

mtDNA variation and human-mediated introgression of indigenous *Sus* populations on several Indonesian islands

Naotaka Ishiguro^{1,*}, Yasuo Inoshima¹, Motoki Sasaki², Akira Matsui³, Hitomi Hongo⁴, Ryouhei Takahashi⁴, Wahono Esthi Prasetyaningtyas⁵, Ita Djuwita⁵, Srihadi Agungpriyono⁵, Supratikno⁵, Kusdiantoro⁵, Elhayat Labiro⁶, Teguh Budipitojo⁷, Woro Danur Wendo⁷, Dewi Kania Musana⁷ and Takdir Saili⁸

¹ Laboratory of Food and Environmental Hygiene, Veterinary Medicine, Faculty of Applied and Biological Sciences, Gifu University, Gifu 501-1193, Japan

² Laboratory of Anatomy, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-5111, Japan

³ Nara National Culture Properties Research Institute, Nara 630-8577, Japan

⁴ Department of Advanced Sciences, Graduate University for Advanced Studies, Hayama, Kanagawa 240-0193, Japan

⁵ Department of Anatomy, Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor 16680, Indonesia

⁶ Department of Biology, Tadulako University, Palu, Sulawesi 94221, Indonesia

⁷ Department of Anatomy, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta 55281, Indonesia

⁸ Laboratory of Animal Science, Faculty of Agriculture, Haluoleo University, Kendari 93232, Indonesia

Abstract. To examine the genetic origin of the domestic pig, the distribution of wild boar, and human-mediated translocation of the domestic pig, we collected 223 samples from domestic pigs and wild boars from eight Indonesian islands, sequenced the control region of mitochondrial DNA (mtDNA) from each sample, and compared these sequences with previously determined sequences from East and Southeast Asian domestic pigs and wild boars. Three *Sus* species (*S. scrofa*, *S. verrucosus*, and *S. celebensis*) were identified on the Indonesian islands. The mtDNA sequences of three Indonesian *Sus* species were diverse, and they clustered into three lineages with low bootstrap values (an *S. scrofa* group including East and Southeast Asian domestic pigs and wild boars, a group including indigenous *S. scrofa* together with *S. verrucosus* from Sumatra and Java Islands, and an *S. celebensis* group from Sulawesi Island). The mtDNA haplotypes of *S. scrofa* wild boars from three (Sumbawa, Flores and New Guinea) islands and domestic pigs from two (Lombok and Timor) islands east of the Wallace Line, and some *S. scrofa* wild boars from Sumatra and Java Islands were related to Vietnamese pig mtDNA sequences in the East and Southeast Asian domestic pig and wild boar clade, supporting that ancient immigrants likely introduced domestic pigs from the Asian continent to east Indonesian islands. The mtDNA haplotypes of *S. celebensis* were broadly divided into three groups, which were distributed in the north and southwest areas, central area and southeast area of Sulawesi Island.

Key words: domestic pig, Indonesia, mtDNA, origin, wild boar.

The origin of domestic pigs has been debated for many years. The genus *Sus* includes ten species: *Sus ahoenobarbus*, *S. barbatus*, *S. bucculentus*, *S. cebifrons*, *S. celebensis*, *S. oliveri*, *S. philippensis*, *S. salvanius*, *S. scrofa*, and *S. verrucosus* (Grubb 2005; Chen et al. 2007). *Sus scrofa* is the most widely distributed of these ten species, inhabiting Asia, Europe and northwestern Africa. The other nine *Sus* species inhabit in limited

continental areas or archipelagos, such as the Indonesian islands or Philippine islands. In Indonesia, *S. barbatus* is endemic to Borneo Island, *S. celebensis* to Sulawesi Island, *S. verrucosus* to Java Island, and *S. scrofa* to Sumatra and Java Islands. Giuffra et al. (2000) proposed that the domestic pig was domesticated independently in Europe and Asia from two distinct ancestral populations of *S. scrofa* wild boar and at multiple sites. Mitochon-

*To whom correspondence should be addressed. E-mail: Ishiguna@gifu-u.ac.jp

Table 1. Particulars of DNA samples isolated from domestic pigs and wild boars in Indonesia

