DOCTORAL THESIS

Evolutionary History of *Gallus gallus* in Southeast Asia and South Pacific: Genetic Insights into its Phylogeography and Population Dynamics

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GENERAL RESEARCH OVERVIEW

1.1 Introduction

The domestication of animals has led to significant shifts in human demographics that helped shape early population communities. Decades of research on when, where, and how domestication took place have led to a better understanding of the complex past societies, though several important questions remain unresolved (Larson & Fuller, 2014). Chickens are the most widely domesticated animal species in the world. It plays a crucial role in human societies as the largest source of animal protein (FAO, 2015; Lawler, 2020) and as a significant factor in socio-cultural development (Sykes, 2012). Since domestication, chickens have been distributed throughout various countries and continents and have resulted in a wide range of chicken breeds today (Groeneveld et al., 2010; Malomane et al., 2019). Even crossing multiple cultural and religious boundaries, chickens are bred across every continent except Antarctica (Lawler, 2015).

In 2020, the global chicken population was over 33 billion, and more than 1,600 different local chicken breeds are internationally recognized (FAO, 2022). Despite their global distribution, studies on their process of domestication and translocation history remain obscure. Modern biological and zooarchaeological approaches suggest that chicken domestication probably occurred across southwest China and Southeast Asia, involving one or more wild progenitors across their native geographical range (Eda et al., 2016; Liu et al., 2006; Miao et al., 2013; Peters et al., 2016; Wang et al., 2020). Subsequently, domestic chickens have been translocated from their domestication centers to every region inhabited by human migration and trade expansion. This led to the evolution of subpopulations of chickens in response to natural selection pressure and selective breeding for adaptation to various agro-ecological conditions (Lawal & Hanotte, 2021).

The genus *Gallus* includes four morphologically and genetically distinct species (Delacour, 1977; McGowan & Kirwan, 2020): (i) *Gallus gallus* (Red junglefowl), which has a

geographic distribution spanning continental South and Southeast Asia; (ii) *Gallus sonneratii* (Grey junglefowl) inhabited South and West India; (iii) *Gallus lafayettii* (Ceylon junglefowl) endemic to Sri Lanka; and (iv) *Gallus varius* (Green junglefowl) endemic to Java and neighboring islands (Delacour, 1977; Lawal et al., 2020) (see geographic distribution in Figure 1). Additionally, the polytypic red junglefowl consists of five subspecies: (i) *G. g. gallus*, (ii) *G. g. spadiceus*, (iii) *G. g. murghi*, (iv) *G. g. jabouillei*, and (v) *G. g. bankiva*, having considerable variations in the plumage color and the shape of male hackles and the color of the earlobe. *G. g. spadiceus* and *G. g. jabouillei* have red earlobes, while *G. g. gallus*, *G. g. murghi*, and *G. g. bankiva* have white (Delacour, 1977; Nishida et al., 1985, 2000).

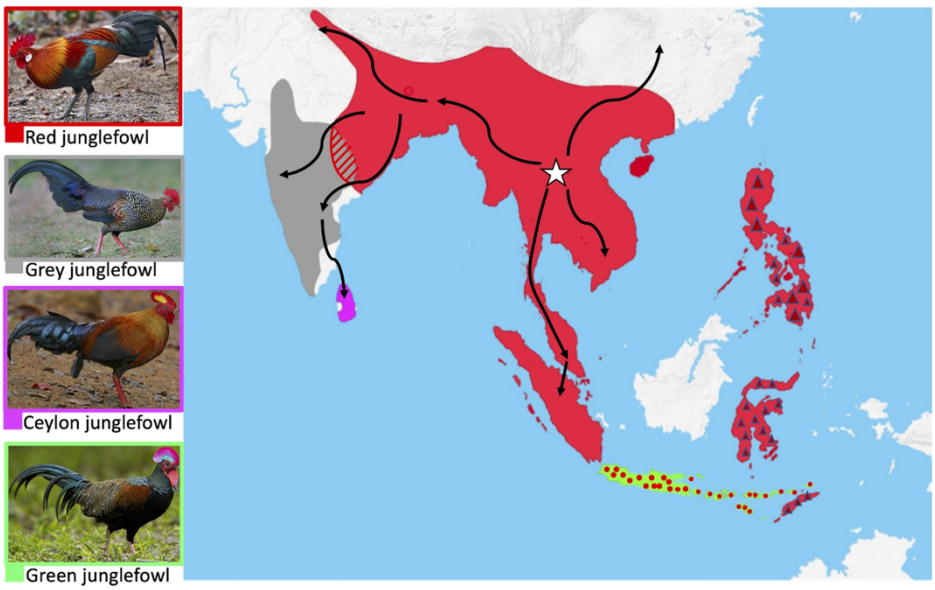

Figure 1.1. Map showing the geographic distribution of the junglefowls species across Asia (adapted from Lawal *et al.* 2020 – http://creativecommons.org/licenses/by/4.0). The white star represents the domestication from *G. g. spadiceus* in Southeast Asia (Wang *et al.* 2020). The arrows illustrate the dispersion of domestic chicken across Asia.

Southeast Asia (SEA), being the most geographically complex tropical region on Earth, has given rise to a diverse and highly endemic avifauna (Lohman et al., 2011; Myers et al., 2000). In particular, the Island Southeast Asia (ISEA) is an exceptional theatre for extraordinary species richness and endemism that exists in one of the world's most biologically rich and geologically dynamic regions. Four leading biodiversity hotspots are in the Malay

Archipelago: Indo-Burma, the Philippines, Sundaland, and Wallacea (Myers et al., 2000). These biodiversity hotspots have long been considered a natural laboratory for studying evolution and biogeography (Lohman et al., 2011).

The rich history of human migrations and settlements in the ISEA provides interesting records of earlier agricultural populations in the Malay Archipelago (Bellwood, 2007; Piper, 2017). The mid-Holocene human migration epoch in the ISEA was believed to have brought varieties of material culture, initial farming communities for rice agriculture, and domestic animals, particularly dogs, pigs, and chickens (Diamond & Bellwood, 2003; Piper, 2017). The emergence of agricultural societies harboring domesticated animals allowed a remarkable expansion of genetically divergent domestic populations, a case seen in chickens that likely followed a commensal route of the domestication process (Larson & Burger, 2013). In addition, the region's favorable seasonal weather patterns and vegetation make it a suitable environment for chicken dispersal (Mittermeier et al., 2004; Peters et al., 2016; Pitt et al., 2016).

Early studies reconstructing the matrilineal history of domestic chickens based on mitochondrial DNA (mtDNA) analysis supported a monophyletic origin of the red junglefowl, which serves as the primary wild ancestor of domestic chickens (Fumihito et al., 1994, 1996). However, in the early 21st century, numerous mtDNA analyses suggested multiple domestication events (Kanginakudru et al., 2008; Liu et al., 2006; Miao et al., 2013) and the possibility of other *Gallus* species contributing to the genetic composition of domestic chickens (Eriksson et al., 2008; Lawal et al., 2020; Mariadassou et al., 2021; Nishibori et al., 2005). Moreover, recent genome-wide data closely linked domestic chickens to the Southeast Asian subspecies *G. g. spadiceus*, which locally interbred with other subspecies across South and Southeast Asia (Wang et al., 2020).

Mitochondrial DNA (mtDNA) D-loop variation has been extensively used to gain a better understanding of chicken populations, types, evolutionary relationships, and

domestication history. Chickens have been classified into eight highly divergent maternal haplogroups (A–G, V) and six rare haplogroups (H–I, W–Z) (Huang et al., 2018; Miao et al., 2013). Major haplogroups A and B were ubiquitously distributed in Asian regions, whereas haplogroup E was widely distributed in Europe, the Middle East, Africa, and South America (Al-Jumaili et al., 2020; Herrera et al., 2020; Miao et al., 2013; Mwacharo et al., 2011). Haplogroup C was distributed over East Asia, whereas haplogroup F was restricted to Yunnan, China and Myanmar (Huang et al., 2018; Miao et al., 2013; Mon et al., 2021). Haplogroup D was mainly found in SEA and Pacific populations (Dancause et al., 2011; Godinez et al., 2021; Miao et al., 2013; Thomson et al., 2014). However, significant challenges from the zooarchaeological perspective remain as only a few reports of chicken bone traces in SEA (Storey et al., 2012) and prehistoric exploitation has yet to be elucidated (Eda et al., 2019). Such evolutionary links would likely provide a better understanding of the evolutionary history and population dynamics of the world's most common farm animal. The knowledge of population studies on genetic diversity, population structures, and demography is essential to understanding the role of past and present evolutionary processes of chickens over the course of domestication.

In this thesis, complete mtDNA D-loop sequences of chickens from mainland SEA (i.e., Cambodia, Laos, Thailand, and Myanmar), ISEA (i.e., the Philippines), and South Pacific (i.e., Fiji) spanning a geographical transect that is believed to encompass possible translocations of this species in the region were generated. By combining these newly generated sequence data with previously published sequences from ISEA (the Philippines and Indonesia), the Pacific, and other neighboring chicken populations in Asia, this study sought to obtain a new perspective on the matrilineal phylogeny, phylogeography, and population dynamics that shaped the diversity of SEA and Pacific chickens.

1.2 General Objectives

This thesis focuses primarily on the evolutionary history and genetic characterization of *Gallus gallus* in Southeast Asia and the South Pacific to better understand and gain a new perspective on their phylogeography, population structure, genetic diversity, demographic history, and population dynamics. The complex geographical and temporal origins of chicken domestication continue to be of most interest in molecular phylogeny and phylogeographic studies as they remain unresolved. As one of the world's most geologically dynamic regions, Southeast Asia has given rise to incredibly rich biodiversity hotspots. However, insufficient evidence links the present-day chickens to their founding lineages due to limited phylogeographic studies of this species in the region. On a broader scale, this thesis is expanded to address the lack of phylogenetic studies by having a large-scale sampling from SEA and the South Pacific spanning a geographical transect that is thought to encompass faunal translocations in the region. The following specific objectives have been set:

1.2.1 Specific Objectives

1. Provide an updated mtDNA dataset of RJFs and domestic chickens from ISEA and the South Pacific and characterize their genetic diversity and population structure.
2. Elaborate on the evolutionary history, phylogeography, and translocation scenarios of the ISEA chicken populations.
3. Elucidate finer resolution of the matrilineal phylogeny and population dynamics of *Gallus gallus* in SEA and the Pacific, together with other available sequence data of Asian chickens.
4. Estimate the lineage-specific divergence times and evaluate the genetic similarities and differentiation between continental and island chicken populations.

1.3 Ethics Statement

Animal care and experimental procedures were approved by the Institutional Animal Care and Used Committee Guidelines of Hiroshima University as established by the Laboratory of Animal Genetics, Graduate School of Integrated Sciences for Life (Approval No. 015A170426).

1.4 Review of Related Literatures

1.4.1 The spread of agriculture in Southeast Asia

The initial postulated domestication of plants and animals began around 12,000-11,000 years ago at the end of the Pleistocene (Diamond & Bellwood, 2003; Larson et al., 2014). The subsequent independent domestication process occurred at different times, between 8,500 and 2,500 years ago, over all inhabited continents except for Australia (Diamond & Bellwood, 2003). The development of agriculture over the past millennia, generally due to human forethought and activities and eventually exodus, was undeniably driven by several geological, ecological, biological factors, and human cultural exchanges (Bellwood, 2005; Larson et al., 2014). Such development provides almost all the world's food has occurred over the past thousand years and continues apace.

In mainland Southeast Asia, the advent of agriculture was mainly derived from the expansion of farming communities originally from South China (i.e., Yangtze Valley) (Liu et al., 2007) and spread south, via the coast and the major rivers, to enter the broad riverine plains of Southeast Asia (Higham, 2021; Higham & Higham, 2009). Consequently, the recorded cultural markers of Austroasiatic-speaking populations settled in the village communities were thought to move further southwards to Laos, south Vietnam, Cambodia, and Thailand. Rice and millet farming was documented in the region from about 4,500 to 3,700 years BP, with its ancestry traced back to Southern China (Bellwood, 2005; Castillo et al., 2016; Gutaker et al.,

2020; Higham, 2021). As being raised for food and some secondary products, animal domestication followed the emergence of mixed-crop farming societies roughly around the Neolithic period (Zeder, 2012). As a result, numerous animals domesticated became incorporated into human cultures, including but not limited to dogs, pigs, and chickens (Frantz et al., 2020; Piper, 2017).

Meanwhile, in ISEA, the agricultural expansion of early farmers in South China also reached Taiwan and led to the "Out-of-Taiwan" diaspora of the Austronesian-speaker populations to the rest of the island archipelago and as far as Remote Oceania (Bellwood, 2007; Diamond & Bellwood, 2003). The dispersal of rice farming to ISEA took place later, after about 2,500 years BP, as rice populations established themselves in Indonesia, Borneo, and the Philippines (Gutaker et al., 2020). However, there are contending views of prior settlements in the island archipelago before the arrival of rice agriculture in the area (Larena et al., 2021). Recent genomic analysis of rice likewise indicates that tropical rice that arrived in the insular was identified as a subpopulation from mainland SEA rather than Taiwan (Alam et al., 2021). Similarly, multiple translocation events probably resulted in two or more lineages of pigs, dogs, and chickens entering the ISEA from northeast and west via MSEA.

Undoubtedly, studying domestication processes is preeminently multidisciplinary. Many hypotheses suggest that episodes of population expansion occurred as dependence on farming and herding and further correlated with the spread of cultures, languages, and genes. Of course, this study bid the limitations of not claiming that the findings of this research work provide a definite resolution to what was a seemingly perplexing topic. But could potentially contribute a piece to a new independent line of evidence as a biological proxy to infer the origin and development of agriculture.

1.4.2 Translocations of animal domesticates in SEA and Oceania

The multistage process of animal domestication exemplifies ways in which animals respond to anthropogenic niches. The seminal paper of Darwin (1868) initially recognized the pervasiveness of a wide variety of traits that differentiate animal domesticates from their wild progenitors. Later, Zeder et al. (2012) described three separate domestication scenarios that animals followed into domestication: a commensal pathway, a prey pathway, and a directed pathway. These characterized animal domestication trajectories are crucial for defining appropriate population genetic models for the inference of the origins and subsequent evolution and demography of domestic animals (Larson & Burger, 2013).

The introduction of domestic animals, particularly dogs, pigs, and chickens, into the MSEA and ISEA was deliberately linked with the dispersal of agricultural societies. Pigs, dogs, and chickens follow the framework establishing a commensal relationship with humans. This association labeled domesticates attracted to the anthropogenic environments consciously manipulated by humans (for example, wild animals feed on human refuse). At some point in this association, these animals take advantage of the resources of the human hosts and would likely develop evolutionary adaptation along the way.

1.4.2.1 Dogs

Dogs were the classic example of a domestic animal that likely followed a commensal pathway (Zeder, 2012). They were exclusively derived from gray wolves (Lindblad-Toh et al., 2005) and were the first domestic animal before the advent of agriculture (Larson et al., 2012). Genomic and archaeological evidence on the origins of dogs indicate that they have been domesticated independently in East Asia (Frantz et al., 2016; Wang et al., 2016) and Western Eurasia (Frantz et al., 2016) from distinct wolf populations. The origins and routes of translocations of the domestic dog in MSEA and ISEA have focused on dingo lineage (Larson et al., 2012) and likely originated in southern China and translocated through Southeast Asia and Indonesia (Oskarsson et al., 2011).

1.4.2.2 Pigs

Pigs, just like dogs, also followed a commensal route of the domestication process because they are opportunistic scavengers that feed on refuse around human habitats and are drawn to anthropogenic environments (Larson & Fuller, 2014; Zeder, 2012). However, pig domestication in MSEA is fairly complex. It appears to have undergone domestication multiple times (Larson et al., 2010), and long-term gene flow between domestic pigs and wild boars during and after domestication was evident throughout Eurasia (Frantz et al., 2015). One of the lineages (i.e., Pacific clade) likely originated in Southeast Asia, provided an interesting line of evidence of pig translocations in the region. The Pacific clade signature of pigs can be traced from southern China/northern Vietnam through Laos and into peninsular Malaysia, Sumatra, Java, and as far as Melanesia (Larson et al., 2005, 2007), but has never been documented in pigs from Taiwan or the Philippines (Larson et al., 2007). In another study, Larson et al. (2010) detected wild boars containing Pacific clade signature in Laos and Yunnan province, China. More recent findings documented one haplotype of the Philippine pigs (i.e., PHL11 from Bohol Island) that has the same mutational signature as Pacific clade wild boar. This signal is likely introduced from MSEA via peninsular SEA (Layos et al., 2022). These expanded the geographic distribution of pigs having Pacific clade signatures and supported the hypothesis of this lineage's peninsular Southeast Asia origin. This evidence supports the postulated Neolithic expansion of the Austroasiatic agricultural population along the major Southeast Asian rivers (Larson et al., 2010; Piper, 2017).

Another indigenous *Sus* lineage exclusively found in the Indo-Burma Biodiversity Hotspots (IBBH), the so-called MC3 or previously MTSEA distributed in Southeast Asia, consists of wild and domestic pigs (Larson et al., 2010; Tanaka et al., 2008). This clade possessed unique mtDNA signatures that are restricted to the IBBH. Although, a subsequent report using mtDNA samples of Philippine pigs identified at least 20 unique haplotypes of D7

or MC3 (previously MTSEA) detected in major islands of the Philippines (Palawan, Cebu, Samar, Panay, and Bohol) (Layos et al., 2022). This recently disclosed evidence that D7 (MTSEA) lineage in the context of the Philippine pigs had a significantly larger ancestral population size than all other classified lineages in the region. The potential route of translocation of this lineage entering the Philippines likely dispersed from MSEA via the Sunda region during the Pleistocene through the Sulu archipelago and Palawan, and thereafter spread throughout the Philippine islands (Layos et al., 2022).

Domestic pigs that became synonymous with the Austronesian expansion into the Pacific had signatures found in wild boar indigenous to peninsular Southeast Asia (Larson et al., 2007). However, some Philippine native pigs carry a unique lineage of Lanyu-type referred to as the Philippine Lanyu subclade (Layos et al., 2022), which appeared as a sister clade from the identified Lanyu pigs initially from the island of Lanyu off the coast of Taiwan (Li et al., 2017). This indicates that the Lanyu haplotype might have been introduced to the northern Philippines by the Austronesians sometime in the past (Bellwood, 2017; Piper, 2017) or vice versa (Layos et al., 2022). Perhaps, these scenarios hint at the complexity of pig domestication in the MSEA and ISEA.

1.4.2.3 Chickens

The association of chickens to the agricultural societies from anthropophilic wildfowl, which was attracted to kitchen scraps, animal dung, and crop-processing wastes, made this species a critical commensal domesticated animal (Larson & Fuller, 2014). Since their domestication, chickens have been dispersed globally across multiple cultural and religious boundaries. The initial proposal of Darwin (1868) discussed that domestic chickens are probably descendants of the Malayan or Indian junglefowl (*Gallus bankiva*, later replaced with *Gallus gallus*) based on several lines of evidence: (1) that the birds resemble each other; (2) can make fertile offspring; (3) tameness characteristics of the wild progenitor. Darwin's first

claim on the monophyletic origin of chickens was widely accepted and even supported by the evidence of continental junglefowl (from Thailand and its adjacent region) as the matriarchic origin of domestic chickens (Fumihito et al., 1994, 1996). However, with the increase of genetic data and resolving power of sequence data in recent years, other succeeding studies suggested a polyphyletic origin of domestic chickens which descended from two or more of the four extant wild junglefowl (Kanginakudru et al., 2008; Lawal et al., 2020; Liu et al., 2006; Wang et al., 2020).

Indeed, the domestication of chickens seems to have a long history. West and Zhou (1988) suggested that chickens were first domesticated in Southeast Asia and were taken north to China and south to peninsular SEA and as far as Sumatra. Other recent genetic evidence validated this claim hinting at the origin of chicken domestication across southwestern China and Southeast Asia, involving one or more wild progenitors across their native geographical range (Lawal et al., 2020; Peters et al., 2016; Wang et al., 2020). Chickens colonized the world from continental Southeast Asia through human-mediated translocation and terrestrial and maritime trading routes. Genetic diversity studies suggested that red junglefowl and indigenous chickens in Thailand (Hata et al., 2021; Teinlek et al., 2018), Laos (Kawabe et al., 2014), Vietnam (Cuc et al., 2011), Cambodia (Ren et al., 2022), and Myanmar (Mon et al., 2021) have large gene pool and diverse matriline with extensive genetic diversity that has been conserved in the populations for a long time and that some were a subset of the red junglefowl population involved in the domestication.

The course of chicken introduction in ISEA continues to be a research area of interest, considering the rich history of human diaspora and colonization in the insular archipelago. Previous data sources argued that chicken populations containing haplogroup E matriline first arrived in the Pacific (Storey et al., 2012), but later studies found that most modern and all ancient chickens in the Pacific were haplogroup D rather than haplogroup E (Herrera et al.,

2020; Thomson et al., 2014). A recent study documented the presence of a distinct island chicken population subgroup, the "Philippine-Pacific subclade" (Godinez et al., 2021), classified elsewhere as "Polynesian D" (Thomson et al., 2014), represents a unique genetic uniformity between the Philippines and the Pacific despite their geographical isolation. This latterly expanded matriline is unique to this region, suggesting a human-mediated scenario on its translocation. Their phylogeographic signal likely corresponds to the initial introduction pattern of its founding matrilines (i.e., sub-haplogroup D2) from MSEA (Godinez et al., 2021). This translocation pattern was presumably influenced by the migration of the Negritos, the Manobo ancestry, and the Sama ancestry across the continental landmass of Sundaland, entering the southern Philippines and Palawan (Jinam et al., 2012; Larena et al., 2021; Lipson et al., 2014). Contrarily, the unidirectional north-to-south translocation of East Asian chicken haplotypes (from Taiwan) does not support the genetic relatedness with the island chicken populations from either the Philippines or the Pacific region (Chang et al., 2012; Godinez et al., 2021). Instead, Taiwan indigenous chickens (e.g., Ju-Chi) and gamecock (Hua-Tung) share genetic similarities with the East Asian chicken haplotypes from China, Japan, and the Indian subcontinent.

1.4.3 Zooarchaeological and paleoenvironment evidence of chickens

Archaeological evidence elucidated by West and Zhou (1988) suggests that chickens were first domesticated in Southeast Asia from the red junglefowl *Gallus gallus* well before the sixth millennium BC. They identified 90 archaeological sites containing chicken bone remains in Europe and Asia, 29 were predating the recovered material in the region of Indus Valley *c.* 2000 BC from the evidence at Mohenjo-Daro (West & Zhou, 1988; Zeuner, 1963). The archaeological sites from Neolithic Peiligang (Henan Province; 5935 ± 480 to 5495 ± 200 BC) and Neolithic Cishan (Hebei Province; 5405 ± 100 to 5285 ± 105 BC) were thought to be the world's earliest records of domestic chickens, dated by calibrated radiocarbon analysis

(West & Zhou, 1988). Initially, Crawford (1984) noted that the radiation of junglefowl reached the early sites in northern China rather than through domestication. Delacour (1977) recognized that the junglefowl avoids alluvial plains, hindering their further expansion. Other types of vegetation seem to provide barriers to their northward diffusion: high mountains and steppe to the north and west of the western distribution area, and subtropical forest (dry and wet hardleaf evergreen) north of the eastern area (West & Zhou, 1988). The concordance of archaeological (West & Zhou, 1988) and paleoenvironmental evidence (Crawford, 1984; Ho, 1977) defined little changes in the biogeographical distribution of wild junglefowl in Southeast Asia and China during the past 10 millennia.

Ancient mitogenetic signatures of galliform bones recovered at the Neolithic sites in northern China concluded an earlier Holocene chicken domestication event (Xiang et al., 2014). However, these ancient chicken sequences fell into the major haplogroups of modern domestic chickens, suggesting they are derived from domesticated populations. Perhaps, West & Zhou (1988) argument nearly three decades ago remains relevant, citing those chickens from Neolithic sites in northern China were domestic birds introduced by humans. Archaeological sites of Non Nok Tha, Ban Chiang, and Ban Na Di in Thailand also recorded culturally important evidence of stock-raising around *ca*. 4,000-3,000 BP (Higham, 1989). Bones of animals (e.g., pig, cattle, dog, deer, and chicken) and clay animal figurines were excavated in the human burial sites, suggesting that animals were part of the ritual practices during prehistoric inhumation (Higham, 1989). Recent morphological bone identification further documented the existence of chicken remains from other known archaeological sites in Thailand as early as 4,000 BP (Eda et al., 2019). Evidence from modern mitogenome analyses of domestic chickens in China, focusing on certain haplogroups restricted to modern-day chickens, supported a recent domestic chicken expansion in northern China and denied the scenario of early Holocene chicken domestication in this region (Huang et al., 2018).

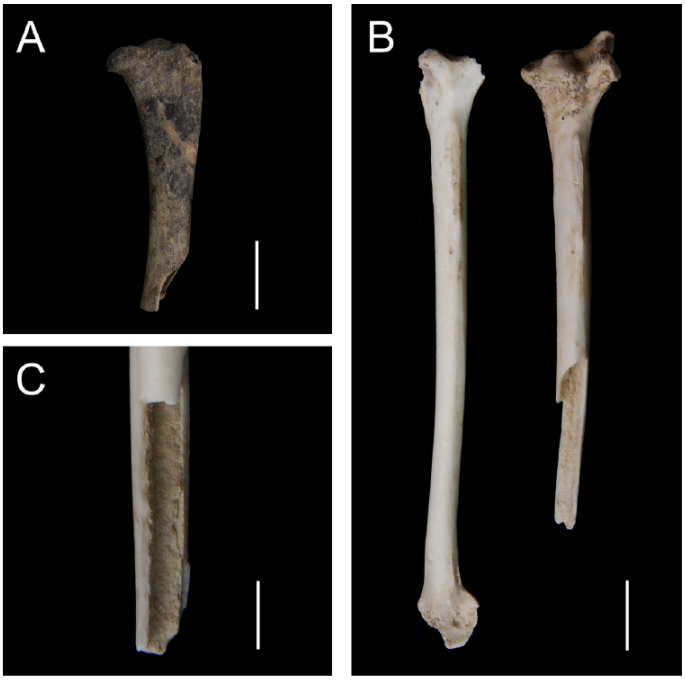

Figure 1.2. Candidate chicken bones from Xiawanggang (A) and Zaoshugounao (B and C). **A**: femur of immature candidate chicken; **B**: tibiotarsi of candidate chicken; **C**: medullary bone found in **B** (adapted from Eda *et al.* 2016). Scale bars: 1 cm.

Following the paleoenvironmental evidence, habitat requirements of red junglefowl were absent during the early and middle Holocene of northern China (Peters et al., 2016; Pitt et al., 2016). Red junglefowl is inhabitants of warm habitats at low and moderate altitudes. They are non-migratory birds with limited flight ability and aboriginals of forested habitats. In case the *Gallus* are dispersed to northern China, they forcibly need to cross major ecogeographical barriers such as the vast East-West running water courses bordered by steep rock formations, extensive floodplains, and swampy vegetations (Peters et al., 2016). Instead, they suggested that domestication in an area of poor environmental suitability would be unlikely to succeed. This posit contending views from the report of Xiang et al. (2014) on domestic chickens in northern China during the Holocene, that the purported chicken bones in this region are instead derived from a different species (Peters et al., 2016; Pitt et al., 2016).

The reappraisal of Eda et al. (2016) based on osteomorphological identification criteria of 280 Phasianidae hindlimbs bones (femur, tibiotarsus, and tarsometatarsus) from 11 Neolithic

sites in China extensively distinguished chicken bones from other indigenous Phasianidae species. This morphological re-assessment revealed that these unearthed bones were represented by pheasants (Deng et al., 2013; Eda et al., 2016). Instead, Eda et al. (2016) noted a candidate chicken bone from the Neolithic Xiawanggang (~5.0 to 4.0 cal. BP) in the Yangtze River basin and two potential chicken bones identified from the early Bronze Age Zaoshugounao (3.2 to 3.0 ka cal. BP) located in the Wei River valley (Peters et al., 2016) (Figure 1.2). These concluded that red junglefowl was not extensively distributed, nor domestic chickens were not widely kept throughout central and northern China during the early and middle Holocene (Eda, 2021; Eda et al., 2016).

The earliest evidence of chicken bones in Near Oceania is associated with the initial development of the Lapita Cultural Complex, dated between 3,350-3,300 BP (Bedford et al., 2006; Piper, 2017; Storey et al., 2008; Summerhayes, 2007). Direct dates of two chicken bones from archaeological deposits across the Teouma site in Vanuatu were calibrated between *ca.* 3,100-2,700 cal. BP, indicating that domestic chickens were introduced during the earliest phase of site occupation (Petchey et al., 2015). Ancient mtDNA of Polynesian chickens recorded at Anakena site in Rapa Nui Island, Makauwahi site in Hawaii, and Anatoloa in Nuie Island, support a domestic chicken dispersion towards the east, up to Hawaii and as far as Easter Island (Thomson et al., 2014). These ancient chicken samples showed a high level of genetic continuity relative to their founding mitochondrial lineage from the Philippines despite extensive European settlement over the past centuries (Thomson et al., 2014). It is likely that the introduction of chickens in the Philippines must have been prior to the earliest recorded archaeological remains in the Pacific. However, reports of chicken traces in the Philippines (or in Southeast Asia in general) are scarce and prehistoric exploitation has yet to be explored (Eda et al., 2019; Storey et al., 2012). Factors that may include butchering practice, consumption, toolmaking, and predation of pigs, dogs, and rats are likely the reason why almost nothing is

known about the population history of chickens in ISEA (Storey et al., 2008). Therefore, the discovery of archaeological data in ISEA will likely provide evolutionary links to better understand the population dynamics of the world's most common farm animal.

1.4.4 Phylogeny, introgression, and divergence between *Gallus* species and between domestic chickens and wild extant RJF (sub)species

Modern biological approaches documented that other red junglefowl subspecies and wild junglefowl species contributed to the domestic chicken genetic profile. Darwin (1868) initially thought that domestic chickens descend from *Gallus bankiva*, later replaced with *Gallus gallus* because they resemble each other and can reproduce progeny. However, the mtDNA and genome-wide analyses suggested multiple *Gallus gallus* subspecies as the wild progenitors of the domestic chickens, where they locally interbred across their natural geographical range in Southeast Asia and southwestern China (Lawal et al., 2020; Liu et al., 2006; Miao et al., 2013; Wang et al., 2020). It was previously reported that hybridization occurred among red junglefowl subspecies following their overlapping distribution, e.g., between *G. g. gallus*, *G. g. spadiceus*, and *G. g. jabouillei* in Southeast Asia (Delacour, 1977). Molecular evidence further indicated that inter-species hybridization occurred between *Gallus sonneratii* and *Gallus gallus* and between *Gallus sonneratii* and *Gallus lafayettii*. Although red junglefowls are considered to have largely contributed to the establishment of contemporary chicken, grey junglefowl and ceylon junglefowl also contributed to a lesser extent (Nishibori et al., 2005).

In particular, the yellow skin phenotype remains the most compelling example that linked to a *Gallus* species introgression into the domestic chicken (Eriksson et al., 2008). The yellow skin pigmentation in chickens is under the genetic control of a recessive regulatory mutation(s) in the dermal β-carotene dioxygenase 2 (*BCDO2*). Inhabiting the expression of this locus could led to a yellow skin pigmentation in chickens. The phylogenetic analysis done by Eriksson et al. (2008) showed that yellow-skinned breeds clustered with grey junglefowl, thereby

suggesting that red junglefowl is not the sole wild ancestor of the domestic chickens. Other diverse morphological variations in domestic chickens may have started from similar introgression episodes.

A recent genome-wide evolutionary study suggested that the domestic chickens diverged from red junglefowl around 8,093 years ago (CI: 7,014-8,768) (Lawal et al., 2020). The same study documented an extensive bidirectional introgression between the grey junglefowl and domestic chickens, a few estimated introgression signatures between domestic chickens and Ceylon junglefowl, and a single introgression signature between domestic chickens and green junglefowl (Lawal et al., 2020). Recently, an autosomal genome-wide investigation involving 863 genomes from a worldwide sampling of chickens, representing all four wild junglefowl species and all five wild extant subspecies of red junglefowl, shows strong support for *G. g. spadiceus* as the closest progenitor of contemporary Asian domestic chickens (Wang et al., 2020). In their phylogenetic and principal component analysis, all domestic chickens formed a monophyletic clade and showed a closer genetic affinity with *G. g. spadiceus* (Figure 1.3). Wang et al. (2020) once again disclosed that there was evidence of low admixture between other jungle species and domestic chickens, and they interbred locally across their native geographical ranges in southwestern China and Southeast Asia.

1.4.5 Mitochondrial DNA as a molecular marker

Mitochondrial DNA (mtDNA) is the genetic material in the mitochondrion that is maternally inherited (i.e., the descendants of the same maternal ancestor have almost identical mitochondria). The evolution of mtDNA occurred primarily as a single base pair substitution, with a higher mutation rate, high copy number in animals, and lack of recombination (Avise et al., 1987; Zhang & Hewitt, 1996). This maternal marker has been instrumental in identifying wild ancestors, localizing domestication centers, reconstructing colonization, and tracking population movements and trading routes (Groeneveld et al., 2010; Liu et al., 2006). The D-

loop region of mtDNA does not encode for protein and evolves much faster than other regions of the mtDNA genome, so it is the most valuable and sensitive region for investigating genetic variation within species. Although mtDNA data has several advantages and are sufficient for differentiating between broadly defined populations, they are somehow limited to quantifying degrees of admixture between populations (Larson & Burger, 2013; Zhang & Hewitt, 1996).

However, with the increasing genetic data and resolving power of computer simulation methods (e.g., coalescence simulation) in recent years, sequence data have been tested for explicit hypothesis-testing inference. Modeling methods have shown efficiency at testing different evolutionary and demographic models of expanding and migrating populations generated from ancient and modern sources (Larson & Burger, 2013).

