Was the Lanyu pig independently domesticated in the Philippines? A meta-analysis review on the prehistoric expansion of the unique Lanyu pigs

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Abstract

Due to the paucity of molecular studies, the origin of the unique Lanyu pigs has been described as “cryptic.” Recent evidence of the presence of Lanyu pigs with Type I signatures spanning from Northern Luzon (NL) through the western and central Philippine regions suggests the possibility of independent domestication of Lanyu pigs in the Philippines. To clarify the spatio-temporal dispersal of Lanyu pigs, this study meta-analyzed all D-loop sequences of Lanyu pigs with Type I signatures (n=323) from Taiwan and the Philippines and elucidated the role of humans in their expansion. The result supported the existence of two subclades of Type I Lanyu pigs, the Taiwanese Lanyu and the Philippine Lanyu subclades. While the two subclades shared certain morphological traits, the latter had signs of morphological patterns that had undergone feralization. Long-distance movement of these pigs during the post-Neolithic era may have been facilitated by the known back-and-forth migration between the islanders of Lanyu (Orchid Island) and the Batanes Archipelago, the northernmost region of the Philippines. Due to the absence of Lanyu pig signatures in Borneo and the fascinating presence of Lanyu signatures in Palawan, two possible scenarios are being proposed. First, the Lanyu pig originated in Taiwan and was introduced by humans (e.g., through trade after domestication into the Philippines) moving to the west of the Philippines where a new population later became established. Secondly, the ancestral origin of the Lanyu pig radiated from mainland Southeast Asia into the Philippines but was extirpated, leaving the subsequent population. The latter suggests the allopatric expansion of the unique Lanyu pigs, which could likely support that Sus scrofa is native to the Philippines. This finding provides new perspectives on the complex evolutionary and anthropogenic history of pig dispersal into Island Southeast Asia.

Keywords: biogeography, dispersal, Lanyu pigs, mitochondrial DNA, phylogenetics

Introduction

The Eurasian wild boar (Sus scrofa L.) has a wide geographic distribution throughout Eurasia that consists of many isolated and wild populations. The divergence between European and Asian pig populations has been estimated at 1.2 Mya (Groenen et al., 2012; Frantz et al., 2013) that has resulted in many fixed molecular differences (Bosse, 2018), and has adapted to a variety of new environments in a relatively short evolutionary time frame (Frantz et al., 2016). Independent domestication of these two populations in East Asia and Western Eurasia has resulted in distinct domesticated populations (Giuffra et al., 2000; Larson et al., 2005; Megens et al., 2008; Bosse et al., 2012, 2014a). Because of the widespread distribution of S. scrofa, the profound patterns of gene flow in domestic pigs have long been the subject of debate (Tanaka et al., 2008). Furthermore, due to widespread hybridization and introgression among domestic pigs from highly divergent wild populations, modern mitochondrial genomes have emerged that exhibit a mosaic of different haplotypes (Giuffra et al., 2000; Goedbloed et al., 2013; Bosse et al., 2014b).

One of the domestic pigs whose origin has been described as “cryptic” is the Lanyu pig from Taiwan (Larson et al., 2010) due to its unique molecular and morphological characteristics that differ from East Asian pig breeds (Luetkemeier et al., 2010). Mitochondrial DNA (mtDNA) D-Loop studies have shown that the Lanyu pig has two distinct lineages, one closely related to Asian pigs (referred as the Type II Lanyu pig) and the other distinct from Asian and European wild and domestic pigs (referred as the Type I Lanyu pig) (Wu et al., 2007). Unlike other wild and domestic pigs, where the centers of domestication are relatively well known (e.g., haplogroups D1 (Central Europe), D2 (China), and D7 (Southeast Asia), the evolutionary history and dispersal of the distinct Lanyu pigs are still unclear. The few molecular studies suggest that these pigs were descended from Formosan wild boar (FWB) (Li et al., 2017) or were likely domesticated from local wild boars through early dispersal from Eurasia (Zhang et al., 2021). While these conclusions are speculative due to the lack of phylogenetic representation, research on neighboring countries such as the Philippines is imperative to provide a clearer picture of the evolutionary history of these unique animals.