Year	Sample Name	Species	Island ¹⁾	Location	Elements	Status	bp	Haplotype	Accession No.	
2006	E1, 3, 4, 10	<i>S. scrofa</i>	Sumatra	Bengkulu	Muscle	Wild	572	2006E1, 4, 10	AB564327~9	
	E2, 5~9	<i>S. scrofa</i>	Sumatra	Bengkulu	Muscle	Wild	573	2006E2, 5, 7~9	AB564332~6	
	E11	<i>S. scrofa</i>	Java	Bandung	Muscle	Wild	573	2006E11	AB564337	
	E12, 13, 15, 16	<i>S. scrofa</i>	Java	Sumedang	Muscle	Wild	573	2006E11, 15, 16	AB564338, 9	
	E14	<i>S. scrofa</i>	Java	Sumedang	Muscle	Wild	572	2006E4		
	W1, 7, 8, 22	<i>S. scrofa</i>	Java	Banjarnegara	Muscle	Wild	572	2006W1, 22	AB564330, 1	
	W2, 5, 6, 9	<i>S. scrofa</i>	Java	Banjarnegara	Muscle	Wild	573	2006W2, 6, 9	AB564340~2	
	W3, 4	<i>S. scrofa</i>	Java	Purbalingga	Muscle	Wild	572	2006W1		
	W10	<i>S. scrofa</i>	Java	Pacitan	Muscle	Wild	573	2006W10	AB564343	
	W11~16, 19~21	<i>S. scrofa</i>	Java	Purwokerto	Muscle	Wild	573	2006W11, 19, 21	AB564344, 6, 7	
	W17	<i>S. scrofa</i>	Java	Garut	Muscle	Wild	573	2006W17	AB564345	
	W18	<i>S. verrucosus</i>	Java	Cilacap	Muscle	Wild	573	2006W11		
	W23~25	<i>S. scrofa</i>	Java	Wonosobo	Muscle	Wild	573	2006W19, 21		
	W26	<i>S. verrucosus</i>	Java	Cilacap	Hair	Wild	573	2006W6		
	W27	<i>S. scrofa</i>	Sumatra	Jambi	Hair	Wild	573	2006W27	AB564348	
	2007	E17~20	<i>S. scrofa</i>	Java	Bandung	Muscle	Wild	573	2006E11	
		E21	<i>S. scrofa</i>	Java	Bandung	Muscle	Wild	572	2007E21	AB564349
E22		<i>S. scrofa</i>	Sumatra	Banda Aceh	Muscle	Wild	573	2007E22	AB564357	
E23		<i>S. scrofa</i>	Sumatra	Banda Aceh	Muscle	Wild	572	2007E21		
E24~29, 47, 53~57		<i>S. scrofa</i>	Sumbawa	Sunbawa Besar	Muscle	Wild	572	2006E4, 2007E21, 26, 53	AB564350, 4	
E30, 32~39		<i>S. scrofa</i>	Timor	Kupang	Muscle	Domestic	572	2007E21		
E31		<i>S. scrofa</i>	Timor	Kupang	Muscle	Domestic	573	2007E31	AB564358	
E40, 43		<i>S. scrofa</i>	Flores	Larantuka	Muscle	Wild	573	2007E31		
E41, 42, 44		<i>S. scrofa</i>	Flores	Larantuka	Muscle	Wild	572	2006E4, 2007E21		
E45, 46, 58~68		<i>S. scrofa</i>	Lombok	Mataran	Muscle	Domestic	572	2007E21, 45, 46, 53, 59	AB564352, 3, 5	
E48~52		<i>S. scrofa</i>	Lombok	Mataran	Hair	Domestic	572	2007E45		
N1~10		<i>S. scrofa</i>	Lombok	Jembatan Kembar	Hair	Domestic	572	2007E45		
N11~18		<i>S. scrofa</i>	Lombok	Karang Anyar	Hair	Domestic	572	2007E45, 53		
N19~30	<i>S. scrofa</i>	Lombok	Tanjung	Hair	Domestic	572	2007E21, 45, 46			
2008	G1	<i>S. celebensis</i>	Sulawesi (N)	Buloita	Muscle	Wild	573	2008G1	AB564359	
	G2, 4, 7, 9	<i>S. celebensis</i>	Sulawesi (N)	Popalo	Muscle	Wild	573	2008G1, 7, 11	AB564362, 4	
	G3	<i>S. celebensis</i>	Sulawesi (N)	Monano	Muscle	Wild	573	2008G3	AB564361	
	G5	<i>S. celebensis</i>	Sulawesi (N)	Atingola	Muscle	Wild	573	2008G1		
	G6	<i>S. celebensis</i>	Sulawesi (N)	Tolango	Muscle	Wild	573	2008G1		
	G8, 14	<i>S. celebensis</i>	Sulawesi (N)	Pontolo	Muscle	Wild	573	2008G11, 14	AB564360	
	G10	<i>S. celebensis</i>	Sulawesi (N)	Paguyaman	Muscle	Wild	573	2008G1		
	G11	<i>S. celebensis</i>	Sulawesi (N)	Alo	Muscle	Wild	573	2008G11		
	G12	<i>S. celebensis</i>	Sulawesi (N)	Labanu	Muscle	Wild	573	2008G1		
	G13	<i>S. celebensis</i>	Sulawesi (N)	Tibawa	Muscle	Wild	573	2008G1		