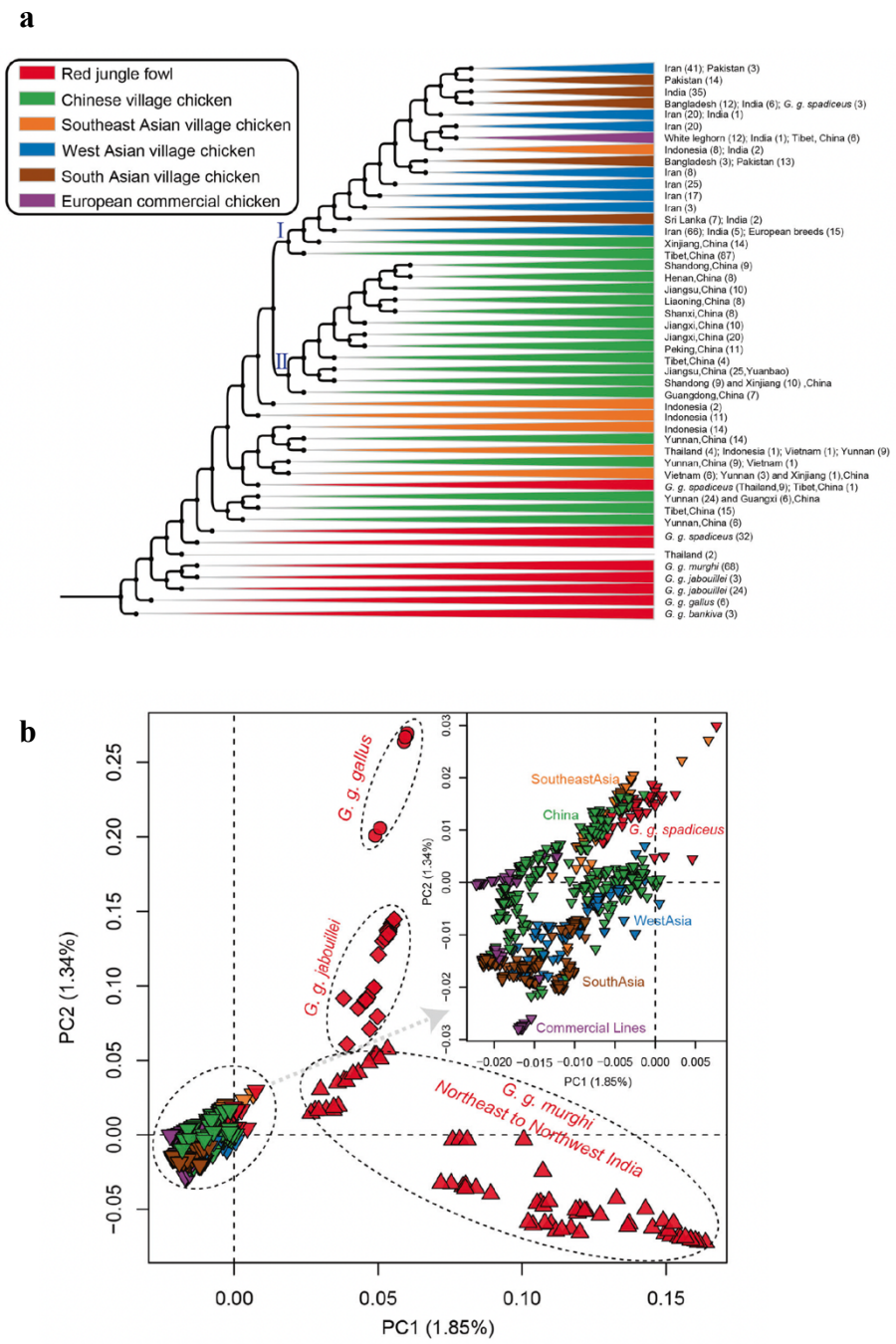

Figure 1.3. (**a**) Maximum-likelihood phylogenetic tree showing that domestic chickens form a monophyletic clade, with *G. g. spadiceus* being the closest wild progenitor. Black dots at nodes indicate ≥ 99% bootstrap support. Domestic chicken and RJF clades are collapsed and colored according to their geographic ranges and subspecies classifications. (**b**) PCA showing a closer genetic affinity between domestic chickens and *G. g. spadiceus*. RJF subspecies are denoted within rings (Wang et al., 2020).

CHAPTER II

Evolutionary history and population genetic structure of the Philippine-Pacific chickens inferred by mitochondrial DNA

Abstract

The Philippines is considered one of the biodiversity hotspots for animal genetic resources. In spite of this, the population genetic structure, genetic diversity, and past population history of Philippine chickens are not well studied. In this study, phylogeny reconstruction and estimation of population genetic structure were based on 107 newly generated mtDNA complete D-loop sequences and 37 previously published sequences of Philippine chickens, consisting of 34 haplotypes. Philippine chickens showed high haplotypic diversity (*Hd*=0.915± 0.011) across Southeast Asia and Oceania. The phylogenetic analysis and median-joining network revealed predominant maternal lineage haplogroup D classified throughout the population, while support for the Philippine-Pacific subclade was evident, suggesting a Philippine origin of Pacific chickens. Interestingly, the Philippine red junglefowls were clustered at the basal position of the tree within Haplogroup D, indicating an earlier introduction into the Philippines, potentially via mainland Southeast Asia. Another observation was significantly low genetic differentiation of Philippine chickens and Pacific chicken populations. The negative Tajima's *D* and Fu's *Fs* neutrality tests revealed that Philippine chickens exhibited an expansion signal. The analyses of mismatch distribution and neutrality tests were consistent with the presence of weak phylogeographic structuring and evident population growth of Philippine chickens (haplogroup D) in the Islands of Southeast Asia. Furthermore, the Bayesian Skyline Plot analysis showed an increase in the effective population size of Philippine chickens relating to human settlement and expansion events. The high genetic variability of Philippine chickens demonstrates conservation significance and thus, must be explored in the future.

Keywords: demographic history, *Gallus gallus*, genetic structure, mtDNA, Philippine-Pacific subclade, phylogeography

2.1 Introduction

The rich history of colonization in the Islands of Southeast Asia (ISEA) provides interesting records of earlier agricultural populations in the Malay Archipelago (Bellwood, 2007; Piper, 2017). The two-wave hypothesis of peopling in the ISEA provides varying interpretations of the prehistoric evolution of the indigenous populations in the insular (Jinam et al., 2012). The mid-Holocene migration epoch of the Taiwan-centered Austronesian speakers into the ISEA was believed to have deliberately brought varieties of material culture, initial farming communities for rice agriculture, and domestic animals, particularly dogs, pigs, and chickens (Diamond & Bellwood, 2003; Piper, 2017). However, recent genetic data documented an earlier introduction from mainland Southeast Asia (MSEA) to the insular, predating the mid-Holocene human migration model (Arenas et al., 2020; Jinam et al., 2012; Lipson et al., 2014; Soares et al., 2016). Evidence of diverse migration routes and dispersal events documented rapid human population expansion in the Philippines and the Pacific (Arenas et al., 2020; Bellwood, 2007). These human movement scenarios linking domestic animal translocation present broad interests in understanding the chicken domestication events in the ISEA.

The Philippines presents essential models for understanding Southeast Asia's evolutionary processes and species diversification. This terrestrial island faunal laboratory is considered one of the most biologically rich regions globally in animal genetic resources and offers opportunities for elucidating evolutionary and ecological processes (Brown et al., 2013; Myers et al., 2000). However, insufficient evidence links the present-day chickens to their founding lineages due to an unclear timeline of translocations and routes of dispersal across the ISEA. Unlike chickens, the route of introduction of earlier founding lineages of domestic dogs and pigs into the ISEA corresponds to the proposed origins from Southern China and across parts of MSEA and East Asia via the "out-of-Taiwan" expansion (Ding et al., 2012;

Larson et al., 2007; Oskarsson et al., 2011; Pang et al., 2009; Piper et al., 2014; Savolainen et al., 2004). Modern biological and zooarchaeological approaches suggest that chicken domestication probably occurred across southwest China and Southeast Asia, involving one or more wild progenitors in their natural biogeographic range (Eda et al., 2016; Liu et al., 2006; Miao et al., 2013; Peters et al., 2016; Wang et al., 2020; West & Zhou, 1988). Previous mtDNA studies have corroborated multiple origins for domestic chickens where both wild progenitors and their divergent descendants co-exist (Kanginakudru et al., 2008; Liu et al., 2006; Miao et al., 2013). Subsequently, domestic chickens have been translocated to every inhabited region by human migration, trade expansion, and cultural exchange. This led to the evolution of subpopulations of chickens in response to natural selection pressure and selective breeding for adaptation to a variety of agro-ecological conditions (Lawal & Hanotte, 2021).

In the Philippines, little is known whether early human populations of MSEA ancestry mediated the earlier introduction of ancestral lineage for island chickens. Although ancient DNA recovered from Polynesian chickens documented potential traces of origin from the Philippines (Thomson et al., 2014), there is no direct evidence of the earlier introduction of domestic chickens to the Philippines before 4,500 *cal.* BP. Prehistoric exploitation of chicken remains in ISEA is yet to be discovered. Perhaps, the multiple waves of human translocations most likely influenced the earlier lineages of domestic chickens introduced in the Philippines (Jinam et al., 2012; Larena et al., 2021; Piper, 2017).

Therefore, the present study extensively characterized complete mtDNA D-loop sequences of native chickens (NCs) from the Philippines and the South Pacific and Philippine RJFs to assess their matrilineal phylogeny and genetic diversity, and population genetic structure across ISEA and Oceania. In addition, the existing complete mtDNA D-loop sequences publicly available in the GenBank repository were also included in the analyses.

2.2 Materials and Methods

2.2.1 Sampling site and sample collection

Blood samples were collected from the brachial vein of the wing of RJFs (*n*=7) and NCs (*n*=100) from selected areas of Samar and Leyte Provinces, Philippines (Appendix Figure S2.1). RJFs were captured in the wild by the locals. All samples were collected following the Experimental Animal Care Guidelines established by the Laboratory of Animal Genetics, Hiroshima University (Approval No. 015A170426). Animal owners consented to the inclusion of their animals in the study.

Pursuant to the provisions of Republic Act No. 9147 (Wildlife Resources Conservation and Protection Act) and its implementing Rules and Regulations (Administrative Order No. 2004-01), biological sample collection was approved with Gratuitous Permit DENR-GP No. R08 2017-39 issued by the Department of Environmental and Natural Resources – Region VIII (DENR-R08), Philippines.

The details of the sampled animals are listed in Appendix Table S2.1. The final dataset was integrated with published complete mtDNA D-loop sequences archived in the GenBank database (Appendix Table S2.1; Table S2.3).

2.2.2 DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from stored whole blood samples of Philippine RJFs and NCs using the phenol-chloroform method following the recommended protocol described by (Green & Sambrook, 2012).

About 5.0 kbp mtDNA D-loop fragments were amplified using a long and accurate – PCR (LA-PCR) kit (KOD-FX Neo Polymerase, TOYOBO, Osaka, Japan) with chicken DNA as a template and LA-PCR primer sets: *Cytb-Forward*: 5′-TACACGAATCAGGCTCAAACAACCCCCTAGGCATC-3′, *16S-Reverse*: 5′-TGCACCATTAGGTTGTCCTGATCCAACATCGAGGT-3′ recommended by (Nishibori et

al., 2003). The reaction began with a preliminary denaturation at 94 °C for 2 min, followed by 30 cycles of DNA denaturation at 98 °C for 10 sec, annealing of primers at 57 °C for 30 sec, and primer extension at 68 °C for 2 min and 30 sec, using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, USA). Amplified fragments were used for segmental amplification of the complete mtDNA D-loop region (1.3 kbp) following the primer sets: *Gal1F* 5′-AGGACTACGGCTTGAAAAGCCATTG-3′ and *Gal1R* 5′-GCTGAGTACCCGTGGGGGTGTGGCT-3′ in 20 µl reaction volume containing 2x PCR buffer, 0.4 mM dNTPs, 0.3 µM concentrations of each primer, 0.4 U of KOD-FX Neo DNA Polymerase, and 15-25 ng of amplified fragment DNA as template. The PCR cycling condition began with a preliminary denaturation at 94 °C for 2 min, followed by 30 cycles of DNA denaturation at 98 °C for 10 sec, annealing of primers at 59 °C for 30 sec, and primer extension at 68 °C for 30 sec, using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, USA). The DNA fragments obtained from the segmental amplification were cleaned and purified using Exonuclease I (ExoI) and Shrimp Alkaline Phosphatase (SAP) to degrade the residual PCR primers and dephosphorylate the remaining dNTPs, respectively. The two PCR primers and one internal primer: *Gal1-2F* 5′ -TCCACCTCACGAGAGATCAGCAACCC-3′ (Nishibori et al., 2001) were used for the sequencing reaction. Subsequently, the mtDNA D-loop fragments were directly sequenced using 3130/3130xl Genetic Analyzers (Applied Biosystems, Foster City, USA).

2.2.3 DNA sequence editing and alignment

The 107 complete mtDNA D-loop sequences generated in this study were edited initially using the GeneStudio Pro tool (GeneStudio, Inc., http://genestudio.com/) and were aligned together with other complete mtDNA D-loop sequences across Asia using ClustalW (Thompson et al., 1994). Previous sequences of Samar RJFs (n=3) (MK085033-MK085035) and Samar NCs (n=17) (MK085038-MK085054) (Godinez et al., 2019) and other Philippine

chicken complete D-loop sequences retrieved from GenBank (n=17) were also included in the analysis (Appendix Table S2.1). Aligned nucleotide sequences (corresponding to the chicken mtDNA reference sequence, accession no. NC_040970) were edited and viewed using the BioEdit sequence alignment editor (Hall, 1999). All complete mtDNA D-loop sequences of RJFs and representative sequences from identified haplotypes of Philippine native chickens were deposited in the GenBank database with accession numbers MN986370-MN986403 (Appendix Table S2.1).

2.2.4 Genetic diversity and phylogenetic reconstruction

Intra-population level genetic diversity indices such as the number of polymorphic (segregating) sites (S), haplotype diversity (Hd), nucleotide diversity (π), and mean number of pairwise difference were estimated using the DnaSP v. 6.0 software (Librado & Rozas, 2009).

The phylogenetic tree was inferred by IQ-TREE using the maximum likelihood (ML) method (Nguyen et al., 2015) to estimate the genealogy of Philippine chickens together with the other complete mtDNA D-loop sequences from Indonesian and Pacific chickens and other sequences of RJFs and NCs across Asia retrieved from GenBank (Appendix Table S2.1). The best-fit substitution model was determined based on the Bayesian Information Criterion using jModeltest v2.1.10 (Darriba et al., 2012). Node support was estimated using 1,000 ultrafast bootstrap replicates (Hoang et al., 2018). The nomenclatures of the 13 haplogroups (Haplogroups A to I and Haplogroups W to Z) reported by Miao et al. (2013) and haplogroup V (Huang et al., 2018) were used as references for the haplogroup notations. The list of haplotypes used, and the corresponding GenBank accession numbers are provided in the supplementary data (Appendix Table S2.1). Median-joining (MJ) network was constructed to infer the evolutionary relationships among chicken haplotypes using NETWORK 4.6 software (Bandelt et al., 1999). This method calculates the net divergence of each taxon from all other

taxa as the sum of the individual distances from variance within and among groups. The number and assignment of haplotypes were determined using DnaSP v. 6.0 software.

Truncated partial sequences (764-bp fragment) were also analyzed for a wider phylogeographic analysis of chicken population in the ISEA and the Pacific region, together with other partial sequences (Appendix Table S2.3) from Indonesian and Pacific chickens (Herrera et al., 2017; Thomson et al., 2014). Bootstrap values were estimated with 1,000 repetitions.

2.2.5 Population genetic structure and demographic history

The population pairwise net genetic distance based on population pairwise F_{ST} (significant values were accepted at $p<0.05$) and Slatkin's linearized F_{ST} was estimated using Arlequin v. 3.5.2.2 software (with 10,000 permutation) (Excoffier & Lischer, 2010). The level of significance was evaluated based on 1,023 random permutations. To visualize the pattern of genetic relationships among the populations across geographical distribution, the population pairwise F_{ST} values for both complete and partial sequences were plotted into the principal coordinate analysis (PCoA) using GenAlEx v. 6.503 (Peakall & Smouse, 2006). With the current available mtDNA D-loop sequence data (both from this study and those archived in the GenBank repository), this study generated finer PCoA plots using truncated 764 bp length D-loop sequence. To further estimate the genetic structure of each population among geographic groups, an analysis of molecular variance was performed as implemented by Arlequin v. 3.5.2.2 software. Significance testing was evaluated using 10,000 coalescent simulations.

Past demographic parameters were inferred by the analysis of the distribution of the number of site differences (mismatch distribution) using the program DnaSP v. 6.0 software (Librado & Rozas, 2009). Expected (simulated) values under expanding population model were calculated and plotted against the observed values. Populations that have undergone recent demographic growth tend to show a unimodal distribution without large differences in

the frequency of the ranked pairwise differences, while those populations at demographic equilibrium present a multimodal distribution (Rogers & Harpending, 1992). Raggedness statistics, *r* (Harpending, 1994) was used to quantify the smoothness of the mismatch distributions, and the confidence intervals were provided by coalescent algorithm simulations using DnaSP v. 6.0 software. The sum of squared deviations (SSD), as implemented in Arlequin v. 3.5.2.2, was used to further evaluate the sudden expansion model (Rogers, 1995; Rogers & Harpending, 1992). More powerful neutrality statistical tests, such as Tajima's *D* (Tajima, 1989) and Fu's *Fs* statistics (Fu, 1997), were used to further support the inference for the population growth model. These population expansion tests measure haplotype frequencies under neutrality and panmixis. Statistical tests and confidence intervals were based on a coalescent simulation algorithm under a neutral infinite-site model.

The coalescent-based methods had been widely used to quantify the relationship between the genealogy of the sequences and the demographic history of the population. Bayesian Skyline Plot (BSP) (Drummond et al., 2005) was estimated to infer deeper insight into the demographic history of Philippine chickens as implemented in BEAST v. 2.6.3 (Bouckaert et al., 2019). The BSP was generated with a strict molecular clock model and setting with 3.13 $\times 10^{-7}$ mutations/site/year rate (Alexander et al., 2015). The piecewise constant function and HKY nucleotide substitution model was used for the analysis. The Markov chain Monte Carlo (MCMC) chain was run for 5×10^{7} generations, with a sampling of parameters every 5,000 steps and 5×10^{6} generations served as burn-in. Convergence of the posterior estimates of the effective population size (N_e) to the likelihood stationary distribution was evaluated using Tracer v. 1.7.1 software (Rambaut et al., 2018).

2.3 Results

2.3.1 Mitochondrial DNA variation and genetic diversity

A total of 144 complete mtDNA D-loop sequences (1,231-1,232 bp) of Philippine chickens were analyzed in this study of which 107 were newly generated. There were 34 haplotypes (18 parsimony-informative sites) identified, 29 of which were possessed by native chickens with 1 haplotype (Hap_68) shared with red junglefowl, while 5 haplotypes are unique in the red junglefowl samples. The overall haplotypes of Philippine chickens (RJFs and NCs) had 32 polymorphic sites (all transition substitutions). The distribution of the nucleotide positions and sequence variations of each haplotype are presented in Appendix Table S2.2.

The haplotype (gene) diversity (*Hd*) was relatively high ranging from 0.884 ± 0.103 in RJFs to 0.904 ± 0.012 in NCs (overall; *Hd* = 0.915± 0.051). The overall gene diversity concurred with the previously described intra-population genetic diversity of chicken populations in Samar, Philippines (Godinez et al., 2019). Nucleotide diversity (π) varied between 0.0017 ± 0.0003 to 0.0044 ± 0.0002 in RJFs and NCs, respectively (overall; π = 0.0043 ± 0.0002). The total mean number of pairwise difference was 4.256 ± 2.358, with the higher value observed among Philippine native chickens (Table 2.1).

Table 2.1. Genetic diversity indices of red junglefowl and native chicken populations (complete mtDNA D-loop sequence) and their haplogroup distributions.

Population	N	S	Ht	*Hd*	π	P_d
Philippine RJFs	10	6	6	0.844 ± 0.103	0.00189 ± 0.00035	2.622 ± 1.528
Philippine NCs	134*	31	29	0.904 ± 0.012	0.00447 ± 0.00027	5.890 ± 2.830
Overall	*144*	*32*	*34*	*0.915± 0.011*	*0.00434 ± 0.00026*	*4.256 ± 2.358*
Indonesian NCs[a]	14	18	9	0.901± 0.062	0.00320±0.00102	2.560 ± 1.461
Pacific chickens[b]	15	8	7	0.724±0.121	0.00096±0.00026	0.978 ± 0.704

* Included 17 sequences derived from GenBank (Herrera et al., 2018 – direct submission).
[a, b] Sequence data from Herrera et al., (2018) – GenBank direct submission.
N, number of sequences; S, number of polymorphic (segregating) sites; Ht, number of haplotypes; Hd, haplotype (gene) diversity; π, nucleotide diversity; Pd, mean number of pairwise difference.
Italic values indicate combined values for Philippine RJFs and Philippine NCs (all samples).

2.3.2 Phylogeography and distribution of Philippine chicken haplogroups

The phylogenetic analysis of Philippine chickens, together with the other chicken populations in the Pacific, Indonesia, MSEA, and sequences derived from the global mtDNA phylogeographic study (Huang et al., 2018; Miao et al., 2013) were investigated. Hypervariable region (HVR) was also analyzed to accommodate other D-loop partial sequences (764 bp) for a finer phylogeographic analysis of chicken populations in the ISEA and Pacific region (Appendix Table S2.3). The rooted ML tree revealed four haplogroups (A, B, D, and E) of Philippine chickens. The majority (70.13%) of the samples using the complete mtDNA D-loop region belonged to haplogroup D with 23 haplotypes, and the rest belonged to haplogroups A, B, and E (Figure 2.1 and Appendix Figure S2.2). It was found that the Philippine RJFs clustered to haplogroup D. There were 4 RJF samples (i.e., MN986398-MN986400, MN986403) in Hap_25 unprecedentedly clustered to sub-haplogroup D2 together with the fighting cock Tulufan from Xinjiang, China (as the reference sub-haplogroup D2 nomenclature) (Miao et al., 2013) and Tosa-Jidori from Japan (Oka et al., 2007) which is reported to be related to Philippine RJFs for the first time in the present study (Figure 2.1 and Appendix Figure S2.2). The other 3 RJFs were unclassified to any previously identified sub-haplogroup nomenclatures for haplogroup D as patterned from the global profile (Miao et al., 2013) but appeared to be a sister-group to the sub-haplogroup D3 or sub-haplogroup D3b (mutational motif T220C; A281G) (Huang et al., 2018) (Figure 2.1 and Appendix Figure S2.2). The Philippine native chickens with predominant haplogroup D showed a close genetic relationship to the Pacific and Indonesian chickens compared to chicken populations from the MSEA. Within haplogroup D, rooted ML tree interestingly revealed a subclade for Philippine-Pacific chickens and another subclade for Philippine-Indonesian chickens (Figure 2.1).

The median-joining (MJ) network further revealed a consistent distinction of four maternal haplogroups (A, B, D, and E) of Philippine chickens (Figure 2.2.A). Clearly, within

haplogroup D, there were two dominant haplotypes that distinguished the two subclades between Philippine-Pacific chickens (H_57) and Philippine-Indonesian chickens (H_49). Haplotype H_57 grouped with Philippine RJF (NC_007236) (Nishibori et al., 2005) along with other samples of Philippine native chickens and Pacific chickens. The genetic distance clarified two unprecedented mutation signatures of the Philippine-Pacific subclade with transition substitutions at the nucleotide positions C296T and G686A while diverging to the Philippine-Indonesian subclade with the absence of those identified mutational motifs (Appendix Table S2.2). These findings agreed with the previously defined diagnostic motifs (SNPs A281G, C296T, T306C, and A342G) from the ancient Pacific chicken sequences relative to the Philippine chickens (Thomson et al., 2014). However, the present study accounted for the complete mtDNA D-loop sequences and found diagnostic motif of SNP at the G686A nucleotide position. Furthermore, a wider analysis of Haplogroup D using HVR (*n*=849) consistently showed distinct subclades of these chicken populations in the ISEA and Pacific region (Figure 2.2.B).

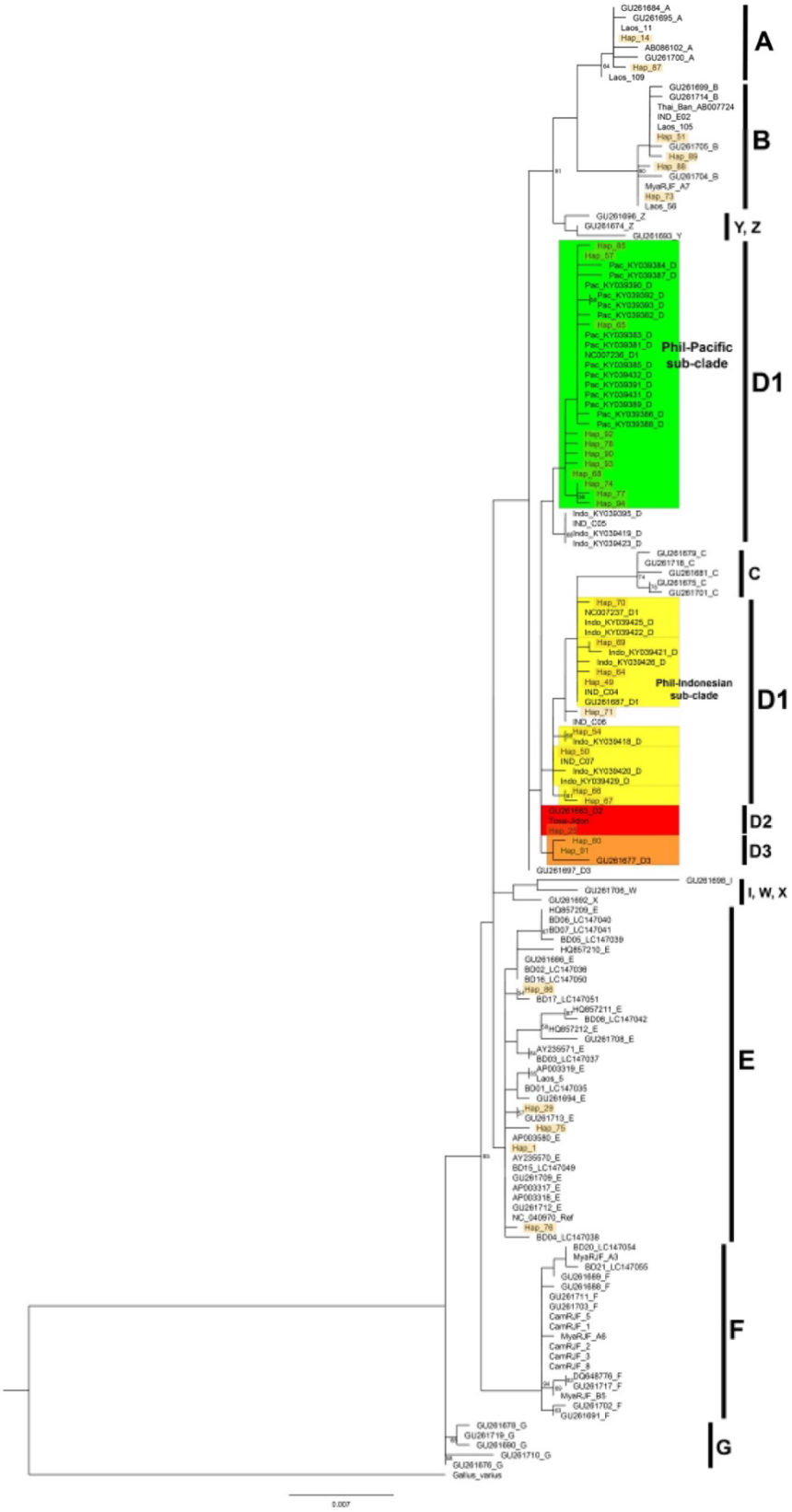

Figure 2.1. Maximum-likelihood phylogenetic tree for complete mtDNA D-loop nucleotide sequences of Philippine chickens. Node labels correspond to bootstrap support values evaluated with 1,000 ultrafast bootstrap replicates in IQ-TREE. The scale bar (0.007) indicates the genetic distance (substitution per site). Bootstrap values under 50% are not shown.

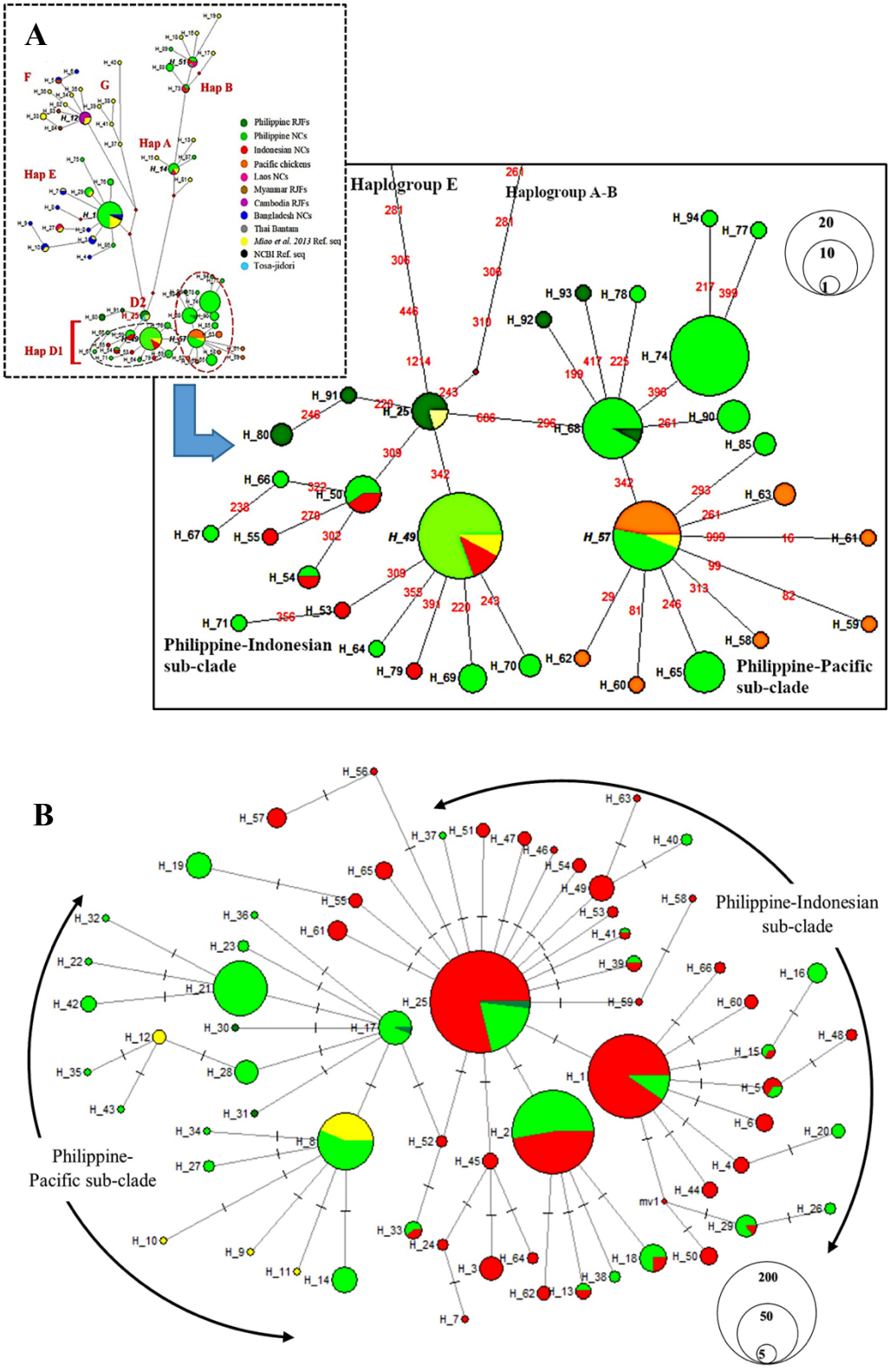

Figure 2.2. Median-joining network (**A**) of the complete mtDNA D-loop region depicting relationship of Philippine chickens, Indonesian chickens, and Pacific chickens. The area of each circle is proportional to the frequency of the corresponding haplotypes. The length of the branch connecting to other haplotypes correspond to mutational positions. (**B**) MJ network of mtDNA hypervariable region illustrating genealogical relationships of chickens from the Philippines (green), Indonesia (red), and the Pacific (yellow).

2.3.3 Population genetic structure and demography

The previous mtDNA study on Philippine chickens was limited only to matrilineal phylogenetic analyses (Godinez et al., 2019). Here, the population genetic differentiation was calculated using pairwise divergence (F_{ST}), Slatkin's linearized F_{ST}, and pairwise differences among Philippine chickens (RJFs and NCs), Pacific chickens, Indonesian chickens and MSEA chickens. Low genetic differentiation was observed between Philippine and Indonesian chickens (pairwise F_{ST}; 0.1540 and Slatkin's F_{ST}; 0.1821) and between Philippine and Pacific chickens (pairwise F_{ST}; 0.2681 and Slatkin's F_{ST}; 0.3663), which suggest that chicken populations in these regions were not isolated from each other (Table 2.2). Furthermore, the F_{ST} values between Philippine and Indonesian chickens (F_{ST} = 0.1540; 0.180) were lower than Philippine and Pacific chickens (F_{ST} = 0.2681; 0.3663), suggesting a genetic closeness in the former populations due to geographical proximity and closer maritime ranges. However, interesting findings documented high genetic divergence (F_{ST} = 0.4788) between Pacific and Indonesian chickens, while the MSEA chickens were the most remotely related to the Pacific chicken populations (F_{ST} = 0.5916). The F_{ST} values and population pairwise differences are consistent with the previous analysis that showed the Philippines as potentially the key contributor to Pacific chicken's diversity and genetic characteristics. All the F_{ST} values and population pairwise comparisons were significant at the 5% level.

The result of multivariate ordination using all haplogroups reveals broad geographical structuring across Asia and Oceanic regions (Figure 2.3A). The distribution of these populations on the first two principal coordinates is relative to their haplogroup structure. Close genetic relatedness between Philippine and Pacific chickens is observed, while the latter is distantly linked to Indonesian and MSEA chickens (Figure 2.3A; Appendix Figure S2.3). The same observation is derived when only haplogroup D populations (within ISEA) are plotted (Figure 2.3B). Meanwhile, there is no significant subgrouping among chicken populations in

all haplogroups in the Greater and the Lesser Sunda Islands, but tendency of population subdivision within haplogroup D-lineage can be observed in West Papua and Java populations (Figure 2.3B). The PCoA plot clearly showed genetic structuring between continental and island chicken populations, while MSEA and East Asian populations have weak genetic differentiation except for Cambodian chickens. South Asian and the Middle East chickens appeared to have genetically differentiated from the eastern population groups (Figure 2.3A).

