

Insular East Asia pig dispersal and vicariance inferred from Asian wild boar genetic evidence¹

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ABSTRACT: The Formosan wild boar (*Sus scrofa taiwanus*) is an endemic subspecies in Taiwan. Understanding the origins and spread of the Formosan wild boar could help clarify East Asian wild boar dispersal. Although in situ domestication of the wild boar occurred at a number of domestication centers across East Asia, corroborating archaeological and genetic evidence of pig domestication on Taiwan is lacking, leading to domestication being described as cryptic. This characterization applies to the Lanyu pig—a domestic pig breed found on Taiwan. To better understand pig domestication, this study examines the sympatric Formosan wild boar and domestic Lanyu pig to build a model of potential wild boar domestication on Taiwan and elucidate wild boar domestication patterns in the region. To this end, a comprehensive phylogenetic study of the Formosan wild boar and the Lanyu pig was conducted on animals sourced from Taiwan, Lanyu, and the Philippines. Phylogenetic analyses were conducted using full mitochondrial control-region sequences from 345 wild boars and domestic pigs. These were studied in concert with existing reports on 206 Asian wild boars. Genetic characteristics and Bayesian phylogenetic tree

results identified 2 wild boar lineages of remote phylogenetic relationship. These were Formosan wild boar lineage (FWBL) and Formosan wild boar with Lanyu sign lineage (FWBLYL). Molecular clock analyses indicate that FWBLYL diverged earlier than other insular East Asia wild boars and show that FWBLYL and FWBL diverged approximately 0.60 million years ago. This result supports boars of FWBLYL being the earliest wild boars to have spread and become isolated in insular East Asia. In addition, the study proposes 6 Asian wild boar dispersion routes during glacial periods. At least 3 of these events occurred in insular East Asia with subsequent geographical isolation after glacial recession. This isolation potentially led to allopatric differentiation of wild boar subspecies. Also, the similar genetic signature and phylogenetic uniqueness of Lanyu pigs to wild boars of FWBLYL suggests such wild boars were the wild ancestor of domestic Lanyu pigs. This result indicates potential in situ domestication occurring on Taiwan. Finally, pigs possessing FWBLYL's genetic signatures were continuously distributed among Taiwan, Lanyu, and the Philippines. This pattern may signify human-mediated pig dispersal routes.

Key words: Asian wild boar, Formosan wild boar, Lanyu pig, mitochondrial DNA, phylogeography

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doi:10.2527/jas2016.1117

¹This work was supported by grants from the Ministry of Science and Technology of Taiwan (NSC 98-2313-B-002-023 and MOST 103-2923-B-002-006-MY3), Yushan National Park of Taiwan (98-1185), the Council of Agriculture of Taiwan, Republic of China (105AS-2.7.6-AD-U1 and 105AS-2.1.4-AD-U1), and the Department of Science and

Technology of the Republic of the Philippines (PRP #2093). We thank Daniel Flynn of Planet Editing for editing the manuscript.

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Received October 11, 2016.

Accepted December 11, 2016.

INTRODUCTION

Taiwan is a centrally located island among the western Pacific's island arcs. It is home to the Formosan wild boar (*Sus scrofa taiwanus*; Grubb, 2005). Recent phylogenetic studies reveal contradictory evidence of East Asian wild boar dispersion routes throughout the region. This evidence is based on polymorphism of partial mitochondrial DNA (mtDNA) sequences (Watanobe et al., 1999, 2003; Cho et al., 2009). Given Taiwan's geographical location, studying the origin and spread of the Formosan wild boar should provide insight into the dispersion routes of the East Asian wild boar.

Furthermore, although archaeological and genealogical evidence supports pig domestication events having occurred independently at a number of sites in East Asia (Larson et al., 2005, 2010; Wu et al., 2007b; Yang et al., 2011; Jin et al., 2012), in many regions, such evidence is lacking and the dispersion and domestication of the East Asian wild boar is unclear. Besides the Formosan wild boar, Taiwan is home to a domestic pig breed called the Lanyu pig. This breed has remote genetic distance to Asian and European pig breeds (Wu et al., 2007a). There is no archaeological or genealogical evidence of Lanyu pig domestication, and the wild ancestor of the Lanyu pig has yet to be discovered or may already be extinct. It is among a number of pig breeds whose domestication is described as cryptic (Larson et al., 2010). To help provide a model of in situ Lanyu pig domestication and East Asian wild boar dispersal, this paper studies the phylogenetic relationship among the sympatric Formosan wild boar and Lanyu pig as well as the East Asian wild boar.

To this end, this paper comprehensively examines 1) genetic characteristics and differentiation of the Formosan wild boar, 2) phylogeny and divergence times among the East Asian wild boar, and 3) the Lanyu pig's wild ancestry. The study should elucidate aspects of pig domestication on Taiwan and offer greater insight into East Asian wild boar dispersion patterns.

MATERIALS AND METHODS

Taiwan's Geography

Taiwan is described by wide plains in the west and rugged forest-covered mountains in the east. There are 5 mountain ranges. The predominant mountain range is the Central Mountain Range, which runs from Suao in the northeast to Eluanbi at the southern tip of the island. It extends 330 km from north to south and approximately 80 km from east to west. Furthermore, there are 268 peaks over 3,000 m above sea level in Taiwan. This forest-clad mountain range is characterized by deep gorges and sharp valleys.

Sample Collection and Genomic DNA Extraction

Individual samples from 278 Formosan wild boars (Fig. 1a) were collected at 6 different mountain areas (north to south) designated populations A to F throughout Taiwan based on home range and activity patterns of the wild boar (Singer et al., 1981; Boitani et al., 1994). High genetic differentiation among the 6 populations was confirmed by an analysis of molecular variance (AMOVA) test. For sampling, the Central Mountain Range was divided into 5 areas as follows: the north part (Population A), north-central part (Population B), central part (Population C), south-central part (Population D), and south part (Population E). Populations A, C, and D also include mountain areas of the Xue Mountain Range, Yushan Mountain Range, and Coastal Mountain Range, respectively (Table 1). In addition, Population F is located in the mountain area of Hengchun Peninsula. All geographic positing information was recorded by a global positioning system receiver (Fig. 1c). Furthermore, 57 Lanyu pigs (Fig. 1b) were obtained. Twenty Lanyu pigs were obtained from the Taitung Animal Propagation Station in Taitung, Taiwan. Control-region sequences of 28 individuals from the Taitung Animal Propagation Station, 5 individuals from National Taiwan University (Taipei, Taiwan), and 4 individuals from Lanyu Islet previously described as Type I Lanyu pigs were also used in this study (Wu et al., 2007a; Jiang et al., 2008). Ten Philippine native pigs were collected from the Batan Archipelago and Luzon Island in the Philippines. All sampling protocols were reviewed and approved by the Institutional Animal Care and Use Committee of National Taiwan University (NTU-102-EL-33). Genomic DNA was extracted and purified from whole blood and muscle tissue using Qiagen's QIAamp DNA Blood Maxi Kit (Qiagen Inc., Valencia, CA) and DNeasy Blood and Tissue Kit (Qiagen Inc.). The procedure for genomic DNA isolation from feces samples followed the protocols described by Zhang et al. (2006). Genomic DNA extraction from teeth or bones followed the method described by Kalmár et al. (2000).