Year	Sample Name	Species	Island ¹⁾	Location	Elements	Status	bp	Haplotype	Accession No.
	G15	<i>S. celebensis</i>	Sulawesi (N)	Kuandang	Muscle	Wild	573	2008G15	AB564363
	P1	<i>S. celebensis</i>	Sulawesi (C)	Taripa	Muscle	Wild	573	2008P1	AB564367
	P2, 16	<i>S. celebensis</i>	Sulawesi (C)	Saloya	Muscle	Wild	574	2008P16	AB564377
	P3	<i>S. celebensis</i>	Sulawesi (C)	Taripa	Muscle	Wild	574	2008P16	
	P4, 24-26	<i>S. celebensis</i>	Sulawesi (C)	Donggala	Muscle	Wild	573	2008P22, 24	AB564374
	P5, 17, 19, 20	<i>S. celebensis</i>	Sulawesi (C)	Batusuya	Muscle	Wild	573	2008P11, 15, 19	AB564375
	P6-10, 15, 21, 31	<i>S. celebensis</i>	Sulawesi (C)	Saloya	Muscle	Wild	573	2008P10, 15, 21, 24, 28, 32	AB564365, 68, 71, 72
	P11	<i>S. celebensis</i>	Sulawesi (C)	Lero	Muscle	Wild	573	2008P11	AB564369
	P12	<i>S. celebensis</i>	Sulawesi (C)	Dalika	Muscle	Wild	574	2008P16	
	P13, 14	<i>S. celebensis</i>	Sulawesi (C)	Sumari	Muscle	Wild	574	2008P16	
	P18	<i>S. celebensis</i>	Sulawesi (C)	Batusuya	Muscle	Wild	574	2008P16	
	P22, 23	<i>S. celebensis</i>	Sulawesi (C)	Toaya	Muscle	Wild	573	2008P22, 23	AB564370, 3
	P27	<i>S. celebensis</i>	Sulawesi (C)	Kasimbar	Muscle	Wild	573	2008P24	
	P28	<i>S. celebensis</i>	Sulawesi (C)	Palasa	Muscle	Wild	573	2008P28	AB564366
	P29	<i>S. celebensis</i>	Sulawesi (C)	Towale	Muscle	Wild	573	2008P28	
	P30, 35	<i>S. celebensis</i>	Sulawesi (C)	Sesere	Muscle	Wild	573	2008P15, 35	AB564376
	P32	<i>S. celebensis</i>	Sulawesi (C)	Dalaka	Muscle	Wild	573	2008P32	
	P33	<i>S. celebensis</i>	Sulawesi (C)	Tambarana	Muscle	Wild	573	2008P21	
	P34	<i>S. celebensis</i>	Sulawesi (C)	Sulewana	Muscle	Wild	573	2008P24	
2009	B1-9	<i>S. celebensis</i>	Sulawesi (SE)	Kendari (Maligano, Muna)	Muscle	Wild	572	2009B1-4, 8	AB564383-7
	B10	<i>S. celebensis</i>	Sulawesi (SE)	Kendari (Wakalambe, buton)	Muscle	Wild	572	2009K10	AB564381
	B11-13	<i>S. celebensis</i>	Sulawesi (SE)	Kendari (Kambara, Muna)	Muscle	Wild	572	2009K4, B12, 13	AB564379, 88, 89
	B14	<i>S. celebensis</i>	Sulawesi (SE)	Kendari (Wakuli, Buton)	Muscle	Wild	572	2009B14	AB564390
	B15	<i>S. celebensis</i>	Sulawesi (SE)	Kendari (Labundo, Buton)	Muscle	Wild	574	2009B15	AB564404
	B16	<i>S. celebensis</i>	Sulawesi (SE)	Kendari (Pure Buten)	Muscle	Wild	574	2009B15	
	I1, 2	<i>S. scrofa</i>	New Guinea	Papua (Irian Jaya)	Muscle	Wild	573	2009K19	AB564398
	I3, 4	<i>S. scrofa</i>	New Guinea	Papua (Irian Jaya)	Muscle	Wild	572	2009I3	AB564399
	K1	<i>S. celebensis</i>	Sulawesi (SE)	Kendari (Lalowaru, Moramo)	Muscle	Wild	573	2009K1	AB564391
	K2	<i>S. celebensis</i>	Sulawesi (SE)	Kendari (Lalowaru, Moramo)	Muscle	Wild	572	2009K2	AB564378
	K3	<i>S. celebensis</i>	Sulawesi (SE)	Kendari (Lameuru, West Ronomeeto)	Muscle	Wild	573	2009K3	AB564392
	K4	<i>S. celebensis</i>	Sulawesi (SE)	Kendari (Kolono, South Konawe)	Muscle	Wild	572	2009K4	
	K5	<i>S. celebensis</i>	Sulawesi (SE)	Kendari (Tanjung Hitam, Moramo)	Muscle	Wild	573	2009K5	AB564393
	K6	<i>S. celebensis</i>	Sulawesi (SE)	Kendari (Kolono)	Muscle	Wild	572	2009K6	AB564380
	K7-9, 11-17, 19	<i>S. celebensis</i>	Sulawesi (SE)	Kendari (North Konawe)	Muscle	Wild	573	2009K7-9, 11, 19	AB564394-7
	K10, 18	<i>S. celebensis</i>	Sulawesi (SE)	Kendari (North Konawe)	Muscle	Wild	572	2009K10, 18	AB564382
	M1, 2, 4	<i>S. celebensis</i>	Sulawesi (N)	Mamado	Muscle	Wild	573	2009G1, M2, SS1	AB564400, AB564403
	M3	<i>S. celebensis</i>	Sulawesi (SE)	Kendari (Wakuli, Buton)	Muscle	Wild	574	2009M3	AB564405
	SS1-5	<i>S. celebensis</i>	Sulawesi (SW)	Makasar	Muscle	Wild	573	2009SS1, 4	AB564401
Total	223								