The analysis of molecular variance supports the low genetic differentiation of chicken populations in the ISEA, with 79.18% of genetic variation show significance within populations. Consistently, Philippine-Indonesian (i.e., Group B) and Philippine-Pacific (i.e., Group C) showed lower among-group variances with 9.43% and 20.97%, respectively, while higher among-group variance is observed in the Philippine-MSEA (i.e., Group D) with 34.17% (Table 2.3). However, the analysis showed higher genetic differentiation within the Pacific-Indonesian group (55.05% within-population variance, p=0.000) than within the Philippine-Pacific group (69.61% within-population variance, p=0.000) at haplogroup D (Table 2.3).

The mismatch distributions of Philippine chickens (haplogroup D), Philippine RJFs, and Pacific chickens were unimodal (Figure 2.4), characteristics of a population that has undergone expansion. Support for the smoothness of the observed distributions was statistically fit for Philippine chickens haplogroup D and RJFs as quantified by raggedness statistics and coalescent algorithm simulations. In agreement, the observed distributions from all populations did not significantly deviate (SSD values >5%) from the simulated values under the assumption of population expansion (Table 2.4). However, Philippine native chickens (including all haplogroups in the dataset) and the Indonesian chickens and MSEA chickens exhibited a ragged mismatch distribution with high raggedness statistics (r) values. Both Tajima's D and Fu's *Fs* neutrality tests further indicated that chickens from the ISEA and Pacific region deviated from neutrality except for chickens from MSEA, which support a model of

demographic expansion. The negative and significant Fu's *Fs* statistical values in Philippine chickens (haplogroup D) and Pacific chickens provided strong evidence of population growth signatures of these populations in the region (Table 2.4). Evidence for an excess of recent mutations or rare nucleotide site variants has been observed in the Philippine chickens considering all other haplogroups A, B, and E and Indonesian chickens under the selective neutrality model, but the excess was statistically non-significant (Fu, 1997).

In an attempt to obtain a better inference for the demographic history of the Philippine chickens, this study evaluated the changes in maternal effective population size (N_e) at different points along the genealogical timescale. The Bayesian Skyline Plot showed evidence of Philippine chickens experiencing a long period of relatively constant N_e during the early Holocene period, followed by a gradual increase which started at approximately 3,000 BP, while the episode of eminent population growth commenced at about 2,500 BP (Figure 2.5). Taken together, these analyses of population pairwise F_{ST}, mismatch distributions, and BSP were consistent in showing that the Philippines was the main contributor to the diversity and genetic characteristics of Pacific chickens (Figure 2.6), related to the eastward movement of the Austronesian speakers from the Philippines approximately 3.0 thousand years ago (kya) (Bellwood, 2007; Soares et al., 2016).

Table 2.2. Genetic divergence between populations of Philippine chickens (RJFs and NCs), Indonesian, Pacific, and MSEA chickens at complete mtDNA D-loop sequences

(a) Population pairwise F_{ST} and Slatkin's Linearized F_{ST} (Population)	(1)	(2)	(3)	(4)
(1) Philippine chickens[a]		0.1821	0.3663	0.6076
(2) Indonesian native chicken[b]	0.1540**		0.9188	0.6695
(3) Pacific chickens[b]	0.2681**	0.4788**		1.4487
(4) MSEA chickens[c]	0.3779**	0.4010**	0.5916**	
(b) Population average pairwise differences				
(1) Philippine chickens[a]		6.1231*	5.3178*	11.0679**
(2) Indonesian native chicken[b]	1.0373		5.1777**	12.1250**
(3) Pacific chickens[b]	2.0207	2.3606		13.1833**
(4) mSEA chickens[c]	3.5101	5.0469	7.8940	

[a] RJFs and NCs combined populations; * Significant F_{ST} at $P < 0.05$; ** highly significant F_{ST} at $P < 0.001$
[b] direct submission sequences retrieved from Genbank; [c] sequences from Osman and Nishibori, 2014
(a) Population pairwise genetic distance. Lower triangular matrix: population pairwise estimates of F_{ST}; Upper triangular matrix: slatkin's Linearized F_{ST}. (b) Population average pairwise genetic differences. Upper triangular matrix: average number of pairwise differences between populations (PiXY); Lower triangular matrix: corrected average pairwise difference (PiXY-(PiX+PiY)/2)

Table 2.3. Population genetic structure estimated from the AMOVA based on complete mtDNA D-loop sequences from (1) Philippine Red Junglefowls, (2) Philippine Native Chickens, (3) Indonesian Native Chickens, (4) Pacific Chickens, and (5) MSEA Chickens

Group	No. of popula-tions	No. of groups	Source of variation (%)		
			Among groups	Among populations within group	Within populations
no groupings	181	1	-	29.27	70.73
Group A (1,2 versus 3,4)	165	2	2.23	18.58**	79.18**
Group B (1,2 versus 3)	150	2	9.43	6.60*	83.97**
Group C (1,2 versus 4)	153	2	20.97	6.37*	72.66**
Group D (1,2 versus 5)	154	2	34.17	4.19*	61.63**
Haplogroup D only					
no groupings	131	2	-	26.56	73.44
Group E (1,2 versus 3)	116	2	13.84	11.57**	74.60**
Group F (1,2 versus 4)	116	2	17.58	12.80**	69.61**
Group G (3 versus 4)	29	2	47.05	-2.11**	55.05**

* Significant F_{ST} at $P < 0.05$; ** Significant F_{ST} at $P < 0.01$; grey-shaded area corresponds to analysis for Haplogroup D only

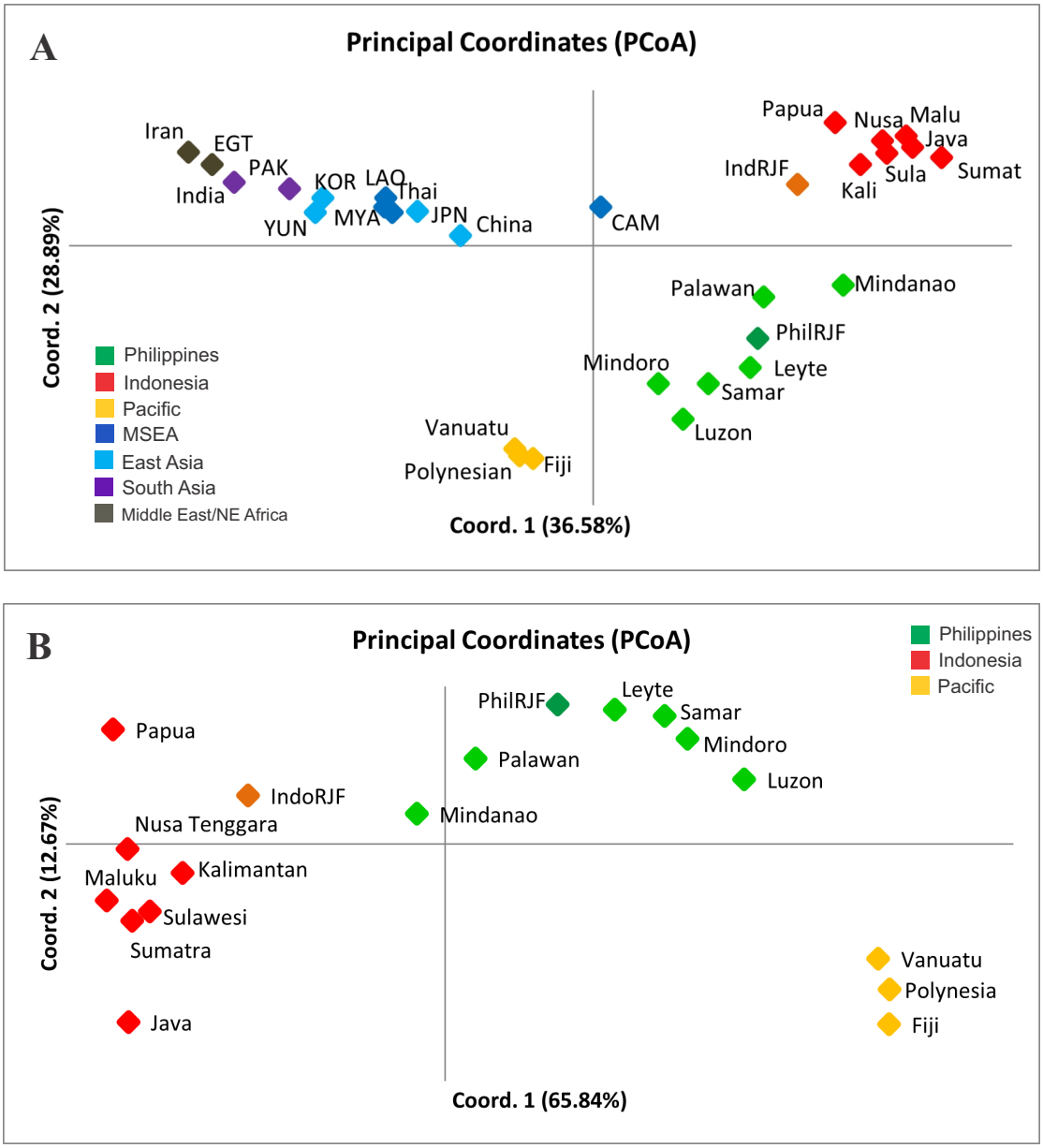

Figure 2.3. Principal coordinate analysis (PCoA) plots of the population pairwise F_{ST} (A) for chicken populations from ISEA, MSEA, Pacific, East Asia, South Asia, and Middle East/Northeast Africa using partial mtDNA D-loop region (764 bp). Populations are assigned the following colors (green: Philippines; yellow: Pacific; red: Indonesia; dark blue: MSEA; light blue: East Asia; purple: South Asia; black: Middle East/Northeast Africa. Populations are abbreviated as follows: PhilRJF, Philippine red junglefowl; IndRJF, Indonesian red junglefowl; Kali, Kalimantan; Sula, Sulawesi; Nusa, Nusa Tenggara; Malu, Maluku; Sumat, Sumatra; CAM, Cambodia; LAO, Laos; MYA, Myanmar; Thai, Thailand; JPN, Japan; KOR, Korea; YUN, Yunnan; PAK, Pakistan; EGT, Egypt; others remained spell out. (B) Haplogroup D across ISEA and Pacific chicken populations (color legend is the same).

Table 2.4. Neutrality tests and mismatch analysis sums of squared deviation (SSD) and Harpending's raggedness index for Philippines RJFs and NCs complete mtDNA D-loop sequence

Population	Tajima's *D*	Fu's *Fs*	SSD (*p*)	Raggedness, *r*
Philippine RJFs	-0.1063	-1.1431	0.0214 (0.440)	0.0479*
Philippine NCs	-0.1126	-3.5575	0.0374 (0.064)	0.1090
combined (haplogroup D)	*-0.9398*	*-8.9218***	*0.0293 (0.125)*	*0.0210**
Indonesian NCs[a]	-1.2120	-1.2768	0.0342 (0.200)	0.0775
Pacific chickens[a]	-1.5494*	-4.1899**	0.0150 (0.200)	0.0592*
MSEA chickens	1.1233	1.1029	0.0430 (0.100)	0.0437
Mean (p)	*-0.3366*	*-1.8129*	0.0323 (0.200)	

*p-value <0.05; **p-value <0.01; [a] direct submission sequences retrieved from Genbank

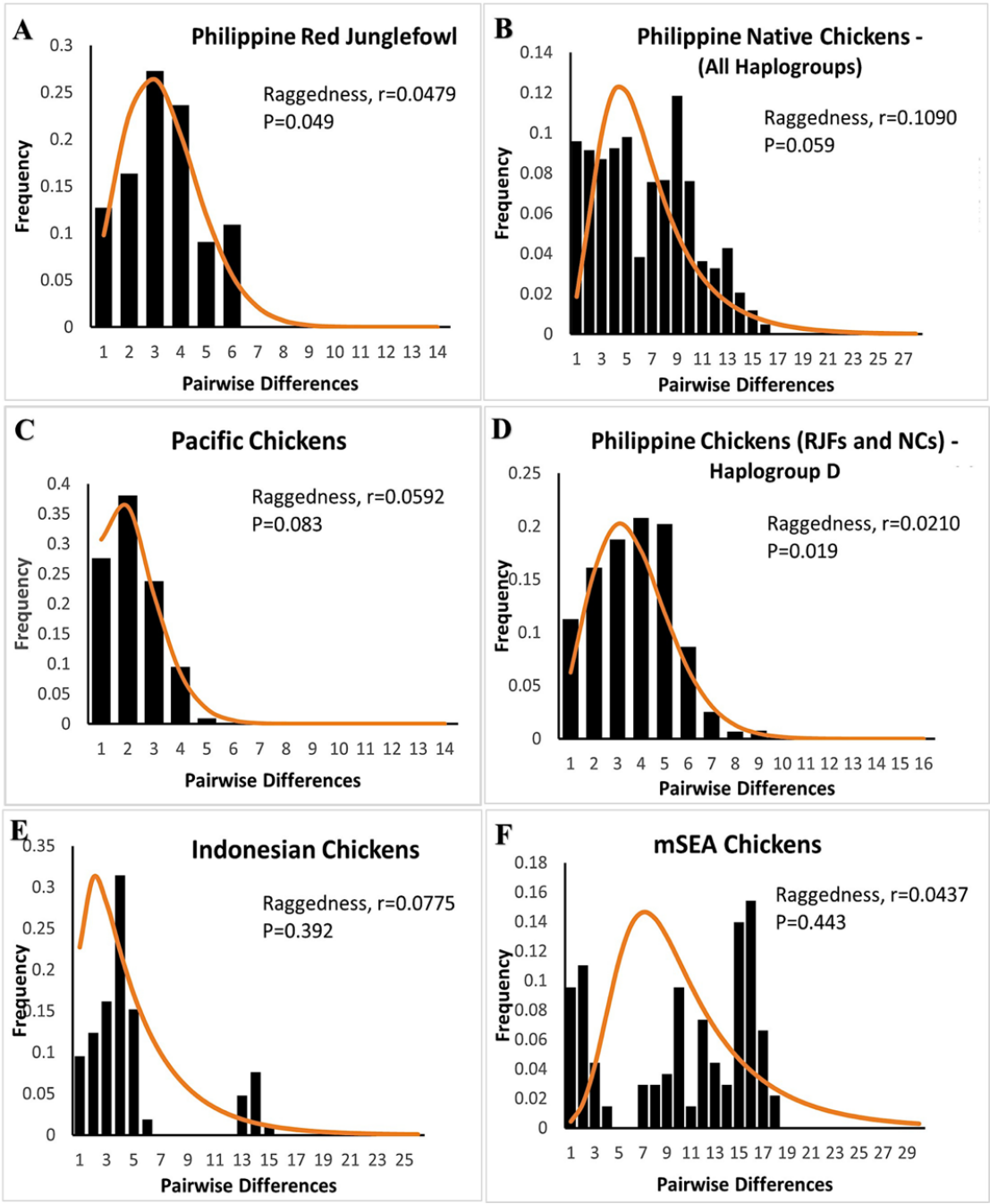

Figure 2.4. Mismatch distribution of the complete mtDNA D-loop sequences of (**A, B, D**) Philippine chickens (RJFs and NCs), (**C**) Pacific, (**E**) Indonesia, and (**F**) mainland Southeast Asia chickens based on pairwise nucleotide site differences. The solid line indicates the theoretical distribution under population expansion model. The raggedness statistics and corresponding *p*-values for (**A**) Philippine RJFs; $r = 0.0479$, $p = 0.049$ and (**D**) RJFs-NCs-haplogroup D; $r = 0.0210$, $p = 0.019$, provided statistical support for the smoothness of the observed distributions.

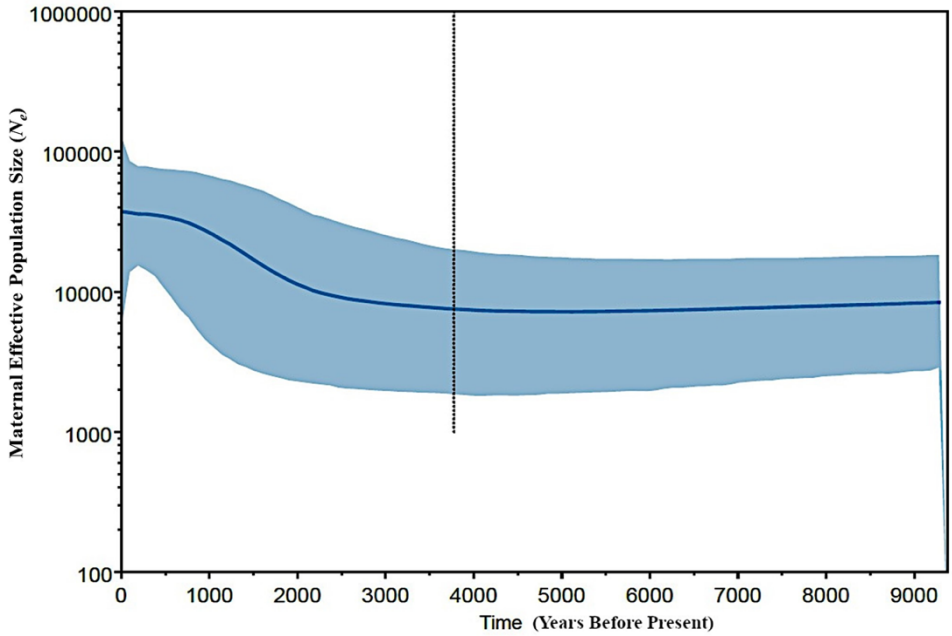

Figure 2.5. Bayesian coalescent skyline plot showing estimated demographic history of Philippine chickens (haplogroup D). The central blue line is the median estimate effective population size. The shaded area shows the upper and lower estimates of 95% credibility interval. The vertical dotted line represents the median estimate of time to the most recent common ancestor. The *x*-axis is the time (in years before present), and the *y*-axis indicates population size (as the product of N_e and the generation length in years).

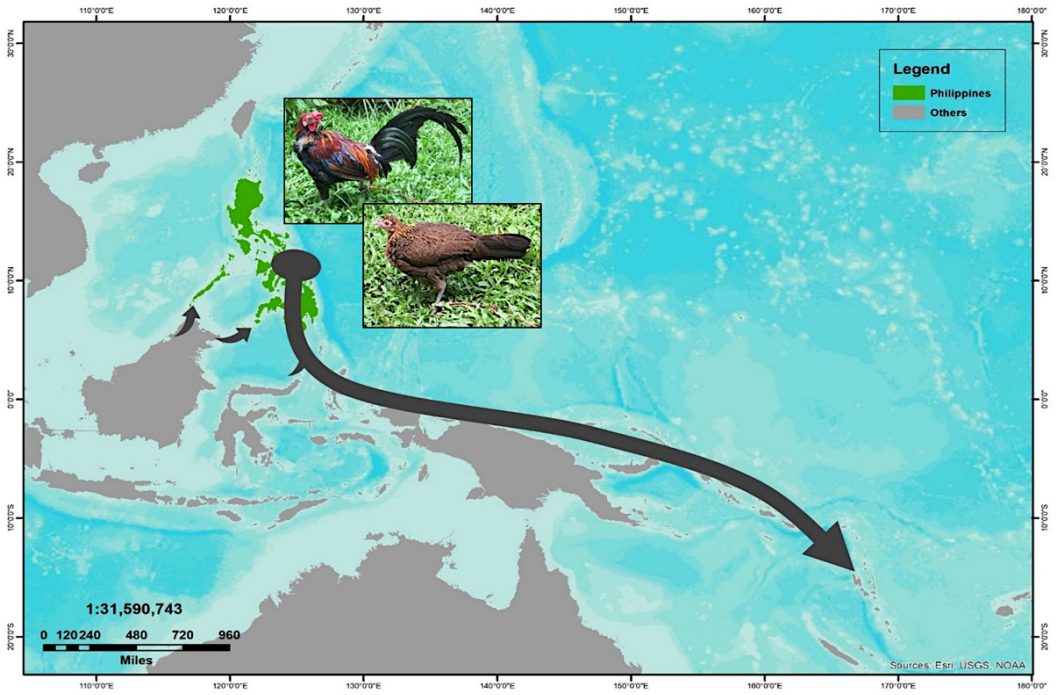

Figure 2.6. The proposed routes of translocation scenario of Philippine chickens expanding to the Pacific relating to the Austronesian-speakers movement, as supported by the population pairwise genetic divergence (F_{ST}) estimates, mismatch distribution analyses, and demographic inference based on coalescent simulation of Bayesian skyline plot (BSP).

2.4 Discussion

Philippine chickens have been shown to have high haplotypic diversity with a relatively large proportion of low-frequency haplotypes in the predominant haplogroup D (gene diversity = 0.915± 0.011). They show higher genetic diversity than Indonesian crowing chickens (Ulfah et al., 2017), Thai indigenous chickens (Teinlek et al., 2018), Laotian (Kawabe et al., 2014), and Vietnamese chickens (Cuc et al., 2011), North African native chickens (Osman et al., 2016), West Africa native chickens (Adebambo et al., 2010), Central Africa chickens (Hassaballah et al., 2015) and all East African chickens combined (Mwacharo et al., 2011), except Chinese chickens (*Hd* =0.916; π=00591) (Gao et al., 2017; Guo et al., 2017). The high genetic diversity in Philippine chickens resulted from the presence of abundant haplotype signatures (13 parsimony-informative sites and 10 singletons) in the predominant haplogroup D, which points that the population is large and expanding. Since genetic diversity is linked to the processes of adaptation and extinction (Evans & Sheldon, 2008), high population-level genetic diversity and mean heterozygosity provide greater evolutionary potential for Philippine chickens. Increased population growth rate decreases the loss of genetic variation (Austerlitz et al., 1997), thus diversity indices are an essential foundation for potential genetic improvement and selection of species.

Previous fine-grained mtDNA phylogeographic study of chickens across the world revealed 13 divergent haplogroups (A-I and W-Z) (Liu et al., 2006; Miao et al., 2013) with the recent addition of haplogroup V (Huang et al., 2018). Haplogroups C and D are among the most diverse chicken haplogroups inhabiting East Asia and ISEA, respectively (Liu et al., 2006; Miao et al., 2013). They coalesced to form macro-haplogroup CDV at approximately 8.1 kya with a common ancestral motif at 306 nucleotide position, but haplogroup D diverged ~ 4.4 kya harboring an ancestral mutational motif at 342 nucleotide position (Huang et al., 2018). Most RJF subspecies and their descendants are classified in haplogroup D, which is widely

observed in the continental subclade while few are represented in the island clade (Liu et al., 2006).

This present work investigated the phylogeography of Philippine chickens and their possible dispersal to the Pacific. Phylogenetic analysis and median-joining network revealed four distinct maternal haplogroups (A, B, D, and E) of Philippine chickens, with a predominant haplogroup D throughout the population. This confirms previous genetic evidence that haplogroup D is the maternal lineage largely concentrated in the ISEA-Pacific region (Liu et al., 2006; Miao et al., 2013) and distinctively traced as a specific signature for the Pacific sequence motif potentially found in the Philippines (Thomson et al., 2014). Both analyses from the complete and HVR mtDNA D-loop region of Philippine chickens together with Indonesian and Pacific chickens formed two subclades within sub-haplogroup D1. The divergence pattern of the Philippine-Pacific subclade harbored two mutational diagnostic motifs, C296T and G686A, while undetected in the Philippine-Indonesian subclade concordant to the genealogical mitogenome classification reported by Huang et al. (2018) albeit using few samples of Philippine chickens in the previous report. The newfound basal position patterns of Philippine RJFs in sub-haplogroup D2 are grouped with one of the early recorded Chinese gamecocks - Tulufan and the oldest Jidori-type breed in Japan - the Tosa-Jidori. The ancestral origin of Tosa-Jidori is suggested from the ISEA (Oka et al., 2007), while the ancestral origin of haplogroup D Tulufan gamecock in Northwest China is ambiguous because its diversity of distribution is mostly concentrated in haplogroups A and C (Miao et al., 2013) and independent admixture among gamecock breeds is evident (Luo et al., 2020). However, recent reports using whole-genome sequences showed the possible contribution of the local RJF subspecies or the earlier admixed domestic lineages from Yunnan Province, China and MSEA (Luo et al., 2020; Wang et al., 2020). The geographical distribution of sub-haplogroup D2 is still unclear due to limited representation from other chicken populations across Southeast Asia. On the other

hand, sub-haplogroup D3 has geographical distribution in East China (Miao et al., 2013), South China, and Thailand (Huang et al., 2018), which likely suggests an earlier introduction pattern to the Philippines from Indochina via early human migration movement (i.e., Negrito or First Sundaland people) around Holocene period (Jinam et al., 2012; Lipson et al., 2014). The identified 5 haplotypes (n=31) of Philippine native chickens assigned to haplogroup E are believed to be the result of interbreeding between present-day chickens and commercial or show breeds. This haplogroup is widely represented in European domestic chickens and commercial lines with distinct haplotypes dispersed in the Middle Eastern and Indian subcontinents (Liu et al., 2006). This study also found two haplotypes in haplogroup A (n=5) and four haplotypes in haplogroup B (n=7), which are believed to have been introduced from neighboring countries, including South and East China, Japan, and some countries in the MSEA (Liu et al., 2006; Oka et al., 2007).

Although Neolithic archaeological records of Philippine chickens are still enigmatic (Piper, 2017), mtDNA evidence of the Philippine-Pacific subclade provided strong inference for the Philippine origin of Pacific chickens, especially when both ancient and modern Pacific haplotype D (Polynesian motif) chickens (Thomson et al., 2014) clustered along with the Philippine chickens forming a subclade. The antiquity of the ancestral Polynesian haplotype previously described by Thomson et al. (2014) has been confirmed by its identification in Lapita contexts in Vanuatu (Petchey et al., 2015), which likely suggests an initial pattern of gene flow in the Melanesian populations before reaching Polynesia. The absence of Indonesian chicken sequences grouped with the ancestral Polynesian chicken motif (Thomson et al., 2014) and Philippine-Pacific subclade suggests a possible direct introduction from the Philippines. The most probable dispersal processes of Philippine chickens that might have contributed to the genetic characteristics of the Pacific chickens reflect the Austronesian speaker's movement or the "out of Taiwan" migration model (Bellwood, 2007; Hung et al., 2011). This follows the

Malayo-Polynesian dispersal in the Philippines about 2,200 BCE and their continuous movement eastward through North Maluku to Island Melanesia before reaching Remote Oceania (Bellwood, 2007; Piper, 2017). In view of linguistic evidence, the Proto-Malayo-Polynesian term for domestic chickens appears to be widely recognized as far as Remote Oceania while distantly acquainted with the Proto-Austronesian speakers (Blust, 1995; Piper, 2017). Both genome-wide and mitogenome analyses of Austronesian speakers support an eastward movement harboring substantial aboriginal Taiwan-related ancestry, approximately 4.4 kya (Lipson et al., 2014; Soares et al., 2016). However, Taiwanese indigenous chickens (e.g., Ju-Chi) and gamecock (Hua-Tung) do not exhibit haplotypes patterned for ISEA signatures, instead influenced mainly by Chinese haplotypes and populations introgressed from the Indian subcontinent (Chang et al., 2012). This likely suggests that Austronesian speakers did not carry chickens (i.e., Haplogroup D matriline) during their movement to the Philippines but potentially took hold of earlier domestic lineages upon expanding eastward.

The result indicated low genetic differentiation among the ISEA and Pacific chicken populations. High genetic closeness (F_{ST} <0.2) was observed between Philippine and Pacific chickens (*P=0.001*) than between Indonesian and Pacific chickens, which suggests little or no genetic difference among the former populations. This weak genetic structure is confirmed by the AMOVA analysis with 69.61% within-population variance (P=0.000) for the Philippine-Pacific group, rejecting geographical-based isolation. This potentially reflects regional translocation from the Philippines going eastward to the Pacific by Austronesian speakers around <3,000 years ago (Soares et al., 2016). Support for the Philippine chicken expansion was provided by the unimodal mismatch distribution observed in the Philippine RJFs and Philippine NCs classified in haplogroup D. Conversely, Indonesian chickens and MSEA chickens appeared to be statistically unfit to satisfy the population growth model with large fractions of zero difference in the pairwise differences and with observed ragged mismatch

distribution despite having high population diversity (Ray et al., 2003). This indicates that the population had undergone stability and population subdivision (Slatkin & Hudson, 1991). Philippine-Pacific subclade, especially Pacific populations, exhibited a star-like gene genealogy with more singleton sites (low-frequency variants) and long terminal branches, characteristics that populations had undergone recent population growth across Oceania (Harpending et al., 1998; Rogers & Harpending, 1992).

It has been argued that the sudden demographic expansion model and raggedness statistics have limitations in detecting population expansion and estimating demographic parameters (Harpending, 1994; Ramos-Onsins & Rozas, 2002; Rogers & Harpending, 1992). Therefore, Tajima's *D* and Fu's *Fs* statistics were substantiated to further infer possible population growth signals. This study ruled out past population expansion signatures of Philippine RJFs and native chickens (haplogroup D), which validated our previous analysis inferring the contributions of Philippine chickens to the genetic characteristics of the Pacific chickens. On the other hand, the negative and significant Fu's *Fs* statistical test (Fu, 1997) provided strong evidence for the past population growth of Philippine chickens (haplogroup D) in the ISEA. Interestingly, the BSP analysis indicated demographic expansion of the Philippine chickens predating the recovered ancient DNA samples of Pacific chickens in the Anatoloa site, Niue Island, and the Anakena site, Rapa Nui (~1,200-600 BP) (Thomson et al., 2014). This finding corroborated the eastward expansion of the Austronesian speakers from the Philippines before reaching the Pacific region. Overall, the Philippine-Pacific subclade is congruent with the evidence of increased maternal effective population size of Philippine chickens while concordant with the demographic signals imprinted in DNA genealogies and timing of introduction brought by human dispersal (Figures 2.5 and 2.6).

Estimates of genetic diversity, phylogeography, and population structure of Philippine chickens obtained in this study are characterized by a high level of genetic variability,

especially influenced by the chicken populations identified as the haplogroup signatures for Philippine chickens. Although Philippine RJFs appear to have a fairly diverse population, flexibility for conservation efforts must not be neglected (Evans & Sheldon, 2008). An important direction for future work is to increase the density of sample populations from identified localized chicken breeds within the Philippines and neighboring countries.

2.5 Conclusion and Recommendations

This study provides an in-depth understanding of the matrilineal phylogeny, genetic diversity, and population dynamics of Philippine chickens. This explains the genetic relatedness of Philippine chickens with other chicken populations widespread in ISEA, especially the Philippine contribution to the genetic characteristics of Pacific chickens. The significantly low genetic differentiation of Philippine chickens and Pacific chickens indicates a high level of gene flow among these chicken populations, which are mainly impacted by human-assisted movement around 3,000-2,500 years ago.

The genetic information of these indigenous poultry resources is essential for conservation efforts, and these data serve as a baseline for monitoring to avoid further loss of genetic diversity. This asserts great potential for genetic improvement and selection of valuable traits for developing sustainable chicken production systems in the Philippines.

2.6 Data availability

The compete mtDNA D-loop sequences are deposited and available in GenBank database (accession numbers: MN986370-MN986403).

2.7 Acknowledgement

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Sports, Science and Technology (MEXT), and partially assisted by the Department of Science and Technology Human Resource Development Program (DOST-ASTHRDP), Philippines, through the previous Master of Science Thesis Grant. In addition, the author thanks the local farmers in Samar and Leyte Provinces for providing samples.

2.8 Appendices

Appendix Table S2.1. Complete mtDNA control region information of Philippine chickens including sequences from Pacific, Indonesia, Mainland Southeast Asia, East Asia, South Asia, and references taken from Miao *et al.,* 2013.

