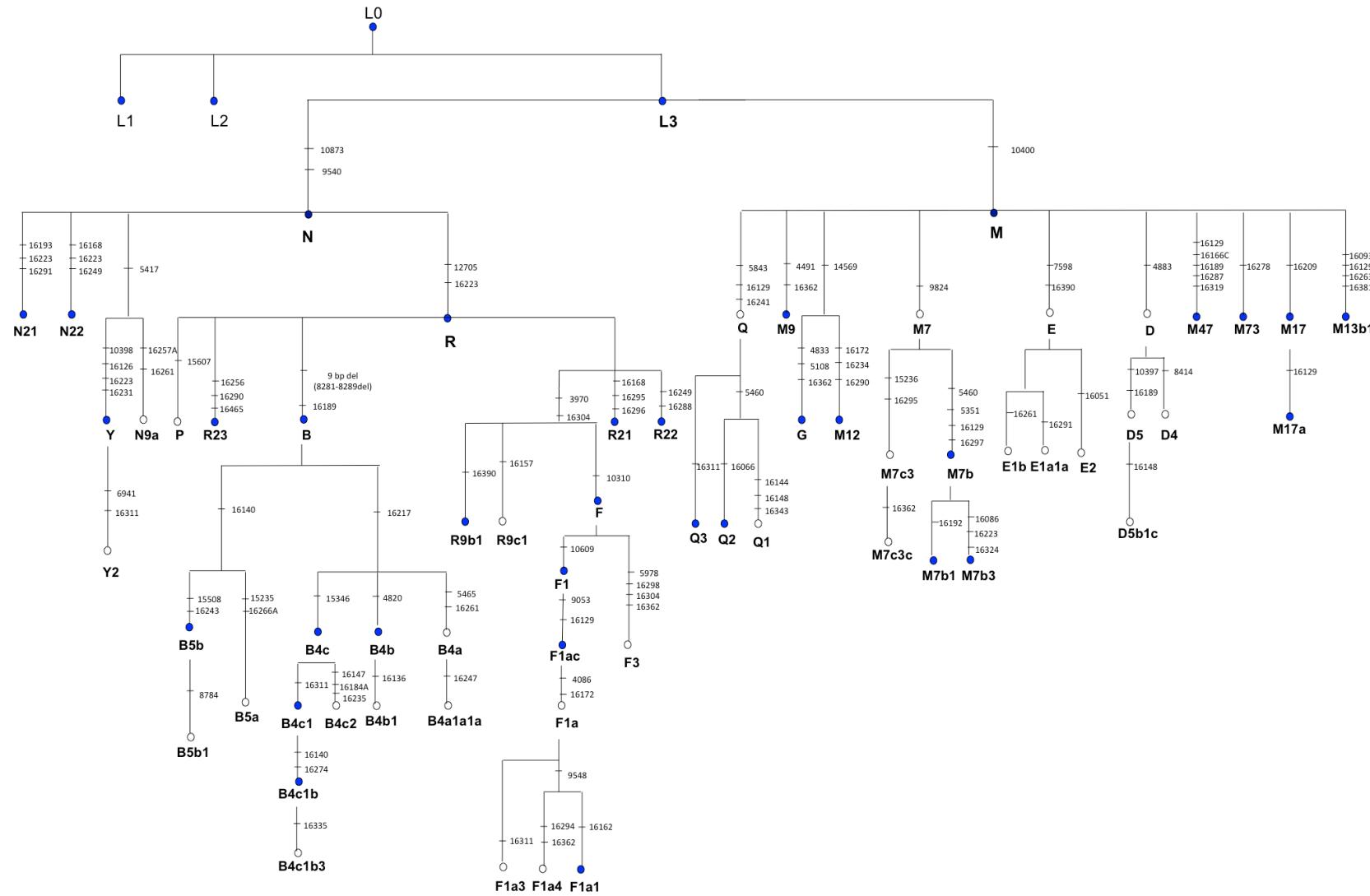


Supplementary Figure 2 *continued*

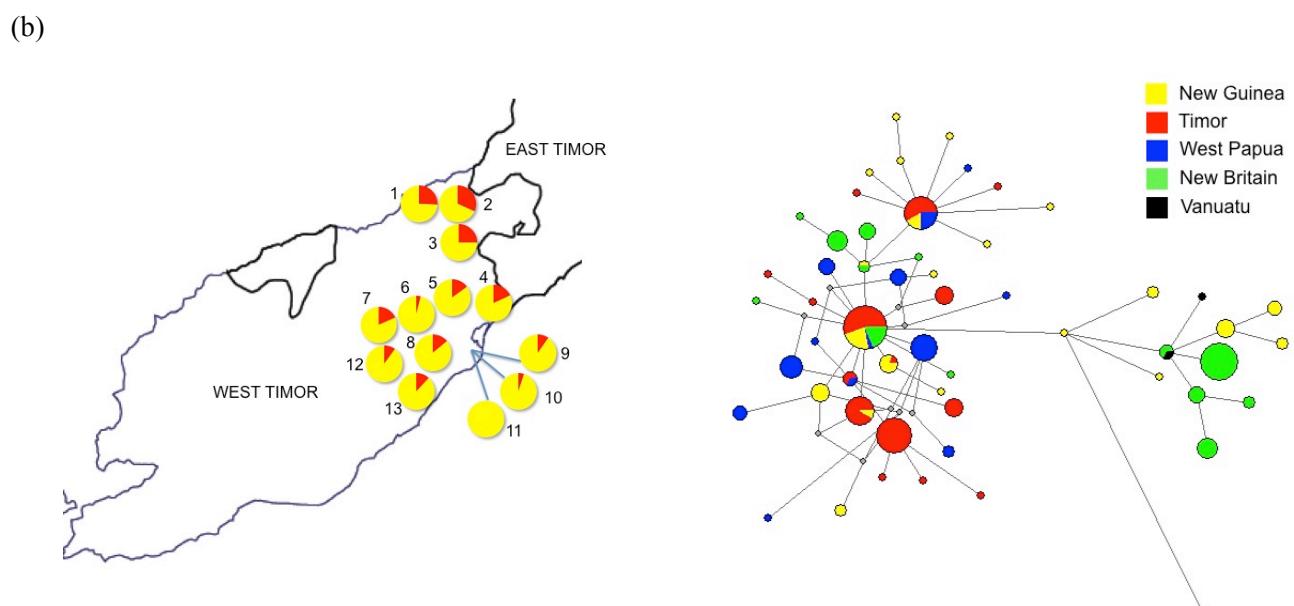