¹⁾ (N), North Sulawesi; (C), Central Sulawesi; (SE), Southeast Sulawesi; (SW), Southwest Sulawesi.

drial DNA (mtDNA) analyses provided compelling evidence for independent domestication events in Europe and Asia from *S. scrofa* wild boars (Watanabe et al. 1985; Giuffra et al. 2000). Larson et al. (2005) systematically collected modern and ancient DNA samples derived primarily from wild-caught specimens from museum collections, examined mtDNA lineages phylogenetically and revealed that pig domestication from wild boars took place at several sites. Recently, Larson et al. (2010) reported that there are five potential independent domestication sites of indigenous *S. scrofa* wild boars: one in India; three in peninsular Southeast Asia (including northern Vietnam); and one off the coast of Taiwan. These results indicate that the Southeast Asia peninsula is the main domestication site for Asian domestic pigs.

Independently from Larson's studies (Larson et al. 2005, 2007, 2010), we analyzed mtDNA samples of *S. scrofa* from Japan and neighboring countries, as well as Vietnam, from 2003 to 2005 in order to explore the genetic origin of domestic pigs (Hongo et al. 2002; Ishiguro et al. 2008a, b). We also visited several Indonesian islands to collect samples over a 4-year period for the present study. The sequences of mtDNA control regions determined from DNA samples of Indonesian domestic pigs and wild boars were compared with previ-

ously determined sequences from East and Southeast Asian domestic pigs and wild boars.

In this study, we examined the mtDNA diversity of domestic pigs and wild boars in Indonesia to trace the genetic origin of the domestic pig. The phylogenetic analysis using the data set suggested that the *S. scrofa* from Indonesian islands have high mtDNA diversity, and that indigenous wild boars with unique haplotypes inhabited each island. Some mtDNA haplotypes from *S. scrofa* wild boar samples from Sumbawa, Flores and New Guinea Islands, the islands east of the Wallace Line, and from *S. scrofa* wild boar samples from Sumatra and Java Islands were closely related to Vietnamese pig mtDNA lineages.

Materials and methods

Samples

Tissue samples (223 total: 37 hair samples from domestic pigs and 186 muscle samples from wild boars) were collected from domestic pigs and wild boars in Indonesia from 2006 to 2009 (43 samples in 2006, 82 samples in 2007, 50 samples in 2008, and 48 samples in 2009). Detailed information (island, type of tissue samples, and wild or domestic) for the 223 samples is shown in Table 1. Sample collection sites are shown in Fig. 1.