(D-loop) Fragment Length	Species/Breed	Haplo-type assignments (used in this study)	Haplo-group assignment	GenBank accession number	Number of individuals	Collection locality	Source
Complete Sequences	Red junglefowl	Hap_25	D2	MN986398	1	Leyte, Philippines	This study
	Red junglefowl	Hap_25	D2	MN986399	1	Leyte, Philippines	This study
	Red junglefowl	Hap_25	D2	MN986400	1	Leyte, Philippines	This study
	Red junglefowl	Hap_80	*D3**	MN986401	1	Leyte, Philippines	This study
	Red junglefowl	Hap_80	*D3**	MN986402	1	Leyte, Philippines	This study
	Red junglefowl	Hap_25	D2	MN986403	1	Leyte, Philippines	This study
	domestic chicken	Hap_1	E1	MN986370	8	Samar, Philippines	This study
	domestic chicken	Hap_88	B	MN986371	2	Samar, Philippines	This study
	domestic chicken	Hap_74	D1	MN986372	4	Samar, Philippines	This study
	domestic chicken	Hap_68	D1	MN986373	3	Samar, Philippines	This study
	domestic chicken	Hap_90	D1	MN986374	3	Samar, Philippines	This study
	domestic chicken	Hap_1	E1	MN986375	1	Samar, Philippines	This study
	domestic chicken	Hap_94	D1	MN986376	1	Samar, Philippines	This study
	domestic chicken	Hap_49	D1	MN986377	6	Samar, Philippines	This study
	domestic chicken	Hap_49	D1	MN986378	1	Samar, Philippines	This study
	domestic chicken	Hap_14	A	MN986379	3	Samar, Philippines	This study
	domestic chicken	Hap_1	E1	MN986380	12	Leyte, Philippines	This study
	domestic chicken	Hap_74	D1	MN986381	16	Leyte, Philippines	This study
	NC/RJF	Hap_68	D1	MN986382	6**	Leyte, Philippines	This study
	domestic chicken	Hap_1	E1	MN986383	1	Leyte, Philippines	This study
	domestic chicken	Hap_49	D1	MN986384	12	Leyte, Philippines	This study
	domestic chicken	Hap_49	D1	MN986385	1	Leyte, Philippines	This study
	domestic chicken	Hap_29	E1	MN986386	1	Leyte, Philippines	This study
	domestic chicken	Hap_76	E1	MN986387	1	Leyte, Philippines	This study
	domestic chicken	Hap_69	D1	MN986388	2	Leyte, Philippines	This study
	domestic chicken	Hap_65	E1	MN986389	4	Leyte, Philippines	This study
	domestic chicken	Hap_75	E1	MN986390	1	Leyte, Philippines	This study
	domestic chicken	Hap_50	E1	MN986391	3	Leyte, Philippines	This study
	domestic chicken	Hap_73	B	MN986392	1	Leyte, Philippines	This study

domestic chicken	Hap_68	D1	MN986393	1	Leyte, Philippines	This study
domestic chicken	Hap_77	D1	MN986394	1	Leyte, Philippines	This study
domestic chicken	Hap_29	E1	MN986395	1	Leyte, Philippines	This study
domestic chicken	Hap_74	D1	MN986396	1	Leyte, Philippines	This study
domestic chicken	Hap_78	D1	MN986397	1	Leyte, Philippines	This study
Junglefowl		*D3**	MK085033		Samar, Philippines	Godinez et al., 2019
Junglefowl		D1	MK085034		Samar, Philippines	Godinez et al., 2019
Junglefowl		D1	MK085035		Samar, Philippines	Godinez et al., 2019
domestic chicken		A	MK085038		Samar, Philippines	Godinez et al., 2019
domestic chicken		A	MK085039		Samar, Philippines	Godinez et al., 2019
domestic chicken		B	MK085040		Samar, Philippines	Godinez et al., 2019
domestic chicken		B	MK085041		Samar, Philippines	Godinez et al., 2019
domestic chicken		B	MK085042		Samar, Philippines	Godinez et al., 2019
domestic chicken		D1	MK085043		Samar, Philippines	Godinez et al., 2019
domestic chicken		D1	MK085044		Samar, Philippines	Godinez et al., 2019
domestic chicken		D1	MK085045		Samar, Philippines	Godinez et al., 2019
domestic chicken		D1	MK085046		Samar, Philippines	Godinez et al., 2019
domestic chicken		D1	MK085047		Samar, Philippines	Godinez et al., 2019
domestic chicken		D1	MK085048		Samar, Philippines	Godinez et al., 2019
domestic chicken		D1	MK085049		Samar, Philippines	Godinez et al., 2019
domestic chicken		E1	MK085050		Samar, Philippines	Godinez et al., 2019
domestic chicken		E1	MK085051		Samar, Philippines	Godinez et al., 2019
domestic chicken		E1	MK085052		Samar, Philippines	Godinez et al., 2019
domestic chicken		E1	MK085053		Samar, Philippines	Godinez et al., 2019
domestic chicken		E1	MK085054		Samar, Philippines	Godinez et al., 2019
		D1	KY039417		Mindoro, Philippines	Herrera et al., 2018 (direct submission)
		D1	KY039402		Zamboanga, Philippines	Herrera et al., 2018 (direct submission)
		D1	KY039401		Tugop, Philippines	Herrera et al., 2018 (direct submission)
		D1	KY039409		Ifugao, Philippines	Herrera et al., 2018 (direct submission)
		D1	KY039414		Palawan, Philippines	Herrera et al., 2018 (direct submission)
		D1	KY039408		Asipulo, Philippines	Herrera et al., 2018 (direct submission)
		D1	KY039396		Manticao, Philippines	Herrera et al., 2018 (direct submission)
		D1	KY039412		Palawan, Philippines	Herrera et al., 2018 (direct submission)
		D1	KY039411		Palawan, Philippines	Herrera et al., 2018 (direct submission)
		D1	KY039400		Tugop, Philippines	Herrera et al., 2018 (direct submission)
		D1	KY039413		Palawan, Philippines	Herrera et al., 2018 (direct submission)
		D1	KY039437		Palawan, Philippines	Herrera et al., 2018 (direct submission)

	D1	KY039404	Ifugao, Philippines	Herrera et al., 2018 (direct submission)
	D1	KY039405	Ifugao, Philippines	Herrera et al., 2018 (direct submission)
	D1	KY039416	Mindoro, Philippines	Herrera et al., 2018 (direct submission)
	D1	KY039407	Ifugao, Philippines	Herrera et al., 2018 (direct submission)
	D1	KY039403	Zamboanga, Philippines	Herrera et al., 2018 (direct submission)
	D1	KY039386	Easter Island	Herrera et al., 2018 (direct submission)
	D1	KY039387	Easter Island	Herrera et al., 2018 (direct submission)
	D1	KY039388	Easter Island	Herrera et al., 2018 (direct submission)
	D1	KY039389	Easter Island	Herrera et al., 2018 (direct submission)
	D1	KY039390	Easter Island	Herrera et al., 2018 (direct submission)
	D1	KY039384	Hawaii	Herrera et al., 2018 (direct submission)
	D1	KY039391	Fiji	Herrera et al., 2018 (direct submission)
	D1	KY039385	Hawaii	Herrera et al., 2018 (direct submission)
	D1	KY039383	Hawaii	Herrera et al., 2018 (direct submission)
	D1	KY039381	Marquesas	Herrera et al., 2018 (direct submission)
	D1	KY039382	Niue	Herrera et al., 2018 (direct submission)
	D1	KY039392	Vanuatu	Herrera et al., 2018 (direct submission)
	D1	KY039393	Vanuatu	Herrera et al., 2018 (direct submission)
	D1	KY039431	New Caledonia	Herrera et al., 2018 (direct submission)
	D1	KY039432	New Caledonia	Herrera et al., 2018 (direct submission)
	D1	KY039429	Sulawesi, Indonesia	Herrera et al., 2018 (direct submission)
	D1	KY039428	Sulawesi, Indonesia	Herrera et al., 2018 (direct submission)
	D1	KY039427	Sulawesi, Indonesia	Herrera et al., 2018 (direct submission)
	D1	KY039426	Lombok, Indonesia	Herrera et al., 2018 (direct submission)
	D1	KY039425	Maluku, Indonesia	Herrera et al., 2018 (direct submission)
	D1	KY039395	Maluku, Indonesia	Herrera et al., 2018 (direct submission)
	D1	KY039424	Manokwari, Indonesia	Herrera et al., 2018 (direct submission)
	D1	KY039423	Manokwari, Indonesia	Herrera et al., 2018 (direct submission)
	D1	KY039422	Kalimantan, Indonesia	Herrera et al., 2018 (direct submission)
	D1	KY039421	Kalimantan, Indonesia	Herrera et al., 2018 (direct submission)
	D1	KY039420	Java, Indonesia	Herrera et al., 2018 (direct submission)
	D1	KY039418	Java, Indonesia	Herrera et al., 2018 (direct submission)
	D1	KY039419	Java, Indonesia	Herrera et al., 2018 (direct submission)
NCBI Ref. Seq.	D1	NC_040970.1	Tibetan, autonomous	Liu et al, 2018
domestic chicken	A	AB086102	Japan: Hiroshima	Wada *et al.,* 2004
domestic chicken	A	GU261684	China: Yunnan	Miao *et al.,* 2013
junglefowl	A	GU261695	China: Yunnan	Miao *et al.,* 2013
junglefowl	A	GU261700	Myanmar	Miao *et al.,* 2013
junglefowl	B	NC_007235	Laos: Vientiane	Nishibori *et al.,* 2005

junglefowl	B	GU261704	China: Yunnan	Miao *et al.,* 2013
domestic chicken	B	GU261705	China: Yunnan	Miao *et al.,* 2013
domestic chicken	B	GU261714	China: Yunnan	Miao *et al.,* 2013
domestic chicken	B	GU261699	China: Yunnan	Miao *et al.,* 2013
junglefowl	Z	GU261674	China: Hainan	Miao *et al.,* 2013
junglefowl	Z	GU261696	China: Hainan	Miao *et al.,* 2013
junglefowl	Y	GU261693	China: Yunnan	Miao *et al.,* 2013
domestic chicken	C1	GU261701	China: Henan	Miao *et al.,* 2013
domestic chicken	C1	GU261675	China: Hunan	Miao *et al.,* 2013
domestic chicken	C1	GU261681	China: Hunan	Miao *et al.,* 2013
domestic chicken	C1	GU261718	China: Yunnan	Miao *et al.,* 2013
domestic chicken	C1	GU261679	China: Henan	Miao *et al.,* 2013
domestic chicken	C2	GU261680	Southern India	Miao *et al.,* 2013
junglefowl	C3	GU261716	Myanmar	Miao *et al.,* 2013
junglefowl	C3	GU261707	India	Miao *et al.,* 2013
junglefowl	D1	NC_007236	Philippine: Manila	Nishibori *et al.,* 2005
junglefowl	D1	NC_007237	Indonesia: Bali	Nishibori *et al.,* 2005
domestic chicken	D1	GU261687	Laos	Miao *et al.,* 2013
domestic chicken	D1	GU261682	Laos	Miao *et al.,* 2013
domestic chicken	D2	GU261683	China: Xinjiang	Miao *et al.,* 2013
domestic chicken	D3	GU261677	China: Zhejiang	Miao *et al.,* 2013
domestic chicken	D3	GU261697	Southern India	Miao *et al.,* 2013
domestic chicken	D3	GU261685	Northeast India	Miao *et al.,* 2013
domestic chicken	E1	GU261686	China: Henan	Miao *et al.,* 2013
domestic chicken	E1	GU261713	China: Yunnan	Miao *et al.,* 2013
domestic chicken	E1	AP003317	Commercial Line	Nishibori *et al.,* 2003
domestic chicken	E1	AY235571	Commercial Lines	Froman and Kirby, 2005
domestic chicken	E1	AP003318	Commercial Line	Nishibori *et al.,* 2003
domestic chicken	E1	GU261712	China: Yunnan	Miao *et al.,* 2013
domestic chicken	E1	GU261709	India	Miao *et al.,* 2013
domestic chicken	E1	AY235570	Commercial Line	Froman and Kirby, 2005
domestic chicken	E1	AP003580	Commercial Line	Nishibori *et al.,* 2003
domestic chicken	E1	GU261694	China: Hebei	Miao *et al.,* 2013
domestic chicken	E1	AP003319	Laos: Vientiane	Nishibori *et al.,* 2005
domestic chicken	E1	HQ857210	Northeast India	Miao *et al.,* 2013
domestic chicken	E2	HQ857209	Northeast India	Miao *et al.,* 2013
junglefowl	E3	GU261708	India	Miao *et al.,* 2013
domestic chicken	E3	HQ857212	Northeast India	Miao *et al.,* 2013
domestic chicken	E3	HQ857211	Northeast India	Miao *et al.,* 2013
junglefowl	F	GU261691	Myanmar	Miao *et al.,* 2013
junglefowl	F	GU261702	China: Yunnan	Miao *et al.,* 2013

domestic chicken	F	GU261688	China: Yunnan	Miao *et al.,* 2013
domestic chicken	F	GU261711	China: Yunnan	Miao *et al.,* 2013
domestic chicken	F	GU261689	China: Yunnan	Miao *et al.,* 2013
junglefowl	F	GU261703	Myanmar	Miao *et al.,* 2013
domestic chicken	F	GU261717	China: Yunnan	Miao *et al.,* 2013
domestic chicken	F	DQ648776	China: Yunnan	Tong *et al.,* 2006
domestic chicken	G	GU261678	China: Henan	Miao *et al.,* 2013
domestic chicken	G	GU261710	China: Yunnan	Miao *et al.,* 2013
domestic chicken	G	GU261676	China: Yunnan	Miao *et al.,* 2013
domestic chicken	G	GU261719	China: Yunnan	Miao *et al.,* 2013
junglefowl	G	GU261690	China: Yunnan	Miao *et al.,* 2013
domestic chicken	H	GU261715	China: Yunnan	Miao *et al.,* 2013
junglefowl	W	GU261706	China: Yunnan	Miao *et al.,* 2013
junglefowl	X	GU261692	China: Yunnan	Miao *et al.,* 2013
domestic chicken	I	GU261698	Northeast India	Miao *et al.,* 2013
domestic chicken		AB268540	Indonesia	Oka *et al,* 2007
domestic chicken		AB268528	Indonesia	Oka *et al,* 2007
domestic chicken		AB268527	Indonesia	Oka *et al,* 2007
domestic chicken		AB268526	Indonesia	Oka *et al,* 2007
domestic chicken		AB268525	Indonesia	Oka *et al,* 2007
domestic chicken		AP003319	Laos	Nishibori *et al,* 2005
domestic chicken		LC146470	Laos	Osman and Nishibori, 2014
domestic chicken		LC146469	Laos	Osman and Nishibori, 2014
domestic chicken		LC146468	Laos	Osman and Nishibori, 2014
domestic chicken		LC146467	Laos	Osman and Nishibori, 2014
domestic chicken		LC146466	Laos	Osman and Nishibori, 2014
junglefowl		LC146458	Cambodia	Osman and Nishibori, 2014
junglefowl		LC146456	Cambodia	Osman and Nishibori, 2014
junglefowl		LC146455	Cambodia	Osman and Nishibori, 2014
junglefowl		LC146454	Cambodia	Osman and Nishibori, 2014
junglefowl		LC146453	Cambodia	Osman and Nishibori, 2014
junglefowl		LC146451	Myanmar	Osman and Nishibori, 2014
junglefowl		LC146450	Myanmar	Osman and Nishibori, 2014
junglefowl		LC146449	Myanmar	Osman and Nishibori, 2014
junglefowl		LC146447	Myanmar	Osman and Nishibori, 2014
domestic chicken		LC147055	Bangladesh	Islam *et al,* 2019
domestic chicken		LC147054	Bangladesh	Islam *et al,* 2019
domestic chicken		LC147053	Bangladesh	Islam *et al,* 2019
domestic chicken		LC147052	Bangladesh	Islam *et al,* 2019
domestic chicken		LC147051	Bangladesh	Islam *et al,* 2019
domestic chicken		LC147050	Bangladesh	Islam *et al,* 2019
domestic chicken		LC147049	Bangladesh	Islam *et al,* 2019

domestic chicken		LC147048	Bangladesh	Islam *et al,* 2019
domestic chicken		LC147047	Bangladesh	Islam *et al,* 2019
domestic chicken		LC147046	Bangladesh	Islam *et al,* 2019
domestic chicken		LC147045	Bangladesh	Islam *et al,* 2019
domestic chicken		LC147044	Bangladesh	Islam *et al,* 2019
domestic chicken		LC147043	Bangladesh	Islam *et al,* 2019
domestic chicken		LC147042	Bangladesh	Islam *et al,* 2019
domestic chicken		LC147041	Bangladesh	Islam *et al,* 2019
domestic chicken		LC147040	Bangladesh	Islam *et al,* 2019
domestic chicken		LC147039	Bangladesh	Islam *et al,* 2019
domestic chicken		LC147038	Bangladesh	Islam *et al,* 2019
domestic chicken		LC147037	Bangladesh	Islam *et al,* 2019
domestic chicken		LC147036	Bangladesh	Islam *et al,* 2019
domestic chicken		LC147035	Bangladesh	Islam *et al,* 2019
domestic chicken		AB007724	Thailand	Direct submission
Tosa-Jidori		AB268522	Japan	Oka et al., 2007
Tosa-Jidori	D	LC507814	Japan	Osman et al., 2021
Tosa-Jidori	D	LC507816	Japan	Osman et al., 2021

* sister-group for sub-haplogroup D3 (Miao *et al*., 2013)
** 5 Native Chickens and 1 Red junglefowl

Appendix Table S2.2. Control region diagnostic motifs and sequence variation of Philippine chicken haplogroups relative to the reference sequence.

Haplotype notation in Fig. 1 and Fig. 2	*n*	Haplo-group	Variation v.s. NCBI new reference sequence NC_040970	Source
	2			
Hap_1	4	E1	*same as reference sequence*	domestic chicken
Hap_14	4	A	T167C, C217T, C225T, C243T, C256T, T261C, T310C, T446C, T1214C	domestic chicken
Hap_25	4	D2	C217T, A281G, T306C, T446C, T1214C	red junglefowl
Hap_29	3	E1	T199C,	domestic chicken
	2			
Hap_49	1	D1	C217T, A281G, T306C, A342G, T446C, T1214C	domestic chicken
Hap_50	3	D1	C217T, A281G, T306C, T309C, T446C, T1214C	domestic chicken
Hap_51	2	B	G212A, C217T, C243T, C246T, C256T, T261C, T310C, C315T, T446C, G792A, T1214, G1215A	domestic chicken
Hap_54	1	D1	C217T, A281G, C302T, T306C, T309C, T446C, T1214C	domestic chicken
Hap_57	8	D1	C217T, A281G, **C296T**, T306C, A342G, T446C, **G686A**, T1214C	domestic chicken
Hap_64	1	D1	C217T, A281G, T306C, A342G, T355C, T446C, T1214C	domestic chicken
Hap_65	6	D1	C217T, C246T, A281G, **C296T**, T306C, A342G, T446C, **G686A**, T1214C	domestic chicken
Hap_66	1	D1	C217T, A281G, T306C, T309C, T322C, T446C, T1214C	domestic chicken
Hap_67	1	D1	C217T, G238A, A281G, T306C, T309C, T322C, T446C, T1214C	domestic chicken
	1			
Hap_68	2	D1	C217T, A281G, **C296T**, T306C, T446C, **G686A**, T1214C	domestic chicken
Hap_69	3	D1	C217T, T220C, A281G, T306C, A342G, T446C, T1214C	domestic chicken
Hap_70	2	D1	C217T, C243T, A281G, T306C, A342G, T446C, T1214C	domestic chicken
Hap_71	1	D1	C217T, A281G, T306C, T309C, A342G, T446C, T1214C	domestic chicken
Hap_73	1	B	G212A, C217T, C243T, C246T, C256T, T261C, T310C, C315T, T446C, T1214C, G1215A	domestic chicken
	2			
Hap_74	2	D1	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	domestic chicken
Hap_75	1	E1	G238A, T322C,	domestic chicken
Hap_76	2	E1	T190C	domestic chicken

Hap_77	1	D1	C217T, A281G, **C296T**, T306C, T396C, G399A, T446C, **G686A**, T1214C	domestic chicken
Hap_78	1	D1	C217T, C225T, A281G, **C296T**, T306C**,** T446C, **G686A**, T1214C	domestic chicken
Hap_80	2	D3*	C217T, T220C, C246T, A281G, T306C, T446C, T1214C	red junglefowl
Hap_85	2	D1	C217T, A281G, T293C, **C296T**, T306C, A342G, T446C, **G686A**, T1214C	domestic chicken
Hap_86	1	E1	G399A,	domestic chicken
Hap_87	1	A	T167C, C217T, C225T, C243T, C256T, T261C, T310C, G399A, T446C, T1214C	domestic chicken
Hap_88	3	B	G212A, C217T, C243T, C246T, C256T, T261C, A281G, T310C, C315T, T446C, T1214C, G1215A	domestic chicken
Hap_89	1	B	G212A, C217T, C243T, C246T, C256T, T261C, T310C, C315T, G399A, T446C, G792A, T1214C, G1215A	domestic chicken
Hap_90	4	D1	C217T, T261C, A281G, **C296T**, C306T, T446C, **G686A**, T1214C	domestic chicken
Hap_91	1	D3*	C217T, T220C, A281G, T306C, T446C, T1214C	red junglefowl
Hap_92	1	D1	T199C, C217T, A281G, **C296T**, T306C, T446C, **G686A**, T1214C	red junglefowl
Hap_93	1	D1	C217T, A281G, **C296T**, T306C, C417T, T446C, **G686A**, T1214C	red junglefowl
Hap_94	1	D1	A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	domestic chicken

Note: * Philippine red junglefowl sequences (*novel for this present study*) that does not have any specific haplogroup signatures from previously identified global haplogroup classification (*Miao et al., 2013*), but appear to be sister-group with sub-haplogroup D3.

the unique mutation signatures for Philippine-Pacific subclade are in **BOLD**.

indel observed at 859 nucleotide position.

1

Appendix Table S2.3. mtDNA D-loop hypervariable region information of Philippine chickens, Indonesian chickens, Pacific chickens, and chicken populations across Asia, the Middle East and Africa.

(D-loop) Fragment Length	Species	Haplo group assign ment	GenBank accession number	Number of individu als	Collection locality	Source
Partial Sequence [764bp]	RJF	D2	MN986398	1	Leyte, Philippines	This study
	RJF	D2	MN986399	1	Leyte, Philippines	This study
	RJF	D2	MN986400	1	Leyte, Philippines	This study
	RJF	D3	MN986401	1	Leyte, Philippines	This study
	RJF	D3	MN986402	1	Leyte, Philippines	This study
	RJF	D2	MN986403	1	Leyte, Philippines	This study
	domestic chicken	D1	MN986372	4	Samar, Philippines	This study
	domestic chicken	D1	MN986373	3	Samar, Philippines	This study
	domestic chicken	D1	MN986374	3	Samar, Philippines	This study
	domestic chicken	D1	MN986376	1	Samar, Philippines	This study
	domestic chicken	D1	MN986377	6	Samar, Philippines	This study
	domestic chicken	D1	MN986378	1	Samar, Philippines	This study
	domestic chicken	D1	MN986381	16	Leyte, Philippines	This study
	domestic chicken	D1	MN986382	6	Leyte, Philippines	This study
	domestic chicken	D1	MN986384	12	Leyte, Philippines	This study
	domestic chicken	D1	MN986385	1	Leyte, Philippines	This study
	domestic chicken	D1	MN986388	2	Leyte, Philippines	This study
	domestic chicken	D1	MN986393	1	Leyte, Philippines	This study
	domestic chicken	D1	MN986394	1	Leyte, Philippines	This study
	domestic chicken	D1	MN986396	1	Leyte, Philippines	This study
	domestic chicken	D1	MN986397	1	Leyte, Philippines	This study
	Junglefowl	D3	MK085033		Samar, Philippines	Godinez et al., 2019
	Junglefowl	D1	MK085034		Samar, Philippines	Godinez et al., 2019
	Junglefowl	D1	MK085035		Samar, Philippines	Godinez et al., 2019
	domestic chicken	D1	MK085043		Samar, Philippines	Godinez et al., 2019
	domestic chicken	D1	MK085044		Samar, Philippines	Godinez et al., 2019
	domestic chicken	D1	MK085045		Samar, Philippines	Godinez et al., 2019
	domestic chicken	D1	MK085046		Samar, Philippines	Godinez et al., 2019
	domestic chicken	D1	MK085047		Samar, Philippines	Godinez et al., 2019
	domestic chicken	D1	MK085048		Samar, Philippines	Godinez et al., 2019
	domestic chicken	D1	MK085049		Samar, Philippines	Godinez et al., 2019
		D1	KY039417		Mindoro, Philippines	Herrera et al., 2018
		D1	KY039402		Zamboanga, Philippines	Herrera et al., 2018
		D1	KY039401		Tugop, Philippines	Herrera et al., 2018
		D1	KY039409		Ifugao, Philippines	Herrera et al., 2018
		D1	KY039398		Cotabato, Philippines	Herrera et al., 2018
		D1	KY039414		Palawan, Philippines	Herrera et al., 2018
		D1	KY039408		Asipulo, Philippines	Herrera et al., 2018
		D1	KY039396		Manticao, Philippines	Herrera et al., 2018
		D1	KY039412		Palawan, Philippines	Herrera et al., 2018

D1	KY039411	Palawan, Philippines	Herrera et al., 2018
D1	KY039397	Alubijid, Philippines	Herrera et al., 2018
D1	KY039406	Ifugao, Philippines	Herrera et al., 2018
D1	KY039400	Tugop, Philippines	Herrera et al., 2018
D1	KY039413	Palawan, Philippines	Herrera et al., 2018
D1	KY039437	Palawan, Philippines	Herrera et al., 2018
D1	KY039415	Cagayan Valley, Philippines	Herrera et al., 2018
D1	KY039404	Ifugao, Philippines	Herrera et al., 2018
D1	KY039405	Ifugao, Philippines	Herrera et al., 2018
D1	KY039416	Mindoro, Philippines	Herrera et al., 2018
D1	KY039407	Ifugao, Philippines	Herrera et al., 2018
D1	KY039410	Batanes, Philippines	Herrera et al., 2018
D1	KY039403	Zamboanga, Philippines	Herrera et al., 2018
D1	KY039386	Easter Island	Herrera et al., 2018
D1	KY039387	Easter Island	Herrera et al., 2018
D1	KY039388	Easter Island	Herrera et al., 2018
D1	KY039389	Easter Island	Herrera et al., 2018
D1	KY039390	Easter Island	Herrera et al., 2018
D1	KY039384	Hawaii	Herrera et al., 2018
D1	KY039391	Fiji	Herrera et al., 2018
D1	KY039385	Hawaii	Herrera et al., 2018
D1	KY039383	Hawaii	Herrera et al., 2018
D1	KY039381	Marquesas	Herrera et al., 2018
D1	KY039382	Niue	Herrera et al., 2018
D1	KY039392	Vanuatu	Herrera et al., 2018
D1	KY039393	Vanuatu	Herrera et al., 2018
D1	KY039431	New Caledonia	Herrera et al., 2018
D1	KY039432	New Caledonia	Herrera et al., 2018
D1	KY039429	Sulawesi, Indonesia	Herrera et al., 2018
D1	KY039428	Sulawesi, Indonesia	Herrera et al., 2018
D1	KY039427	Sulawesi, Indonesia	Herrera et al., 2018
D1	KY039426	Lombok, Indonesia	Herrera et al., 2018
D1	KY039425	Maluku, Indonesia	Herrera et al., 2018
D1	KY039395	Maluku, Indonesia	Herrera et al., 2018
D1	KY039424	Manokwari, Indonesia	Herrera et al., 2018
D1	KY039423	Manokwari, Indonesia	Herrera et al., 2018
D1	KY039422	Kalimantan, Indonesia	Herrera et al., 2018
D1	KY039421	Kalimantan, Indonesia	Herrera et al., 2018
D1	KY039420	Java, Indonesia	Herrera et al., 2018
D1	KY039418	Java, Indonesia	Herrera et al., 2018
D1	KY039419	Java, Indonesia	Herrera et al., 2018
D	KX642695	Ifugao, Philippines	Herrera et al., 2017
D	KX642490	Bataan, Philippines	Herrera et al., 2017
D	KX642491	Bataan, Philippines	Herrera et al., 2017
D	KX642986	Manticao, Philippines	Herrera et al., 2017
D	KX642891	Lurugan, Philippines	Herrera et al., 2017
D	KX643088	Palawan, Philippines	Herrera et al., 2017
D	KX643089	Palawan, Philippines	Herrera et al., 2017
D	KX642492	Batanes, Philippines	Herrera et al., 2017
D	KX642493	Batanes, Philippines	Herrera et al., 2017
D	KX642494	Batanes, Philippines	Herrera et al., 2017
D	KX642495	Batanes, Philippines	Herrera et al., 2017
D	KX642469	Asipulo, Philippines	Herrera et al., 2017
D	KX642701	Ifugao, Philippines	Herrera et al., 2017
D	KX642683	Ifugao, Philippines	Herrera et al., 2017
D	KX642684	Ifugao, Philippines	Herrera et al., 2017
D	KX642692	Ifugao, Philippines	Herrera et al., 2017
D	KX642693	Ifugao, Philippines	Herrera et al., 2017
D	KX642697	Ifugao, Philippines	Herrera et al., 2017
D	KX642699	Ifugao, Philippines	Herrera et al., 2017
D	KX642702	Ilocos, Philippines	Herrera et al., 2017

D	KX643034	Naawan, Philippines	Herrera et al., 2017
D	KX643014	Mindoro, Philippines	Herrera et al., 2017
D	KX643018	Mindoro, Philippines	Herrera et al., 2017
D	KX643023	Mindoro, Philippines	Herrera et al., 2017
D	KX643133	Sorsogon, Philippines	Herrera et al., 2017
D	KX642464	Alubijid, Philippines	Herrera et al., 2017
D	KX642465	Alubijid, Philippines	Herrera et al., 2017
D	KX643196	Talakag, Philippines	Herrera et al., 2017
D	KX643200	Talakag, Philippines	Herrera et al., 2017
D	KX642894	Lurugan, Philippines	Herrera et al., 2017
D	KX643122	San Juan, Philippines	Herrera et al., 2017
D	KX643317	Zamboanga, Philippines	Herrera et al., 2017
D	KX643318	Zamboanga, Philippines	Herrera et al., 2017
D	KX642547	Cebu, Philippines	Herrera et al., 2017
D	KX643029	Mindoro, Philippines	Herrera et al., 2017
D	KX643070	Palawan, Philippines	Herrera et al., 2017
D	KX642694	Ifugao, Philippines	Herrera et al., 2017
D	KX642698	Ifugao, Philippines	Herrera et al., 2017
D	KX642680	Ifugao, Philippines	Herrera et al., 2017
D	KX642681	Ifugao, Philippines	Herrera et al., 2017
D	KX642682	Ifugao, Philippines	Herrera et al., 2017
D	KX643131	Sorsogon, Philippines	Herrera et al., 2017
D	KX642487	Basilan, Philippines	Herrera et al., 2017
D	KX642471	Aya-aya, Philippines	Herrera et al., 2017
D	KX642777	Kalupnayan, Philippines	Herrera et al., 2017
D	KX642599	Davao Oriental, Philippines	Herrera et al., 2017
D	KX643201	Talakag, Philippines	Herrera et al., 2017
D	KX643202	Talakag, Philippines	Herrera et al., 2017
D	KX643204	Talakag, Philippines	Herrera et al., 2017
D	KX642592	Damulog, Philippines	Herrera et al., 2017
D	KX642593	Damulog, Philippines	Herrera et al., 2017
D	KX642542	Caraga, Philippines	Herrera et al., 2017
D	KX642543	Caraga, Philippines	Herrera et al., 2017
D	KX642544	Caraga, Philippines	Herrera et al., 2017
D	KX642545	Caraga, Philippines	Herrera et al., 2017
D	KX642716	Intabas, Philippines	Herrera et al., 2017
D	KX642714	Intabas, Philippines	Herrera et al., 2017
D	KX642715	Intabas, Philippines	Herrera et al., 2017
D	KX642984	Manticao, Philippines	Herrera et al., 2017
D	KX642588	Cotabato, Philippines	Herrera et al., 2017
D	KX642849	Kibawe, Philippines	Herrera et al., 2017
D	KX642850	Kibawe, Philippines	Herrera et al., 2017
D	KX643123	San Juan, Philippines	Herrera et al., 2017
D	KX643320	Zamboanga, Philippines	Herrera et al., 2017
D	KX643322	Zamboanga, Philippines	Herrera et al., 2017
D	KX643331	Zamboanga, Philippines	Herrera et al., 2017
D	KX643332	Zamboanga, Philippines	Herrera et al., 2017
D	KX642708	Iloilo, Philippines	Herrera et al., 2017
D	KX642709	Iloilo, Philippines	Herrera et al., 2017
D	KX642710	Iloilo, Philippines	Herrera et al., 2017
D	KX643071	Palawan, Philippines	Herrera et al., 2017
D	KX643073	Palawan, Philippines	Herrera et al., 2017
D	KX643075	Palawan, Philippines	Herrera et al., 2017
D	KX643080	Palawan, Philippines	Herrera et al., 2017
D	KX643086	Palawan, Philippines	Herrera et al., 2017
D	KX643112	Samar, Philippines	Herrera et al., 2017
D	KX642466	Asipulo, Philippines	Herrera et al., 2017
D	KX642467	Asipulo, Philippines	Herrera et al., 2017
D	KX643134	Sorsogon, Philippines	Herrera et al., 2017
D	KX642592	Damulog, Philippines	Herrera et al., 2017
D	KX642531	Butuan, Philippines	Herrera et al., 2017

D	KX643333	Zamboanga, Philippines	Herrera et al., 2017
D	KX643090	Palawan, Philippines	Herrera et al., 2017
D	KX642595	Davao Oriental, Philippines	Herrera et al., 2017
D	KX643078	Palawan, Philippines	Herrera et al., 2017
D	KX643084	Palawan, Philippines	Herrera et al., 2017
D	KX643085	Palawan, Philippines	Herrera et al., 2017
D	KX643087	Palawan, Philippines	Herrera et al., 2017
D	KX642533	Cagayan Valley, Philippines	Herrera et al., 2017
D	KX642468	Asipulo, Philippines	Herrera et al., 2017
D	KX642703	Ilocos, Philippines	Herrera et al., 2017
D	KX642704	Ilocos, Philippines	Herrera et al., 2017
D	KX642719	Isabela, Philippines	Herrera et al., 2017
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D	OM240181	Fiji, Pacific	Godinez et al. (in press)
D	OM240182	Fiji, Pacific	Godinez et al. (in press)
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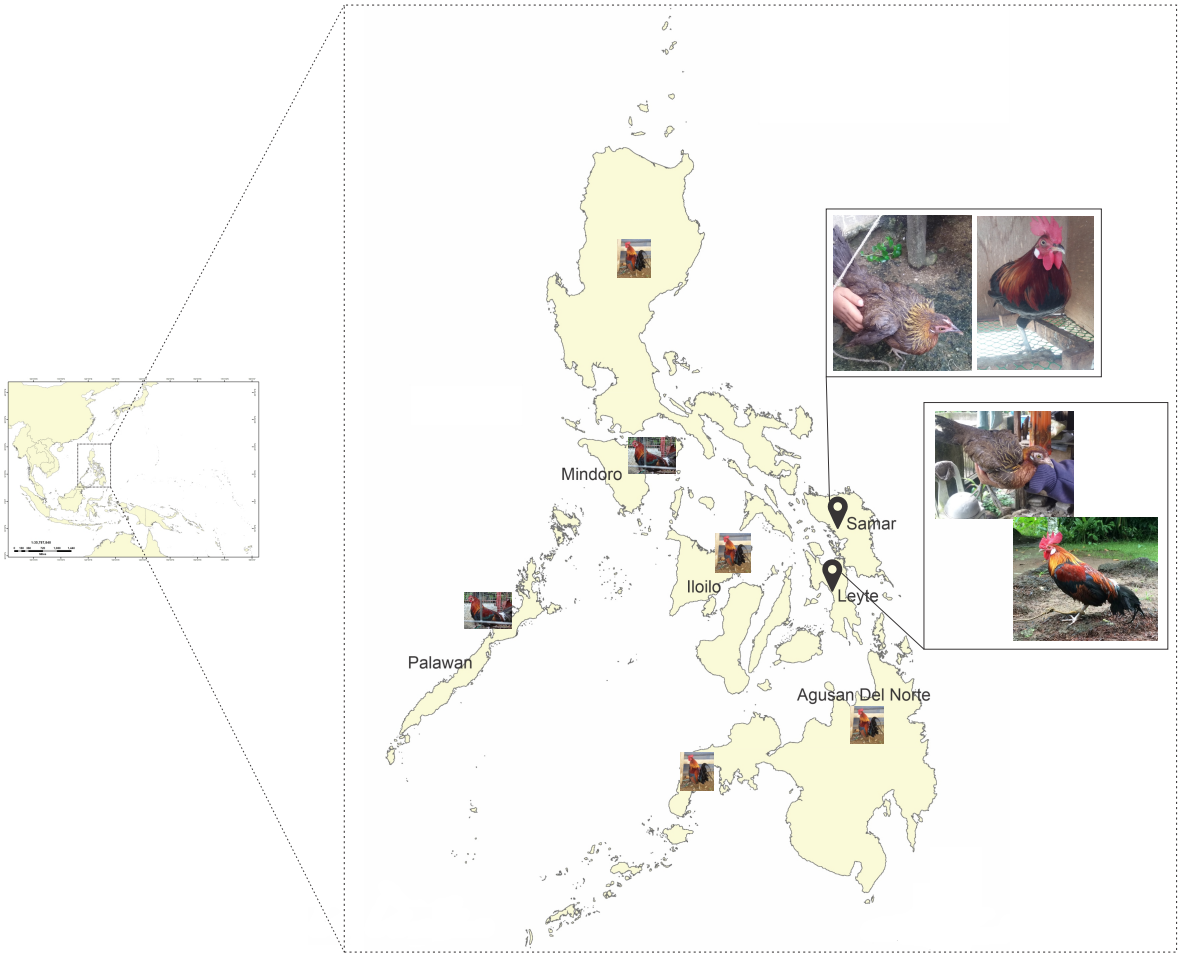
	AB829477	Egypt	Osman et al., 2016
	AB829478	Egypt	Osman et al., 2016
	AB829479	Egypt	Osman et al., 2016
	AB829480	Egypt	Osman et al., 2016
	AB829481	Egypt	Osman et al., 2016
	AB829482	Egypt	Osman et al., 2016
	AB829483	Egypt	Osman et al., 2016
	AB829484	Egypt	Osman et al., 2016