Primer Design, Amplification of Full Mitochondrial DNA Control Regions, and Sequencing

Amplification of the full mtDNA control-region (D-loop) sequence (1,044 bp) was divided into 3 overlapping fragments, and PCR was performed using a PTC-200 DNA Engine thermal cycler (MJ Research, Inc., Waltham, MA). According to the pig mtDNA sequence (GenBank accession number EF375877), the following primer sets were designed and amplified using PCR: D-loop L1, 5'-CCAAGACTCAAGGAAGGAGA-3' (position 16428–16447); D-loop H1, 5'-GGTCCTGAAGTAAGAACCAG-3' (position 425–

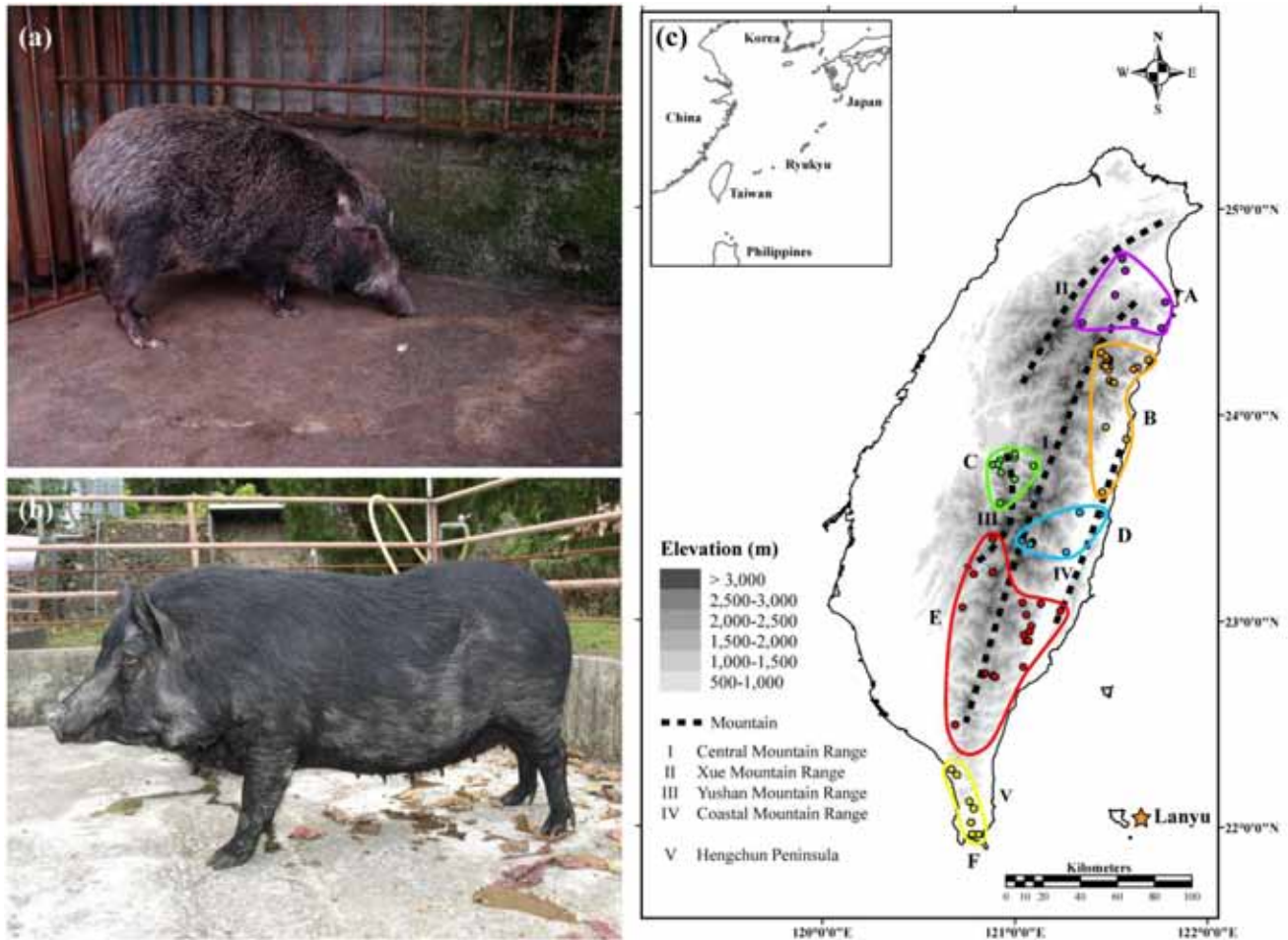


Figure 1. Photos and sampling localities of pigs: (a) the Formosan wild boar, (b) the Lanyu pig, and (c) the sampling localities of 278 Formosan wild boars in Taiwan. Six different populations are designated populations A to F with different colors across mountain areas from north to south in Taiwan. Bold dotted lines show the major mountain ranges in Taiwan. The orange star indicates Lanyu Islet. The inset in the upper left corner indicates the location of Taiwan relative to East Asia.

444); D-loop L2, 5'-TGATCGTACATAGCACAT-3' (position 246–263); D-loop H2, 5'-TTATGTC CYGTA ACCATTGACTG-3' (position 677–699); D-loop L3, 5'-CGCGCATATAAGCAGGTAAA-3' (position 710–729); and D-loop H3, 5'-TGTGTTTATGGGGCTGTGAG-3' (position 1115–1134). The D-loop region divides into fragments A, B, and C. Fragments A (516 bp), B (454 bp), and C (425 bp) were amplified in PCR for different primer pairs D-loop L1–H1, D-loop L2–H2, and primer D-loop L3–H3 (Supplemental Table S1; see the online version of the article at <http://journalofanimalscience.org>). In addition, primers D-loop L1 and D-loop H4, 5'-GGCGCGGATACTTGCATGTG-3' (position 1177–1196), were used for amplifying the full-length control region (Supplemental Table S1; see the online version of the article at <http://journalofanimalscience.org>). Polymerase chain reaction was performed in 50- μ L volumes using the Advantage 2 PCR enzyme system (Clontech Laboratories, Inc., Mountain View,

CA), each reaction containing 100 ng of genomic DNA, 10 mM Tris-HCl pH 8.5, 2 mM MgCl₂, 0.4 μ M of each primer, 200 μ M of each deoxyribonucleotide triphosphate, and 1 μ L 50X Advantage 2 polymerase mix. Polymerase chain reaction performance conditions for amplifying the fragments of control regions were 94°C for 5 min, 35 cycles of 94°C for 30 s, 55°C for 30 s, and 68°C for 35 s with a final extension at 68°C for 10 min. Polymerase chain reaction performance conditions for amplifying the full-length control region were 94°C for 5 min, 35 cycles of 94°C for 30 s, 63°C for 30 s, and 68°C for 80 s with a final extension at 68°C for 10 min (Supplemental Table S1; see the online version of the article at <http://journalofanimalscience.org>). All the sequences were bidirectionally sequenced by PCR primers using an Applied Biosystems 3730 DNA sequencer and analyzed with SeqEd software (Applied Biosystems, Inc., Foster City, CA). Each control-region sequence was generated by overlapping forward and reverse sequences using EditSeq software (DNASTAR, Inc., Madison, WI).

Table 1. Sample localities, sample size (*n*), number of haplotypes (Nh) and polymorphic sites (Ph), number of private haplotypes (Np) and private polymorphic sites (Pp), haplotype diversity (*h*), nucleotide diversity (π), and neutrality tests among populations of the Formosan wild boar

Lineage ¹	Population/ region	Sample localities	<i>n</i>	Nh	Np	Ph	Pp	<i>h</i>	π	Tajima's <i>D</i>	Fu's <i>F_S</i>
FWBL	Northern-Central Region		108	18	14	17	7	0.903 ± 0.017	0.00303 ± 0.00012	-0.05896	-3.55916
	Population A	North part of Central Mountain Range and Xue Mountain Range	39	8	4	9	3	0.861 ± 0.025	0.00281 ± 0.00021	1.10359	0.50975
		Population B	North-central part of Central Mountain Range	37	9	3	9	0	0.778 ± 0.057	0.00256 ± 0.00019	0.71174
	Population C	Central part of Central Mountain Range and Yushan Mountain Range	32	7	5	8	3	0.835 ± 0.032	0.00287 ± 0.00024	1.52413	0.99277
		Central-Southern Region		97	15	10	13	2	0.900 ± 0.013	0.00303 ± 0.00009	0.68481
	Population D	South-central part of Central Mountain Range and Coastal Mountain Range	30	7	4	10	1	0.777 ± 0.050	0.00256 ± 0.00040	0.19029	0.48126
		Population E	South part of Central Mountain Range	67	9	6	10	1	0.846 ± 0.024	0.00267 ± 0.00011	0.90475
	Hengchun Peninsula Region		44	4	3	4	3	0.663 ± 0.039	0.00191 ± 0.00006	2.72997	3.04976
	Population F	Hengchun Peninsula	44	4	3	4	3	0.663 ± 0.039	0.00191 ± 0.00006	2.72997	3.04976
	Total	–	249	32	–	23	–	0.951 ± 0.004	0.00356 ± 0.00008	-0.03916	-10.22580*
FWBLYL Total	Central Mountain Range and Yushan Mountain Range	29	4	–	5	–	0.200 ± 0.098	0.00057 ± 0.00029	–	–	

¹FWBL = Formosan wild boar lineage; FWBLYL = Formosan wild boar with Lanyu sign lineage.