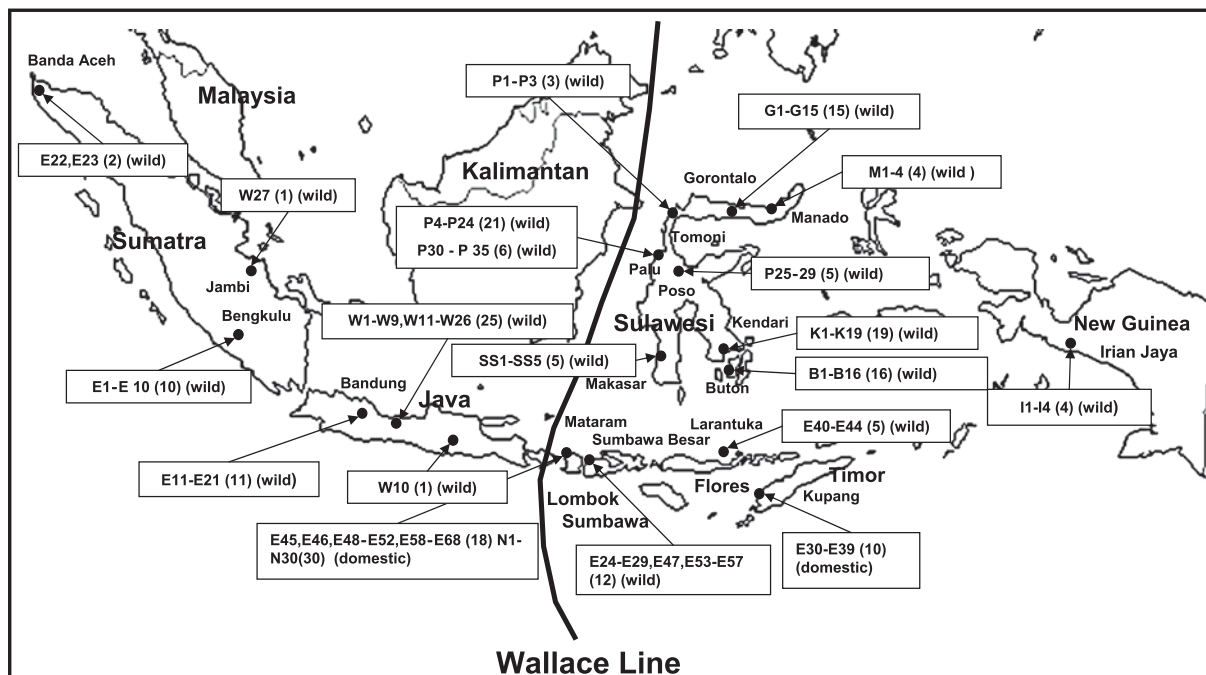


Fig. 1. Sampling sites in Indonesia. Numbers in parentheses indicate the number of samples collected from each site. The Wallace line indicates a geographical division between Australian and Southeast Asian fauna. wild, wild boar; domestic, domestic pig.

Muscle samples from wild boars on each island were obtained from native hunters. Identification of *Sus* species for each sample was conducted based on morphological information (e.g., body size) provided by native hunters.

DNA extraction and mtDNA analysis

Total DNA was extracted from the 37 hair samples and 186 muscle samples using the DNeasy Tissue kit (Qiagen) according to the manufacturer's instructions. The mtDNA D-loop region (572 to 574 bp) was amplified using two primers, mit112 and mit106 (Watanobe et al. 2003). PCR products were purified using a QIAquick PCR Purification kit (Qiagen), as described by Ishiguro and Nishimura (2005). Sequence reaction of the purified PCR products was then performed using two primers (mit11 and mit12) and a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems), followed by automated sequencing on an ABI 310 Genetic Analyzer. Novel control region sequences from this study were deposited in GenBank with Accession Nos. AB564327 to AB564405.

Alignment of DNA sequences and phylogenetic analysis

mtDNA sequences (572 to 574 bp) were aligned with haplotypes published previously (Hongo et al. 2002; Watanobe et al. 2003; Ishiguro and Nishimura 2005; Ishiguro et al. 2008a) using Clustal W (gap opening penalty 15, gap extension penalty 6.66) (Thompson et al. 1994). Genetic distance was estimated by the proportion (P)-distance method in the MEGA4 (Tamura et al. 2007). The corresponding regional sequence of *Phacochoerus aethiopicus* (Cape and Somali Warthog, AB046876, Okumura et al. 2001) was used as an outgroup. Phylogenetic trees were constructed using the neighbor joining (NJ) method (Saitou and Nei 1987) and the maximum parsimony (MP) method in MEGA4. The confidence of each branch in the phylogeny was estimated using 1,000 bootstrap replications (Felsenstein 1985).

Results

Nucleotide sequence diversity and phylogenetic relationships

All wild boars sampled from Sumatra, Java, Sumbawa, Flores and New Guinea Islands were identified as *S. scrofa*, except for two *S. verrucosus* individuals from Java Island. All wild boar samples from Sulawesi Island

were identified as *S. celebensis* (Table 1).

We detected 77 different mtDNA haplotypes in the 223 Indonesian DNA samples. When the 77 mtDNA haplotypes were compared with previously reported mtDNA sequences, the haplotype of sample No. 2007E45, isolated from Lombok Island, was identical to the haplotype M33 from domestic pig (Ishiguro and Nishimura 2005).