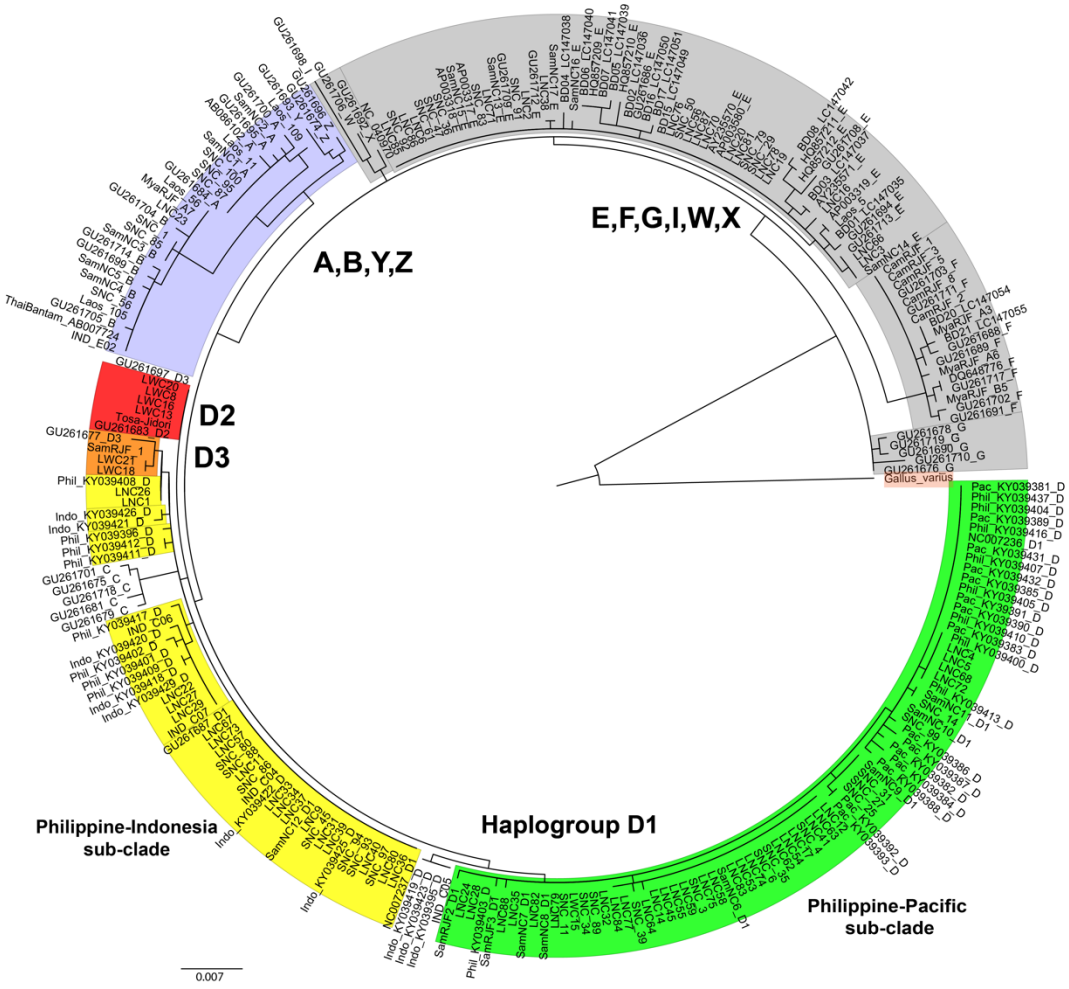
AB829485	Egypt	Osman et al., 2016
AB829486	Egypt	Osman et al., 2016
AB829487	Egypt	Osman et al., 2016
AB829488	Egypt	Osman et al., 2016
AB829490	Egypt	Osman et al., 2016
AB829491	Egypt	Osman et al., 2016

-sequences from this study were truncated to accommodate partial sequence length
-sequences and accession no. for MSEA chicken populations were listed in Supp. Table S3.1 in Chapter III
-sequences for Chinese chickens were personally gifted by Prof. Xunhe Huang (Jiaying University) and were listed listed in Supp. Table S3.4 in Chapter III

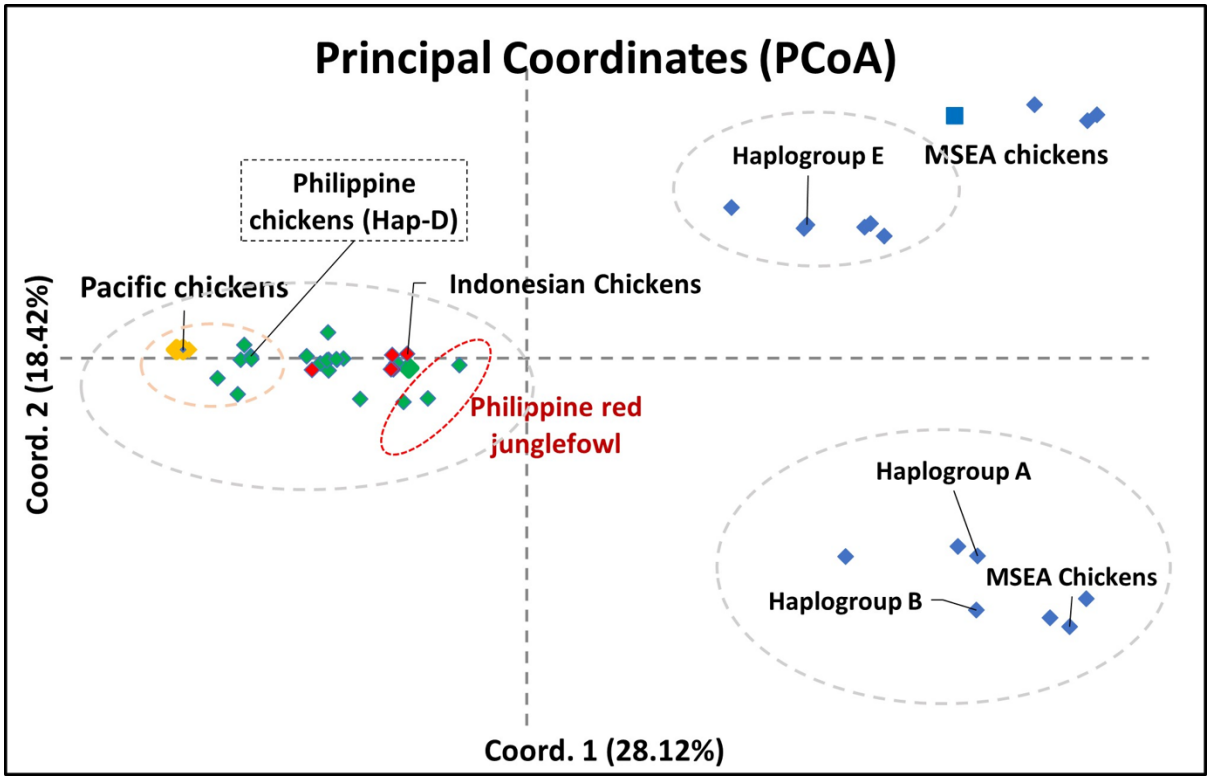
Appendix Figure S2.1. Sampling locations used in this study. The geographical distribution of Philippine chickens was complemented with published sequences retrieved from GenBank database (in small picture).



Appendix Figure S2.2. Maximum likelihood (ML) phylogenetic tree showing four haplogroup classifications (predominant haplogroup D) of Philippine chickens and different classifications from other neighboring countries. Node labels correspond to bootstrap support values evaluated with 1,000 ultrafast bootstrap replicates in IQ-TREE. The scale bar (0.007) indicates the genetic distance (substitution per site).



Appendix Figure S2.3. Principal coordinate analysis (PCoA) plots of the population pairwise inter-haplotypic distance matrix of chicken populations in the ISEA and MSEA using complete mtDNA D-loop region. Populations are assigned the following colors (green: Philippine RJFs and native chickens; yellow: Pacific chickens; red: Indonesian chickens; blue: MSEA chickens and other haplogroups).



CHAPTER III

Origin and domestication of Southeast Asian chickens: Insights into the phylogeography, geographical distribution, and population dynamics

Abstract

The complex geographic and temporal origins of chicken domestication have attracted wide interest in molecular phylogeny and phylogeographic studies as they continue to be debated up to this day. In particular, the unresolved question remains whether the founding lineages of chickens introduced in the island archipelago arrived as descendants of wild endemic populations or descendants of domestic chickens from mainland Southeast Asia. This study analyzed 519 complete mitochondrial DNA control region sequences and identified 133 haplotypes with 70 variable sites. The result documented 82.7% geographically unique haplotypes distributed across major haplogroups except for haplogroup C, suggesting high polymorphism among studied individuals. Mainland SEA (MSEA) chickens have higher overall genetic diversity than island SEA (ISEA) chickens. Divergent sub-haplogroups that retained ancestral mutational motifs observed among continental chicken populations suggest geographic proximity to the center of domestication. Phylogenetic trees and median-joining network revealed a new divergent matrilineage (i.e., haplogroup V) as a sister-clade of haplogroup C. Interestingly, this potential ancestral matriline sub-haplogroup D2 and newfound matriline haplogroup V were identified in sampling areas along the Lower Mekong subregion. Genetic differentiation of populations and PCoA analyses revealed genetic substructure between geographically isolated populations, i.e., between MSEA and ISEA chickens, reflecting deep phylogeographic diversification. The coalescent-based Bayesian demographic analyses detected earlier effective population size expansion in MSEA chickens, while island populations showed more recent demographic growth signatures. This study suggests human-mediated translocation of the haplogroup D ancestral matriline (i.e., haplogroup D2) from MSEA, which gave rise to the island populations and expanded into a new sub-haplogroup D1b (i.e., Philippine-Pacific subclade).

Keywords: domestication, *Gallus* gallus, genetic diversity, Lower Mekong, phylogeography, Southeast Asia

3.1 Introduction

The domestication of animals has led to important shifts in human demographics that helped shape early human societies. Chickens are the most widely domesticated animal species in the world. As a result, they play a crucial role in human societies as the largest source of animal protein (FAO, 2015; Lawler, 2020) and as a significant factor in socio-cultural development (Sykes, 2012). Since domestication, chickens have been distributed throughout various countries and continents and have resulted in a wide range of chicken breeds today (Groeneveld et al., 2010; Malomane et al., 2019). However, studies on the chicken domestication process and translocation history remain obscure despite their global distribution. Modern biological and zooarchaeological approaches suggest that chicken domestication probably occurred across southwestern China and Southeast Asia, involving one or more wild progenitors across their native geographical range (Eda et al., 2016; Liu et al., 2006; Miao et al., 2013; Peters et al., 2016; Wang et al., 2020; West & Zhou, 1988). Subsequently, domestic chickens have been translocated from domestication centers to every region inhabited by human migration and trade expansion. This led to the evolution of subpopulations of chickens in response to natural selection pressure and selective breeding for adaptation to a variety of agro-ecological conditions (Lawal & Hanotte, 2021).

Being the most geographically complex tropical region on Earth, Southeast Asia (SEA) has given rise to a diverse and highly endemic avifauna (Lohman et al., 2011; Myers et al., 2000). The emergence of agricultural societies harboring domesticated animals allowed a remarkable expansion of genetically divergent domestic populations, a case seen in chickens that likely followed a commensal route of the domestication process (Larson & Burger, 2013).

Several DNA sources and molecular strategies were recently used to resolve chicken phylogeny and their genetic expansion from their wild progenitors (Hata et al., 2021; Herrera et al., 2020; Lawal et al., 2020; Mariadassou et al., 2021; Tiley et al., 2020; Wang et al., 2020). However, significant challenges from the zooarchaeological perspective remain as only a few reports of chicken remains in SEA (Storey et al., 2012), and prehistoric exploitation has yet to be elucidated (Eda et al., 2019). Such evolutionary links would likely provide a better understanding of the evolutionary history and population dynamics of the world's most common farm animal.

Early studies reconstructing the evolutionary history of domestic chickens based on mitochondrial DNA (mtDNA) analysis supported a monophyletic origin of the red junglefowl, which serves as the primary wild ancestor of domestic chickens (Fumihito et al., 1994, 1996). However, in the early 21st century, numerous mtDNA analyses suggested multiple domestication events (Kanginakudru et al., 2008; Liu et al., 2006; Miao et al., 2013) and the possibility of different *Gallus* species contributing to the genetic characteristics of domestic chickens (Eriksson et al., 2008; Lawal et al., 2020; Mariadassou et al., 2021; Nishibori et al., 2005). Moreover, recent genome-wide data linked domestic chickens most closely to the Southeast Asian subspecies *G. g. spadiceus*, which locally interbred with other subspecies across southwestern China and Southeast Asia (Wang et al., 2020). Mitochondrial DNA D-loop variation has been extensively used to understand chicken populations, types, evolutionary relationships, and domestication history. Chickens have been classified into eight highly divergent maternal haplogroups (A–G, V) and six rare haplogroups (H–I, W–Z) (Huang et al., 2018; Miao et al., 2013). Major haplogroups A and B were ubiquitously distributed in Asian regions, whereas haplogroup E was widely distributed in Europe, the Middle East, Africa, and South America (Al-Jumaili et al., 2020; Herrera et al., 2020; Miao et al., 2013; Mwacharo et al., 2011). Haplogroup C was distributed over East Asia, whereas haplogroup F

was restricted to Yunnan, China and Myanmar (Huang et al., 2018; Miao et al., 2013; Mon et al., 2021). Haplogroup D was mostly found in SEA and Pacific populations (Dancause et al., 2011; Godinez et al., 2021; Thomson et al., 2014). The knowledge of population studies on genetic diversity, population structures, and demography is essential to understanding the role of past and present evolutionary processes of chickens throughout domestication.

This study generated complete mitochondrial DNA D-loop sequences of chickens from mainland SEA (Cambodia, Laos, Thailand, and Myanmar), the Philippines, and Fiji, spanning a geographical transect that is believed to encompass possible translocations of this taxon in the region. These newly generated sequence data were combined with previously published data of ISEA chickens (the Philippines and Indonesia), Pacific chickens, and neighboring chicken populations in Asia. Furthermore, this study sought to obtain an updated perspective of the matrilineal phylogeny and demographic events that shaped the genetic diversity of SEA and Pacific chickens.

3.2 Materials and Methods

3.2.1 Sampling and DNA extraction

Blood samples were collected from a total of 369 individuals from Cambodia (n=173, domestic chickens), Laos (n=63, domestic chickens), Myanmar (n=75, domestic chickens; n-=3, red junglefowls), Thailand (n=18, red junglefowls; n=7, domestic chickens), the Philippines (n=6, red junglefowl), and Fiji (n=24, domestic chickens) (Appendix Figure S3.1). Details of the sampled animals and their geographical distribution are listed in Appendix Table S3.1. Precautions were taken to avoid sampling from the related individuals. Genomic DNA was extracted from stored whole blood samples using the phenol-chloroform method (Green & Sambrook, 2012).

The final dataset was complemented with previously published sequences of Philippine chickens (*n*=129) (Godinez et al., 2021) and directly submitted sequences of Indonesian (*n*=10) and Pacific chickens (*n*=11) retrieved from GenBank (Appendix Table S3.1).

3.2.2 PCR amplification and sequencing

The target complete mitochondrial control region (1,231-1,232 bp) was amplified in two procedures. First, about 5.0 kbp mtDNA D-loop fragments were amplified using a long and accurate – PCR (LA-PCR) kit (KOD-FX Neo Polymerase, TOYOBO, Osaka, Japan) with chicken DNA as a template and LA-PCR primer sets: *Cytb-Forward*: 5′-TACACGAATCAGGCTCAAACAACCCCCTAGGCATC-3′, *16S-Reverse*: 5′-TGCACCATTAGGTTGTCCTGATCCAACATCGAGGT-3′ recommended by Nishibori et al. (2003). The reaction began with a preliminary denaturation at 94 °C for 2 min, followed by 30 cycles of DNA denaturation at 98 °C for 10 sec, annealing of primers at 57 °C for 30 sec, and primer extension at 68 °C for 2 min and 30 sec, using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, USA). Second, the amplified fragments were used for segmental amplification of the complete mtDNA D-loop region (1.3 kbp) following the primer sets: *Gal1F* 5′-AGGACTACGGCTTGAAAAGCCATTG-3′ and *Gal1R* 5′-GCTGAGTACCCGTGGGGGTGTGGCT-3′ in 20 µl reaction volume containing 2x PCR buffer, 0.4 mM dNTPs, 0.3 µM concentrations of each primer, 0.4 U of KOD-FX Neo DNA Polymerase, and 15-25 ng of amplified fragment DNA as template. The PCR cycling condition began with a preliminary denaturation at 94 °C for 2 min, followed by 30 cycles of DNA denaturation at 98 °C for 10 sec, annealing of primers at 59 °C for 30 sec, and primer extension at 68 °C for 30 sec, using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, USA). The DNA fragments obtained from the segmental amplification were cleaned and purified using Exonuclease I (ExoI) and Shrimp Alkaline Phosphatase (SAP) to degrade the residual PCR primers and dephosphorylate the remaining dNTPs, respectively. The two PCR

primers and one internal primer: *Gal1-2F* 5′ -TCCACCTCACGAGAGATCAGCAACCC-3′ (Nishibori et al., 2001) were used for the sequencing reaction. Subsequently, the mtDNA D-loop fragments were directly sequenced using 3130/3130xl Genetic Analyzers (Applied Biosystems, Foster City, USA).

3.2.3 Sequence alignment

Three hundred sixty-nine complete mtDNA control region sequences generated in this study were initially edited using GeneStudio Pro tool (GeneStudio, Inc., http://genestudio.com/). Ambiguous sites were trimmed and cleaned sequences were aligned in MEGAX (Kumar et al., 2018) with ClustalW (Thompson et al., 1994). Aligned nucleotide sequences were viewed using BioEdit 7.2.5 software (Hall, 1999). All newly generated sequences were deposited in the GenBank database with accession numbers OM240181-OM240549 (Appendix Table S3.1).

3.2.4 Genetic diversity and phylogenetic inference

Intrapopulation level and intraclade genetic diversity indices such as the number of haplotypes (Ht), haplotype diversity (*Hd*), and nucleotide diversity (π) were estimated using the DnaSP v6.0 software (Librado & Rozas, 2009).

Phylogenetic analyses were inferred using two different model-based approaches: maximum-likelihood (ML) and Bayesian inference (BI). Maximum-likelihood analysis was performed in IQ-TREE (Nguyen et al., 2015) with the best-fit 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. Bayesian inference was performed using BEAST2 v2.6.6 (Bouckaert et al., 2019) under uncorrelated relaxed clock log-normal distribution setting a clock rate of 3.13×10^{-7} mutations/site/year rate (Alexander et al., 2015). A general time reversible (GTR) nucleotide

substitution site model was used with assumed rate heterogeneity among sites modeled under gamma distribution and a coalescent-based model as a tree prior. The posterior distributions of parameters were estimated via Markov chain Monte Carlo (MCMC) with duplicate runs of 50 million generations and sampling every 10,000 steps. The initial 10% trees of each MCMC run were discarded as burn-in. Convergence of MCMC chains was assessed using Tracer v.1.7.1, and sufficient sampling was verified with all estimated parameters exceeding 200 ESS values. A Maximum Clade Credibility (MCC) tree (target tree) was obtained from a sample of trees using TreeAnnotator v2.6.3 (Bouckaert et al., 2019). Phylogenetic trees were visualized and edited in FigTree v1.4.4. (http://tree.bio.ed.ac.uk/software/figtree/).

Median-joining (MJ) network was 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. The definition of haplogroups was employed in DomeTree (http://dometree.kiz.ac.cn/) and MitoToolPy (http://mitotool.kiz.ac.cn/) (Peng et al., 2015).

3.2.5 Population genetic structure and demographic inference

The population pairwise net genetic distance based on population pairwise F_{ST} (significant values were accepted at $p<0.05$) was estimated using Arlequin v3.5.2.2 software (with 10,000 permutations) (Excoffier & Lischer, 2010). To visualize the pattern of genetic relationship between geographical populations, population pairwise F_{ST} values were plotted into the principal coordinate analysis (PCoA) using GenAlEx v6.503 (Peakall & Smouse, 2006). Estimation of the genetic structures was calculated by the analysis of molecular variance (AMOVA) as implemented by Arlequin v3.5.2.2 software. The level of significance was evaluated based on 1,000 random permutations.

Inference for the population growth model was initially estimated by neutrality statistical tests, such as Tajima's *D* (Tajima, 1989) and Fu's *Fs* statistics (Fu, 1997). These population

expansion tests measure haplotype frequencies under neutrality and panmixis. Statistical tests and confidence intervals were based on a coalescent simulation algorithm under a neutral infinite-site model. To further support the inference for the population expansion signal, coalescent-based Bayesian Skyline Plot (BSP) was used to quantify the relationship between genealogies and the demographic history of the population (Drummond et al., 2005). BSP was simulated to infer deeper insights into the demographic history of MSEA and ISEA chickens as implemented in BEAST v2.6.3 (Bouckaert et al., 2019). BSP was generated with a relaxed molecular clock model and setting with 3.13×10^{-7} mutations/site/year rate (Alexander et al., 2015). The piecewise constant function and HKY+G4 nucleotide substitution model was used for the analysis. The MCMC chain was run for 5×10^{7} generations, with a sampling of parameters every 5,000 steps and 5×10^{6} generations served as burn-in. Convergence of the posterior estimates of the effective population size (N_e) to the likelihood stationary distribution was evaluated using Tracer v1.7.1 software (Rambaut et al., 2018).

3.3 Results

3.3.1 Haplotype variation and genetic diversity

We analyzed complete mtDNA control region sequences of chickens from Cambodia (*n*=173), Laos (*n*=63), Thailand (*n*=25), Myanmar (*n*=78), the Philippines (*n*=6), and Fiji (*n*=24) generated in this study and including previously published sequences from the Philippines (*n*=129), Indonesia (*n*=10), and Pacific (*n*=11). A total of 133 haplotypes were identified, with 70 variable sites consisting of 10 singletons and 60 parsimony informative sites. This study documented 82.7% geographically unique haplotypes, while 17.3% of haplotypes were shared transregionally across SEA, suggesting high polymorphism among the studied individuals. Island populations (i.e., Philippine and Pacific chickens) accounted for 28% of all unique haplotypes identified, while 72% were unique to continental populations.

The summary of observed polymorphic sites and haplotype variations is presented in Appendix Tables S3.1-S3.2.

The indices of genetic diversity for each geographic population are shown in Table 3.1. Undoubtedly, haplotypic and nucleotide diversity was very high in the SEA chicken populations. The MSEA chickens had higher total haplotypic diversity (*Hd*=0.963 ± 0.005) and nucleotide diversity (π=0.00782 ± 0.00398) than the ISEA chickens (*Hd*=0.942 ± 0.009; π=0.00466 ± 0.00249), although no major differences were observed. The highest value of *Hd* and π was found in Thai chickens (72% RJFs in our data set), whereas the least was observed in Pacific chickens. These results should be taken with caution given the relatively small sample size of Pacific chickens, meanwhile, the Thai chicken data set was predominated with RJFs (18/25). Thus, the genetic diversity is usually higher than that of the domestic chicken populations. Remarkably, the Thai chickens had a high number of haplotypes (Ht=19) in 25 individuals examined, suggesting a diverse population in the region. Similarly, intraclade diversity indices indicated high haplotype and nucleotide diversity of haplogroup D than all other major haplogroups classified in SEA and Pacific chickens (Supp. Table S3.3).

Table 3.1. Genetic diversity indices of Southeast Asian and Pacific chicken populations estimated using complete mtDNA D-loop sequences

Region	Molecular diversity indices				Neutrality tests	
	N	Ht	*Hd*	π	Tajima's *D*	Fu's F_S
Cambodia	173	54	0.889 ± 0.019	0.00585 ± 0.00030	-0.3677	-22.826**
Laos	63	27	0.935 ± 0.013	0.00678 ± 0.00028	0.7429	-4.4319
Thailand	25	19	0.953 ± 0.029	0.00912 ± 0.00051	0.5534	-4.0472*
Myanmar	78	35	0.922 ± 0.016	0.00837 ± 0.00020	0.7659	-6.6700
Philippines	135	36	0.900 ± 0.013	0.00435 ± 0.00027	0.0318	-11.1450**
Fiji	24	7	0.725 ± 0.077	0.00380 ± 0.00036	1.1958	2.1475
Pacific[a]	35	15	0.862 ± 0.047	0.00386 ± 0.00215	-0.5632	-2.8581
MSEA *overall*[b]	339	91	0.963 ± 0.005	0.00782 ± 0.00398	-0.2515*	-24.037**

ISEA *overall*[c]	145	41	0.942 ± 0.009	0.00466 ± 0.00249	-0.2570	-16.235**

[a] include published sequences of Pacific chickens (*n*=11)
[b] combined sequences representing MSEA (Cambodia, Laos, Thailand, and Myanmar chickens)
[c] combined sequences representing ISEA (Philippine chickens and database sequences of Indonesian chickens)
N – number of sequences; Ht – number of haplotypes; *Hd* - haplotype (gene) diversity; π – nucleotide diversity;
* *p-value* <0.05; ** *p-value* <0.01

3.3.2 Phylogeography and genetic affinities of continental and island SEA chickens

The sequences generated in the present study and the reference sequences representing chicken mtDNA control region-based haplogroup nomenclatures were used to reconstruct the matrilineal phylogeny (Appendix Tables S3.1, S3.4). Pioneering molecular phylogenetic studies based on mtDNA control regions and mitogenomes revealed fourteen haplogroups (A-I and V-Z) of chicken worldwide (Herrera et al., 2020; Huang et al., 2018; Liu et al., 2006; Miao et al., 2013). Divergent haplogroups D and V showed enigmatic phylogeny resolution and previously claimed to have been distributed in ISEA and Thailand, respectively (Godinez et al., 2021; Huang et al., 2018; Liu et al., 2006; Thomson et al., 2014).

In this study, model-based maximum likelihood and Bayesian phylogenetic analyses produced concordant topologies and comparable branch lengths of the tree (Figure 3.1a; Appendix Figures S3.2-S3.3). Major clades have strong SH-aLRT and UFBoot supports for the ML tree and significant posterior probability support for the Bayesian tree. Minor differences involved only some rearrangements of terminals for haplotypes: Hap_60, Hap_61, Hap_62, Hap_66, and Hap_122, as they clustered with haplogroup D1 (SEA subclade) in the ML tree, while grouped with haplogroup D2 in the Bayesian tree.

Phylogenetic analyses grouped the MSEA chickens into major haplogroups A, B, D, E, and F, with the evidence of newfound haplogroup V as a sister-clade of haplogroup C (Figure 3.1a; Appendix Figure S3.2). Haplogroup V, classified by ancestral mutation motifs A281G, T355C, and C363T, was further subdivided into two sub-haplogroups (Figure 3.1b). Here, evidence of sub-haplogroup V2 lineage (classified by unique mutation motifs: C228T, A237G,

C391T) was only identified in Cambodian and Laotian domestic chickens and one haplotype of Thai RJFs (*G. g. gallus*) (Hap_103) at the basal position of the subclade. On the other hand, evidence of sub-haplogroup V1 observed predominantly in Thai RJFs (in our dataset) shared commonality with the reclassified haplogroup V of RJFs in Thailand and Cambodia (Huang et al., 2018). Interestingly, both model-based phylogenetic trees revealed ancestral lineage of haplogroup D2 from MSEA chickens, primarily observed in Cambodian chickens (38.7%) and some low frequency of Laotian (7.9%) and Thai chickens (8.0%), while remaining undetected in Myanmar chickens. Haplogroups A and B have wide geographical distribution all over SEA, while Haplogroup F was prevalent among Myanmar chickens (34.6%), with some low frequency detected in Thai chickens (12.0%).

Likewise, ISEA chickens (i.e., the Philippines and Pacific) have a shared genetic affinity of predominant haplogroup D1 (here classified as sub-haplogroup D1b). Godinez et al. (2021) previously characterized this island sub-group as the "Philippine-Pacific subclade." This subclade is defined by five unique mutation motifs, A281G, C296T, T306C, A342G, and G686A, and includes diagnostic motifs from the downstream region of the complete mtDNA control region sequence (Figure 3.1b; Appendix Table S3.1). These findings also correspond to the diagnostic motifs (SNPs: A281G, C296T, T306C, A342G) of Polynesian chicken ancient DNA (Thomson et al., 2014).

Consistent classification of the major mitochondrial lineages of SEA chickens was also depicted in the median-joining network analysis (Figure 3.2). Notably, haplogroup V lineage was separated from haplogroup D and haplogroup C with nine and seven mutational sites, respectively. Within the haplogroup V lineage, newly classified sub-haplogroup V2 was separated from sub-haplogroup V1 with four mutational signatures. The geographical-specific MJ network analysis exhibited a close transregional evolutionary relationship of MSEA chickens in major haplogroups except for haplogroup F, which was predominated in Myanmar

chickens (Appendix Figure S3.4a-d). Similarly, the Philippine and Pacific chickens also shared closely related haplotypes classified under sub-haplogroup D1 (Appendix Figure S3.4e-f).

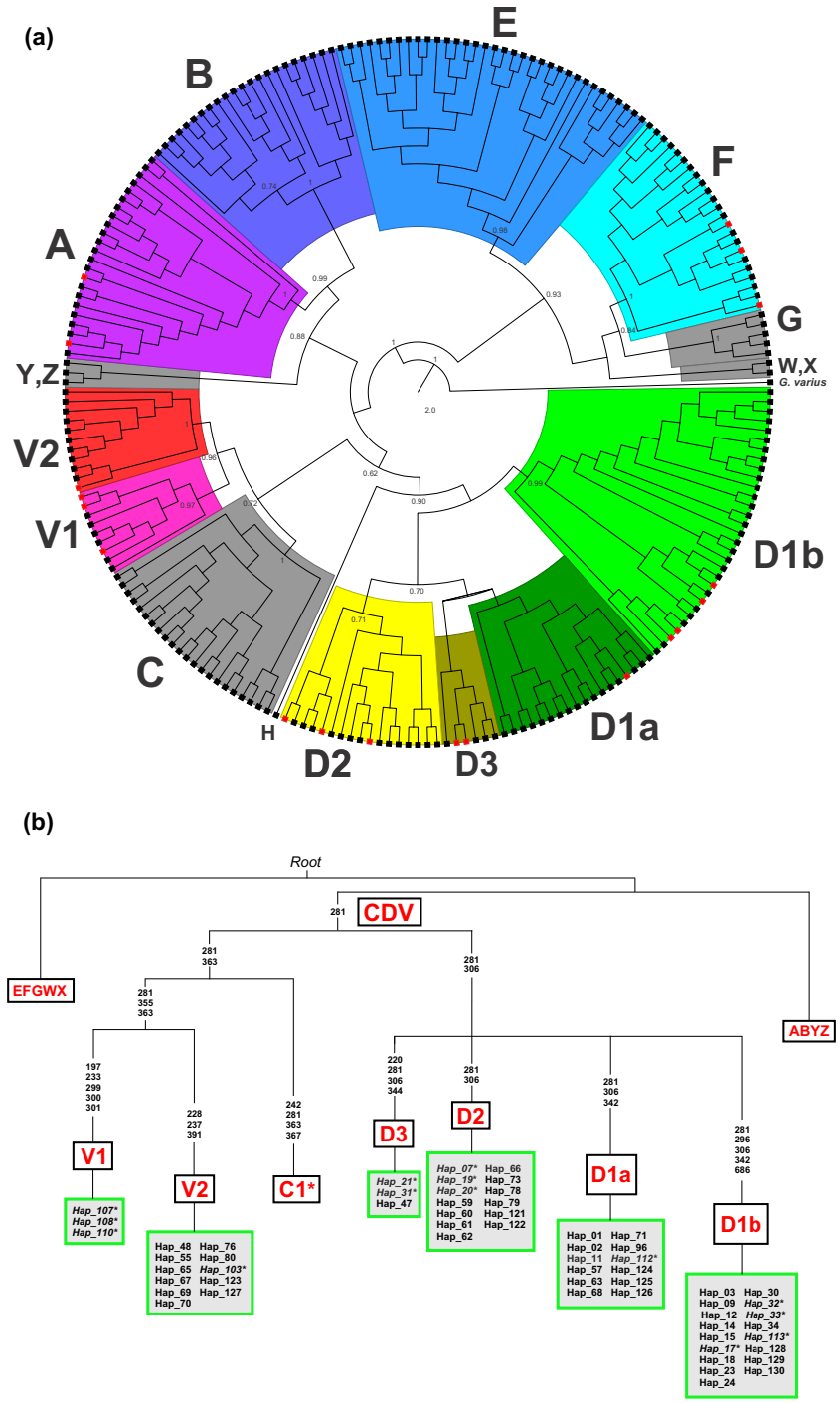


Figure 3.1. (**a**) Bayesian phylogenetic tree of complete mtDNA D-loop nucleotide sequences of Southeast Asian and Pacific chickens. The tree was constructed together with database sequences defined by Huang et al. (2018) (Appendix Table S4). Node labels correspond to posterior probability support values. Tips highlighted in red indicate red junglefowl. (**b**) Schematic classification tree showed reclassified macrohaplogroup CDV. The nucleotide positions were scored relative to the reference sequence NC_040970. Mutational motifs (transitions) are shown on the branches.