Due to problematic archeological evidence, where pig remains were mostly attributed to extant wild pigs in the archipelago (Piper et al., 2011; Ochoa, 2019), it has been...
speculated that the wild boar S. scrofa was introduced into the Philippines as a domestic animal from the Mainland Southeast Asia (MSEA) only in the late Holocene (Larson et al., 2005; Dobney et al., 2008; Cucchi et al., 2009; Piper et al., 2009; Layos et al., 2022a). Using mtDNA studies, it was recently reported that the prehistoric dispersal of domestic pigs in the Philippines was due to multiple maternal lineages that spread via two possible routes. One parallels the Neolithic expansion in Island Southeast Asia (ISEA) and Oceanica via Northeast Asia (NEA) and the other from the MSEA to Palawan and the Sulu Archipelago south of the Philippines (Layos et al., 2022a). The former has been linked to the only potentially identified archaeological records of domestic pigs in the Philippines from the Neolithic (4000-3000 cal. BP) and Early Metal Age (3000-2000 cal. BP) at Nagsabaran in Northern Luzon (NL), confirming the clear distinction between the domesticated pigs and the Philippine warty pigs (Piper et al., 2009; Amano et al., 2013; Layos et al., 2022a). Although there is no historical record of geographic contact between the Philippines and Taiwan, it has been hypothesized that the migration of these pigs may have been assisted by humans, consistent with the presumed movements of Austronesians from Taiwan to the Philippines about 3,000 years ago (Bellwood & Dizon, 2005; Larson et al., 2005).

However, several studies have disputed the links between Taiwan and the Philippines concerning this hypothesized pig dispersal. Li and colleagues (2015), for instance have challenged this hypothesis, arguing that there were no domestic pigs in Taiwan at a similar time questioning the possible Neolithic introduction of domestic pigs into the Philippines. In addition, the gene flow of indigenous rice from Northern Luzon (NL) to Taiwan was documented only recently, about 1,300 years ago (Alam et al., 2021), which could argue for the south-to-north colonization. Thus, all these information have led to the enigma of the exact trajectory of Lanyu pigs which has remained the subject of scientific debate due to conflicting literature on the prehistoric introduction of domestic pigs into the Philippines (Li et al., 2015; Layos et al., 2022a). Recently, the existence of a diverse Philippine Lanyu subclade has been proposed, extending its range from Northern Luzon (NL) to Palawan and further into the central Philippines (Layos et al., 2022a). In addition, Dichoso et al. (2022) reported the wide distribution of domestic pigs with Lanyu signatures in Batanes Islands, Luzon, and Marinduque off the coast of Luzon Island. If domestic pigs with Type I Lanyu pig signatures with high haplotype variability are widely distributed in various Philippine islands, can the Philippines be considered the center of domestication of these unique animals? Thus, to shed light on the spatio-temporal distribution of Lanyu pigs, this study analyzed the mtDNA variation of the previously reported mtDNA D-Loop sequences of domestic pigs with Lanyu signatures from Taiwan and the Philippines.

Materials and Methods

From our previously reported 166 original sequences (Layos et al., 2022a, 2022b), 157 complete mtDNA D-Loop sequences from the GenBank were pooled, comprising 70 from the Philippines (Basilio et al., 2016) and 87 sequences from Taiwan (58 Lanyu pigs and 29 Formosan wild boars-FWB) (Li et al., 2017). All samples from Taiwan included in this study comprise all known Lanyu pig sequences recorded in the literature to date. Thus, this study focuses only on individuals with the Lanyu pig signatures. These datasets were then complemented with previously published D-Loop sequences representing Asian and European pig lineages retrieved from GenBank. Using MEGA X (Kumar et al., 2018), ambiguous sites were trimmed, cleaned, and aligned using the ClustalW (Thompson et al., 1994). After the sequences were aligned, the screening and detection of the ancestral mutational motifs were viewed using BioEdit 7.2.5 (Hall, 1999). Phylogenetic analyses were inferred using two different models based on probabilistic and parametric approaches: maximum-likelihood (ML) and Bayesian inference (BI). Maximum-likelihood analysis was performed in IQ-TREE (Nguyen et al., 2015) with the best-fitted substitution model, TIM2+F+I+G4 based on the Bayesian Information Criterion (BIC) determined by Modelfinder (Kalyaanamoorthy et al., 2017). Statistical node support was calculated using ultrafast bootstrap support (Hoang et al., 2018) and SH-aLRT (Guindon et al., 2010) with 1,000 replicates. Meanwhile, the BI was performed using BEAST2 v2.6.6 (Bouckaert et al., 2019) under uncorrelated relaxed clock log-normal distribution, setting a clock rate 1.36×10-8 mutations/site/year rate (Pesole et al., 1999), with HKY+G+I as the best-fitted model of molecular evolution. The posterior distributions of parameters were estimated using the Markov Chain Monte Carlo (MCMC) with duplicate runs of 20 million generations, sampling every 2,000 steps, and the initial 10% trees of each MCMC run were discarded as burn-in. Using Tracer v.1.7.1, the convergence of MCMC chains was assessed making sure that the sufficient sampling with all estimated parameters exceeded 200 effective sample size (ESS) values. The median-joining (MJ) network was also constructed to infer the evolutionary relationships between haplotypes using PopArt v1.7 software (Leigh & Bryant, 2015). The number and assignment of haplotypes were determined using DnaSP v6.0 software (Librado & Rozas, 2009).