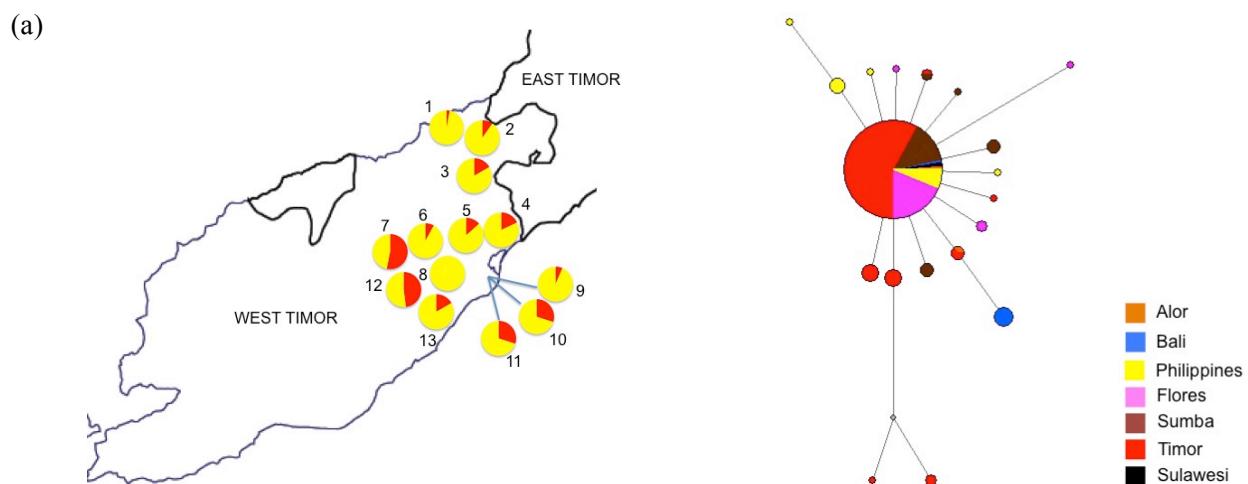
d) Lineages O, M and S (including a newly reported haplogroup in red)