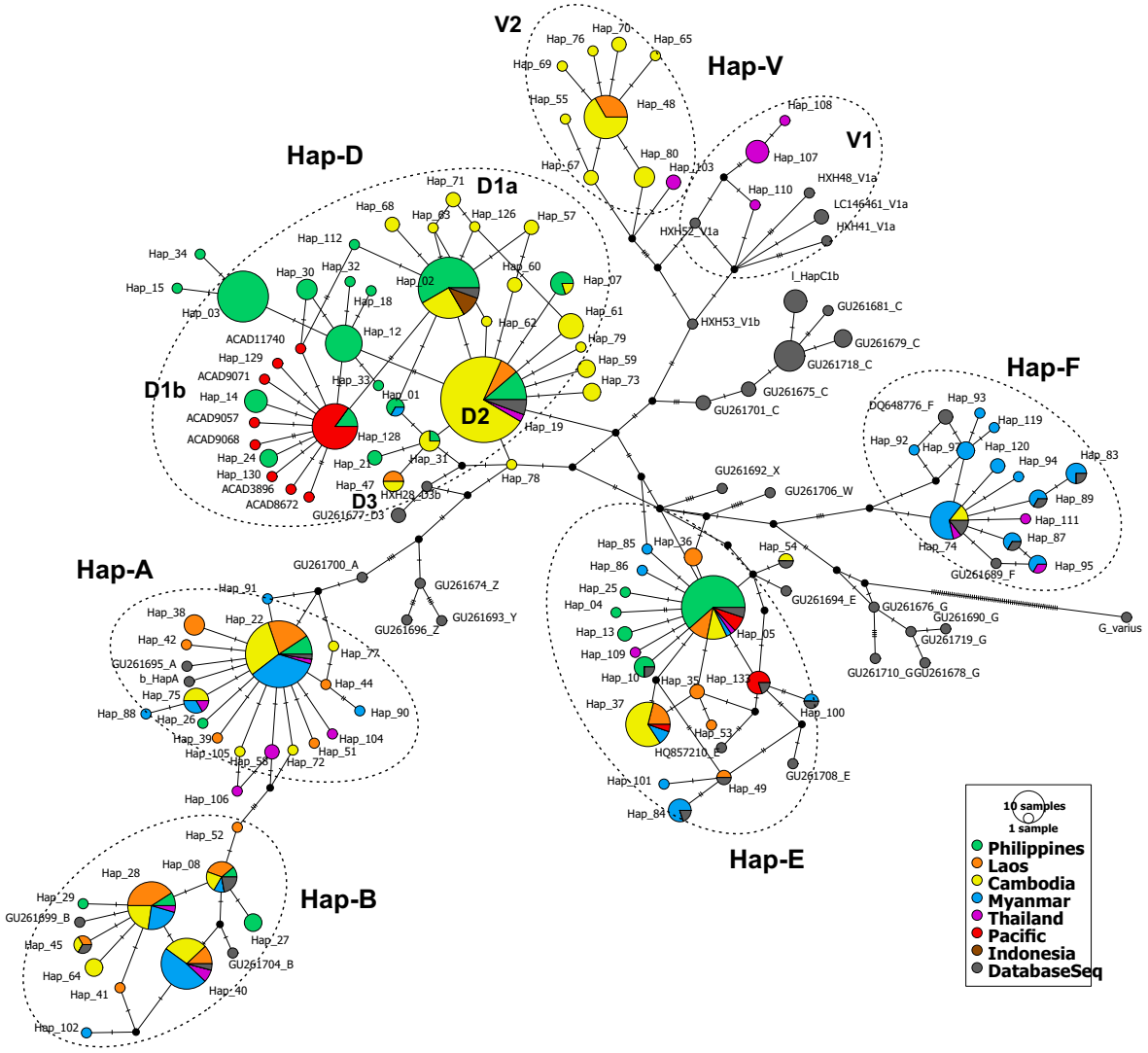


Figure 3.2. Median-joining network of the complete mtDNA D-loop region (1,232 bp) depicting evolutionary relationship of MSEA and ISEA chicken populations. The area of each circle is proportional to the frequency of the corresponding haplotypes. The length of branch connecting to other haplotypes corresponds to mutational positions.

3.3.3 Population structure and genetic differentiation

This study carried out a multivariate approach to complement the phylogenetic analysis to further assess the relationships among and between geographical populations, including database sequences of East Asia, South Asia, and Middle East chickens (Appendix Table S3.5). The result of the PCoA distinguished population substructure between mainland and island SEA chickens along the first two axes, which accounted for 52.09% or 52.53% variation

(Figures 3.3a-b). A homogenous subgroup was observed within island populations, particularly among the Philippine and Pacific chickens (F_{ST} = 0.06936). In contrast, MSEA populations showed a more diverse assemblage, consistent with the phylogenetic analyses and haplogroup variations. In addition, the study documented close relationships between Myanmar chickens and Yunnan chickens than any other Chinese chicken population. The pairwise F_{ST} value confirmed that Myanmar and Yunnan chickens were not differentiated from each other (F_{ST} = 0.00816; *p-value* < 0.01). Meanwhile, within MSEA chickens, transregional population substructures were observed ranging from 0.06895 between Laos and Thailand to 0.19202 between Cambodia and Myanmar (Appendix Table S3.6). Interestingly, Cambodian chickens were situated halfway between other continental populations and ISEA chickens, supporting the basal affiliations of identified ancestral matriline (i.e., sub-haplogroup D2) depicted in both ML and BI phylogenetic trees. The PCoA plot also indicates a significant genetic differentiation and population substructure between East Asian chickens and South Asian-Middle Eastern chickens, ranging from 0.14938 to 0.77115 (Figure 3.3a; Appendix Table S3.6). Similarly, results indicated a close genetic affinity of Japanese and Korean chickens to the Chinese and MSEA chicken populations after removing South Asian and Middle Eastern chickens from the dataset (Figure 3.3b).

Hierarchical AMOVA revealed that the majority of the variations (i.e., 79.21% between ISEA and MSEA chickens and 79.74% between MSEA and EA chickens) could be attributed to within-population differentiation, specifically chickens distributed across Southeast and East Asia (Table 3.2). Higher within-population variation was also observed within ISEA and Cambodian chickens. Likewise, no significant population genetic differentiation was found among groups of the island and mainland SEA chickens and among groups of MSEA and East Asian chickens. These observed patterns of genetic differentiation from the partitioned

variances among hierarchical groups reflect consistency established in the previous phylogenetic and PCoA analyses.

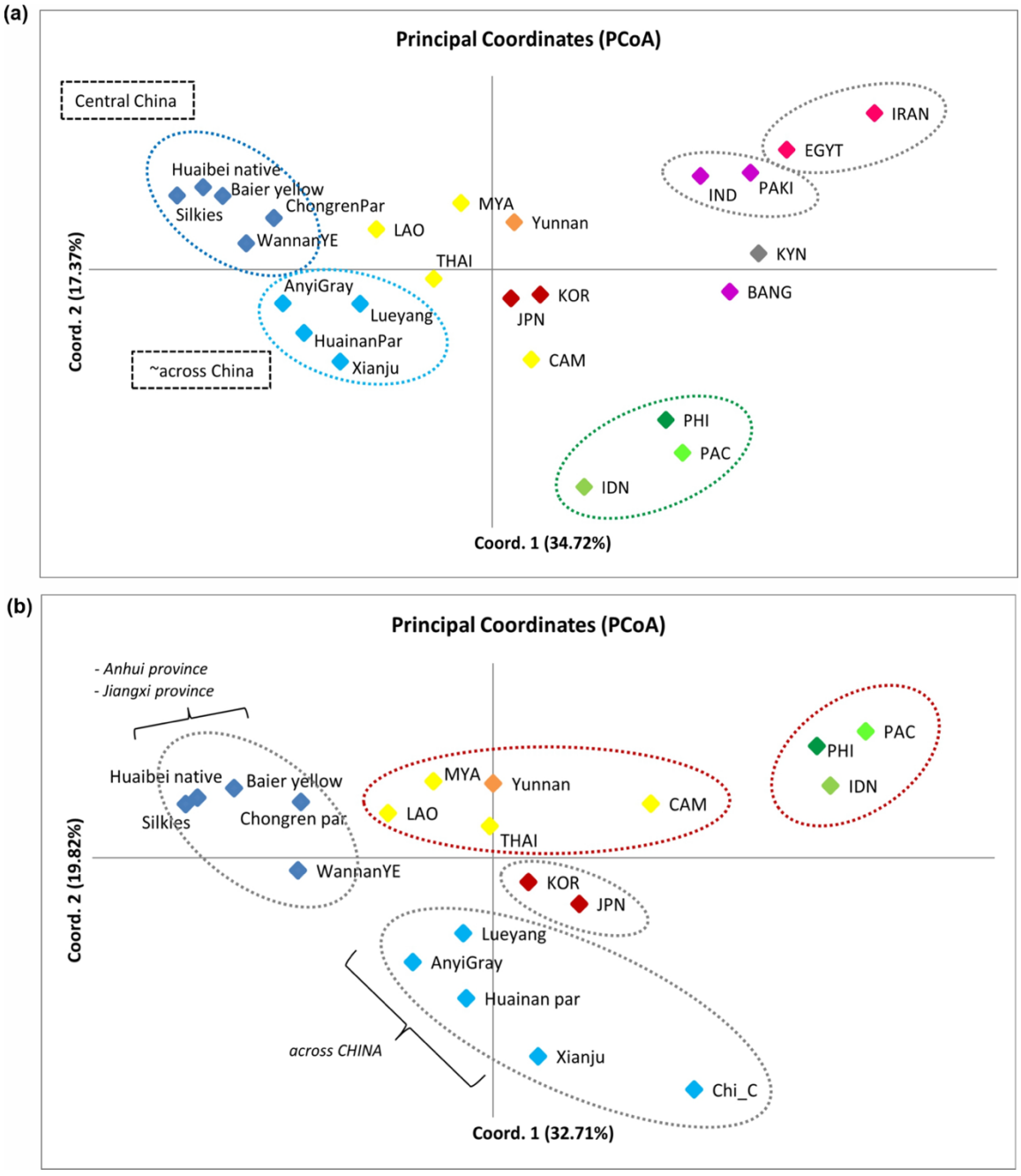


Figure 3.3. PCoA plots of population pairwise F_{st} values of SEA and Pacific chickens, together with other chicken populations from East Asia (i.e., China, Japan, and Korea), South Asia (i.e., Bangladesh, Pakistan, and India), Africa (i.e., Egypt and Kenya), and Middle East (Iran). The geographic origins of populations are shown by different colors (yellow: MSEA, green: ISEA and Pacific, blue: China, red: Japan and Korea, purple: South Asia, pink: Egypt and Iran, and gray: Kenya).

Table 3.2. Population genetic structure estimated from the AMOVA based on complete mtDNA D-loop sequences from (1) Philippine chickens, (2) Pacific chickens, (3) Indonesian chickens, (4) Cambodian chickens, (5) Laotian chickens, (6) Thailand chickens, (7) Myanmar chickens, and database sequences from East Asia (EA), South Asia (SA), and Middle East (ME).

Group	*N*	No. of popul-ation	No. of groups	Source of variation (%)		
				Among groups	Among populations within group	Within populations
no grouping	526	7	1	-	17.70*	82.30
A (ISEA-1,2,3 vs. MSEA-4,5,6,7)	526	7	2	9.06	11.73**	79.21**
a.1 (1,2,3 vs 4)	360	4	2	5.92	7.38**	86.70**
a.2 (1,2,3 vs 5)	250	4	2	24.37	6.06**	69.57**
a.3 (1,2,3 vs 6)	212	4	2	22.59	6.32**	71.09**
a.4 (1,2,3 vs 7)	265	4	2	26.12	5.20**	68.68**
B (ISEA vs EA)	677	15	2	15.66*	18.63**	65.71**
C (ISEA vs SA)	259	6	2	18.53*	8.14**	73.33**
D (ISEA vs ME)	244	5	2	34.25*	5.63**	60.12**
E (MSEA vs EA[a])	829	16	2	1.17	19.09**	79.74**
F (MSEA vs SA[a])	411	7	2	10.86*	11.39**	77.75**
G (MSEA vs ME[a])	396	6	2	19.93*	10.57**	69.51**

significant fixation indices at * $p < 0.05$; ** $p < 0.05$; [a] database sequences retrieved from GenBank

3.3.4 Demographic history

The simulations for neutrality tests indicated both MSEA and ISEA chickens deviated from neutrality (Table 3.1), which supported a demographic expansion. The negative and significant Tajima's *D* and Fu's *Fs* statistical values of MSEA chickens and significantly negative Fu's *Fs* value of ISEA chickens provided evidence for population growth signatures in the Asia-Pacific region. To obtain a better inference of the demographic history of MSEA and ISEA chickens, changes in maternal effective population size (N_e) at the different points along the genealogical timescale were estimated. The Bayesian Skyline Plot (BSP) showed evidence of MSEA chicken populations experiencing an episode of population stasis during the early Holocene period, but N_e started to increase around 4,000 years BP, and imminent

population growth commenced about 3,000-3,500 years BP (Appendix Figures S3.5a-c). On the other hand, the Philippine and Pacific chickens later started to increase their N_e around 2,500 years BP and 1,500 years BP, respectively (Appendix Figures S3.5d-e). Looking into the individual geographical population, BSP indicated earlier population growth of Myanmar chickens (~ 4.0 kya) than the Cambodian and Laotian chickens (~ 3.0 kya). Similarly, among within-island populations, Philippine chickens were observed to show increased N_e around 2.5 kya, while Pacific chickens have a much recent population growth expansion estimated at 1.0 to 1.5 kya.

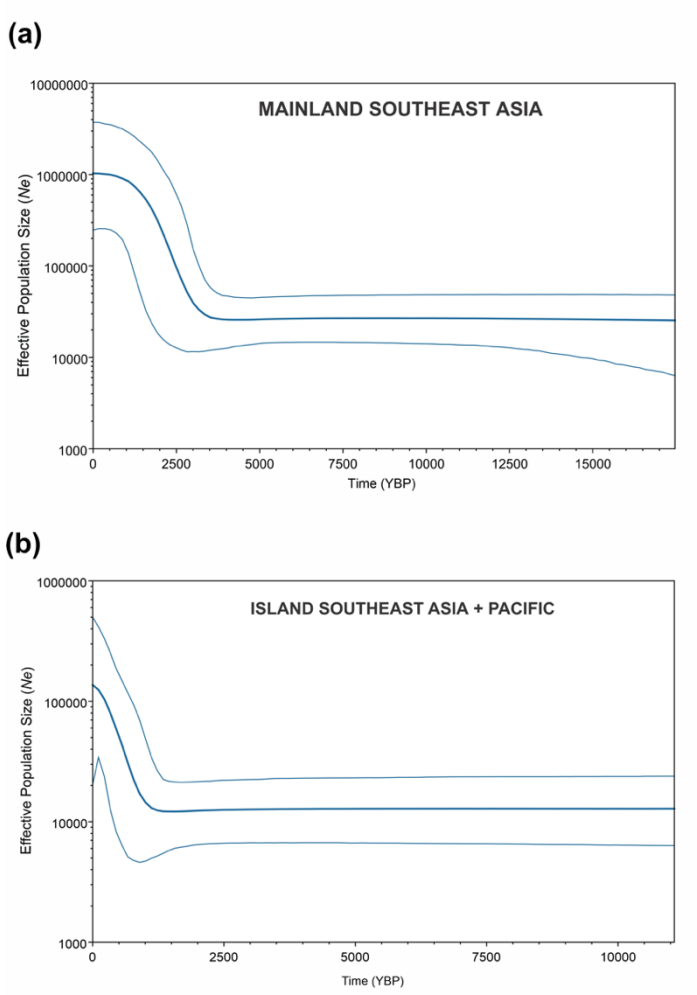


Figure 3.4. Bayesian coalescent skyline plot showing estimated demographic history of MSEA chickens (**a**), ISEA and Pacific chickens (**b**). The central blue line is the median estimate effective population size. The shaded area shows the upper and lower estimates of 95% credibility interval. The x-axis is the time (in years before present) and y-axis indicates population size (as the product of N_e and the generation length in years).

3.4 Discussion

The timing and location of chicken domestication have been the subject of protracted debate worldwide and have stimulated several molecular studies using modern biological and zooarchaeological data (Eda, 2021; Eda et al., 2016; Huang et al., 2018; Lawal et al., 2020; Lawal & Hanotte, 2021; Miao et al., 2013; Wang et al., 2020). Consensus among researchers and several molecular studies confirmed that domestic chickens evolved from red junglefowls somewhere in South and Southeast Asia (Huang et al., 2018; Kanginakudru et al., 2008; Liu et al., 2006; Miao et al., 2013; Wang et al., 2020) but identifying their exact geographic center of origin has been challenging (Eda, 2021; Eda et al., 2016; Lawal & Hanotte, 2021). Here, a comprehensive resolution of mitochondrial lineage diversity and phylogenetic analyses, population differentiation, and demographic inference of chickens in Southeast Asia and the Pacific region is presented. Patterns of sequence variation indicated that chickens in the MSEA region have higher intrapopulation genetic diversity than island populations. The average genetic diversity values of Southeast Asian chickens (MSEA: Hd=0.963 ± 0.005; π=0.00782 ± 0.00398; ISEA: Hd=0.942 ± 0.009; π=0.00466 ± 0.00249) observed in this study were higher than those of Chinese chickens (Gao et al., 2017; Huang et al., 2018), Japanese chickens (Hata et al., 2020; Oka et al., 2007), South Asian chickens (i.e., India, Bangladesh, and Pakistan) (Huang et al., 2018; Islam et al., 2019; Kanginakudru et al., 2008; Nisar et al., 2018), Egyptian chickens, (Osman et al., 2016) and East African chickens (Mwacharo et al., 2011). The substantial diversity of SEA chickens reflects the high matrilineal genetic variation documented in the major haplogroups, particularly haplogroup D with a large number of divergent haplotypes, and haplogroup V which has been detected only in Thailand, Cambodia, and Laos (Appendix Tables S3.2; S3.3). However, the influence of RJFs samples on the overall genetic diversity cannot be invalidated as they reflect ancestral genetic variations. Divergent sub-haplogroups that retained ancestral variations were also observed in these lineages, likely

due to geographic proximity to the center of domestication. These defined indices of biodiversity offer great opportunities for the development of genetic improvement strategies, trait selection, effective management of genetic resources, and future conservation efforts (Boettcher et al., 2010; Food and Agriculture Organization, 2007; Groeneveld et al., 2010; Toro et al., 2009).

Pioneering molecular studies and DNA sources based on the hypervariable region (partial sequence) (Liu et al., 2006), complete mtDNA control region (Miao et al., 2013; Oka et al., 2007), mitogenome (Huang et al., 2018; Miao et al., 2013), and whole genome data (Lawal et al., 2020; Mariadassou et al., 2021; Wang et al., 2020) provided important insights in resolving the chicken phylogeny. Recent genome-wide phylogenetic inferences provided a new perspective of wild species ancestry (i.e., *G. g. spadiceus*) of domestic chickens somewhere in southwestern China and Southeast Asia (Wang et al., 2020). However, topological discrepancies have also been documented in genome-wide data, often explained by differences in data sources and taxon sampling (Lawal et al., 2020; Mariadassou et al., 2021; Reddy et al., 2017). The scope of the present study defines new evidence for modern chicken genetic information with increased data sources spanning Southeast Asia and Oceania. Clearly, zooarchaeological DNA analysis can further clarify the evolutionary history of chickens in this region (Eda, 2021; Frantz et al., 2020).

Population genetic and phylogenetic analyses of more than 500 complete mtDNA control region sequences unveiled new perspectives on the population dynamics of SEA and Pacific chickens. Consistent with reports from various population genetic analyses, haplogroups A and B were widely distributed in East Asia and Southeast Asia, while haplogroup E had the widest global distribution (Hata et al., 2020; Herrera et al., 2020; Huang et al., 2018; Liu et al., 2006; Miao et al., 2013). Haplogroup F was primarily represented in Myanmar chickens and shared this matriline with chicken populations in adjacent Yunnan Province, China (Huang et al.,

2018; Liu et al., 2006; Mon et al., 2021). Consistent with the phylogenetic analyses, the pairwise F_{ST} value of Myanmar chickens was not genetically different from those of Yunnan chicken populations (Figures 3.3a-b; Appendix Table S3.6). This can be explained by the geographic proximity and the course of the Burma Road, which connects Myanmar and Yunnan Province (le Bail & Tournier, 2010). Genetic differentiation of populations and PCoA analyses revealed genetic substructure between geographically isolated populations, i.e., between MSEA and ISEA chickens, between South Asian and East Asian chickens, and between South Asian and ISEA chickens (Figure 3.3a; Appendix Table S3.6). Transregional population substructure was also observed within Southeast Asian chickens, reflecting deep phylogeographic diversification. Strong topological supports consistently define major haplogroup nomenclatures and provide evidence for the presence of a haplogroup D ancestral lineage (i.e., sub-haplogroup D2) from MSEA populations. A new matrilineage (i.e., sub-haplogroup V2) gave rise to the population of domestic chickens sampled in Cambodia, Laos, and Thailand, whereas its ancestral lineage (i.e., sub-haplogroup V1) was represented in Thai red junglefowl (i.e., *G. g. gallus*). The previously reconstructed mtDNA phylogenetic tree described by Huang et al. (2018) assigned some of the previously identified haplogroup C samples to haplogroup V and linked them as a sister clade to the macrohaplogroup CD. However, because of the expanded sample distribution and increase in samples, this present study characterized haplogroup V as a sister group to haplogroup C only (Figures. 3.1, 3.2; Appendix Figure S3.2). This resulted in a clearer reclassification of macrohaplogroup CDV (Figure 3.1b). Interestingly, the ancestral matrilines classified under sub-haplogroup D2 and haplogroup V were identified in sampling areas along the Lower Mekong subregion, for example, in Champasak and Bolikhamsai provinces in Laos, in Kampong Cham, Mondulkiri, Stung Treng, and Kratie provinces in Cambodia, and Sakhon Nakhon province in Thailand (Appendix Table S3.1). The favorable climatic conditions and vegetation in this area are

suitable for the red junglefowl (and its earlier descendants) to diversify and expand their distribution within its native range (Higham, 1989; Peters et al., 2016; West & Zhou, 1988). In addition, migratory junglefowl has been sighted in the areas closer to the Mekong River, apparently attempting to cross it: *"In crossing, the birds fly up as high as they can go, and then attempt to glide across… This movement does not seem to be caused by lack of food as the birds are extraordinary plump and in good condition. It is not easy to understand why it is taking place, as conditions on both sides of the Mekong seem the same"* (Giles, 1932). As one of the most geologically dynamic regions in the world, the Indo-Burma Biodiversity Hotspot has the highest recorded bird species (> 1,200) in the entire Asia-Pacific region (Mittermeier et al., 2004; Myers et al., 2000). The favorable seasonal weather patterns (i.e., dry northeast monsoon) and vegetation in much of the south, central, and west of the hotspot (Mittermeier et al., 2004) make it a suitable habitat for chicken dispersal (Eda, 2021; Peters et al., 2016; Pitt et al., 2016).

Meanwhile, the presence of sub-haplogroup D1b (Philippine-Pacific subclade) is well documented in the present study and strongly supported by bootstrap and posterior probabilities. This matriline represents genetic uniformity and shows no significant signals of population structure despite geographic isolation between the Philippines and the Pacific region (Godinez et al., 2021; Thomson et al., 2014). This recently expanded lineage is unique to this region, suggesting a human-mediated scenario of its phylogeography. This may be due to the dispersal of Austronesian speakers to the Philippines (ca. 4000 cal. BP) and continued movement eastward to the Melanesian islands (ca. 3300-3150 cal. BP) and as far as Remote Oceania (Bellwood, 2017; Hung et al., 2011; Piper, 2017). This translocation route has been reliably defined by the recovered ancient DNA of Polynesian chickens, which identified the Philippines as a homeland for the diversity of Pacific chickens (Petchey et al., 2015; Thomson et al., 2014). Similarly, the phylogeographic dispersal of sub-haplogroup D1b, which first

diversified in the Philippine archipelago, likely corresponds to the initial introduction pattern of its ancestral matrilines (i.e., sub-haplogroup D2) from MSEA. This translocation pattern may have been influenced by the numerous waves of human migration to the Philippines brought by the Negritos across the continental landmass of Sundaland (Jinam et al., 2012; Larena et al., 2021; Lipson et al., 2014). The introduction of the Manobo and the Sama ancestry into the southern Philippines and Palawan cannot be ruled out, as they showed high genetic relatedness to MSEA-affiliated populations (Larena et al., 2021). The timing of migration of people of Manobo ancestry (> 12,000 years ago) and people of Sama ancestry (~ 8,000 to 12,000 years ago) is the closest possible translocation scenario (Larena et al., 2021), which is consistent with archaeological evidence suggesting that domestication of chickens in Southeast Asia occurred long before 8,000 BP (West & Zhou, 1988). However, there are few reports of chicken remains in Southeast Asia (Storey et al., 2012), and prehistoric exploitation has yet to be discovered (Eda et al., 2019). Therefore, zooarchaeological and paleoclimatic studies are essential to reliably identify their exact geographic center of origin. On the contrary, this study cannot assume a unidirectional north-to-south translocation of chickens from Taiwan because Taiwan's indigenous chickens (e.g., Ju-Chi) and gamecock (Hua-Tung) share genetic similarities with East Asian chicken haplotypes and populations introduced from Japan and the Indian subcontinent (Chang et al., 2012).

The coalescent-based Bayesian demographic analyses detected earlier effective population size expansion in MSEA chickens, while island populations showed more recent demographic growth signatures (Figure 3.4a). Although BSP results consider relevant sampling schemes with high sample sizes per demes, we still carefully acknowledge the potential impact of population structure on demographic estimates (Heller et al., 2013). The timing of the demographic expansion of MSEA chickens observed in this study can be explained by the cultural importance of stock-raising in the archaeological sites of Non Nok

Tha and Ban Chiang in Thailand around ca. 4,000-3,000 BP (Higham, 1989). Bones of animals (e.g., pig, cattle, dog, deer, and chicken) and clay animal figurines were excavated in the human burial sites, suggesting that animals were part of the ritual practices during prehistoric inhumation (Higham, 1989). It was well documented that agriculture and animal-raising were among the subsistence activities of domestic communities during prehistoric settlements in the broad valleys of the Lower Mekong (Higham, 1989). In addition, the ancient DNA of Thai chickens recovered in Ban Non Wat dated back to around 2,500 BP supported the demographic expansion of MSEA chickens (Storey et al., 2012). Recent morphological bone identification further documented the existence of chicken remains from other known archaeological sites in Thailand as early as 4,000 BP (Eda et al., 2019). On the other hand, the demographic expansion pattern of the island chicken population seems to suggest the timeline of Austronesian settlement in the region (Bellwood, 2007; Soares et al., 2016).

3.5 Conclusion and Recommendation

In conclusion, this study provides a comprehensive insight into the genetic diversity and unique population dynamics of Southeast Asian chickens. High-resolution matrilineal phylogeny sheds new light on the evolutionary history of globally acknowledged haplogroups of SEA and Pacific chickens and provides evidence of a new divergent matrilineage (i.e., haplogroup V) that is distributed across its native range in the Lower Mekong subregion. The phylogeographic and time tree phylogeny suggests human-mediated translocation of the haplogroup D ancestral matriline (i.e., haplogroup D2) from MSEA, which later diversified, forming a divergent sub-haplogroup D1b distinct to the island populations (i.e., Philippine-Pacific subclade). Future integrated genome-wide and environmental adaptation studies are required to unravel new elements of genomic evolution of SEA chickens for sustainable genetic improvement for climate resilience, effective management strategies, and future conservation endeavors.

3.6 Data availability

The compete mtDNA D-loop sequences are deposited and available in GenBank database (accession numbers: OM240181 - OM240549).

3.7 Acknowledgement

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3.8 Appendices

Appendix Table S3.1. mtDNA control region information of SEA and Pacific chicken samples

Type	Breed/Species	Sample ID	Accession No.	Region	Location	Haplo-group	Control Region mutational motif	Age of aDNA (years BP)*	Reference
Indigenous		Laos14	OM240211	MSEA	Vientiane Province, Laos	A	167 217 225 239 243 256 261 310 446 1214		this study
Indigenous		Laos20	OM240216	MSEA	Vientiane Province, Laos	A	167 217 225 239 243 256 261 310 446 1214		this study
Indigenous		Laos27	OM240220	MSEA	Vientiane Province, Laos	A	167 217 225 243 256 261 280 310 446 1214		this study
Indigenous		Laos33	OM240222	MSEA	Vientiane Province, Laos	A	167 217 225 243 256 261 310 446 1214		this study
Indigenous		Laos36	OM240224	MSEA	Vientiane Province, Laos	A	167 217 225 243 256 261 310 361T 446 1214		this study
Indigenous		Laos38	OM240225	MSEA	Vientiane Province, Laos	A	167 217 225 243 256 261 310 446 1214		this study
Indigenous		Laos41	OM240226	MSEA	Vientiane Province, Laos	A	167 217 225 243 256 261 310 446 1214		this study
Indigenous		Laos45	OM240229	MSEA	Vientiane Province, Laos	A	167 217 225 243 256 261 310 446 686 1214		this study
Indigenous		Laos59	OM240235	MSEA	Xiengkhuang, Laos	A	167 217 225 243 256 261 310 446 1214		this study
Indigenous		Laos67	OM240238	MSEA	Champasak Province, Laos	A	167 217 225 243 256 261 310 446 1214		this study
Indigenous		Laos90	OM240250	MSEA	Bolikhamsai Province, Laos	A	167 217 225 239 243 256 261 310 446 1214		this study
Indigenous		Laos91	OM240251	MSEA	Bolikhamsai Province, Laos	A	167 217 225 239 243 256 261 310 446 1214		this study
Indigenous		Laos95	OM240255	MSEA	Bolikhamsai Province, Laos	A	167 217 225 243 256 261 310 446 1214		this study
Indigenous		Laos102	OM240258	MSEA	Bolikhamsai Province, Laos	A	167 217 225 243 256 261 310 446		this study
Indigenous		Laos103	OM240259	MSEA	Bolikhamsai Province, Laos	A	167 217 225 243 256 261 310 446 1214		this study
Indigenous		Laos104	OM240260	MSEA	Bolikhamsai Province, Laos	A	167 217 225 243 256 261 310 446 1214		this study
Indigenous		Laos107	OM240266	MSEA	Bolikhamsai Province, Laos	A	167 217 225 243 256 261 310 446 1214		this study
Indigenous		Laos32	OM240221	MSEA	Vientiane Province, Laos	B	212 217 243 246 256 261 296 310 315 446 792 1214 1215		this study
Indigenous		Laos34	OM240223	MSEA	Vientiane Province, Laos	B	212 217 243 246 256 310 315 792 1214 1215		this study
Indigenous		Laos42	OM240227	MSEA	Vientiane Province, Laos	B	212 217 243 246 256 261 310 315 446 792 1214 1215		this study
Indigenous		Laos43	OM240228	MSEA	Vientiane Province, Laos	B	212 217 243 246 256 261 310 315 446 792 1214 1215		this study
Indigenous		Laos46	OM240230	MSEA	Vientiane Province, Laos	B	212 217 243 246 256 261 310 315 446 686 792 1214 1215		this study
Indigenous		Laos50	OM240263	MSEA	Xiengkhuang, Laos	B	212 217 243 246 256 261 310 315 446 792 1214 1215		this study
Indigenous		Laos53	OM240232	MSEA	Xiengkhuang, Laos	B	212 217 243 246 256 261 310 315 446 792 1214 1215		this study
Indigenous		Laos54	OM240233	MSEA	Xiengkhuang, Laos	B	212 217 243 246 256 261 310 315 446 1214 1215		this study
Indigenous		Laos58	OM240234	MSEA	Xiengkhuang, Laos	B	212 217 243 246 256 261 310 315 446 1214 1215		this study
Indigenous		Laos61	OM240236	MSEA	Xiengkhuang, Laos	B	212 217 243 246 256 261 310 315 446 1214 1215		this study
Indigenous		Laos66	OM240237	MSEA	Champasak Province, Laos	B	212 217 243 246 256 261 310 315 446 792 1214 1215		this study
Indigenous		Laos76	OM240264	MSEA	Champasak Province, Laos	B	212 217 243 246 256 261 310 315 446 792 1214 1215		this study
Indigenous		Laos92	OM240252	MSEA	Bolikhamsai Province, Laos	B	212 217 243 246 256 261 310 315 446 792 1214 1215		this study
Indigenous		Laos97	OM240256	MSEA	Bolikhamsai Province, Laos	B	212 217 243 246 256 261 296 310 315 446 792 1214 1215		this study
Indigenous		Laos98	OM240257	MSEA	Bolikhamsai Province, Laos	B	212 217 243 246 256 261 310 315 446 792 1214 1215		this study
Indigenous		Laos101	OM240265	MSEA	Bolikhamsai Province, Laos	B	212 217 243 246 256 261 296 310 315 446 792 1214 1215		this study
Indigenous		Laos106	OM240261	MSEA	Bolikhamsai Province, Laos	B	212 217 243 246 256 261 310 315 446 1214		this study
Indigenous		Laos111	OM240267	MSEA	Bolikhamsai Province, Laos	B	212 217 243 246 256 261 310 315 446 792 1214 1215		this study
Indigenous		Laos51	OM240231	MSEA	Xiengkhuang, Laos	D2	217 281 306 446 1214		this study
Indigenous		Laos72	OM240241	MSEA	Champasak Province, Laos	D2	217 281 306 446 1214		this study
Indigenous		Laos73	OM240242	MSEA	Champasak Province, Laos	D2	217 281 306 446 1214		this study
Indigenous		Laos74	OM240243	MSEA	Champasak Province, Laos	D2	217 281 306 446 1214		this study
Indigenous		Laos77	OM240245	MSEA	Champasak Province, Laos	D2	217 281 306 446 1214		this study