Results

Of the 323 sequences, 58 haplotypes were identified, 15 of which formed the unique Lanyu clade comprising 44.27% (n=143) of the total dataset. Six haplotypes (with 6 variable sites), which accounted for 26.93% of the dataset, occurred exclusively in the Central Mountain Range, South-Central Mountain Range, and Yushan Mountain Range in Taiwan. Nine haplotypes (with 17 variable sites), which accounted for 17.34% of the total sequences, were distributed throughout the Philippines, with increasing frequency mainly in mainland Luzon, Palawan in the west, and Bohol in the central Philippines. Hap 14 was the most common haplotype with 83 sequences containing 57 Lanyu pigs and 26 FWB. Sharing of haplotypes between the two countries was not observed. Haplotype diversity was highest among Philippine Lanyu

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Table 1. Analysis of molecular variance of Lanyu pigs based on mtDNA D-loop

<table>
<thead>
<tr>
<th>Group</th>
<th>Sources of variation (%)</th>
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<tr>
<td></td>
<td>Among populations</td>
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<td>PHL vs. TWN</td>
<td>79.66**</td>
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**highly significant value at p<0.01; PHL-Philippines; TWN-Taiwan
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Figure 1. A) Geographical distribution of all the Lanyu pigs identified so far. Morphological characteristics of the Lanyu pigs are shown on the right corner. Numbers on the map are number of sequences that constitute one haplotype. B) Bayesian phylogenetic tree (MCMC=20,000,000) of all available complete mitochondrial DNA D-loop sequences from Lanyu pigs, including selected Asian and European domestic pigs and wild boars. Numbers shown in Figure 1A are number of sequences per haplotypes, while numbers shown on branches in Figure 1B are posterior probability values higher than 75%.

pigs with 0.743±0.039, while the Taiwanese Lanyu pigs with 0.090±0.042 (data not shown). Meanwhile, the AMOVA revealed that the majority of the variations were observed among the populations with 79.66%, while 20.34% could be attributed to within-population differentiation (Table 1).

To clarify the phylogenetic relationships of these individuals with greater certainty, maximum likelihood analysis (ML) and Bayesian inference (BI) (both model-based approaches) and median-joining (MJ) network analyses were performed. In addition, Asian and European domestic pigs and wild boars were included in the phylogenetic analyses to further infer the relationships and demographic distribution of our tested Lanyu pig population. To avoid overestimating the analysis, only two of the Lanyu pig diagnostic motifs “ACACAAACC” were included in the analysis to complement the rest of the sequences, including those from the GenBank. The result revealed that the Lanyu pigs formed a separate clade from the major Asian and European domestic and wild boars (with a difference of 12 mutational steps), with significant posterior probability for the BI (Fig. 1) and strong SH-aLRT and UFBoot support for the ML tree (not shown). Meanwhile, the FWB was positioned at the base of the tree, suggesting it as the oldest evolutionary branch of the Lanyu clade. This result is consistent with Li et al. (2017), who found that the FWB and Taiwanese Lanyu shared similar substitution patterns and diagnostic motif repetition. Similarly, the FWB showed two distinct lineages, one with pure FWB and the other FWB with Lanyu pig signatures. Interestingly, two subclades of
Lanyu pigs were clearly visible in the tree, the Taiwanese Lanyu subclade (TLSC) and the Philippine Lanyu subclade (PLSC), with significant probability support of 75% and 98%, respectively. Although the TLSC population exhibited morphological characteristics that differ from the PLSC (e.g., Figs. 1A,B between 1A-D), similarities between the two populations remain evident, such as the ear orientation, coat color, size and body structures (Fig. 1a). However, based on the morphological characteristics such as having tusks and long snout features, the PLSC inhabiting Palawan may have experienced feralization.