**P* < 0.05.

Genetic Characteristics and Diversities

The full length control-region sequences of 278 Formosan wild boars, 57 Lanyu pigs, and 10 Philippine native pigs were obtained, and then these sequences were edited until 1 common highly variable tandem repeat (5'-CGTGCCTACA-3') remained. After editing the sequences, complete control-region sequences were deposited into National Center for Biotechnology Information GenBank (KP987268–KP987307 and KT895077; Supplemental Table S2 [see the online version of the article at <http://journalofanimalscience.org>]). Sequence alignment was performed by MegAlign software (DNASTAR, Inc.) for clarifying genetic characteristics among these pigs. The Type I Lanyu diagnostic motif (5'-ACACAAACC-3') was edited until there remained 1 repeat for further phylogenetic analysis (Wu et al., 2007a). Haplotype diversity (*h*) and nucleotide diversity (π) were calculated to examine genetic diversity within each population using DNA Sequence Polymorphism (DnaSP) software version 5.10 (Librado and Rozas, 2009). In addition, neutrality tests, including Tajima's *D* and Fu's *F_S*, were implemented in Arlequin software version 3.5.1.2 (Excoffier et al., 2005) for each population.

Population Differentiation and Haplotype Phylogeny

A hierarchical AMOVA test among and within regions and localities was performed by Arlequin software version 3.5.1.2 based on the nucleotide substitutions between haplotypes (Excoffier et al., 1992, 2005). This procedure was executed to search for a best-fit grouping pattern via maximization of the proportion of genetic variance among groups. This analysis calculated the standard variance components of each hierarchic characteristic at 3 levels: among groups defined a priori, among populations within groups, and among localities within populations (Excoffier et al., 1992). Fixation index (Φ) statistics of population genetics were calculated, and the significance of the variance components and Φ -statistics were tested using the 10,000 random-permutation procedure. Φ_{CT} is the difference among the groups of total haplotypes, Φ_{SC} is the difference among populations within groups, and Φ_{ST} is the difference among localities within populations (Excoffier et al., 1992). In addition, haplotype network cladograms were executed with 95% parsimonious connections using the TCS version 1.21 software to indicate haplotype phylogeny (Clement et al., 2000).

Bayesian Phylogenetic Tree and Molecular Clock Analysis

Two data sets were constructed and analyzed under phylogenetic tree analysis. The first data set contained partial control-region sequences (652 bp) of 552 Asian wild boars, Lanyu pigs, and Philippine native pigs. All sequences were cut into the same fragment. The second data set contained full control-region sequences (1,044 bp) of the 41 pig haplotypes in this study and 60 wild boar haplotypes in insular East Asia and the Korean Peninsula (Supplemental Table S2; see the online version of the article at <http://journalofanimalscience.org>). jModelTest version 2.1.5 software (Darriba et al., 2012) was used to select a model of best fit for nucleotide substitution under the Bayesian information criterion. For both data sets, a Bayesian phylogenetic inference tree was constructed using a Hasegawa–Kishino–Yano model of nucleotide substitution with gamma-distributed rate variation among sites and a proportion of invariant sites identified by jModelTest software. In addition, the Bayesian Markov chain Monte Carlo (MCMC) method was performed for Bayesian evolutionary analysis by sampling trees (BEAST) software package version 2.3.0 (Bouckaert et al., 2014). Under Bayesian inference analysis, 4 MCMC chains were implemented for 30,000,000 generations and sampled every 1,000 generations. The first 10% of tree samples was omitted as burn-in, and the consensus tree was generated in a maximum clade credibility tree in TreeAnnotator version 2.3.0 (Bouckaert et al., 2014). Divergence times among wild boars in insular East Asia and the Korean Peninsula were estimated in BEAST version 2.3.1 software based on fossil calibration. The analysis applied a Bayesian random local clock model and a calibrated Yule tree prior for relaxed phylogenetics and divergence-time estimations (Drummond and Suchard, 2010; Heled and Drummond, 2012). According to Frantz et al. (2013), the divergence time between *Sus verrucosus* and *Sus cebifrons* was at 2.8 million yr ago (MYA) based on fossil calibration and whole-genome sequence analyses. We specified a normal prior as 2.8 MYA on the node of *S. verrucosus* and *S. cebifrons* for divergence-time estimation. All priors were set to default value, estimated, and specified. The values of effective sample size (>100) for all parameter estimates were assessed to confirm the convergence of MCMC runs in Tracer version 1.6 (<http://tree.bio.ed.ac.uk/software/tracer/>; Accessed 11 December 2013). The Bayesian consensus tree was edited using FigTree software version 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>; Accessed 9 July 2014).

RESULTS

Genetic Characteristics and Diversities of the Formosan Wild Boar

To understand the genetic characteristics of control-region sequences and diversity among the Formosan wild boar, full length control-region sequences from 278 Formosan wild boars were obtained and aligned. Thirty-two haplotypes (FWB01–FWB32) sharing 23 polymorphic sites including 22 transition sites and 1 transversion site were identified (Supplemental Table S3; see the online version of the article at <http://journalofanimalscience.org>). Interestingly, 29 individuals of 4 wild-boar haplotypes (FWBLY01–FWBLY04) were obtained from the Central Mountain Range and Yushan Mountain Range. Their control-region sequences possessed 6 transitions (at positions 90, 279, 302, 535, 575, and 657) and 1 transversion (at position 741). These results were different from those of 32 FWB haplotypes. Wu et al. (2007a) found 6 unique substitution sites (at positions 302, 391, 535, 542, 657, and 741) as indicators of Type I Lanyu pig (here after referred to as Type I Lanyu pig sign). Type I Lanyu pig is a domestic miniature pig. It has a control-region sequence indicating maternal genetic lineage distinct from Asian and European pigs (Wu et al., 2007a). Furthermore, after aligning 3 haplotypes (LY01–LY03) from 57 Lanyu pig individuals, the genetic characteristics of the control-region sequences of 29 Formosan wild boars were very similar to those of Type I Lanyu pigs. Significantly, these wild boars possessed 3, 4, or 6 repeated “ACACAAACC” motifs (Supplemental Table S4; see the online version of the article at <http://journalofanimalscience.org>). This motif was identified as a diagnostic motif of Type I Lanyu pigs (Wu et al., 2007a). Notably, the FWBLY04 haplotype shared a haplotype identical to Type I Lanyu. To emphasize genetic discrimination, FWB01 through FWB32 haplotypes of 249 Formosan wild boars were used to denominate Formosan wild boar lineage (FWBL), and FWBLY01 through FWBLY04 haplotypes of 29 Formosan wild boars possessing Type I Lanyu pig sign and repeating motifs were used to denominate the Formosan wild boar with Lanyu sign lineage (FWBLYL). Additionally, the 57 Lanyu pigs can be divided into 3 haplotypes (LY01–LY03) using a transition site (adenine in LY02 at position 294) and distinct Type I Lanyu pig diagnostic motif repeats (Supplemental Tables S3 and S4; see the online version of the article at <http://journalofanimalscience.org>).

To clarify the geographical distribution of Lanyu pigs, pig samples from Taiwan, Lanyu Islet, and the Philippines were collected. The control-region sequences of Philippine native pigs were obtained and

aligned to FWBL and FWBLYL. Two haplotypes twice possessing Type I Lanyu diagnostic motif repeats were obtained from 10 Philippine native pigs. These were designated as haplotype PHL01 through PHL02. Consequently, all FWBLY, LY, and PHL haplotypes similarly harbored Type I Lanyu pig sign (Supplemental Tables S3 and S4; see the online version of the article at <http://journalofanimalscience.org>). These genetic characteristics are strong evidence of FWBLY, LY, and PHL haplotypes being of the same genealogy (FWBLYL). Significantly, FWBLY haplotypes shared similar substitution patterns and diagnostic motif repeats corresponding to domestic Type I Lanyu pigs.