Indigenous	Laos68	OM240239	MSEA	Champasak Province, Laos	D3	217 220 281 306 344 446 1214	this study
Indigenous	Laos75	OM240244	MSEA	Champasak Province, Laos	D3	217 220 281 306 344 446 1214	this study
Indigenous	Laos3	OM240205	MSEA	Vientiane Province, Laos	E1	330	this study
Indigenous	Laos4	OM240262	MSEA	Vientiane Province, Laos	E1	330	this study
Indigenous	Laos6	OM240206	MSEA	Vientiane Province, Laos	E1	521	this study
Indigenous	Laos9	OM240207	MSEA	Vientiane Province, Laos	E1	222 330	this study
Indigenous	Laos10	OM240208	MSEA	Vientiane Province, Laos	E1	330	this study
Indigenous	Laos11	OM240209	MSEA	Vientiane Province, Laos	E1	same as reference	this study
Indigenous	Laos13	OM240210	MSEA	Vientiane Province, Laos	E1	222 330	this study
Indigenous	Laos15	OM240212	MSEA	Vientiane Province, Laos	E1	521	this study
Indigenous	Laos16	OM240213	MSEA	Vientiane Province, Laos	E1	same as reference	this study
Indigenous	Laos18	OM240214	MSEA	Vientiane Province, Laos	E1	same as reference	this study
Indigenous	Laos19	OM240215	MSEA	Vientiane Province, Laos	E1	222 330	this study
Indigenous	Laos21	OM240217	MSEA	Vientiane Province, Laos	E1	521	this study
Indigenous	Laos22	OM240218	MSEA	Vientiane Province, Laos	E1	same as reference	this study
Indigenous	Laos23	OM240219	MSEA	Vientiane Province, Laos	E1	222 330	this study
Indigenous	Laos78	OM240246	MSEA	Champasak Province, Laos	E3	222 249 355	this study
Indigenous	Laos70	OM240240	MSEA	Champasak Province, Laos	V2	212 217 228 237 246 256 261 281 355 363 391 446 1214	this study
Indigenous	Laos82	OM240247	MSEA	Champasak Province, Laos	V2	212 217 228 237 246 256 261 281 355 363 391 446 1214	this study
Indigenous	Laos87	OM240248	MSEA	Bolikhamsai Province, Laos	V2	212 217 228 237 246 256 261 281 355 363 391 446 1214	this study
Indigenous	Laos89	OM240249	MSEA	Bolikhamsai Province, Laos	V2	212 217 228 237 246 256 261 281 355 363 391 446 1214	this study
Indigenous	Laos93	OM240253	MSEA	Bolikhamsai Province, Laos	V2	212 217 228 237 246 256 261 281 355 363 391 446 1214	this study
Indigenous	Laos94	OM240254	MSEA	Bolikhamsai Province, Laos	V2	212 217 228 237 246 256 261 281 355 363 391 446 1214	this study
Indigenous	Cam31	OM240288	MSEA	Kampong Cham Province, Cambodia	A	217 225 243 356 261 310 446 1214	this study
Indigenous	Cam32	OM240289	MSEA	Kampong Cham Province, Cambodia	A	217 225 243 256 261 310 446 1214	this study
Indigenous	Cam49	OM240302	MSEA	Kampong Cham Province, Cambodia	A	167 199 217 225 243 256 261 310 446 1214	this study
Indigenous	Cam59	OM240308	MSEA	Kampong Cham Province, Cambodia	A	167 217 225 243 256 261 310 446 1214	this study
Indigenous	Cam61	OM240373	MSEA	Kampong Cham Province, Cambodia	A	167 217 225 243 256 261 310 1214	this study
Indigenous	Cam63	OM240311	MSEA	Kampong Cham Province, Cambodia	A	167 217 225 243 256 261 310 446 1214	this study
Indigenous	Cam91	OM240334	MSEA	Mondulkiri Province, Cambodia	A	167 217 225 243 256 261 310 446 1214	this study
Indigenous	Cam100	OM240339	MSEA	Mondulkiri Province, Cambodia	A	167 217 225 243 256 261 281 310 446 1214	this study
Indigenous	Cam104	OM240343	MSEA	Mondulkiri Province, Cambodia	A	167 217 225 243 256 261 310 446 1214	this study
Indigenous	Cam121	OM240433	MSEA	Mondulkiri Province, Cambodia	A	167 217 225 243 256 261 310 1214	this study
Indigenous	Cam126	OM240381	MSEA	Mondulkiri Province, Cambodia	A	167 217 225 243 256 261 310 1214	this study
Indigenous	Cam146	OM240360	MSEA	Kratie Province, Cambodia	A	217 225 243 256 261 310 446 1214	this study
Indigenous	Cam152	OM240364	MSEA	Kratie Province, Cambodia	A	167 217 225 243 256 261 310 446 1214	this study
Indigenous	Cam160	OM240369	MSEA	Kratie Province, Cambodia	A	167 217 225 243 256 261 310 446 1214	this study
Indigenous	Cam162	OM240384	MSEA	Kratie Province, Cambodia	A	167 217 225 243 256 261 310 1214	this study
Indigenous	Cam174	OM240398	MSEA	Kratie Province, Cambodia	A	167 217 225 243 256 261 310 446 1178 1214	this study
Indigenous	Cam185	OM240403	MSEA	Kratie Province, Cambodia	A	167 217 225 243 256 261 310 446 1178 1214	this study
Indigenous	Cam193	OM240407	MSEA	Stung Treng, Cambodia	A	167 217 225 243 256 261 310 391A 446 686 1214	this study

Indigenous	Cam205	OM240413	MSEA	Stung Treng, Cambodia	A	167 217 225 243 256 261 310 446 1178 1214	this study
Indigenous	Cam1	OM240371	MSEA	University Farm, RUA, Cambodia	B	199 212 217 243 246 256 261 310 315 792 1214 1215	this study
Indigenous	Cam35	OM240291	MSEA	Kampong Cham Province, Cambodia	B	212 217 243 246 256 261 310 315 446 1214 1215	this study
Indigenous	Cam40	OM240296	MSEA	Kampong Cham Province, Cambodia	B	212 217 243 246 256 261 310 315 446 1214 1215	this study
Indigenous	Cam43	OM240298	MSEA	Kampong Cham Province, Cambodia	B	212 217 243 246 256 261 310 315 446 792 1214 1215	this study
Indigenous	Cam47	OM240300	MSEA	Kampong Cham Province, Cambodia	B	212 217 243 246 256 261 310 315 446 792 1214 1215	this study
Indigenous	Cam53	OM240304	MSEA	Kampong Cham Province, Cambodia	B	212 217 243 246 256 261 310 315 446 792 1214 1215	this study
Indigenous	Cam66	OM240313	MSEA	Kampong Cham Province, Cambodia	B	212 217 243 246 256 261 296 310 315 446 792 1214 1215	this study
Indigenous	Cam92	OM240374	MSEA	Mondulkiri Province, Cambodia	B	199 212 217 243 246 256 261 310 315 792 1214 1215	this study
Indigenous	Cam98	OM240337	MSEA	Mondulkiri Province, Cambodia	B	199 212 217 243 246 256 261 310 315 446 792 1214 1215	this study
Indigenous	Cam102	OM240341	MSEA	Mondulkiri Province, Cambodia	B	212 217 243 246 256 261 310 315 446 792 1214 1215	this study
Indigenous	Cam103	OM240342	MSEA	Mondulkiri Province, Cambodia	B	212 217 243 246 256 261 296 310 315 446 792 1214 1215	this study
Indigenous	Cam110	OM240430	MSEA	Mondulkiri Province, Cambodia	B	212 217 243 246 256 261 310 315 792 1214 1215	this study
Indigenous	Cam111	OM240431	MSEA	Mondulkiri Province, Cambodia	B	212 217 243 246 256 261 310 315 792 1214 1215	this study
Indigenous	Cam118	OM240380	MSEA	Mondulkiri Province, Cambodia	B	212 217 243 246 256 261 296 310 315 792 1214 1215	this study
Indigenous	Cam159	OM240368	MSEA	Kratie Province, Cambodia	B	212 217 243 246 256 261 296 310 315 446 792 1214 1215	this study
Indigenous	Cam235	OM240419	MSEA	Stung Treng, Cambodia	B	212 217 243 246 256 261 296 310 315 446 792 1214 1215	this study
Indigenous	Cam248	OM240420	MSEA	Ratanakiri Province, Cambodia	B	212 217 243 246 256 261 310 315 446 686 792 1214 1215	this study
Indigenous	Cam285	OM240395	MSEA	Ratanakiri Province, Cambodia	B	212 217 243 246 256 261 296 310 315 792 1214 1215	this study
Indigenous	Cam38	OM240294	MSEA	Kampong Cham Province, Cambodia	D1 (SEA subclade)	217 281 306 342 446 1214	this study
Indigenous	Cam44	OM240299	MSEA	Kampong Cham Province, Cambodia	D1 (SEA subclade)	217 281 306 342 446	this study
Indigenous	Cam48	OM240301	MSEA	Kampong Cham Province, Cambodia	D1 (SEA subclade)	217 281 306 342 446 1214	this study
Indigenous	Cam54	OM240305	MSEA	Kampong Cham Province, Cambodia	D1 (SEA subclade)	217 281 306 342 446 1214	this study
Indigenous	Cam58	OM240424	MSEA	Kampong Cham Province, Cambodia	D1 (SEA subclade)	217 281 306 342 1214	this study
Indigenous	Cam94	OM240335	MSEA	Mondulkiri Province, Cambodia	D1 (SEA subclade)	217 281 306 342 446 1214	this study
Indigenous	Cam112	OM240348	MSEA	Mondulkiri Province, Cambodia	D1 (SEA subclade)	217 229 281 306 342 446 1214	this study
Indigenous	Cam119	OM240432	MSEA	Mondulkiri Province, Cambodia	D1 (SEA subclade)	217 281 306 342 1214	this study

Indigenous	Cam122	OM240434	MSEA	Mondulkiri Province, Cambodia	D1 (SEA subclade)	217 229 281 306 342 1214	this study
Indigenous	Cam133	OM240438	MSEA	Mondulkiri Province, Cambodia	D1 (SEA subclade)	217 221 281 306 342 1214	this study
Indigenous	Cam137	OM240356	MSEA	Kratie Province, Cambodia	D1 (SEA subclade)	217 221 281 306 342 446 1214	this study
Indigenous	Cam147	OM240361	MSEA	Kratie Province, Cambodia	D1 (SEA subclade)	217 221 281 306 342 446 1214	this study
Indigenous	Cam141	OM240357	MSEA	Kratie Province, Cambodia	D1 (SEA subclade)	217 281 306 342 446 1214	this study
Indigenous	Cam156	OM240366	MSEA	Kratie Province, Cambodia	D1 (SEA subclade)	217 281 306 342 446 1214	this study
Indigenous	Cam157	OM240367	MSEA	Kratie Province, Cambodia	D1 (SEA subclade)	217 281 306 342 446 1214	this study
Indigenous	Cam161	OM240370	MSEA	Kratie Province, Cambodia	D1 (SEA subclade)	217 281 306 342 446 1214	this study
Indigenous	Cam189	OM240406	MSEA	Stung Treng, Cambodia	D1 (SEA subclade)	217 281 306 342 446 1214	this study
Indigenous	Cam28	OM240285	MSEA	University Farm, RUA, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam30	OM240287	MSEA	Kampong Cham Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam34	OM240290	MSEA	Kampong Cham Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam36	OM240292	MSEA	Kampong Cham Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam37	OM240293	MSEA	Kampong Cham Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam50	OM240303	MSEA	Kampong Cham Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam55	OM240306	MSEA	Kampong Cham Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam56	OM240307	MSEA	Kampong Cham Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam57	OM240372	MSEA	Kampong Cham Province, Cambodia	D2	217 281 306 1214	this study
Indigenous	Cam62	OM240310	MSEA	Kampong Cham Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam65	OM240425	MSEA	Kampong Cham Province, Cambodia	D2	217 281 306 1214	this study
Indigenous	Cam67	OM240314	MSEA	Kampong Cham Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam68	OM240315	MSEA	Kampong Cham Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam69	OM240426	MSEA	Kampong Cham Province, Cambodia	D2	217 281 306 1214	this study
Indigenous	Cam70	OM240316	MSEA	Kampong Cham Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam71	OM240317	MSEA	Kampong Cham Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam72	OM240318	MSEA	Kampong Cham Province, Cambodia	D2	217 281 306 446 1214	this study

Indigenous	Cam74	OM240320	MSEA	Kampong Cham Province, Cambodia	D2	174 217 281 306 446 1214	this study
Indigenous	Cam75	OM240321	MSEA	Kampong Cham Province, Cambodia	D2	174 217 281 306 446 1214	this study
Indigenous	Cam78	OM240427	MSEA	Kampong Cham Province, Cambodia	D2	217 281 306 1214	this study
Indigenous	Cam80	OM240325	MSEA	Mondulkiri Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam81	OM240326	MSEA	Mondulkiri Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam85	OM240329	MSEA	Mondulkiri Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam86	OM240330	MSEA	Mondulkiri Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam87	OM240331	MSEA	Mondulkiri Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam88	OM240332	MSEA	Mondulkiri Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam89	OM240333	MSEA	Mondulkiri Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam93	OM240375	MSEA	Mondulkiri Province, Cambodia	D2	217 244 281 306 1214	this study
Indigenous	Cam95	OM240336	MSEA	Mondulkiri Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam105	OM240344	MSEA	Mondulkiri Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam109	OM240347	MSEA	Mondulkiri Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam116	OM240378	MSEA	Mondulkiri Province, Cambodia	D2	217 281 306 1214	this study
Indigenous	Cam117	OM240379	MSEA	Mondulkiri Province, Cambodia	D2	217 281 306 1214	this study
Indigenous	Cam127	OM240351	MSEA	Mondulkiri Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam129	OM240353	MSEA	Mondulkiri Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam131	OM240436	MSEA	Mondulkiri Province, Cambodia	D2	217 281 306 1214	this study
Indigenous	Cam132	OM240437	MSEA	Mondulkiri Province, Cambodia	D2	217 281 306 1214	this study
Indigenous	Cam139	OM240383	MSEA	Kratie Province, Cambodia	D2	217 281 306 1214	this study
Indigenous	Cam144	OM240358	MSEA	Kratie Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam145	OM240359	MSEA	Kratie Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam149	OM240363	MSEA	Kratie Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam153	OM240365	MSEA	Kratie Province, Cambodia	D2	174 217 281 306 446 1214	this study
Indigenous	Cam163	OM240385	MSEA	Kratie Province, Cambodia	D2	217 281 306 1214	this study
Indigenous	Cam166	OM240386	MSEA	Kratie Province, Cambodia	D2	217 281 306 1214	this study
Indigenous	Cam168	OM240387	MSEA	Kratie Province, Cambodia	D2	217 281 306 1214	this study
Indigenous	Cam170	OM240388	MSEA	Kratie Province, Cambodia	D2	217 281 306 1214	this study
Indigenous	Cam171	OM240439	MSEA	Kratie Province, Cambodia	D2	217 281 306 1214	this study
Indigenous	Cam172	OM240397	MSEA	Kratie Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam178	OM240389	MSEA	Kratie Province, Cambodia	D2	217 281 306 1214	this study

Indigenous	Cam188	OM240405	MSEA	Stung Treng, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam195	OM240408	MSEA	Stung Treng, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam198	OM240410	MSEA	Stung Treng, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam199	OM240390	MSEA	Stung Treng, Cambodia	D2	217 244 281 306 1214	this study
Indigenous	Cam202	OM240411	MSEA	Stung Treng, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam203	OM240412	MSEA	Stung Treng, Cambodia	D2	217 228 281 306 446 1214	this study
Indigenous	Cam204	OM240391	MSEA	Stung Treng, Cambodia	D2	217 281 306 1214	this study
Indigenous	Cam206	OM240414	MSEA	Stung Treng, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam228	OM240417	MSEA	Stung Treng, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam253	OM240421	MSEA	Ratanakiri Province, Cambodia	D2	217 281 306 446 1214	this study
Indigenous	Cam264	OM240423	MSEA	Ratanakiri Province, Cambodia	D2	217 244 281 306 446 1214	this study
Indigenous	Cam278	OM240393	MSEA	Ratanakiri Province, Cambodia	D2	217 281 306 1214	this study
Indigenous	Cam282	OM240394	MSEA	Ratanakiri Province, Cambodia	D2	217 281 306 1214	this study
Indigenous	Cam196	OM240409	MSEA	Stung Treng, Cambodia	D2	217 306 446 1214	this study
Indigenous	Cam79	OM240324	MSEA	Kampong Cham Province, Cambodia	D2	217 281 306 309 446 1214	this study
Indigenous	Cam82	OM240327	MSEA	Mondulkiri Province, Cambodia	D2	217 221 281 306 446 1214	this study
Indigenous	Cam84	OM240328	MSEA	Mondulkiri Province, Cambodia	D2	217 221 281 306 446 1214	this study
Indigenous	Cam90	OM240428	MSEA	Mondulkiri Province, Cambodia	D2	217 221 281 306 1214	this study
Indigenous	Cam101	OM240340	MSEA	Mondulkiri Province, Cambodia	D2	217 221 281 306 446 1214	this study
Indigenous	Cam125	OM240350	MSEA	Mondulkiri Province, Cambodia	D2	217 221 281 306 446 1214	this study
Indigenous	Cam175	OM240399	MSEA	Kratie Province, Cambodia	D2	217 221 281 306 446 1214	this study
Indigenous	Cam8	OM240271	MSEA	University Farm, RUA, Cambodia	D3	217 220 281 306 446 1214	this study
Indigenous	Cam64	OM240312	MSEA	Kampong Cham Province, Cambodia	D3	217 220 281 306 446 1214	this study
Indigenous	Cam73	OM240319	MSEA	Kampong Cham Province, Cambodia	D3	217 220 281 306 446 1214	this study
Indigenous	Cam106	OM240345	MSEA	Mondulkiri Province, Cambodia	D3	217 220 281 306 344 446 1214	this study
Indigenous	Cam128	OM240352	MSEA	Mondulkiri Province, Cambodia	D3	217 220 281 306 344 446 1214	this study
Indigenous	Cam2	OM240268	MSEA	University Farm, RUA, Cambodia	E1	same as reference	this study
Indigenous	Cam4	OM240269	MSEA	University Farm, RUA, Cambodia	E1	222 330	this study
Indigenous	Cam6	OM240270	MSEA	University Farm, RUA, Cambodia	E1	same as reference	this study
Indigenous	Cam9	OM240272	MSEA	University Farm, RUA, Cambodia	E1	same as reference	this study
Indigenous	Cam10	OM240273	MSEA	University Farm, RUA, Cambodia	E1	222 330	this study

Indigenous	Cam12	OM240274	MSEA	University Farm, RUA, Cambodia	E1	222 330	this study
Indigenous	Cam16	OM240275	MSEA	University Farm, RUA, Cambodia	E1	222 330	this study
Indigenous	Cam17	OM240276	MSEA	University Farm, RUA, Cambodia	E1	222 330	this study
Indigenous	Cam18	OM240277	MSEA	University Farm, RUA, Cambodia	E1	222 330	this study
Indigenous	Cam20	OM240278	MSEA	University Farm, RUA, Cambodia	E1	222 330	this study
Indigenous	Cam21	OM240279	MSEA	University Farm, RUA, Cambodia	E1	222 330	this study
Indigenous	Cam22	OM240280	MSEA	University Farm, RUA, Cambodia	E1	222 330	this study
Indigenous	Cam23	OM240281	MSEA	University Farm, RUA, Cambodia	E1	342 686	this study
Indigenous	Cam24	OM240282	MSEA	University Farm, RUA, Cambodia	E1	222 330	this study
Indigenous	Cam25	OM240283	MSEA	University Farm, RUA, Cambodia	E1	222 330	this study
Indigenous	Cam27	OM240284	MSEA	University Farm, RUA, Cambodia	E1	222 330	this study
Indigenous	Cam97	OM240376	MSEA	Mondulkiri Province, Cambodia	E1	446	this study
Indigenous	Cam261	OM240392	MSEA	Ratanakiri Province, Cambodia	F	217 234 236 254 315 317C 446 904	this study
Indigenous	Cam260	OM240422	MSEA	Ratanakiri Province, Cambodia	F	217 234 236 254 315 317C 446 904	this study
Indigenous	Cam29	OM240286	MSEA	University Farm, RUA, Cambodia	V2	212 217 228 237 238 246 261 281 355 363 391 446 1214	this study
Indigenous	Cam41	OM240297	MSEA	Kampong Cham Province, Cambodia	V2	212 217 228 237 246 256 261 281 355 363 391 446 1214	this study
Indigenous	Cam60	OM240309	MSEA	Kampong Cham Province, Cambodia	V2	212 217 228 237 246 256 261 281 355 363 391 466 1214	this study
Indigenous	Cam76	OM240322	MSEA	Kampong Cham Province, Cambodia	V2	212 217 228 237 246 256 261 281 355 363 391 446 1214	this study
Indigenous	Cam77	OM240323	MSEA	Kampong Cham Province, Cambodia	V2	212 217 228 237 246 256 261 281 355 363 391 446 1214	this study
Indigenous	Cam99	OM240338	MSEA	Mondulkiri Province, Cambodia	V2	212 217 228 237 246 256 261 355 363 391 446 1214	this study
Indigenous	Cam107	OM240429	MSEA	Mondulkiri Province, Cambodia	V2	212 217 228 237 246 256 261 281 355 363 391 1214	this study
Indigenous	Cam108	OM240346	MSEA	Mondulkiri Province, Cambodia	V2	212 217 228 237 246 261 281 355 363 391 446 1214	this study
Indigenous	Cam113	OM240377	MSEA	Mondulkiri Province, Cambodia	V2	212 217 228 237 246 256 261 281 355 363 391 1214	this study
Indigenous	Cam115	OM240349	MSEA	Mondulkiri Province, Cambodia	V2	212 217 228 237 246 261 281 355 363 391 446 1214	this study
Indigenous	Cam123	OM240435	MSEA	Mondulkiri Province, Cambodia	V2	212 217 228 237 246 256 261 281 355 363 391 1214	this study
Indigenous	Cam130	OM240354	MSEA	Mondulkiri Province, Cambodia	V2	212 217 228 237 246 256 261 281 326 355 363 391 446 1214	this study

Indigenous	Cam136	OM240355	MSEA	Kratie Province, Cambodia	V2	217 228 237 246 256 261 281 355 363 391 446 1214	this study
Indigenous	Cam138	OM240382	MSEA	Kratie Province, Cambodia	V2	217 228 237 246 256 261 281 355 363 391 1214	this study
Indigenous	Cam148	OM240362	MSEA	Kratie Province, Cambodia	V2	212 217 228 237 246 256 261 281 355 363 391 446 1214	this study
Indigenous	Cam164	OM240396	MSEA	Kratie Province, Cambodia	V2	212 217 228 237 246 256 261 281 355 363 391 446 1214	this study
Indigenous	Cam179	OM240400	MSEA	Kratie Province, Cambodia	V2	212 217 228 237 246 256 261 281 355 363 391 446 1214	this study
Indigenous	Cam180	OM240401	MSEA	Kratie Province, Cambodia	V2	212 217 228 237 246 256 261 281 355 363 391 446 1214	this study
Indigenous	Cam183	OM240402	MSEA	Kratie Province, Cambodia	V2	212 217 228 237 246 256 261 281 355 363 391 446 1214	this study
Indigenous	Cam186	OM240404	MSEA	Stung Treng, Cambodia	V2	212 217 228 237 246 256 261 281 355 363 446 1214	this study
Indigenous	Cam225	OM240415	MSEA	Stung Treng, Cambodia	V2	212 217 237 246 256 261 281 355 363 391 446 1214	this study
Indigenous	Cam226	OM240440	MSEA	Stung Treng, Cambodia	V2	212 217 237 246 256 261 281 355 363 391 1214	this study
Indigenous	Cam227	OM240416	MSEA	Stung Treng, Cambodia	V2	212 217 237 246 256 261 281 355 363 391 446 1214	this study
Indigenous	Cam229	OM240418	MSEA	Stung Treng, Cambodia	V2	212 217 237 246 256 261 281 355 363 391 446 1214	this study
Indigenous	Cam39	OM240295	MSEA	Kampong Cham Province, Cambodia	I	199 212 217 220 233 246 261 391 447 521 1214	this study
Indigenous	Mya42	OM240453	MSEA	Bago, Myanmar	A	167 217 225 243 256 261 306 310 446 1178 1214	this study
Indigenous	Mya48	OM240457	MSEA	Bago, Myanmar	A	167 210 217 225 243 256 261 310 355 446 686 1214	this study
Indigenous	Mya58	OM240460	MSEA	Kyaingtong, Myanmar	A	167 217 225 243 256 261 310 446 1178 1214	this study
Indigenous	Mya59	OM240461	MSEA	Kyaingtong, Myanmar	A	167 217 225 243 256 261 310 446 1214	this study
Indigenous	Mya61	OM240462	MSEA	Kyaingtong, Myanmar	A	167 217 225 243 256 261 310 446 1178 1214	this study
Indigenous	Mya63	OM240463	MSEA	Kyaingtong, Myanmar	A	167 217 225 243 256 261 310 446 1214	this study
Indigenous	Mya71	OM240468	MSEA	Kyaingtong, Myanmar	A	167 217 225 243 256 261 310 446 1214	this study
Indigenous	Mya81	OM240470	MSEA	Kyaingtong, Myanmar	A	167 217 225 243 256 261 310 391 446 1214	this study
Indigenous	Mya83	OM240472	MSEA	Kyaingtong, Myanmar	A	167 217 225 243 256 261 310 446 1214	this study
Indigenous	Mya99	OM240479	MSEA	Sittwe, Myanmar	A	167 217 225 243 256 261 310 446 1214	this study
Indigenous	Mya116	OM240491	MSEA	Yangon chicken market, Myanmar	A	217 225 243 256 261 310 446 1214	this study
Indigenous	Mya123	OM240505	MSEA	Yangon chicken market, Myanmar	A	167 217 225 243 256 261 310 446 1214	this study
Indigenous	Mya131	OM240501	MSEA	Yangon chicken market, Myanmar	A	167 217 225 243 256 261 310 446 1214	this study
Indigenous	MyaFx1	OM240507	MSEA	Myanmar	A	167 217 225 243 256 261 310 446 1214	this study
Indigenous	MyaFx2	OM240508	MSEA	Myanmar	A	167 217 225 243 256 261 310 446 1214	this study
Indigenous	MyaFx3	OM240509	MSEA	Myanmar	A	167 217 225 243 256 261 310 446 1214	this study
Indigenous	MyaFx4	OM240510	MSEA	Myanmar	A	167 217 225 243 256 261 310 446 1214	this study
Indigenous	MyaFx5	OM240511	MSEA	Myanmar	A	167 217 225 243 256 261 310 446 1214	this study
Indigenous	MyaFx6	OM240512	MSEA	Myanmar	A	167 217 225 243 256 261 310 446 1214	this study
Indigenous	MyaFx7	OM240513	MSEA	Myanmar	A	167 217 225 243 256 261 310 446 1214	this study
Indigenous	Mya32	OM240450	MSEA	Taunggyi, Myanmar	B	212 217 243 246 256 261 310 315 446 792 1214 1215	this study
Indigenous	Mya43	OM240454	MSEA	Bago, Myanmar	B	212 217 243 246 256 261 296 310 315 446 792 1214 1215	this study
Indigenous	Mya64	OM240464	MSEA	Kyaingtong, Myanmar	B	212 217 243 246 256 261 296 310 315 446 792 1214 1215	this study
Indigenous	Mya65	OM240465	MSEA	Kyaingtong, Myanmar	B	212 217 243 246 256 261 296 310 315 446 792 1214 1215	this study
Indigenous	Mya66	OM240466	MSEA	Kyaingtong, Myanmar	B	212 217 243 246 256 261 296 310 315 446 792 1214 1215	this study
Indigenous	Mya70	OM240467	MSEA	Kyaingtong, Myanmar	B	212 217 243 246 256 261 310 315 446 792 1214 1215	this study
Indigenous	Mya76	OM240469	MSEA	Kyaingtong, Myanmar	B	212 217 243 246 256 261 310 315 446 1214 1215	this study
Indigenous	Mya82	OM240471	MSEA	Kyaingtong, Myanmar	B	212 217 243 246 256 261 296 310 315 446 792 1214 1215	this study
Indigenous	Mya84	OM240473	MSEA	Kyaingtong, Myanmar	B	212 217 243 246 256 261 296 310 315 446 792 1214 1215	this study
Indigenous	Mya114	OM240489	MSEA	Yangon chicken market, Myanmar	B	212 217 243 246 256 261 310 315 446 792 1214 1215	this study
Indigenous	Mya120	OM240492	MSEA	Yangon chicken market, Myanmar	B	212 217 243 246 256 261 296 310 315 446 792 1214 1215	this study
Indigenous	Mya124	OM240495	MSEA	Yangon chicken market, Myanmar	B	212 217 243 246 256 261 296 310 315 446 792 1214 1215	this study

Indigenous	Mya125	OM240496	MSEA	Yangon chicken market, Myanmar	B	212 217 243 246 256 261 310 315 446 792 1214 1215	this study
Indigenous	Mya128	OM240499	MSEA	Yangon chicken market, Myanmar	B	212 217 243 246 256 261 296 310 315 446 792 1214 1215	this study
Indigenous	Mya130	OM240500	MSEA	Yangon chicken market, Myanmar	B	212 217 243 246 256 261 296 310 315 446 792 1214 1215	this study
Indigenous	Mya133	OM240506	MSEA	Yangon chicken market, Myanmar	B	212 217 243 246 256 261 296 310 446 792 1214 1215	this study
Indigenous	Mya135	OM240502	MSEA	Yangon chicken market, Myanmar	B	212 217 243 246 256A 296 310 315 446 792 1214 1215	this study
Indigenous	MyaFx8	OM240514	MSEA	Myanmar	B	212 217 243 246 256 261 310 315 446 792 1214 1215	this study
Red junglefowl	MyaJF1	OM240516	MSEA	Myanmar	B	212 217 243 246 256 261 296 310 315 446 792 1214 1215	this study
Indigenous	Mya103	OM240481	MSEA	Sittwe, Myanmar	D1 (SEA subclade)	217 220 281 306 342 446 1214	this study
Indigenous	Mya1	OM240441	MSEA	Bagan, Myanmar	E1	222 330	this study
Indigenous	Mya6	OM240443	MSEA	Bagan, Myanmar	E1	222 330	this study
Indigenous	Mya23	OM240446	MSEA	Heho, Myanmar	E	281	this study
Indigenous	Mya28	OM240448	MSEA	Taunggyi, Myanmar	E1	354	this study
Indigenous	Mya100	OM240480	MSEA	Sittwe, Myanmar	E1	same as reference	this study
Indigenous	Mya121	OM240493	MSEA	Yangon chicken market, Myanmar	E1	224 246 1174	this study
Indigenous	Mya9	OM240444	MSEA	Bagan, Myanmar	E3	222 249 281 355 686	this study
Indigenous	Mya30	OM240503	MSEA	Taunggyi, Myanmar	E3	222 249 281 355 686	this study
Indigenous	Mya40	OM240452	MSEA	Bago, Myanmar	E3	222 249 281 355 686	this study
Indigenous	Mya54	OM240459	MSEA	Khayan, Myanmar	E3	222 249 281 355 686	this study
Indigenous	Mya126	OM240497	MSEA	Yangon chicken market, Myanmar	E3	222 249 265 355	this study
Indigenous	Mya2	OM240442	MSEA	Bagan, Myanmar	F	217 234 236 254 315 317C 347 396 904	this study
Indigenous	Mya16	OM240445	MSEA	Magway, Myanmar	F	217 234 236 254 315 317C 904	this study
Indigenous	Mya27	OM240447	MSEA	Taunggyi, Myanmar	F	217 234 236 254 315 317C 347 386 904	this study
Indigenous	Mya29	OM240449	MSEA	Taunggyi, Myanmar	F	217 234 236 254 265 315 317C 904	this study
Indigenous	Mya36	OM240451	MSEA	Heho, Myanmar	F	217 234 236 254 265 315 317C 904	this study
Indigenous	Mya45	OM240455	MSEA	Bago, Myanmar	F	217 234 236 254 315 317C 396 904	this study
Indigenous	Mya46	OM240456	MSEA	Bago, Myanmar	F	217 234 236 254 315 317C 396 904	this study
Indigenous	Mya50	OM240504	MSEA	Khayan, Myanmar	F	217 234 236 254 315 317C 904	this study
Indigenous	Mya52	OM240458	MSEA	Khayan, Myanmar	F	217 234 236 254 315 317C 904	this study
Indigenous	Mya86	OM240474	MSEA	Kyaingtong, Myanmar	F	217 234 246 315 317C 686 904	this study
Indigenous	Mya89	OM240475	MSEA	Sittwe, Myanmar	F	217 234 254 280 315 317C 686 904	this study
Indigenous	Mya91	OM240476	MSEA	Sittwe, Myanmar	F	217 234 254 315 317C 417 904	this study
Indigenous	Mya93	OM240477	MSEA	Sittwe, Myanmar	F	217 234 254 265 315 317C 342 904	this study
Indigenous	Mya94	OM240478	MSEA	Mrauk-U, Myanmar	F	217 234 254 315 317C 904	this study
Indigenous	Mya104	OM240482	MSEA	Sittwe, Myanmar	F	217 234 254 315 317C 904	this study
Indigenous	Mya106	OM240483	MSEA	Sittwe, Myanmar	F	217 234 254 315 317C 904	this study
Indigenous	Mya107	OM240484	MSEA	Sittwe, Myanmar	F	217 234 254 265 315 317C 342 904	this study
Indigenous	Mya109	OM240485	MSEA	Sittwe, Myanmar	F	217 234 254 315 317C 686 904	this study
Indigenous	Mya111	OM240486	MSEA	Sittwe, Myanmar	F	217 234 254 315 317C 686 904	this study
Indigenous	Mya112	OM240487	MSEA	Sittwe, Myanmar	F	217 234 254 315 317C 686 904	this study
Indigenous	Mya113	OM240488	MSEA	Sittwe, Myanmar	F	217 234 254 315 317C 904	this study
Indigenous	Mya115	OM240490	MSEA	Yangon chicken market, Myanmar	F	217 234 254 315 317C 347 396 904	this study