The phylogenetic distinction of PLSC was further supported using a classification tree that depicted the detailed polymorphic sites of each haplotype presented in Figure 2. Screening and detection of ancestral mutational motifs using BioEdit ver.7.1 (Hall, 1999) revealed that the PLSC has an exclusively rare transversion at site T143A, which is not present in the TLSC. Similarly, a substitution at site C390T was observed only in the TLSC and was absent in the PLSC. However, both subclades shared D-loop motifs at site G965A, a motif that is absent in the FWB. Meanwhile, the MJ networks showed consistent results with the phylogenetic tree (Fig. 3). The low haplotypic diversity of TLSC haplotypes was also apparent, which could be associated with the prevalent inbreeding between the small Lanyu populations (Chang et al., 2009) that has become a conservation concern. This contrast with the PLSC haplotypes that possessed relatively high haplotypic diversity (ranging from 1 to 7 mutational steps) distributed in the different regions in the Philippines (i.e., four haplotypes in Kalinga, three in Ifugao, four in Palawan, two in NL, and one in Bohol), with Hap26 from Palawan and Bohol as the potential ancestral haplotype. Thus, the presence of Lanyu pig signatures in Palawan (west of the Philippines) is intriguing being the only island that was potentially connected to the Asian Continent during the Last Glacial Maximum (Voris, 2000), which could likely point out the continental trajectory of these unique pigs.

**Discussion**

Although wild boars are endemic in Taiwan, archaeological evidence suggests that domestic pigs were not present during Neolithic Taiwan and were introduced only after 1500 BP when Taiwan entered the Metal Age (Li, 2013; Li et al., 2015). This contrasts with the Philippines, where domestic pigs appeared to have been present since the Neolithic and early Metal Age, as evidenced by the presence of the archaeological remains at Nagsabaran in Northern Luzon (NL), which has been described as the earliest record of the introduction of domestic pigs in ISEA (Piper et al., 2009; Larson et al., 2005; Amano et al., 2013). Scholars suspected this as part of the Neolithic package of the so-called “Out of Taiwan” for Austronesian dispersal (Bellwood, 2006) which however, was challenged due to the absence of domestic pigs in Taiwan, arguing for the earlier arrival of domestic pigs into the Philippines. In fact, if domestic pigs were indeed present in Taiwan during the Neolithic and their dispersal corresponded to the migration patterns of the earliest Neolithic people into the Philippines and subsequently to the Pacific Islands, Pacific pigs should have inherited the unique maternal signatures of the Lanyu pigs, which would have been conserved and remained detectible in their mtDNA patterns. However, studies have revealed that the phylogeographic pattern of mtDNA of domestic pigs in the Pacific is dominated by pigs of European and Asian maternal ancestry, particularly of Chinese
origin (Gongora et al., 2004), highlighting the close Neolithic connections between Vietnam and ISEA (Piper et al., 2009). Despite the insufficient comparison data in the Indonesian Archipelago, it cannot be ruled out that Austronesians brought domestic pigs with Lanyu signatures but may have not reached the Pacific, which would refute the contemporaneous eastward trajectory of domestic pigs. Therefore, additional evidence of pigs with Philippine Lanyu signatures in the southern Philippines, particularly in the contact zones between the Mindanao Islands, the Indonesian and Pacific Islands, should be required to establish a clear dispersal trajectory of these unique pigs.