To understand the genetic diversities of the Formosan wild boar, 6 different sampling populations from 3 regions were independently analyzed (Fig. 1c). A summary of genetic diversities and neutrality tests based on the polymorphism of control-region sequences of variable populations is presented in Table 1. The h and π values of total FWBL were 0.951 and 0.00356, respectively (Table 1). In FWBL populations, the value of h ranged between 0.663 (Population F) and 0.861 (Population A) and the value of π ranged between 0.00191 (Population F) and 0.00287 (Population C). All populations showed high h values (>0.5) and low π values (<0.005). The number of haplotypes was 4 to 9 and the number of polymorphic sites was 4 to 10 in populations A to F. In addition, the number of private haplotypes ranged between 3 and 6 and the number of private polymorphic sites ranged between 0 and 3. The fewest haplotypes were in Population F. However, Population F contained a relatively large sample size compared with populations A, B, C, and D. In addition, both populations B and E contained the most haplotypes, but Population E contained over 30 individuals more than Population B (Table 1). The result indicated that the sample size was not necessarily relevant to the number of haplotypes. Among the populations, Population E possessed not only the largest number of haplotypes and polymorphic sites but also the largest number of private haplotypes. On the other hand, the h and π values in FWBLY were 0.200 and 0.00057, respectively (Table 1). Neutrality tests of the mtDNA control region were also executed for inferring demographic history. Tajima's D and Fu's F_s tests showed positive values in most populations of the Formosan wild boar without reaching a statistically significant level. However, the entire FWBL population resulted in a significantly negative value for Fu's F_s test ($P < 0.05$; Table 1). This result indicates that the entire FWBL population underwent population expansion.

Population Differentiation and Haplotype Phylogeny of Formosan Wild Boar Lineage

Genetic characteristics of the Formosan wild boar show 2 different lineages, FWBL and FWBLYL. To reveal population differentiation among FWBL, an AMOVA test was applied to search for a best-fit grouping pattern for FWBL by maximizing the proportion of genetic variance among groups. Simulated groupings for 2 to 4 regions were analyzed, and the results are summarized in Table 2. The fixation indices among individuals within populations (Φ_{ST}) ranged between 0.24700 and 0.46216, and all Φ_{ST} values presented highly significant genetic differentiation ($P < 0.0001$). Furthermore, the fixation indices among populations within groups (Φ_{SC}) ranged between 0.19338 and 0.35650. Highly significant population differentiation was also obvious within groups ($P < 0.0001$). However, the only significant fixation index among groups (Φ_{CT}) occurred in the 3-region analysis (0.20219; $P < 0.05$; Table 2). The 3 regions are denominated as the Northern-Central Region (NCR), the Central-Southern Region (CSR), and the Hengchun Peninsula Region (HPR). The NCR consists of populations A, B, and C; the CSR consists of populations D and E; and the HPR consists of Population F. The results revealed that populations within the same region were relatively genetically homologous whereas populations among other regions possessed relatively high genetic differentiation. In addition, the NCR and CSR had comparatively high haplotype diversities (>0.900) and nucleotide diversities ($>0.3\%$) compared with the HPR. Furthermore, the NCR and CSR also possessed more haplotypes, polymorphic sites, and private haplotypes compared with the HPR (Table 1). The data indicate the NCR and CSR possess higher genetic diversity than the HPR. In addition, a haplotype network cladogram depicting the genealogical relationship of 32 FWBL haplotypes is shown in Supplemental Fig. S1a (see the online version of the article at <http://journalofanimalscience.org>). Haplotypes FWB08, FWB24, and FWB31 show star-like phylogeny, indicating population expansions (Supplemental Fig. S1a; see the online version of the article at <http://journalofanimalscience.org>).

Phylogenetic Relationships among Formosan Wild Boar Lineage, Formosan Wild Boar with Lanyu Sign Lineage, and Asian Wild Boars

The particular evolutionary history and dispersal routes of the Asian wild boar toward Taiwan, resulting in the 2 lineages of the Formosan wild boar (FWBL and FWBLYL), need clarification. If the phylogenetic study is comprehensive and properly conducted, the evolutionary role of FWBL and FWBLYL among Asian wild boars should be confirmed. Owing to the short-

Table 2. Analyses of groupings, percentage variance (%), and fixation indices (Φ) determined by analyses of molecular variance among boars of Formosan wild boar lineage

Analyses of grouping ¹	Source of variation	%	Φ^2	
2-region analysis				
Region 1 (Population A) and region 2 (populations B, C, D, E, and F)	Among groups	-17.02	$\Phi_{CT} = -0.17016$	NS
	Among populations within groups	41.72	$\Phi_{SC} = 0.35650$	***
	Among individuals within populations	75.3	$\Phi_{ST} = 0.24700$	***
Region 1 (populations A, B, and C) and region 2 (populations D, E, and F)	Among groups	5.75	$\Phi_{CT} = 0.05746$	NS
	Among populations within groups	28.29	$\Phi_{SC} = 0.30012$	***
	Among individuals within populations	65.97	$\Phi_{ST} = 0.34034$	***
Region 1 (populations A, B, C, D, E) and region 2 (Population F)	Among groups	31.56	$\Phi_{CT} = 0.31556$	NS
	Among populations within groups	14.66	$\Phi_{SC} = 0.21419$	***
	Among individuals within populations	53.78	$\Phi_{ST} = 0.46216$	***
3-region analysis				
Region 1 (Population A), region 2 (populations B and C), and region 3 (populations D, E, and F)	Among groups	-4.08	$\Phi_{CT} = -0.04076$	NS
	Among populations within groups	35.75	$\Phi_{SC} = 0.34349$	***
	Among individuals within populations	68.33	$\Phi_{ST} = 0.31673$	***
Region 1 (Population A), region 2 (populations B, C, D, and E), and region 3 (Population F)	Among groups	15.92	$\Phi_{CT} = 0.15919$	NS
	Among populations within groups	20.75	$\Phi_{SC} = 0.24678$	***
	Among individuals within populations	63.33	$\Phi_{ST} = 0.36669$	***
Region 1 (populations A, B, and C), region 2 (populations D and E), and region 3 (Population F)	Among groups	20.22	$\Phi_{CT} = 0.20219$	*
	Among populations within groups	15.43	$\Phi_{SC} = 0.19338$	***
	Among individuals within populations	64.35	$\Phi_{ST} = 0.35647$	***
4-region analysis				
Region 1 (Population A), region 2 (populations B and C), region 3 (populations D and E), and region 4 (Population F)	Among groups	13.75	$\Phi_{CT} = 0.13746$	NS
	Among populations within groups	19.9	$\Phi_{SC} = 0.23076$	***
	Among individuals within populations	66.35	$\Phi_{ST} = 0.33650$	***

¹Populations correspond to Table 1.

² Φ_{CT} = the difference among the groups of total haplotypes; Φ_{SC} = the difference among populations within groups; Φ_{ST} = the difference among localities within populations; NS = not significant.

* $P < 0.05$; *** $P < 0.0001$.

ness of control-region sequences (652 bp) located at the 5' end before tandem repeats in most Asian wild boars (Larson et al., 2005), the control-region sequences of all pigs were edited to an identical length of 652 bp to perform phylogenetic analysis. In total, 552 Asian wild boars, Lanyu pigs, and Philippine native pigs were used to construct a Bayesian phylogenetic tree. A phylogenetic tree giving the geographical distribution of each Asian wild boar is presented in Fig. 2. First, wild boars in Island Southeast Asia (ISEA) formed an ancestral clade, whereas remaining Asian wild boars formed another descendant clade. South Asian and West Asian wild boars first split off the descendant clade. The remainder of the tree is divided into 2 major clades separating GEAWB clade from a clade that includes FWBLYL, AWB1, and AWB2. Surprisingly, in this later clade, FWBLYL (which includes FWBLY, LY, and PHLY) is distinct from all other Asian wild boars. The AWB1 and AWB2 clades consist of variable Asian wild boars and cluster together. The AWB1 clade contains the Ryukyu wild boar and the China wild boar. Wild boars in southwest China (Yunnan and Sichuan provinces) mix with Southeast Asian wild boars diverging in the AWB2 clade. Following this pro-

cess, Korean wild boar cluster with some wild boars in Myanmar (Fig. 2). Finally, the GEAWB clade consists of all remaining general East Asian wild boars throughout East Asia, including Taiwan, Japan, Korea, and China. Notably, FWBLYL split off earlier than the succeeding Ryukyu wild boar and Korean wild boar and all the other general East Asian wild boars (Fig. 2). Most importantly, these data indicate FWBLYL being the earliest wild boar that dispersed to insular East Asia.