In order to investigate the phylogenetic relationship among the mtDNA haplotypes in the Indonesian *Sus* samples, an NJ tree was constructed by using pairwise distance matrix calculated by P-distance with the 77 mtDNA haplotypes identified in this study and 81 *S. scrofa* haplotypes reported previously (Fig. 2). A nearly identical phylogenetic tree was obtained using the MP method (data not shown). Two haplotypes, 2007E45 (M33) and 2007E46, from domestic pigs on Lombok Island fell into the East and Southeast Asian domestic pig and wild boar clade. Nine haplotypes (2006E1 and 2006E10 from Sumatra Island, 2006E4 from Sumatra and Flores Islands, 2007E21 from Java, Sumbawa, Flores, Timor and Lombok Islands, 2007E26 from Sumbawa Island, 2007E31 from Timor and Flores Islands, 2007E53 and 2007E59 from Lombok Island, and 2009I3 from New Guinea Island) were related to Vietnamese haplotypes (viet31 and viet12). Among the nine mtDNA haplotypes, seven mtDNA haplotypes (2006E1, 2006E4, 2006E10, 2007E21, 2007E26, 2007E31 and 2009I3) were isolated from *S. scrofa* wild boars, while four mtDNA haplotypes (2007E21, 2007E31, 2007E53 and 2007E59) were isolated from domestic pigs (Table 1). Some mtDNA haplotypes of wild *S. scrofa* samples from Java and Sumatra Islands clustered with the indigenous wild boar groups in each island (Fig. 2). The mtDNA sequences (2006W6 and 2006W11) of *S. verrucosus* isolated from Java Island were difficult to distinguish from those of *S. scrofa*.

The mtDNA haplotypes of *S. celebensis* from Sulawesi Island formed a unique lineage that differed from the *S. scrofa* lineages in the NJ tree (Fig. 2), although the support value was extremely low (20%). The nucleotide variation observed in 45 mtDNA haplotypes from Sulawesi Island is shown in Table 2. The mtDNA haplotypes of *S. celebensis* were divided into three genetic groups, A, B, and C, with low bootstrap values (Table 2 and Fig. 2). The geographical distribution of mtDNA haplotypes of *S. celebensis* was plotted on a map of the Sulawesi Islands in Fig. 3. The haplotypes in group A were found from northern and south-

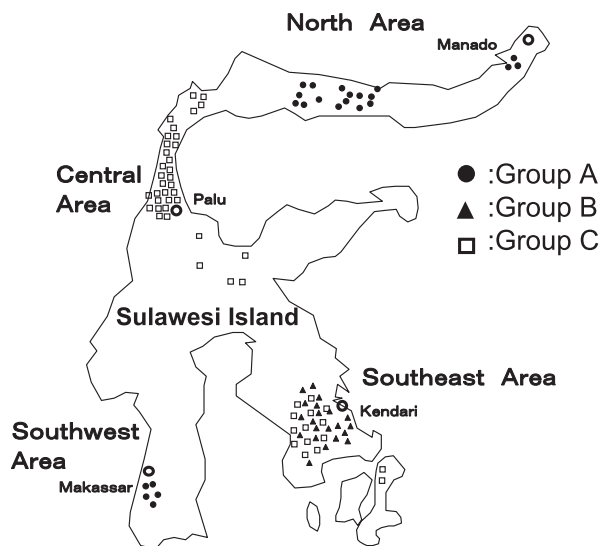


Fig. 3. Geographical distribution of mtDNA haplotypes of *S. celebensis* on Sulawesi Island. Numbers of samples showing different groups are indicated by symbols (●: group A, ▲: group B, and □: group C) in Table 2 and Fig. 2.

western Sulawesi, those in group B were found from southeastern Sulawesi, and those in group C were found from central and southeastern Sulawesi (Fig. 3).

Discussion

In order to explore the genetic origin of domestic pigs, we have investigated the diversity of *S. scrofa* mtDNA sequences isolated from wild boars in Japan, Korea, China and Vietnam. In this study, we analyzed the mtDNA variation of domestic pigs and wild boars from Indonesian Islands. The NJ tree constructed with our data from Indonesian islands revealed that mtDNA haplotypes found in most *S. scrofa* wild boars from Sumatra and Java Islands, *S. verrucosus* from Java Island, and *S. celebensis* from Sulawesi Islands were different from those in the clade encompassing East and Southeast Asian domestic pig and wild boars (Fig. 2). However, the mtDNA haplotypes from domestic pigs on Lombok and Timor Islands were closely related to or within clade comprising East and Southeast Asian domestic pigs and wild boars. These results suggest that major indigenous wild boars in Indonesia were genetically separated from the continental wild boars and domestic pigs, and they did not contribute to Asian *S. scrofa* domestication events.