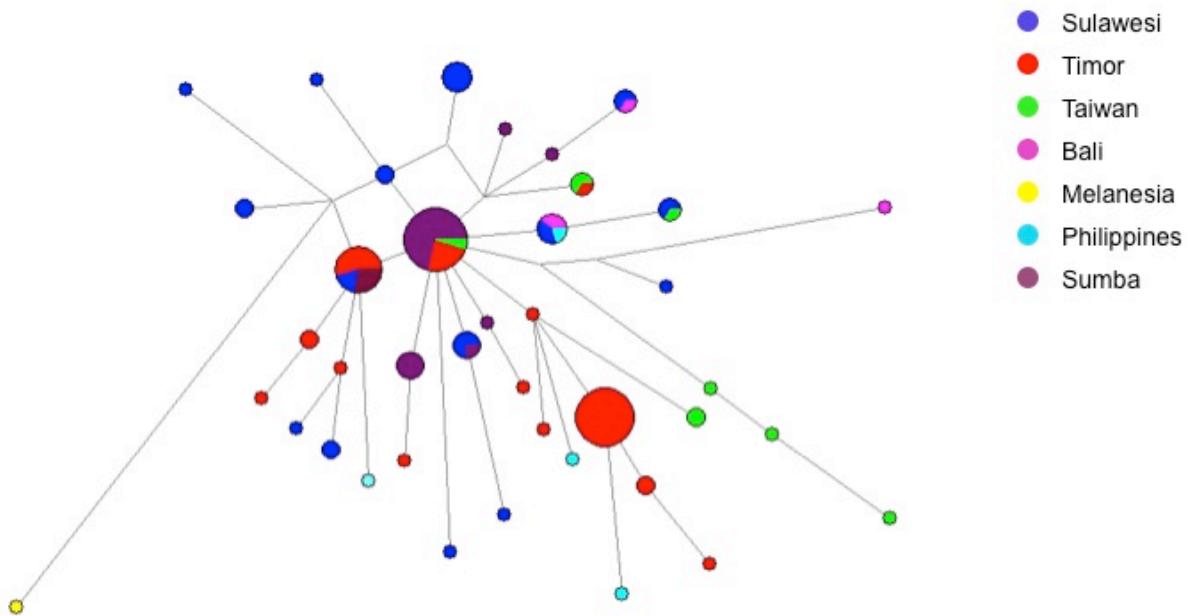
Supplementary Figure 3: Phylogeny of mitochondrial DNA haplogroups observed in Indonesian populations (adapted from Tumonggor et al., 2013). Open circles: mtDNA lineages found in West Timor.



Supplementary Figure 4: Distribution and network of mtDNA haplogroups (a) F1a4 and (b) Q in West Timor. Populations: (1) Fatuketi, (2) Umaklaran, (3) Tialai, (4) Raimanawe, (5) Kamanasa, (6) Kateri, (7) Kakaniuk, (8) Laran, (9) Kletek Rainan, (10) Kletek Suai, (11) Kletek Wefatuk, (12) Umanen Lawalu, and (13) Besikama.



Supplementary Figure 5: Network of Y chromosome haplogroup O-M110



Supplementary Text 1: DNA Extraction and Genetic Screening

DNA was extracted from peripheral blood samples using the salting-out procedure of Miller, Dykes and Polesky⁶⁸. DNA from buccal swabs was extracted using standard phenol-chloroform protocols.

The first hypervariable segment (HVS I) of mitochondrial DNA was amplified using primers L15926 (5'-TCAAAGCTTACACCAGTCTTGTAACCC-3') and H639 (5'-GGGTGATGTGAGCCCCGTCA-3'). PCR amplicons were sequenced in both forward and reverse directions using primers L15965 (5'-CAAGGACAAATCAGAGAA-3') and H11 (5'-GTGGTTAATAGGGTGATAG-3'). Traditional Sanger sequences were aligned and edited with Sequencher® v. 5.0 (Gene Codes Corporation, Ann Arbor, MI, USA; <http://www.genecodes.com>). Polymorphisms were scored relative to the revised Cambridge reference sequence (rCRS)⁶⁹. Haplogroups were initially predicted from HVS I sequences using known reference genomes^{30, 41, 59, 70-73}. Assignments were subsequently confirmed using Taqman and Restriction Fragment Length Polymorphism (RFLP) assays (as described in Tumonggor and colleagues⁶). For mtDNA comparative analysis, we used previously published data from the following geographic regions: surrounding Indonesian islands^{6, 59}, the Philippines⁴¹, indigenous Taiwanese⁷⁴, New Guinea and Melanesian populations⁴², and Australian aborigines⁴⁵.

Polymorphic sites from the non-recombining portion of the human Y chromosome (NRY) included a set of 85 binary markers published previously^{14, 40} and three newly discovered mutations: C-P355, C-P343 and S-P377. Information about these new mutations is provided in **Supplementary Table 12** and their phylogenetic positions are shown in **Supplementary Figure 2**. We use the mutation-based naming system recommended by the Y Chromosome Consortium^{75, 76}. We analyzed 14 Y chromosome short tandem repeats (STRs) (DYS19, DYS385a, DYS385b, DYS388, DYS389I, DYS389CD, DYS390, DYS391, DYS392, DYS393, DYS426, DYS438, DYS457, and TAGA or DYS439+4) using methods described by Redd and colleagues⁷⁷. Additional populations were included for comparative analysis of the Y chromosome data: indigenous Taiwanese ($n = 48$), Filipinos ($n = 47$), Papua New Guineans ($n = 48$), Melanesians ($n = 70$) and Australians ($n = 40$).

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