The prehistoric exchange of culture and geneflow between the Philippines and Taiwan

The frequent interactions between Lanyu (Orchid Island) and the Batanes Archipelago islanders have been documented up to ~300 YBP, as evidenced by the material culture and animal exchanges elicited by these human movements (Chen, 2001; Llorente, 1983; Loo et al., 2011). Similarly, artifacts dating between 2,500 and 1,500 YBP have been discovered on both Lanyu Island and Batanes (Hung et al., 2007). Thus, this migration might be paralleled with the possible long-distance migration of domestic pigs by humans in the post-Neolithic period, which favored the dispersal of the unique Lanyu pigs. This may have occurred in a bidirectional gene flow, similar

Figure 3. Median-joining networks of mitochondrial DNA D-loop sequences of A) Philippine pigs from five major lineages and the B) all the Lanyu pigs identified so far. Color indicates where the sequences originated.
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Taiwan (Lanyu Islet). This may have taken place before the Philippines (likely via Palawan) before finally reaching Borneo since a close relationship of 92% of the non-volant mammal community has been demonstrated between these two islands (Esselstyn et al., 2004). In fact, Jiang et al. (2008) have proposed that the ancestor of the Type I Lanyu pigs may have radiated from MSEA through Sundaland via Palawan as the potential dispersal corridor of Lanyu pigs

The southwestern Philippine island of Palawan is considered the northeastern boundary between the Sundaic biogeographic region and the isolated oceanic islands of Wallacea (Piper et al., 2011). Although it has been previously suggested that Palawan is the likely gateway for the exchange of numerous maternal lineages of domestic pigs (e.g., haplogroups D2 and D7) into the Philippines (Layos et al., 2022a), no evidence for the existence of Lanyu pigs in Sundaland and MSEA has been reported to date.

As a dispersal corridor (Diamond & Gilpin, 1983; Brown et al., 2013), Palawan has demonstrated to be an important geographic region that has essentially provided insights into the evolutionary relationships between some Philippine wild pigs (e.g., *S. barbatus*) and the other endemic ISEA wild pigs (Frantz et al., 2013). Since studies on the genetic diversity of domestic pigs in Borneo are still insufficient, a thorough genetic characterization of the relationships between the two populations in these two important regions should advance the discussion of the spatio-temporal expansion of Lanyu pigs. Nevertheless, it cannot be ruled out that a prehistoric exchange of Lanyu pigs took place between Palawan and Borneo since a close relationship of 92% of the non-volant mammal community has been demonstrated between these two islands (Esselstyn et al., 2004). In fact, Jiang et al. (2008) have proposed that the ancestor of the Type I Lanyu pigs may have radiated from MSEA through Sundaland via the Philippines (likely via Palawan) before finally reaching Taiwan (Lanyu Islet). This may have taken place before the last glacial maximum and may have evolved in isolation in Taiwan and the Lanyu Islet after the last glacial period ended (Meijaard, 2003). This is a phenomenon that reflects increasing evidence of colonization from south to north. For instance, the macaques and viverrids native to the Sunda Islands such as Borneo, Palawan, Java, and Sumatra, as well as MSEA, have been recorded in Neolithic sites in Northern Luzon, suggesting a south-to-north colonization of these taxa (Ochoa, 2019). This trajectory would likely implicate the Philippines as the initial domestication center of these unique pigs and the possibility of Palawan as an epicenter where pig domestication in the Philippines began. However, the need for further evidence on the neighboring island of Borneo is imperative.

Thus far, the lack of signatures of Lanyu pigs in Borneo has allowed us 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 into Palawan Island, where a new population later became established. And finally, the *S. scrofa* wild boar (of Lanyu origin) radiated into the Philippines but was extirpated, leaving the subsequent population. The latter would then justify that the wild boar *S. scrofa* is native to the Philippines. This suggests the allopatric expansion of the unique Lanyu pigs resulting from two subclades, the Taiwanese and the Philippine Lanyu subclades.

Conclusion

In summary, the results of this study and the recent developments in molecular and phylogeographic studies on Philippine domestic pigs have contributed significantly toward elucidating the complex history of animal domestication in the Philippines. However, although it is premature to conclude that the Lanyu pig was independently domesticated in the Philippines because of the limited molecular markers studied, this study suggests that humans played an important role in the expansion of the unique Lanyu pigs, refuting the unidirectional dispersal model (e.g., north to south) for these unique animals. Finally, genome-wide studies and the divergence-time estimates of Lanyu pigs in both the Philippines and Taiwan should be performed which could reconsider the complex evolutionary and anthropogenic history of pig dispersal in ISEA.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Accessibility Statement

All sequences analyzed in this manuscript are available at GenBank database (Accession numbers: OL957183–OL957251; MN625805-MN625830; MW924902-MW92973; KP987300.1-KP987302.1; KT895077.1; KP987304.1; MT904797–MT904875).


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