Next, to further confirm the genealogical role and divergence time of the Formosan wild boar in East Asia, we focused on the wild boars of insular East Asia and the Korean Peninsula. Full length control-region sequences were used to increase the resolving power during Bayesian phylogenetic consensus tree analysis. Bayesian tree topology for East Asian wild boars is similar to that of Asian wild boars (Fig. 2 and 3). All FWBLY, LY, and PHLY haplotypes independently cluster in the FWBLYL clade. Additionally, they were the first to split from other East Asian wild boars including FWBL. Korean wild boars individually formed 3 major clades, termed the KWB1, KWB2, and KWB3 clades. Ryukyu wild boars clustered with

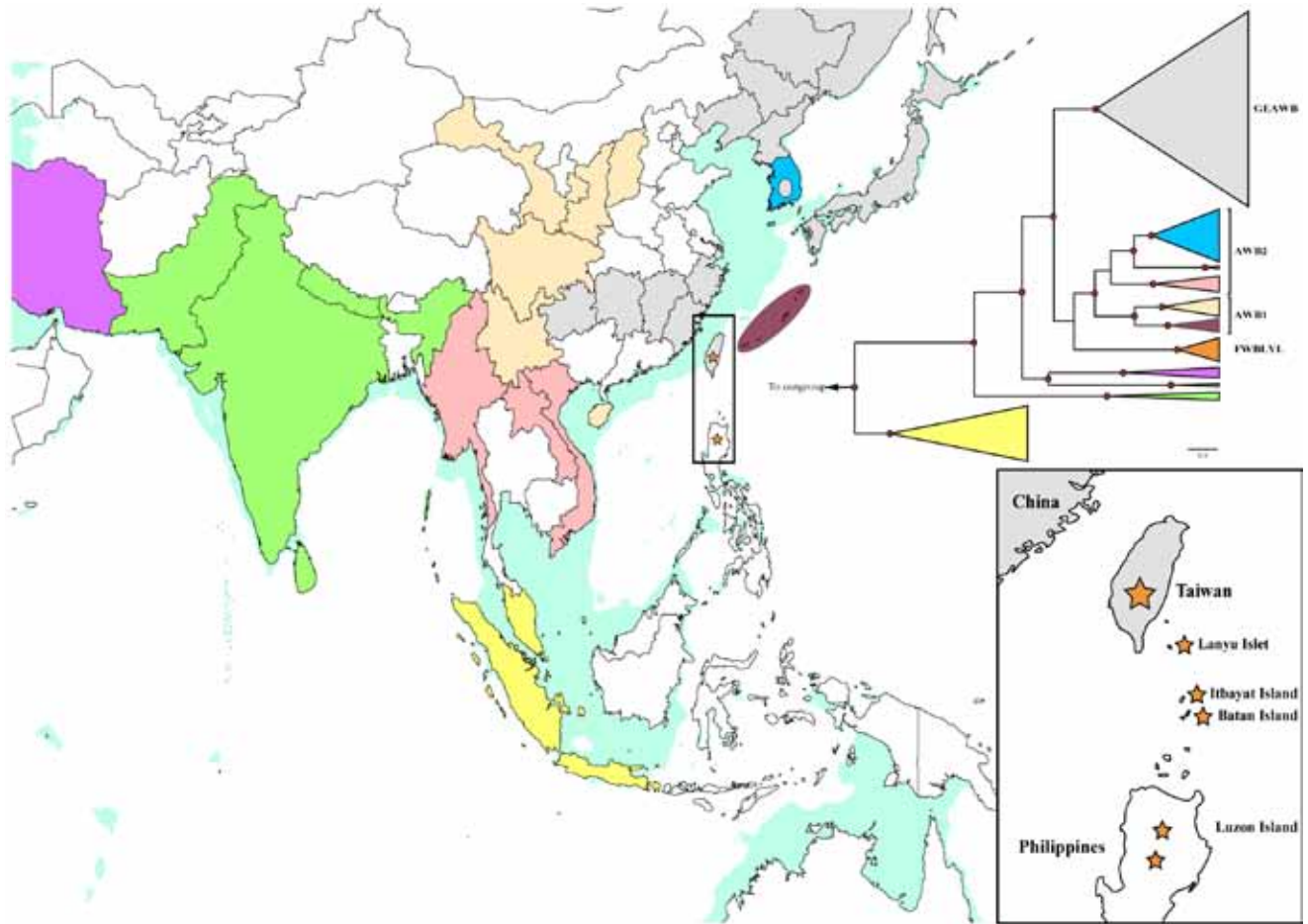


Figure 2. A map of Asia with country and Chinese provincial boundaries, and the Bayesian consensus tree of 552 Asian wild boars, Lanyu pigs, and Philippine native pigs showing phylogenetic relationships among clades in the region. Each clade is represented by a specific color corresponding to its region on the map of Asia. Abbreviations of clades are as follows: GEAWB = general East Asian wild boar; AWB = Asian wild boar; FWBLYL = Formosan wild boar with Lanyu sign lineage. Light blue regions depict depth contours at 130 m below present sea level. These areas could have provided land bridges during glacial periods (Fleming et al., 1998). Color nodes on the phylogenetic tree show posterior probability larger than 0.50. Additional detailed information on haplotypes and posterior probabilities is given in Supplemental Fig. S2 (see the online version of the article at <http://journalofanimalscience.org>). The bold frame at the bottom right corner shows a detailed map of Taiwan, Lanyu Islet, and the Philippines. The orange stars show the sampling sites of pigs of FWBLYL found in Taiwan, Lanyu Islet, and the Philippines.

the KWB1 and KWB2 clades. The FWBL clade cluster with JWB2, JWB1, and KWB3 clades (Fig. 3). Genetic data supporting the maternal lineage of FWBL boars reflects lineage closer to the Japanese wild boar than to the Korean wild boar and Ryukyu wild boar. The divergence times of each clade were estimated to understand the evolutionary history of the East Asian wild boar. The result showed FWBLYL and FWBL diverged at approximately 0.60 MYA (95% highest posterior density [HPD] of 0.36–0.85). The divergence of all East Asian wild boars except FWBLYL was at 0.48 MYA (95% HPD of 0.28–0.70). Divergence time of KWB3, JWB1, JWB2, and FWBL was at 0.31 MYA (95% HPD of 0.17–0.48; Fig. 3). Interestingly, 3 (KWB1, KWB2, and KWB3) and 2 (JWB and RWB) evidently different lineages of wild boars were also identified on the Korean Peninsula and Japan (including the Ryukyu Arc), respectively.

Phylogeny of the Formosan Wild Boar with Lanyu Sign, Lanyu Pigs, and Philippine Native Pigs

In past glacial periods, the Philippine Archipelago was never connected to the Asian continent; hence, *S. scrofa* was unable to reach the Philippine Archipelago (Oliver, 1995; Groves, 1997; Voris, 2000). That *S. scrofa* exists in the Philippines today means it must have anthropogenic origins, likely arriving with some human migration event. In this study, Philippine native pigs were obtained from the Batan Archipelago and Luzon Island and were subjected to parsimony network analysis to depict phylogeny among FWBLY, LY, and PHLY haplotypes (Supplemental Fig. S1b; see the online version of the article at <http://journalofanimalscience.org>). Haplotype network analyses illustrate extremely close phylogeny among FWBLY, LY, and PHLY. Significantly, wild boars of the FWBLY04

