

DOCTORAL THESIS

**Phylogeographic and Evolutionary History
Analyses of Pigs in Southeast Asia**

(Summary)

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Study 1: The Philippine archipelago was believed to have never been connected to the Asian continent, even during the severe Quaternary sea-level drops. As a result, it was hypothesized that the ubiquitous Eurasian wild boar *S. scrofa* is not native to the Philippines, and its dispersal remains controversial and must have some anthropogenic origin associated with human migration events. To date, the only available information suggests that the Philippine domestic pig is a product of indiscriminate interbreeding between numerous domesticated endemic Philippine wild pigs and an introduced domestic pig breed that has been able to survive and reproduce with minimal human intervention. However, this hypothesis remains daunting due to the insufficient molecular studies to support this claim, as the evolution and dispersal of Philippine domestic pigs have yet to be elucidated. In study 1, initial data on the context of origin, dispersal, and the level of genetic introgression in Philippine domestic pigs were analyzed using partial mitochondrial DNA D-loop analysis altogether with domestic pigs and wild boar corresponding to their geographic origin. The results revealed considerable genetic diversity and widespread Asian pig-ancestry in the phylogenetic analysis, with admixed European pig-origin harboring various fractions of ancestry from Berkshire and Landrace. Using parametric and probabilistic approaches, this study uncovered the close genetic connection between the continental wild boars and domestic pigs present in the Philippine domestic pigs. This corroborates our hypothesis of a genetic signal that may be associated with the recently reported waves of human migrations to the Philippines. A high frequency of haplotypes belonging to D7 haplogroups poses an interesting challenge because of the absence of a similar haplotype in the Insular and NEA regions and does not primarily point to the Indo-Burma Biodiversity Hotspots (IBBH) as its direct origin. Moreover, this study reported the first Pacific Clade signature and the ubiquitously distributed D2 haplotypes (Asian major) on several Philippine islands. The analysis of mismatch distribution and neutrality test were consistent with the Bayesian skyline plot, which showed a long stationary period of effective population size. The population decline was consistent with the pronounced population bottleneck in Asian and European pigs during the interglacial periods of the Pleistocene. Therefore, urgent conservation measures and suitable management of their genetic pool are crucial to the management of animal genetic resources at the local and global levels. For future perspectives, Y-specific markers could be performed to assess the extent of male-mediated introgression from European pigs into Philippine domestic pigs.

Study 2: A complete mitochondrial DNA D-loop region from 184 Philippine pigs was analyzed to elucidate their early dispersal history by performing a phylogenetic comparison with wild boars and domestic pigs worldwide. Based on the patterns of mtDNA D-loop variation in Philippine pigs, results were consistent with a clear phylogenetic pattern showing two core lineages of *S. scrofa*, both of Asian and European phylogeographic origin, occurring in the Philippine pig population. By screening and detecting the haplogroup-specific mutational motifs, each haplotype based on the smallest named sub-phylogroups were assigned to which it belongs. Results showed that Philippine pigs could be classified into five phylogenetically distinct haplogroups such as D1, D2, D6, D7, and Lanyu Clade, except for the Philippine wild pig haplotypes. Thus, this study confirmed that the D7 haplogroup (with significantly larger ancestral population sizes) was distinct from the available haplogroup that has been tentatively classified by haplogroup-specific motif recognition in porcine mtDNA. For the first time, this study provides evidence for the presence of the Philippine Lanyu subclade. Demographic signals were also indicative that the genetic exchange of these rare pigs with other domestic pigs in the Philippines may have existed for some time. This is in contrast to the reported scenario in Taiwan, where increasing inbreeding within the small Lanyu population has become a conservation concern. Two dispersal scenarios are being proposed based on the pattern of the current maternal haplotype distribution of Philippine pigs. One is via NEA through Taiwan, in parallel with the Neolithic expansion into ISEA and Oceania, and the other is via Southeast Asia, particularly from the Indochinese Peninsula via the Sundaic Region (Sundaland) to Palawan and the Sulu Archipelago, which spread to the rest of the Philippine islands since prehistoric times. Despite geographic barriers to migration, these animals have expanded their range across the various islands of

the Philippines, and variation in morphological patterns has evolved among these populations. Hints of the discrepancy between the molecular distribution and observed morphological data of the four endemic Philippine wild pigs have been observed in this study. Thus, this study documented the first record of *S. ahoenobarbus* and *S. cebifrons* in GPFR and GPNRF, respectively. Therefore, conservation initiatives based on mutually beneficial conservation measures should be a priority.