Seven mtDNA haplotypes (2006E1, 2006E4, 2006E10,

2007E21, 2007E26, 2007E31, and 2009I3) found in wild boars from Java, Sumatra, Sumbawa, Flores, and New Guinea Islands were related to two Vietnamese mtDNA haplotypes (viet12 and viet31). Although the haplotypes were not identical, the close genetic relationship between mtDNA haplotypes from wild boars between Vietnam and Indonesia found in this study is valuable. Based on archeological and genetic evidence, Larson et al. (2007) reported that domestic pigs bearing Vietnamese mtDNA haplotypes were certainly introduced to several islands east of the Wallace Line, including Lombok, Sumbawa, Flores, Timor and New Guinea Islands, and the so-called wild boars isolated from these islands are most likely feral descendants of domestic pigs introduced by early human immigrants. Based on the mtDNA phylogenies of wild boars from Vanuatu of Micronesia, Lum et al. (2006) reported that wild boars with Vietnamese haplotypes had dispersed during the human colonization of Remote Oceania with the Lapita cultural complex. The human-mediated dispersal routes of domestic pigs on several islands of Micronesia and Polynesia are closely associated with the diffusion of Lapita culture in the Pacific region (Larson et al. 2007). Thus, the close genetic relationship among Vietnam, Java and Sumatra Islands, and the five islands east of the Wallace Line observed in this study may also be associated with the early human dispersals or cultural diffusions as proposed by the other studies (Larson et al. 2005, 2007, 2010; Lum et al. 2006).

Two mtDNA haplotypes (2006W6 and 2006W11) found in *S. verrucosus* were not distinct from those from *S. scrofa*, although only two samples from *S. verrucosus* were examined in this study. Two species, *S. scrofa* and *S. verrucosus*, live in close proximity in a small area on Java Island and have some morphological resemblance. It was recently reported that crossbreeding between *S. verrucosus* and *S. scrofa* occurred on Java Island (Semiadi and Nugraha 2009). To clearly distinguish between *S. verrucosus* and *S. scrofa*, further genetic and morphological investigations would be required.

Larson et al. (2005) reported that two mtDNA clades were found on Sulawesi Island; one clade was associated with a northern population, and the other was associated with a southern population. The existence of two clades of *S. celebensis* on the Sulawesi Island led Larson et al. (2005) to speculate that there were two independent invasions of wild boar onto the Sulawesi Islands. In this study, the mtDNA haplotypes of *S. celebensis* were divided into three mtDNA groups A, B, and C (Fig. 2).

Two mtDNA haplotypes, 2009SS1 and 2009SS4, belonging to the northern *S. celebensis* group A were detected in Makasar, located in the southwest (Fig. 3). In addition to group A, the mtDNA haplotypes of *S. celebensis* group C were widely dispersed in the central and southeastern areas of Sulawesi Island, while those of *S. celebensis* group B were only found in the southeastern area. The mtDNA haplotype analysis of *S. celebensis* revealed that *S. celebensis* populations are genetically composed of at least three mtDNA groups, and the geographical distribution is characteristic on Sulawesi Island. In this study, an *S. scrofa* sample with the same mtDNA haplotype (2009K19) as an *S. celebensis* sample was isolated from New Guinea. The ancestor of this mtDNA haplotype might have also been introduced to New Guinea from Sulawesi by ancient peoples. Similarly, Larson et al. (2007) provided evidence for an early human-mediated translocation of *S. celebensis* to Flores and Timor Islands. Taken together, these observations indicate that human-mediated introgression of wild boars or domestic pigs onto neighboring islands was an important factor in establishing the current geographical distribution of *Sus* populations (Gongora et al. 2004).

The phylogenetic study of mtDNA haplotypes of *S. scrofa* and other related species isolated from Indonesian islands revealed that mtDNA sequences of the *S. scrofa* populations from the Indonesian islands are extremely diverse. Molecular analyses of the origin and dispersal routes of domestic *S. scrofa* in and throughout Indonesia provide helpful information for understanding the activities and skills of ancient people, such as the domestication of pigs from wild boar and the subsequent introduction of livestock to neighboring islands. It is likely that the distribution throughout Pacific islands of current indigenous wild boars that are descendants of feral pigs is closely associated with dispersal of the Lapita cultural complex into Oceania (Lum et al. 2006; Larson et al. 2007). Future genetic analysis of indigenous wild boars from each Indonesian Island may provide detailed information about human-mediated introgression or movement of domestic pigs.

Acknowledgment: This study was supported in part by a Grant-in-Aid (No. 18405034) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