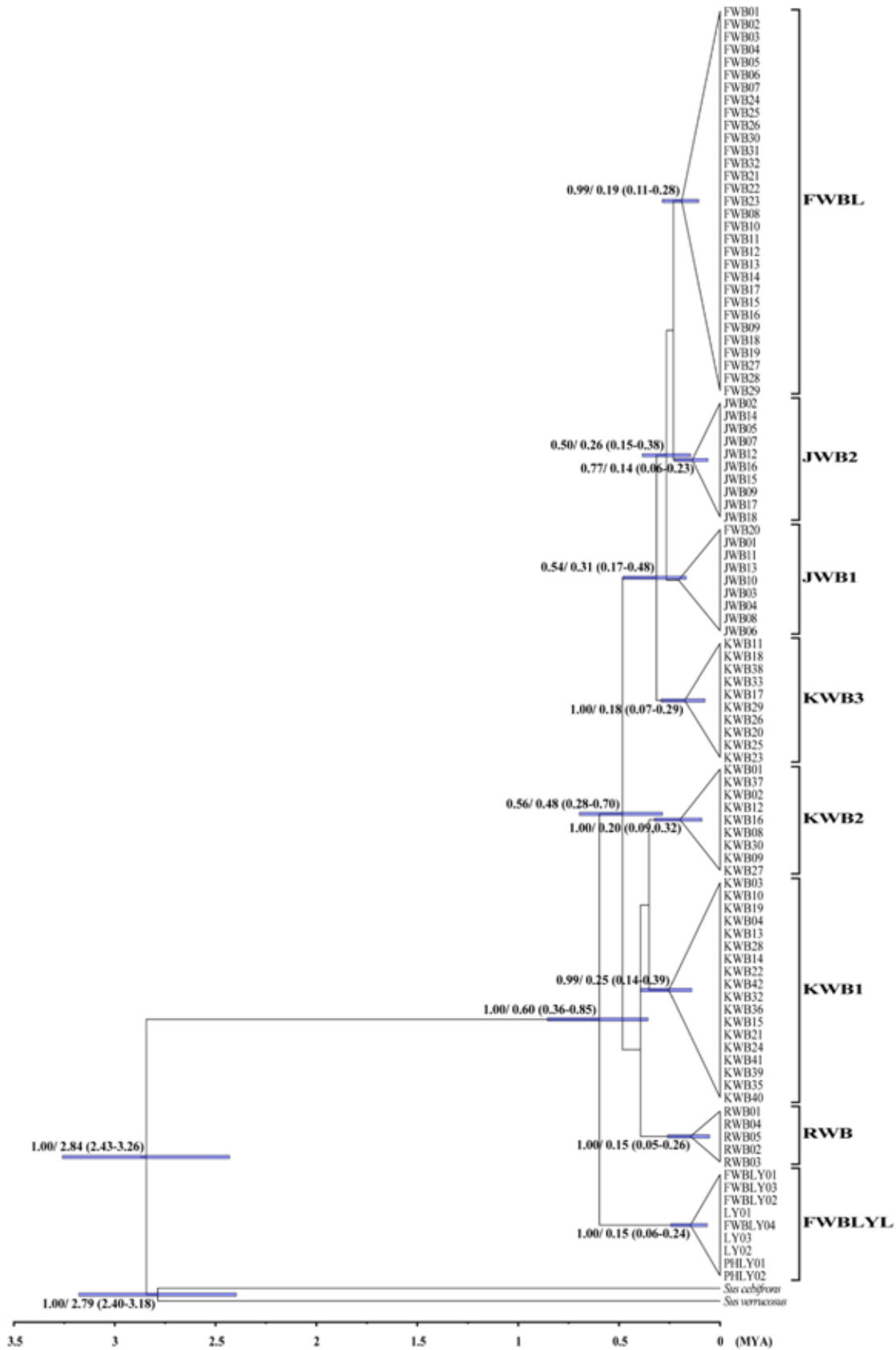


Figure 3. A Bayesian phylogenetic tree was constructed by full mitochondrial DNA control-region haplotypes of 101 wild boars in insular East Asia and the Korean Peninsula, Lanyu pigs, and Philippine native pigs. Warty pigs (*Sus verrucosus* [NC_023536] and *Sus cebifrons* [NC_023541]) are used as the outgroup. Numbers on the nodes are Bayesian posterior probability and divergence time (95% highest posterior density [HPD] in parentheses). Support of Bayesian posterior probabilities larger than 0.50 are presented, and blue bars indicate 95% HPD on the corresponding nodes. Each clade name is listed in the right column. Abbreviations of clades are as follows: FWBL = Formosan wild boar lineage; JWB = Japanese wild boar; KWB = Korean wild boar; RWB = Ryukyu wild boar; FWBLYL = Formosan wild boar with Lanyu sign lineage. MYA = million yr ago.

haplotype and domestic pigs of LY01 and LY03 haplotypes share an identical haplotype (Supplemental Fig. S1b; see the online version of the article at <http://journalofanimalscience.org>). The results of network analyses are consistent with Bayesian phylogenetic tree analyses (Supplemental Fig. S2; see the online version of the article at <http://journalofanimalscience.org>). Therefore, it seems highly likely Philippine native pigs accompanied some human migration event from Taiwan to the Philippines and that its distribution probably reflects human migration patterns. To investigate this very interesting possibility, we further specified the geographical location of pigs possessing FWBLYL. The geographical distribution of wild boars of FWBLYL haplotypes was restricted to the Central Mountain Range and Yushan Mountain Range (Table 1; Supplemental Table S5 [see the online version of the article at <http://journalofanimalscience.org>]). In addition, Lanyu pigs obtained from the Taitung Animal Propagation Station, National Taiwan University, and Lanyu Islet harbored LY haplotypes of FWBLYL. Philippine native pigs with PHL Y haplotypes of FWBLYL were also found in the Batan Archipelago and Luzon Island (Supplemental Tables S2 and S5; see the online version of the article at <http://journalofanimalscience.org>). Obviously, this distribution shows geographical continuity among pigs of FWBLYL between Taiwan, Lanyu, and the Philippines (Fig. 2).

DISCUSSION

Two Evidently Distinct Lineages of the Formosan Wild Boar

This study focuses on the genealogy of the Formosan wild boar. It uses a large sample of wild boars sourced from a wide variety of geographic locations across Taiwan. Full control-region sequences of 278 Formosan wild boars presented 36 haplotypes. Two evidently distinct lineages exist among Formosan wild boars. These have been identified as FWBL (haplotypes FWB01–FWB32) and FWBLYL (haplotypes FWBLY01–FWBLY04) through genetic characteristics and phylogenetic tree analyses. The control-region sequences for boars of FWBL are distinct from boars of FWBLYL, which possess 6 transitions and 1 transversion. Among these substitution sites, 4 are Type I Lanyu pig sign. In addition, the wild boars of FWBLYL harbor 3, 4, or 6 diagnostic repeat “ACACAAACC” motifs. Type I Lanyu pig sign and diagnostic repeat motifs have been used as signatures to trace Lanyu lineage (Wu et al., 2007a). No pig possessing these signatures has been identified among Asian and European pig breeds in previous studies (Wu et al., 2007a; Jiang et al., 2008; Larson et al., 2010). In

addition, microsatellite analysis indicated that the Lanyu pig possesses a unique nuclear genetic structure, and it is distinct from European and Asian domestic pig breeds in Taiwan (Chang et al., 2009) as well as European and Asian wild boars (Luetkemeier et al., 2010).

In the present study, boars of FWBLYL have genetically similar substitution patterns and diagnostic-motif repeats to Lanyu pigs. Importantly, this study found that wild boars possessing haplotype FWBLY04 shared a haplotype identical to Type I Lanyu pigs. In addition, Bayesian phylogenetic tree analysis has FWBLY, LY, and PHL Y haplotypes independently clustering in the FWBLYL clade. These wild boars and boars of FWBL possess only a remote phylogenetic relationship and are assigned to different clades. Lastly, haplotype evidence, phylogenetic uniqueness, and geographical distribution only in Taiwan suggest that boars of FWBLY are the wild ancestors of Type I Lanyu pigs. Hence, this study has produced 2 very important findings. The first is that Taiwan has 2 remote, distinct lineages of wild boars (FWBL and FWBLYL), and the second is that the descendants of the Lanyu pig’s wild ancestors still exist in the mountain regions of Taiwan.

Potential Geographical Barriers Result in Genetic Differentiation of Formosan Wild Boar Lineage