Study 3: One of the domestic pigs whose origin has been described as "cryptic" is the Lanyu pig from Taiwan because it has unique molecular and morphological characteristics that differ from East Asian pig breeds. Recent evidence for the presence of Lanyu pigs with Type I signatures extending from northern Luzon through the western and central Philippines suggests the possibility of independent domestication of Lanyu pigs in the Philippines. To clarify the spatio-temporal dispersal of Lanyu pigs, Study 3 employs model-based phylogenetic approaches to analyze all Lanyu pigs with Type I signatures from Taiwan and the Philippines and understand the role of humans in their dispersal. The result supports the existence of two subclades of the Type I Lanyu pig, the Taiwanese Lanyu subclade and the Philippine Lanyu subclade. This study proposes that the migration of Lanyu pigs may have occurred in a bidirectional gene flow, coinciding with the possible long-distance migrations by humans in the post-Neolithic period, which favored the dispersal of the unique Lanyu pigs. The lack of signatures of the Lanyu pig in Borneo has allowed this study to propose two hypothetical scenarios. First, the Lanyu pig originated in Taiwan and was brought by humans (e.g., through commercial trade after domestication or through migration) from mainland Luzon to Palawan Island in the southwestern Philippines, where a new population later became established. Second, the *S. scrofa* wild boar (of Lanyu wild boar origin) existed in the Philippines but was exterminated, leaving the subsequent population. Therefore, further evidence of pigs with Lanyu signatures in the southern Philippines, particularly in the contact zones between the Mindanao Islands and the Indonesian and Pacific Islands, may further the discussion on the dispersal of these unique animals.

Study 4: This study examined 447 complete mitochondrial DNA D-loop sequences from SEA pigs to infer genetic diversity and structure, phylogeography, population dynamics, and haplogroup-specific divergence. Model-based approaches were employed in the analysis. Results showed a higher overall genetic diversity among SEA pigs compared to other regions. When the population was examined individually, Vietnam and the Philippines showed the highest genetic diversity, while Myanmar showed the lowest, with all samples classified only under the D2 haplogroup. Population-specific pairwise and Slatkin's linearized F_{ST} test revealed highly significant, indicating a limited gene flow between all populations studied. The Bayesian, ML tree, and MJ networks consistently revealed six well-supported mtDNA clades with high posterior probability support. Haplogroups D2 and D7 were the most geographically widespread of the total sequences examined. Based on the geographic distribution of haplotypes, this study supports the clear exchange between domestic pigs from the Mekong region, the adjacent Yangtze River, and southern China. Furthermore, European pigs with Asian maternal signatures have been detected, as it occupies an intermediate phylogenetic position with Asian domestic pigs and wild boars. Results for the neutrality tests and mismatch distribution were consistent with the expected model. Considering individual geographic populations, all sampling sites except BTN and THA exhibited a strong unimodal distribution of pairwise differences, each indicating a major expansion event. The BSP, however, showed a long period of relatively constant N_e for the overall dataset, which the rapid N_e expansion occurred about 250 thousand years ago (kya). Meanwhile, both BSP's predicted a 200 kya increase in N_e for haplogroup D2 while showing a relatively stable population both with and without the isolated ISEA samples. The Bayesian estimates of time to the most recent common ancestor (TMRCA) using the calibration method according to the uncorrelated lognormal relaxed clock model for the various haplogroups were conducted. The oldest divergence event was dated to 2.574 Mya, with 95% highest probability density

(HPD) intervals of 0.650 - 3.250 Mya, the split time between the Asian and European major clades. Haplogroups D1 and D4 diverged ~ 1.490 Mya (95% HPD intervals: 0.754-2.450 Mya). The TMRCA of the Asian major clades was estimated to be ~1.949 (95% HPD intervals: 0.410-2.710 Mya). The TMRA for haplogroup D2 was ~1.176 Mya (95% HPD intervals: 0.210, 1.500 Mya), and the newly identified D7 recently separated from D2 ~0.776 Mya (95% HPD intervals: 0.210, 0.800 Mya).

In conclusion, this study has provided comprehensive insights into the genetic diversity, unique population dynamics, and evolutionary history of domestic pigs in Southeast Asia. The high resolution of matrilineal phylogeny has shed light on the evolutionary history of domestic pigs and wild boars that have contributed significantly towards elucidating the evolutionary diversification of domestic pigs worldwide. Future integrated genome-wide, mitogenome, and environmental adaptation studies are needed for effective management strategies and future conservation efforts.