- Chen, K., Baxter, T., Muir, W. M., Groenen, M. A. and Schook, L. B. 2007. Genetic resources, genome mapping and evolutionary genomics of the pig (*Sus scrofa*). *International Journal of Biological Sciences* 3: 153–165.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.
- Giuffra, E., Kijas, J. M., Amarger, V., Carlborg, O., Jeon, J. T. and Andersson, L. 2000. The origin of the domestic pig: Independent domestication and subsequent introgression. *Genetics* 154: 1785–1791.
- Gongora, J., Fleming, P., Spencer, P. B. S., Mason, R., Garkavenko, O., Meyer, J-N., Droegemueller, C., Lee, J. H. and Moran, C. 2004. Phylogenetic relationships of Australian and New Zealand feral pigs assessed by mitochondrial control region sequence and nuclear *GPII* genotype. *Molecular Phylogenetics and Evolution* 33: 339–348.
- Grubb, P. 2005. *Artiodactyla*. In (D. E. Wilson and D. M. Reeder, eds.) *Mammal Species of the World: A Taxonomic and Geographic Reference*, 3rd ed., pp. 637–722. John Hopkins University Press, Baltimore, U.S.A.
- Hongo, H., Ishiguro, N., Watanobe, T., Shigehara, N., Anezaki, T., Long, V. T., Binh, D. V., Tien, N. T. and Nam, N. H. 2002. Variation in mitochondrial DNA of Vietnamese pigs: relationships with Asian domestic pigs and Ryukyu wild boars. *Zoological Science* 19: 1329–1335.
- Ishiguro, N. and Nishimura, M. 2005. Genetic profile and serosurvey for virus infections of Japanese wild boars in Shikoku Island. *The Journal of Veterinary Medical Science* 67: 563–568.
- Ishiguro, N., Sasaki, M., Iwasa, M., Shigehara, N., Hongo, H., Anezaki, T., Long, V. T., Hao, P. X., Trach, H. X., Nam, N. H. and Thanh, V. N. 2008a. Morphological and genetic analysis of Vietnamese *Sus scrofa* bones for evidence of pig domestication. *Animal Science Journal* 79: 655–664.
- Ishiguro, N., Sasaki, M., Iwasa, M., Shigehara, N., Hongo, H., Anezaki, T., Long, V. T., Lan, D. T. B. and Long, P. T. 2008b. mtDNA variation in Vietnamese pigs, with particular emphasis on the genetic relationship between wild boars from Vietnam and the Ryukyu Islands. *Mammal Study* 33: 51–58.
- Larson, G., Cucchi, T., Fujita, M., Matisoo-Smith, E., Robins, J., Anderson, A., Rolett, B., Spriggs, M., Dolman, G., Kim, T. H., Thuy, N. T., Randi, E., Doherty, M., Due, R. A., Bollt, R., Djubiantono, T., Griffin, B., Intoh, M., Keane, E., Kirch, P., Li, K. T., Morwood, M., Pedriña, L. M., Piper, P. J., Rabett, R. J., Shooter, P., Van den Bergh, G., West, E., Wickler, S., Yuan, J., Cooper, A. and Dobney, K. 2007. Phylogeny and ancient DNA of *Sus* provides insights into neolithic expansion in Island Southeast Asia and Oceania. *Proceedings of the National Academy of Sciences of the United States of America* 104: 4834–4839.
- Larson, G., Dobney, K., Albarella, U., Fang, M., Matisoo-Smith, E., Robins, J., Lowden, S., Finlayson, H., Brand, T., Willerslev, E., Rowley-Conwy, P., Andersson, L. and Cooper, A. 2005. Worldwide phylogeography of wild boar reveals multiple centers of pig domestication. *Science* 307: 1618–1621.
- Larson, G., Liu, R., Zhao, X., Zhu, X., Yuan, J., Fuller, D., Barton, L., Dobney, K., Fan, Q., Gu, Z., Liu, X., Luo, Y., Lv, P., Andersson, L. and Li, N. 2010. Patterns of east Asian pig domestication, migration, and turnover revealed by modern and ancient DNA. *Proceedings of the National Academy of Sciences of the United States of America* 107: 7686–7691.
- Lum, J. K., McIntyre, J. K., Greger, D. L., Huffman, K. W. and Vilar, M. G. 2006. Recent Southeast Asian domestication and Lapita

- dispersal of sacred male pseudohermaphroditic “tuskers” and hairless pigs of Vanuatu. *Proceedings of the National Academy of Sciences of the United States of America* 103: 17190–17195.
- Okumura, N., Kurosawa, Y., Kobayashi, E., Watanobe, T., Ishiguro, N., Yasue, H. and Mitsuhashi, T. 2001. Genetic relationship amongst the major non-coding regions of mitochondrial DNAs in wild boars and several breeds of domesticated pigs. *Animal Genetics* 32: 139–147.
- Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 406–425.
- Semiadi, G. and Nugraha, R. T. P. 2009. Some notes on biological aspects of captive javan warty pig (*Sus verrucosus*). *Biodiversitas* 10: 124–128.
- Tamura, K., Dudley, J., Nei, M. and Kumar, S. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24: 1596–1599.
- Thompson, J. D., Higgins, D. G. and Gibson, T. J. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673–4680.
- Watanabe, T., Hayashi, Y., Ogasawara, N. and Tomita, T. 1985. Polymorphism of mitochondrial DNA in pigs based on restriction endonuclease cleavage patterns. *Biochemical Genetics* 23: 105–113.
- Watanobe, T., Ishiguro, N. and Nakano, M. 2003. Phylogeography and population structure of the Japanese wild boar *Sus scrofa leucomystax*: mitochondrial DNA variation. *Zoological Science* 20: 1477–1489.

Received 25 December 2010. Accepted 17 August 2011.