The biogeographic distribution pattern of FWBL was identified using AMOVA. Boars were distributed among 3 regions: the NCR (populations A, B, and C), the CSR (populations D and E), and the HPR (Population F). These districts showed significant fixation indices among groups (Φ_{CT}). The results of Φ -statistics clearly reveal higher genetic differentiation among regions than within regions. Two potential geographical barriers have been proposed that may have resulted in genetic differentiation among populations in Taiwan. The first geographical barrier is located between populations C and D, and the second between populations E and F. Between populations C and D are a group of high mountain peaks, including Yushan (Jade Mountain [3,952 m]), the highest mountain in Taiwan. Past studies have indicated that the majority of Formosan wild boars do not distribute at high altitudes (Hsu and Agoramorthy, 1997), supporting the 3,000-m peaks surrounding Yushan as being an effective geographical barrier against gene flow. Furthermore, the alluvial plain in southwestern Taiwan developed during glaciations (Yang et al., 2007). This means that low land corridors for the Formosan wild boar would have been limited during glacial periods. The second geographical barrier between populations E and F is at the northern limit of the Hengchun Peninsula. Loss of fauna biodiversity caused by the peninsula effect has

been identified on the Hengchun Peninsula. The peninsula effect describes the decline in species between the base and the tip of a peninsula. The geometry hypothesis of the peninsula effect indicates that species diversity due to migration declines with distance from the base of a peninsular (Jenkins and Rinne, 2008). In the case of Taiwan, reduced wild boar colonization toward the tip of the Hengchun Peninsula may be attributable to peninsular geometry. Furthermore, some amphibians such as frog (*Sylvirana latouchii*) and small mammals such as Pallas's squirrel (*Callosciurus erythraeus*) show unique biogeographic districts on the Hengchun Peninsula (Oshida et al., 2006; Jang-Liaw et al., 2008). Therefore, the peninsula effect is thought to constitute a geographical barrier causing isolation of wild boars and reduced gene flow among populations E and F. On the other hand, the core lineages/haplotypes (FWB08, FWB24, and FWB31) exhibit a star-like pattern, indicating recent population expansions or exponential population growth with common ancestral lineages/haplotypes at their center (Larson et al., 2005; Yang et al., 2011). Furthermore, the core haplotypes locate in the NCR and CSR, which possess higher genetic diversities than the HPR. In addition, FWBL and all populations show large h values (>0.5) and small π values (<0.005). These results indicate that FWBL and regional populations underwent population bottlenecks followed by rapid population growth and accumulation of mutations (Grant and Bowen, 1998). Furthermore, FWBL have a significantly negative value in Fu's F_s test. This result is also interpreted as FWBL undergoing population expansion. Combining genetic diversity, Fu's F_s test, network cladogram, and potential geographical barriers, origin and dispersal scenarios of FWBL are proposed as follows. The ancestor of FWBL first migrated along a land bridge from continental Southeast China to Taiwan. The Taiwan Strait has an average depth of only 60 m and its floor would have been exposed during glacial periods (Jan et al., 2002). This land bridge formation allowed boars to enter Taiwan at an area of central Taiwan through the Formosan bank during the middle Pleistocene (Liao et al., 2008). Over time, boars of FWBL dispersed southward and subjected to the peninsula effect in the HPR. Any northward expansion of wild boars was blocked by high mountains and the absence of a suitable alluvial plain to facilitate their spread. On the other hand, some wild boars dispersed northward, passing or detouring around the mountain barrier with some difficulty. Taiwan's diverse topography meant that original boar populations encountered many natural barriers to their dispersion, resulting in population bottlenecks as well as periods of rapid population growth and expansion.

Multiple Independent Historical Dispersion Events and Between-Island Vicariance Cause Allopatric Differentiation of East Asian Wild Boars

The dispersal routes of East Asian wild boars have been ambiguous in previous studies. Watanobe et al. (1999) indicated that the Ryukyu wild boar had an origin separate from the Japanese wild boar and did not disperse from Kyushu to the Ryukyu Archipelago via the Tokara Channel. Furthermore, Takahashi et al. (2012) proposed wild boars from East or Southeast Asia undergoing multiple migration events to the Ryukyu Archipelago during the Pleistocene. Geological evidence shows that the Ryukyu Arc has been connected to continental Asia via Taiwan since the early Pleistocene (Ota, 1998). Hence, the Ryukyu wild boar likely dispersed using a land bridge across Taiwan to the Ryukyu Arc based on their geographical closeness. However, this hypothesis is still unproven. Watanobe et al. (2003) used partial control-region fragments to identify a haplotype shared by Japanese wild boar and Northeast Asian wild boars and proposed that the ancestor of Japanese wild boar had migrated from continental northeast Asia via the Korean Peninsula to Japan's Kyushu Island across a land bridge in the mid to late Pleistocene. In addition, Cho et al. (2009) proposed a different hypothesis on pig migration from Japan to Korea from that of Watanobe et al. (2003). A median-joining network based on polymorphism of partial control-region sequences indicated that the Korean wild boar's progenitor was introduced from the Ryukyu Arc to Korea through Japan. Another possible dispersion route has the progenitor of the Formosan wild boar dispersing from Okinawa Island westward back toward continental Asia before reaching the Korean Peninsula. They hypothesize the Ryukyu Arc as being the place where Chinese/Ryukyu lineage converged (Cho et al., 2009). Nevertheless, limited samples from the Formosan wild boar, partial control-region sequences, and ignoring the different migration events of East Asian wild boars during glacial epochs meant such studies could not provide clear evidence of actual historical events and, therefore, their study of gene flow was quite asynchronous (Watanobe et al., 1999, 2003; Cho et al., 2009).

The Bayesian phylogenetic tree for Asian wild boars constructed in this study revealed 2 distinct FWBL dispersing "into" and "out of" Taiwan. Two major clades were identified: 1 included wild boars in ISEA consisting of an ancestral clade and the other including all remaining Asian wild boars, which formed the descendant clade. Next, South Asian and West Asian wild boars were the first group to diverge in the descendant clade. It is thought that the *Sus* genus originated in ISEA and followed separate paths to South Asia and West Asia (Larson et al., 2005, 2010;

Cho et al., 2009). Next, FWBLYL, AWB1, and AWB2 split off from the GEAWB clade. In previous studies, the Ryukyu wild boar is presumed to have diverged earliest and was isolated in East Asia (Watanobe et al., 1999; Hongo et al., 2002; Takahashi et al., 2012). Our study proves that boars of FWBLYL split off earlier than the Ryukyu wild boar and supports boars of FWBLYL being the earliest wild boars to have spread and become isolated in insular East Asia. The Ryukyu wild boar is genetically close to China wild boars in the AWB1 clade. The result supports the progenitor of the Ryukyu wild boar dispersing through Southeast Asia to the Ryukyu Archipelago; whether this migration traversed Taiwan requires more proof. This assessment corresponds to the migration hypothesis of the Ryukyu wild boar described by Takahashi et al. (2012). In addition, the AWB2 clade consisted of wild boars in Southeast Asia, southwest China, and Korea. Yang et al. (2011) described Yunnan Province as being the intersection between the Tibetan highlands, southwest China, and Southeast Asia. The Korean wild boar was considered genealogically linked with wild boars in Southeast Asia (Cho et al., 2009). Our results show that the progenitor of the Korean wild boar in the AWB2 clade possibly originated in Southeast Asia and dispersed northward through southwest China and then across China to the Korean Peninsula. Finally, the GEAWB clade formed throughout East Asia, including Taiwan, Japan, Korea, and China. In a previous nuclear genome study, phylogenetic tree analysis constructed by microsatellite markers showed a similar tree topology, supporting our result. This result revealed that wild boars from ISEA and Southeast Asia formed a basal clade, distinct from Northeast Asian wild boars (including Russian, Korean, and Japanese wild boars; Choi et al., 2014). However, widespread wild boar sampling from the numerous countries analyzed in this study provided more detailed and complex dispersion events of wild boars in East Asia.

Full length control-region sequences were used to perform Bayesian phylogenetic tree analysis to further clarify the genealogical role and divergence time of the Formosan wild boar in insular East Asia and the Korean Peninsula. Based on phylogenetic tree analysis and divergence time calculations, boars of FWBLYL split early from East Asian wild boars at approximately 0.60 MYA, during the mid Pleistocene. After that, the the Ryukyu wild boar and Korean wild boar (KWB1 and KWB2) diverged from all other insular East Asian wild boars (except FWBLYL) at 0.48 MYA. We propose these 3 dispersal events of East Asian wild boars as early-stage events of northward dispersal from ISEA onto Taiwan, Ryukyu, and then Korea during the mid Pleistocene. These dispersal events are assumed to be

independent dispersal events in view of the topology of the phylogenetic tree and divergence times. On the other hand, FWBL clusters with JWB1, JWB2, and KWB3, forming a monophyletic clade. This evidence hints at these boars originating from a single progenitor. The divergence time of KWB1, JWB1, JWB2, and FWBL was at 0.31 MYA. This result shows boars of FWBL dispersing to Taiwan during the last dispersal event in insular East Asia; however, this event was distinct from the dispersal of FWBLYL. Finally, between-island vicariance caused allopatric differentiation by geographical isolation after the regression of the ice age. However, more sequence information from China is required to validate each dispersal path proposed in this study. Further whole genome analysis or microsatellite analysis would be helpful to clarify this issue.

The above depiction is a comprehensive scenario describing the multiple dispersal routes of Asian wild boars. The 6 proposed histories of multiple independent dispersal routes in Asia are as follows. First, the ancestor of Asian wild boars originated in ISEA as a differentiation of the *Sus* genus. The first route has wild boars dispersing from ISEA west to West Asia via the Indochina peninsula. The second route has the progenitor of FWBLYL dispersing along a land bridge to Taiwan via the Formosan bank during the mid Pleistocene. The third route has wild boars dispersing northward from ISEA via Southeast Asia and Yunnan to southwest China. The fourth route has progenitors of the Ryukyu wild boar dispersing from ISEA to Southeast Asia/China and later spreading to the Ryukyu Arc via fragmented land bridges connected to Taiwan. The fifth route has wild boars of Southeast Asia moving across continental Asia to the Korean Peninsula, because Southeast Asian wild boars are suggested as the progenitors of the Korean wild boar. The final sixth dispersal has the progenitors of East Asian wild boars potentially dispersing over land bridges to Taiwan and then along land bridges located across the east of the Asian continent northward to Japan and Korea or expanding out from Japan and then reaching the Korean Peninsula. Different wild boar subspecies in insular East Asia potentially resulted from multiple historical dispersal events of Asian wild boars.

Lanyu Pigs “Out of” Taiwan

Taiwan’s pig domestication is considered “cryptic,” as there is a lack of genetic evidence indicating the direct wild ancestor of Lanyu pigs and corroborating archaeological evidence. Previous studies have revealed pig domestication centers for sympatric wild boars and domestic pigs sharing the same mtDNA haplotype (Larson et al., 2005; Yang et al., 2011; Jin et al., 2012).

However, wild boars and domestic pigs belong to the same species, and cross-breeding is possible. Although it has been shown that mitochondrial genome replacement in pigs can occur with some studies proposing genetic introgression from wild boars into domestic pigs through postdomestication hybridization (Frantz et al., 2013; Larson and Burger, 2013; Larson and Fuller, 2014), using mitochondrial approaches to study animal ancestry is still valuable and is widely applied in domestication studies (Achilli et al., 2012; Jin et al., 2012; Mariotti et al., 2013; Miao et al., 2013; Nagarajan et al., 2015). That said, the anomalous possibilities of postdomestication hybridization mean that a rigorous step-by-step approach incorporating mitochondrial analysis of sympatric wild and domestic populations, tracing of wild ancestors, and the use of archaeological evidence is essential to gaining a full understanding of East Asian wild boar dispersal. In our study, the results of haplotype networking and phylogenetic tree analysis indicate that wild boars possessing haplotypes of FWBLY are extremely closely related to domestic pigs with haplotypes of LY and PHL. Significantly, wild boars of FWBLYL possess a similar genetic signature but more genetic diversity of haplotypes than domestic Lanyu pigs. Genetic characteristics and phylogenetic uniqueness suggest wild boars of FWBLY are the wild ancestor of Lanyu pigs. Consequently, these data help offer insight into one of the incidents of “cryptic domestication” described by Larson et al. (2010). Our data provide genetic evidence of extremely close genetic haplotypes being shared by sympatric wild boars and domestic pigs. One wild boar haplotype shared a haplotype totally identical to Lanyu pigs; however, no domestic Lanyu pigs possessed the FWBLY haplotype. This result indicates that wild boars of the FWBLY haplotype did not come from cross-breeding with domestic Lanyu pigs. Instead, the genetic evidence indicates that the domestication process has led to the genetic continuity of the Lanyu pig and its wild ancestors being preserved. Most importantly, the results suggest that the wild ancestor of the Lanyu pig comes from Taiwan and that genetic linkages between the Lanyu pig and its wild ancestors still exist in the gene pool of certain Formosan wild boars to this day. In insular East Asia, it is possible that Taiwan is the only pig domestication center; however, more ancient DNA analysis and archaeological evidence is needed to help verify island domestication on Taiwan. That being said, the number of wild boars with FWBL is extremely large compared with individuals of FWBLYL in modern Taiwan. These results lead us to hypothesize that the progenitor of wild boars of FWBLYL migrated from ISEA and colonized Taiwan, following genetic differentiation caused by island vicariance. After this time, the population size of FWBLYL sharply decreased due to the

mammalian extinction event that occurred in the Late Pleistocene to Holocene. This event probably relates to the Younger Dryas event of approximately 12,900 yr before the present, which caused rapid abrupt environmental change. In the Younger Dryas cooling period, many mammalian and avian taxa suffered catastrophic extinction, and at least 35 mammal genera disappeared in North America (Firestone et al., 2007). Furthermore, this event was a worldwide phenomenon. Pollen stratigraphy and the continuous vegetation history of Taiwan shows evidence of rapid cooling during the Younger Dryas event in central Taiwan (Liew et al., 2006). In addition, *S. scrofa* has been shown to be a dominant large mammal after the mammalian extinction event (between 20,000 and 10,000 yr before the present) in Japan (Watanobe et al., 2003). Taking all this evidence into account, the progenitors of FWBLYL may have sharply decreased due to some anomalous occurrence such as the Younger Dryas event. It is thought that the remaining progenitor population of the Ryukyu wild boar on Taiwan also suffered during this event, resulting in no survivors of wild boars genetically linked to the Ryukyu wild boar existing on Taiwan today.

The dispersal of Austronesian peoples throughout the Pacific and Indian oceans is thought to have been launched from Taiwan over recent millennia. This is based on archaeological, linguistic, and genetic evidence (Diamond, 1988, 2000; Melton et al., 1995; Diamond and Bellwood, 2003). Larson et al. (2007) applied the geographical distribution of pigs representing a Pacific Clade to depict Austronesian dispersal. These pigs likely originated in the Southeast Asian Peninsula and subsequently dispersed to the Sunda Islands, the Moluccas, and New Guinea with the movement of peoples. However, pigs of the Pacific Clade were later reexamined using a larger pig genetic database survey (using network analyses and Bayesian consensus trees), which suggested that pigs of Pacific Clade lineage originated and were domesticated in ISEA, not continental Southeast Asia (Yang et al., 2011; Jin et al., 2012). Despite the controversy surrounding Larson et al. (2007), conceptually, human-mediated dispersal of pigs providing a valuable indicator to trace Austronesian dispersal is still legitimate. Because no Pacific Clade pigs have been found in Taiwan, Larson et al. (2007) suggested that human dispersal from Taiwan via the Philippines to New Guinea (i.e., the “Out of Taiwan” model) was not accompanied by domestic pigs. There are 3 possible reasons for a lack of Pacific Clade pigs on Taiwan. These are the Austronesian people passing through Taiwan from continental Asia not taking any domestic pigs with them, as Larson et al. (2007) proposed. Or the sample size used so few Formosan wild boar samples that an as-yet-undiscovered Pacific Clade of pigs may exist. Alternatively,

the Austronesian people carried domestic pigs distinct from Pacific Clade pigs (i.e., pigs with Lanyu sign lineage) when migrating into and out of Taiwan. In this paper, the geographical distribution of pigs with FWBLYL for Taiwan, Lanyu, and the Philippines depicts potential human-mediated pig dispersal routes. The findings of this study provide genetic evidence in support of this possibility. However, the issue of Austronesian dispersal is quite complicated. Further genetic analysis applied to archaeological *Sus* excavated in Taiwan, Lanyu, and the Philippines would be helpful in providing new insights into Austronesian dispersal routes.

This paper shows different genetic diagnostic motifs for the examination of the Formosan wild boar and investigates genetic diversity and differentiation among Formosan wild boar populations. In addition, we depict the complete dispersal scenario of Asian wild boars and propose 6 possible dispersal routes. Three independent dispersal routes are presented for insular East Asia during glacial periods, following between-island vicariance of wild boars due to geographical isolation after the regression of glacial periods. Clarification of these dispersal routes could be achieved by effectively linking our present database for insular East Asia with additional sampling from southeast and northeast China. Microsatellite analysis in the context of this combined data would assist in clarifying the multiple dispersal route hypothesis. Furthermore, this paper supports the idea that a domestication event occurring in Taiwan with the discovery that the wild ancestor of the Lanyu pig is common to certain Formosan wild boars. By examining the continuous distribution of the Lanyu pig and its wild ancestors, we illustrate human-mediated pig dispersal routes among Taiwan, Lanyu, and the Philippines; however, the exact nature of island domestication of the Lanyu pig is still somewhat ambiguous. Ancient DNA evidence in Taiwan, Lanyu Islet, and the Philippines would be helpful in the investigation of the domestication event that led to the Lanyu pig.

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