Indigenous		Mya122	OM240494	MSEA	Yangon chicken market, Myanmar	F	217 234 254 315 317C 904	this study
Indigenous		Mya127	OM240498	MSEA	Yangon chicken market, Myanmar	F	217 234 254 315 317C 904	this study
Indigenous		MyaFx9	OM240515	MSEA	Myanmar	F	217 234 236 254 315 317C 391 686 904	this study
Red junglefowl		MyaJF2	OM240517	MSEA	Myanmar	F	217 234 236 254 315 317C 521 904	this study
Red junglefowl		MyaJF3	OM240518	MSEA	Myanmar	F	217 234 236 254 315 317C 521 904	this study
Indigenous		Thai_chicken_21	OM240526	MSEA	Khon Kaen, Thailand	A	167 199 217 225 243 246 256 261 310 446 1214	this study
Indigenous		Thai_chicken_23	OM240528	MSEA	Khon Kaen, Thailand	A	167 217 225 243 256 261 310 446 1178 1214	this study
Indigenous		Thai_chicken_24	OM240529	MSEA	Khon Kaen, Thailand	A	167 217 225 243 256 261 310 446 1214	this study
Indigenous		Thai_chicken_25	OM240530	MSEA	Khon Kaen, Thailand	B	212 217 243 246 256 261 310 315 446 792 1214 1215	this study
Indigenous		Thai_chicken_28	OM240531	MSEA	Khon Kaen, Thailand	B	212 217 243 246 256 261 296 310 315 446 792 1214 1215	this study
Indigenous		Thai_chicken_22	OM240527	MSEA	Khon Kaen, Thailand	E1	same as reference	this study
Indigenous		Thai_chicken_30	OM240532	MSEA	Khon Kaen, Thailand	F	217 234 236 254 315 317 904	this study
Red junglefowl		Thai_ChonBuri11	OM240522	MSEA	Chon Buri, Thailand	A	167 217 225 243 256 261 310 446 792 1214	this study
Red junglefowl		Thai_ChonBuri14	OM240523	MSEA	Chon Buri, Thailand	A	167 217 225 243 246 256 261 310 446 1214	this study
Red junglefowl		Thai_ChonBuri20	OM240525	MSEA	Chon Buri, Thailand	A	167 217 225 243 246 256 261 310 446 1214	this study
Red junglefowl	*G. g. gallus*	Thai_ChonBuri8	OM240520	MSEA	Chon Buri, Thailand	B	212 217 243 246 256 261 296 310 315 446 792 1214 1215	this study
Red junglefowl		Thai_ChonBuri9	OM240521	MSEA	Chon Buri, Thailand	D2	217 281 446 1214	this study
Red junglefowl		Thai_Northern45	OM240542	MSEA	Northern, Thailand	D2	217 281 306 446 1214	this study
Red junglefowl	*G. g. gallus*	Thai_ChonBuri4	OM240519	MSEA	Chon Buri, Thailand	V2	211 212 217 237 246 261 281 355 363 391 446 1214	this study
Red junglefowl		Thai_ChonBuri15	OM240524	MSEA	Chon Buri, Thailand	V2	211 212 217 237 246 261 281 355 363 391 446 1214	this study
Red junglefowl	*G. g. gallus*	Thai_SakonNakhon31	OM240533	MSEA	Sakon Nakhon, Thailand	V1	197 212 217 233 246 281 299 300 301 355 363 446 1214	this study
Red junglefowl	*G. g. gallus*	Thai_SakonNakhon32	OM240534	MSEA	Sakon Nakhon, Thailand	V1	197 212 217 233 246 281 299 300 301 355 363 446 1214	this study
Red junglefowl	*G. g. gallus*	Thai_SakonNakhon33	OM240535	MSEA	Sakon Nakhon, Thailand	V1	197 212 217 233 246 281 299 300 301 355 363 446 1214	this study
Red junglefowl	*G. g. gallus*	Thai_SakonNakhon35	OM240537	MSEA	Sakon Nakhon, Thailand	V1	197 212 217 233 246 281 299 300 301 355 363 446 1214	this study
Red junglefowl	*G. g. gallus*	Thai_SakonNakhon36	OM240538	MSEA	Sakon Nakhon, Thailand	V1	197 212 217 233 246 281 299 300 301 355 363 446 1214	this study
Red junglefowl	*G. g. gallus*	Thai_SakonNakhon40	OM240540	MSEA	Sakon Nakhon, Thailand	V1	197 212 217 233 246 281 299 300 301 355 363 446 1214	this study
Red junglefowl	*G. g. spadiceus*	Thai_Northern41	OM240541	MSEA	Northern, Thailand	V1	212 217 233 281 299 300 355 363 446 1214	this study
Red junglefowl		Thai_SakonNakhon37	OM240539	MSEA	Sakon Nakhon, Thailand	E1	391A	this study
Red junglefowl	*G. g. gallus*	Thai_SakonNakhon34	OM240536	MSEA	Sakon Nakhon, Thailand	F	217 234 236 254 265 315 317 342 904	this study

Red junglefowl	*G. g. gallus*	Thai_Northern46	OM240543	MSEA	Northern, Thailand	F	217 234 236 254 315 317 446 904	this study
Red junglefowl	*G. g. gallus*	LeytePHRJF01	OM240544	ISEA	Philippines	D1 (SEA subclade)	C217T, T261C, A281G, T306C, A342G, T446C, T1214C	this study
Red junglefowl	*G. g. gallus*	LeyteRJF19	OM240548	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	this study
Red junglefowl	*G. g. gallus*	LeytePHRJF05	OM240545	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	this study
Red junglefowl	*G. g. gallus*	LeyteRJF17	OM240547	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	this study
Red junglefowl	*G. g. gallus*	LeyteRJF14	OM240546	ISEA	Philippines	D2	C217T, A281G, T306C, T446C, T1214C	this study
Red junglefowl	*G. g. gallus*	LeyteRJF22	OM240549	ISEA	Philippines	D2	C217T, A281G, T306C, T446C, T1214C	this study
Indigenous		LNC1		ISEA	Philippines	D1 (SEA subclade)	C217T, T220C, A281G, T306C, A342G, T446C, T1214C	Godinez et al., 2021
Indigenous		LNC26		ISEA	Philippines	D1 (SEA subclade)	C217T, T220C, A281G, T306C, A342G, T446C, T1214C	Godinez et al., 2021
Indigenous		LNC11		ISEA	Philippines	D1 (SEA subclade)	C217T, A281G, T306C, A342G, T446C, T1214C	Godinez et al., 2021
Indigenous		LNC57		ISEA	Philippines	D1 (SEA subclade)	C217T, A281G, T306C, A342G, T446C, T1214C	Godinez et al., 2021
Indigenous		LNC67		ISEA	Philippines	D1 (SEA subclade)	C217T, A281G, T306C, A342G, T446C, T1214C	Godinez et al., 2021
Indigenous		LNC73		ISEA	Philippines	D1 (SEA subclade)	C217T, A281G, T306C, A342G, T446C, T1214C	Godinez et al., 2021
Indigenous		SNC80		ISEA	Philippines	D1 (SEA subclade)	C217T, A281G, T306C, A342G, T446C, T1214C	Godinez et al., 2021
Indigenous		SNC86		ISEA	Philippines	D1 (SEA subclade)	C217T, A281G, T306C, A342G, T446C, T1214C	Godinez et al., 2021
Indigenous		SNC88		ISEA	Philippines	D1 (SEA subclade)	C217T, A281G, T306C, A342G, T446C, T1214C	Godinez et al., 2021
Indigenous		LNC22		ISEA	Philippines	D1 (SEA subclade)	C217T, A281G, T306C, T309C, T446C, T1214C	Godinez et al., 2021
Indigenous		LNC27		ISEA	Philippines	D1 (SEA subclade)	C217T, A281G, T306C, T309C, T446C, T1214C	Godinez et al., 2021
Indigenous		LNC29		ISEA	Philippines	D1 (SEA subclade)	C217T, A281G, T306C, T309C, T446C, T1214C	Godinez et al., 2021
Indigenous		LNC31		ISEA	Philippines	D1 (SEA subclade)	C217T, A281G, T306C, A342G, T446C, T1214C	Godinez et al., 2021
Indigenous		LNC33		ISEA	Philippines	D1 (SEA subclade)	C217T, A281G, T306C, A342G, T446C, T1214C	Godinez et al., 2021
Indigenous		LNC34		ISEA	Philippines	D1 (SEA subclade)	C217T, A281G, T306C, A342G, T446C, T1214C	Godinez et al., 2021
Indigenous		LNC36		ISEA	Philippines	D1 (SEA subclade)	C217T, A281G, T306C, A342G, T446C, T1214C	Godinez et al., 2021
Indigenous		LNC37		ISEA	Philippines	D1 (SEA subclade)	C217T, A281G, T306C, A342G, T446C, T1214C	Godinez et al., 2021
Indigenous		LNC39		ISEA	Philippines	D1 (SEA subclade)	C217T, A281G, T306C, A342G, T446C, T1214C	Godinez et al., 2021
Indigenous		LNC40		ISEA	Philippines	D1 (SEA subclade)	C217T, A281G, T306C, A342G, T446C, T1214C	Godinez et al., 2021

Indigenous	LNC80	ISEA	Philippines	D1 (SEA subclade)	C217T, A281G, T306C, A342G, T446C, T1214C	Godinez et al., 2021
Indigenous	LNC9	ISEA	Philippines	D1 (SEA subclade)	C217T, A281G, T306C, A342G, T446C, T1214C	Godinez et al., 2021
Indigenous	SamNC12	ISEA	Philippines	D1 (SEA subclade)	C217T, A281G, T306C, A342G, T446C, T1214C	Godinez et al., 2021
Indigenous	SNC45	ISEA	Philippines	D1 (SEA subclade)	C217T, A281G, T306C, A342G, T446C, T1214C	Godinez et al., 2021
Indigenous	SNC93	ISEA	Philippines	D1 (SEA subclade)	C217T, A281G, T306C, A342G, T446C, T1214C	Godinez et al., 2021
Indigenous	SNC94	ISEA	Philippines	D1 (SEA subclade)	C217T, A281G, T306C, A342G, T446C, T1214C	Godinez et al., 2021
Indigenous	SNC97	ISEA	Philippines	D1 (SEA subclade)	C217T, A281G, T306C, A342G, T446C, T1214C	Godinez et al., 2021
Indigenous	LNC4	ISEA	Philippines	D1 (Phil-Pac)	C217T, C246T, A281G, **C296T**, T306C, A342G, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC5	ISEA	Philippines	D1 (Phil-Pac)	C217T, C246T, A281G, **C296T**, T306C, A342G, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC68	ISEA	Philippines	D1 (Phil-Pac)	C217T, C246T, A281G, **C296T**, T306C, A342G, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC72	ISEA	Philippines	D1 (Phil-Pac)	C217T, C246T, A281G, **C296T**, T306C, A342G, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC12	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC14	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC17	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC41	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC45	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC53	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC54	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC55	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC58	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC59	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC62	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC63	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC74	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC75	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC83	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	Godinez et al., 2021

Indigenous	SamNC6	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	SNC3	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	SNC35	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	SNC4	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	SNC6	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC24	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC28	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC32	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC35	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC79	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC82	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	SamNC7	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	SamNC8	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	SNC11	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	SNC34	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	SNC89	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC64	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, G399A, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC77	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC84	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T396C, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	LNC88	ISEA	Philippines	D1 (Phil-Pac)	C217T, C225T, A281G, **C296T**, T306C**,** T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	SamNC10	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, A342G, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	SNC99	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, A342G, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	SamNC11	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, T293C, **C296T**, T306C, A342G, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	SNC14	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, T293C, **C296T**, T306C, A342G, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	SamNC9	ISEA	Philippines	D1 (Phil-Pac)	C217T, T261C, A281G, **C296T**, C306T, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	SNC25	ISEA	Philippines	D1 (Phil-Pac)	C217T, T261C, A281G, **C296T**, C306T, T446C, **G686A**, T1214C	Godinez et al., 2021

Indigenous	SNC27	ISEA	Philippines	D1 (Phil-Pac)	C217T, T261C, A281G, **C296T**, C306T, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	SNC31	ISEA	Philippines	D1 (Phil-Pac)	C217T, T261C, A281G, **C296T**, C306T, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	SNC39	ISEA	Philippines	D1 (Phil-Pac)	C217T, T261C, A281G, **C296T**, C306T, T446C, **G686A**, T1214C	Godinez et al., 2021
Indigenous	SamNC1	ISEA	Philippines	A	T167C, C217T, C225T, C243T, C256T, T261C, T310C, T446C, T1214C	Godinez et al., 2021
Indigenous	SNC100	ISEA	Philippines	A	T167C, C217T, C225T, C243T, C256T, T261C, T310C, T446C, T1214C	Godinez et al., 2021
Indigenous	SNC87	ISEA	Philippines	A	T167C, C217T, C225T, C243T, C256T, T261C, T310C, T446C, T1214C	Godinez et al., 2021
Indigenous	SNC95	ISEA	Philippines	A	T167C, C217T, C225T, C243T, C256T, T261C, T310C, T446C, T1214C	Godinez et al., 2021
Indigenous	SamNC2	ISEA	Philippines	A	T167C, C217T, C225T, C243T, C256T, T261C, T310C, G399A, T446C, T1214C	Godinez et al., 2021
Indigenous	LNC23	ISEA	Philippines	B	G212A, C217T, C243T, C246T, C256T, T261C, T310C, C315T, T446C, T1214C, G1215A	Godinez et al., 2021
Indigenous	SamNC3	ISEA	Philippines	B	G212A, C217T, C243T, C246T, C256T, T261C, A281G, T310C, C315T, T446C, T1214C, G1215A	Godinez et al., 2021
Indigenous	SNC1	ISEA	Philippines	B	G212A, C217T, C243T, C246T, C256T, T261C, A281G, T310C, C315T, T446C, T1214C, G1215A	Godinez et al., 2021
Indigenous	SNC85	ISEA	Philippines	B	G212A, C217T, C243T, C246T, C256T, T261C, A281G, T310C, C315T, T446C, T1214C, G1215A	Godinez et al., 2021
Indigenous	SamNC4	ISEA	Philippines	B	G212A, C217T, C243T, C246T, C256T, T261C, T310C, C315T, T446C, G792A, T1214, G1215A	Godinez et al., 2021
Indigenous	SNC56	ISEA	Philippines	B	G212A, C217T, C243T, C246T, C256T, T261C, T310C, C315T, T446C, G792A, T1214, G1215A	Godinez et al., 2021
Indigenous	SamNC5	ISEA	Philippines	B	G212A, C217T, C243T, C246T, C256T, T261C, T310C, C315T, G399A, T446C, G792A, T1214C, G1215A	Godinez et al., 2021
Indigenous	LNC16	ISEA	Philippines	E	G238A, T322C	Godinez et al., 2021
Indigenous	LNC19	ISEA	Philippines	E	same as reference sequence	Godinez et al., 2021
Indigenous	LNC20	ISEA	Philippines	E	same as reference sequence	Godinez et al., 2021
Indigenous	LNC56	ISEA	Philippines	E	same as reference sequence	Godinez et al., 2021
Indigenous	LNC70	ISEA	Philippines	E	same as reference sequence	Godinez et al., 2021
Indigenous	LNC76	ISEA	Philippines	E	same as reference sequence	Godinez et al., 2021
Indigenous	LNC78	ISEA	Philippines	E	same as reference sequence	Godinez et al., 2021
Indigenous	LNC81	ISEA	Philippines	E	same as reference sequence	Godinez et al., 2021
Indigenous	LNC87	ISEA	Philippines	E	same as reference sequence	Godinez et al., 2021
Indigenous	SNC29	ISEA	Philippines	E	same as reference sequence	Godinez et al., 2021
Indigenous	SNC50	ISEA	Philippines	E	same as reference sequence	Godinez et al., 2021

Indigenous	SNC79		ISEA	Philippines	E	same as reference sequence	Godinez et al., 2021
Indigenous	LNC2		ISEA	Philippines	E	same as reference sequence	Godinez et al., 2021
Indigenous	LNC6		ISEA	Philippines	E	same as reference sequence	Godinez et al., 2021
Indigenous	LNC71		ISEA	Philippines	E	same as reference sequence	Godinez et al., 2021
Indigenous	LNC85		ISEA	Philippines	E	same as reference sequence	Godinez et al., 2021
Indigenous	LNC86		ISEA	Philippines	E	same as reference sequence	Godinez et al., 2021
Indigenous	SamNC13		ISEA	Philippines	E	same as reference sequence	Godinez et al., 2021
Indigenous	SamNC15		ISEA	Philippines	E	same as reference sequence	Godinez et al., 2021
Indigenous	SNC36		ISEA	Philippines	E	same as reference sequence	Godinez et al., 2021
Indigenous	SNC47		ISEA	Philippines	E	same as reference sequence	Godinez et al., 2021
Indigenous	SNC61		ISEA	Philippines	E	same as reference sequence	Godinez et al., 2021
Indigenous	SNC83		ISEA	Philippines	E	same as reference sequence	Godinez et al., 2021
Indigenous	SNC91		ISEA	Philippines	E	same as reference sequence	Godinez et al., 2021
Indigenous	SNC92		ISEA	Philippines	E	same as reference sequence	Godinez et al., 2021
Indigenous	LNC3		ISEA	Philippines	E	T199C	Godinez et al., 2021
Indigenous	SamNC14		ISEA	Philippines	E	T199C	Godinez et al., 2021
Indigenous	LNC38		ISEA	Philippines	E	T190C	Godinez et al., 2021
Indigenous	SamNC17		ISEA	Philippines	E	T190C	Godinez et al., 2021
Indigenous	LNC66		ISEA	Philippines	E	T199C	Godinez et al., 2021
Indigenous	SamNC16		ISEA	Philippines	E	G399A	Godinez et al., 2021
Red junglefowl	LeyteRJF15		ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, T446C, **G686A**, T1214C	Godinez et al., 2021
Red junglefowl	PhilRJF_4	MK085036	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, T293C, **C296T**, T306C, A342G, T446C, **G686A**, T1214C	Godinez et al., 2019
Red junglefowl	PhilRJF_2	MK085034	ISEA	Philippines	D1 (Phil-Pac)	T199C, C217T, A281G, **C296T**, T306C, T446C, **G686A**, T1214C	Godinez et al., 2019
Red junglefowl	PhilRJF_3	MK085035	ISEA	Philippines	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, C417T, T446C, **G686A**, T1214C	Godinez et al., 2019
Red junglefowl	PhilRJF_5	MK085037	ISEA	Philippines	D2	C217T, A281G, T306C, T309C, T446C, T1214C	Godinez et al., 2019
Red junglefowl	LeyteRJF6	MN986403	ISEA	Philippines	D2	C217T, A281G, T306C, T446C, T1214C	Godinez et al., 2021

Red junglefowl		LeyteRJF2	MN986399	ISEA	Philippines	D2	C217T, A281G, T306C, T446C, T1214C	Godinez et al., 2021
Red junglefowl		LeyteRJF3	MN986400	ISEA	Philippines	D2	C217T, A281G, T306C, T446C, T1214C	Godinez et al., 2021
Red junglefowl		LeyteRJF1	MN986398	ISEA	Philippines	D2	C217T, A281G, T306C, T446C, T1214C	Godinez et al., 2021
Red junglefowl		LeyteRJF4	MN986401	ISEA	Philippines	D3	C217T, T220C, C246T, A281G, T306C, T446C, T1214C	Godinez et al., 2021
Red junglefowl		LeyteRJF5	MN986402	ISEA	Philippines	D3	C217T, T220C, C246T, A281G, T306C, T446C, T1214C	Godinez et al., 2021
Red junglefowl		PhilRJF_1	MK085033	ISEA	Samar, Philippines	D3	C217T, T220C, A281G, T306C, T446C, T1214C	Godinez et al., 2019
Indigenous			KY039418	ISEA	Java, Indonesia	D1		Herrera et al., 2018
Indigenous			KY039420	ISEA	Java, Indonesia	D1		Herrera et al., 2018
Indigenous			KY039421	ISEA	Kalimantan, Indonesia	D1		Herrera et al., 2018
Indigenous			KY039422	ISEA	Kalimantan, Indonesia	D1		Herrera et al., 2018
Indigenous			KY039425	ISEA	Maluku, Indonesia	D1		Herrera et al., 2018
Indigenous			KY039426	ISEA	Lombok, Indonesia	D1		Herrera et al., 2018
Indigenous			KY039429	ISEA	Sulawesi, Indonesia	D1		Herrera et al., 2018
Indigenous	Bantam		AB268525	ISEA	Java, Indonesia	D1	212 243 246 256 261 281 306 310 315 342 859d 1215	Oka et al. 2007
Indigenous	Fighting		AB268527	ISEA	Java, Indonesia	D1	212 243 246 256 261 281 306 309 310 315 342 859d 1215	Oka et al. 2007
Indigenous	Fighting		AB268528	ISEA	Java, Indonesia	D1	212 243 246 256 261 281 306 309 310 315 859d 1215	Oka et al. 2007
Indigenous		Fiji1	OM240181	Pacific	Tuvu, Fiji	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, A342G, T446C, **G686A**, T1214C	this study
Indigenous		Fiji3	OM240182	Pacific	Tuvu, Fiji	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, A342G, T446C, **G686A**, T1214C	this study
Indigenous		Fiji4	OM240183	Pacific	Tuvu, Fiji	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, A342G, T446C, **G686A**, T1214C	this study
Indigenous		Fiji5	OM240184	Pacific	Tuvu, Fiji	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, A342G, T446C, **G686A**, T1214C	this study
Indigenous		Fiji6	OM240185	Pacific	Tuvu, Fiji	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, A342G, T446C, **G686A**, T1214C	this study
Indigenous		Fiji7	OM240186	Pacific	Tuvu, Fiji	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, A342G, T446C, **G686A**, T1214C	this study
Indigenous		Fiji9	OM240187	Pacific	Tuvu, Fiji	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, A342G, T446C, **G686A**, T1214C	this study
Indigenous		Fiji11	OM240188	Pacific	Vusu, Fiji	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, A342G, T446C, **G686A**, T1214C	this study
Indigenous		Fiji14	OM240190	Pacific	Vusu, Fiji	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, A342G, T446C, **G686A**, T1214C	this study
Indigenous		Fiji22	OM240191	Pacific	Sigatoka, Fiji	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, A342G, T446C, **G686A**, T1214C	this study

Indigenous	Fiji25	OM240193	Pacific	Sigatoka, Fiji	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, A342G, T446C, **G686A**, T1214C		this study
Indigenous	Fiji26	OM240194	Pacific	Lomaivuna, Fiji	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, A342G, T446C, **G686A**, T1214C		this study
Indigenous	Fiji12	OM240189	Pacific	Vusu, Fiji	D1 (Phil-Pac)	C217T, C233T, A281G, **C296T**, T306C, A342G, T446C, **G686A**, T1214C		this study
Indigenous	Fiji24	OM240192	Pacific	Sigatoka, Fiji	D1 (Phil-Pac)	C217T, A281G, **C296T**, T306C, A342G, T446C, **G686A**, *T869G,* T1214C		this study
Indigenous	Fiji8	OM240195	Pacific	Tuvu, Fiji	E	same as reference sequence		this study
Indigenous	Fiji10	OM240196	Pacific	Tuvu, Fiji	E	same as reference sequence		this study
Indigenous	Fiji18	OM240197	Pacific	Naroro, Fiji	E	859d		this study
Indigenous	Fiji19	OM240198	Pacific	Naroro, Fiji	E	859d		this study
Indigenous	Fiji20	OM240199	Pacific	Naroro, Fiji	E	859d		this study
Indigenous	Fiji21	OM240200	Pacific	Sigatoka, Fiji	E	222 330		this study
Indigenous	Fiji27	OM240201	Pacific	Lomaivuna, Fiji	E	246		this study
Indigenous	Fiji28	OM240202	Pacific	Lomaivuna, Fiji	E	246		this study
Indigenous	Fiji29	OM240203	Pacific	Lomaivuna, Fiji	E	246		this study
Indigenous	Fiji30	OM240204	Pacific	Lomaivuna, Fiji	E	246		this study
Indigenous	ACAD11740	KY039392	Pacific	Vanuatu	D1 (Phil-Pac)			Herrera et al., 2018
Indigenous	ACAD11713	KY039391	Pacific	Fiji (Melanesia)	D1 (Phil-Pac)			Herrera et al., 2018
ancient DNA	ACAD8675	KY039385	Pacific	Hawaii	D1 (Phil-Pac)		534	Herrera et al., 2018
ancient DNA	ACAD8671	KY039383	Pacific	Hawaii	D1 (Phil-Pac)		534	Herrera et al., 2018
ancient DNA	ACAD9073	KY039389	Pacific	Easter Island	D1 (Phil-Pac)		680	Herrera et al., 2018
ancient DNA	ACAD9076	KY039390	Pacific	Easter Island	D1 (Phil-Pac)		600	Herrera et al., 2018
ancient DNA	ACAD3896	KY039382	Pacific	Niue	D1 (Phil-Pac)		1285	Herrera et al., 2018
ancient DNA	ACAD9057	KY039386	Pacific	Easter Island	D1 (Phil-Pac)		660	Herrera et al., 2018
ancient DNA	ACAD9071	KY039388	Pacific	Easter Island	D1 (Phil-Pac)		700	Herrera et al., 2018
ancient DNA	ACAD9068	KY039387	Pacific	Easter Island	D1 (Phil-Pac)		680	Herrera et al., 2018
ancient DNA	ACAD8672	KY039384	Pacific	Hawaii	D1 (Phil-Pac)		534	Herrera et al., 2018

* identified ages of the aDNA samples (in years BP) were adopted from Thomson et al., 2014 [isolate codes: "ACAD"; Acc. Nos. code "KJ"] and complete mtDNA control region sequences where adopted from Herrera et al., 2018 - direct submission [isolate codes: ACAD; Acc. Nos. code "KY"]

Appendix Table S3.2. Accession numbers of mtDNA D-loop sequences identified in the present study.

Species	Region	Location	Sequence name	Accession No.	Haplogroup	Haplotype	Previous Haplotype* (Haplogroup)	Reference
Indigenous	MSEA	Laos	Laos33	OM240222	A	Hap_22		this study
Indigenous	MSEA	Laos	Laos38	OM240225	A	Hap_22		this study
Indigenous	MSEA	Laos	Laos59	OM240235	A	Hap_22		this study
Indigenous	MSEA	Laos	Laos95	OM240255	A	Hap_22		this study
Indigenous	MSEA	Laos	Laos103	OM240259	A	Hap_22		this study
Indigenous	MSEA	Laos	Laos14	OM240211	A	Hap_38		this study
Indigenous	MSEA	Laos	Laos20	OM240216	A	Hap_38		this study
Indigenous	MSEA	Laos	Laos90	OM240250	A	Hap_38		this study
Indigenous	MSEA	Laos	Laos91	OM240251	A	Hap_38		this study
Indigenous	MSEA	Laos	Laos27	OM240220	A	Hap_39		this study
Indigenous	MSEA	Laos	Laos36	OM240224	A	Hap_42		this study
Indigenous	MSEA	Laos	Laos41	OM240226	A	Hap_43		this study
Indigenous	MSEA	Laos	Laos67	OM240238	A	Hap_43		this study
Indigenous	MSEA	Laos	Laos104	OM240260	A	Hap_43		this study
Indigenous	MSEA	Laos	Laos45	OM240229	A	Hap_44		this study
Indigenous	MSEA	Laos	Laos102	OM240258	A	Hap_51		this study
Indigenous	MSEA	Laos	Laos107	OM240266	A	Hap_114		this study
Indigenous	MSEA	Laos	Laos42	OM240227	B	Hap_28		this study
Indigenous	MSEA	Laos	Laos43	OM240228	B	Hap_28		this study
Indigenous	MSEA	Laos	Laos53	OM240232	B	Hap_28		this study
Indigenous	MSEA	Laos	Laos66	OM240237	B	Hap_28		this study
Indigenous	MSEA	Laos	Laos98	OM240257	B	Hap_28		this study
Indigenous	MSEA	Laos	Laos50	OM240263	B	Hap_28		this study
Indigenous	MSEA	Laos	Laos32	OM240221	B	Hap_40		this study
Indigenous	MSEA	Laos	Laos97	OM240256	B	Hap_40		this study
Indigenous	MSEA	Laos	Lao101	OM240265	B	Hap_40		this study
Indigenous	MSEA	Laos	Laos34	OM240223	B	Hap_41		this study
Indigenous	MSEA	Laos	Laos46	OM240230	B	Hap_45		this study
Indigenous	MSEA	Laos	Laos54	OM240233	B	Hap_46		this study
Indigenous	MSEA	Laos	Laos58	OM240234	B	Hap_46		this study
Indigenous	MSEA	Laos	Laos61	OM240236	B	Hap_46		this study
Indigenous	MSEA	Laos	Laos92	OM240252	B	Hap_50		this study
Indigenous	MSEA	Laos	Laos76	OM240264	B	Hap_50		this study
Indigenous	MSEA	Laos	Laos106	OM240261	B	Hap_52		this study
Indigenous	MSEA	Laos	Laos111	OM240267	B	Hap_115		this study
Indigenous	MSEA	Laos	Laos51	OM240231	D2	Hap_19		this study
Indigenous	MSEA	Laos	Laos72	OM240241	D2	Hap_19		this study
Indigenous	MSEA	Laos	Laos74	OM240243	D2	Hap_19		this study
Indigenous	MSEA	Laos	Laos77	OM240245	D2	Hap_19		this study
Indigenous	MSEA	Laos	Laos73	OM240242	D2	Hap_20		this study
Indigenous	MSEA	Laos	Laos68	OM240239	D3	Hap_47		this study
Indigenous	MSEA	Laos	Laos75	OM240244	D3	Hap_47		this study
Indigenous	MSEA	Laos	Laos18	OM240214	E	Hap_05		this study
Indigenous	MSEA	Laos	Laos11	OM240209	E	Hap_06		this study
Indigenous	MSEA	Laos	Laos16	OM240213	E	Hap_06		this study
Indigenous	MSEA	Laos	Laos22	OM240218	E	Hap_06		this study
Indigenous	MSEA	Laos	Laos3	OM240205	E	Hap_35		this study
Indigenous	MSEA	Laos	Laos10	OM240208	E	Hap_35		this study
Indigenous	MSEA	Laos	Laos6	OM240206	E	Hap_36		this study
Indigenous	MSEA	Laos	Laos15	OM240212	E	Hap_36		this study
Indigenous	MSEA	Laos	Laos21	OM240217	E	Hap_36		this study
Indigenous	MSEA	Laos	Laos9	OM240207	E	Hap_37		this study
Indigenous	MSEA	Laos	Laos13	OM240210	E	Hap_37		this study
Indigenous	MSEA	Laos	Laos19	OM240215	E	Hap_37		this study
Indigenous	MSEA	Laos	Laos23	OM240219	E	Hap_37		this study
Indigenous	MSEA	Laos	Laos78	OM240246	E (E3)	Hap_49		this study
Indigenous	MSEA	Laos	Laos4	OM240262	E	Hap_53		this study
Indigenous	MSEA	Laos	Laos70	OM240240	V2	Hap_48		this study
Indigenous	MSEA	Laos	Laos82	OM240247	V2	Hap_48		this study
Indigenous	MSEA	Laos	Laos87	OM240248	V2	Hap_48		this study
Indigenous	MSEA	Laos	Laos89	OM240249	V2	Hap_48		this study
Indigenous	MSEA	Laos	Laos93	OM240253	V2	Hap_48		this study
Indigenous	MSEA	Laos	Laos94	OM240254	V2	Hap_48		this study
Indigenous	MSEA	Cambodia	Cam31	OM240288	A	Hap_22		this study
Indigenous	MSEA	Cambodia	Cam32	OM240289	A	Hap_22		this study
Indigenous	MSEA	Cambodia	Cam59	OM240308	A	Hap_22		this study
Indigenous	MSEA	Cambodia	Cam63	OM240311	A	Hap_22		this study
Indigenous	MSEA	Cambodia	Cam100	OM240339	A	Hap_22		this study
Indigenous	MSEA	Cambodia	Cam104	OM240343	A	Hap_22		this study

Indigenous	MSEA	Cambodia	Cam152	OM240364	A	Hap_22	this study
Indigenous	MSEA	Cambodia	Cam160	OM240369	A	Hap_22	this study
Indigenous	MSEA	Cambodia	Cam61	OM240373	A	Hap_22	this study
Indigenous	MSEA	Cambodia	Cam126	OM240381	A	Hap_22	this study
Indigenous	MSEA	Cambodia	Cam162	OM240384	A	Hap_22	this study
Indigenous	MSEA	Cambodia	Cam91	OM240334	A	Hap_43	this study
Indigenous	MSEA	Cambodia	Cam49	OM240302	A	Hap_58	this study
Indigenous	MSEA	Cambodia	Cam146	OM240360	A	Hap_72	this study
Indigenous	MSEA	Cambodia	Cam174	OM240398	A	Hap_75	this study
Indigenous	MSEA	Cambodia	Cam185	OM240403	A	Hap_75	this study
Indigenous	MSEA	Cambodia	Cam205	OM240413	A	Hap_75	this study
Indigenous	MSEA	Cambodia	Cam193	OM240407	A	Hap_77	this study
Indigenous	MSEA	Cambodia	Cam121	OM240433	A	Hap_114	this study
Indigenous	MSEA	Cambodia	Cam35	OM240291	B	Hap_08	this study
Indigenous	MSEA	Cambodia	Cam40	OM240296	B	Hap_08	this study
Indigenous	MSEA	Cambodia	Cam47	OM240300	B	Hap_28	this study
Indigenous	MSEA	Cambodia	Cam53	OM240304	B	Hap_28	this study
Indigenous	MSEA	Cambodia	Cam43	OM240298	B	Hap_40	this study
Indigenous	MSEA	Cambodia	Cam66	OM240313	B	Hap_40	this study
Indigenous	MSEA	Cambodia	Cam103	OM240342	B	Hap_40	this study
Indigenous	MSEA	Cambodia	Cam159	OM240368	B	Hap_40	this study
Indigenous	MSEA	Cambodia	Cam118	OM240380	B	Hap_40	this study
Indigenous	MSEA	Cambodia	Cam285	OM240395	B	Hap_40	this study
Indigenous	MSEA	Cambodia	Cam102	OM240341	B	Hap_50	this study
Indigenous	MSEA	Cambodia	Cam98	OM240337	B	Hap_64	this study
Indigenous	MSEA	Cambodia	Cam1	OM240371	B	Hap_64	this study
Indigenous	MSEA	Cambodia	Cam92	OM240374	B	Hap_64	this study
Indigenous	MSEA	Cambodia	Cam235	OM240419	B	Hap_81	this study
Indigenous	MSEA	Cambodia	Cam248	OM240420	B	Hap_82	this study
Indigenous	MSEA	Cambodia	Cam110	OM240430	B	Hap_115	this study
Indigenous	MSEA	Cambodia	Cam111	OM240431	B	Hap_115	this study
Indigenous	MSEA	Cambodia	Cam38	OM240294	D1 (SEA subclade)	Hap_02	this study
Indigenous	MSEA	Cambodia	Cam54	OM240305	D1 (SEA subclade)	Hap_02	this study
Indigenous	MSEA	Cambodia	Cam141	OM240357	D1 (SEA subclade)	Hap_02	this study
Indigenous	MSEA	Cambodia	Cam156	OM240366	D1 (SEA subclade)	Hap_02	this study
Indigenous	MSEA	Cambodia	Cam157	OM240367	D1 (SEA subclade)	Hap_02	this study
Indigenous	MSEA	Cambodia	Cam161	OM240370	D1 (SEA subclade)	Hap_02	this study
Indigenous	MSEA	Cambodia	Cam189	OM240406	D1 (SEA subclade)	Hap_02	this study
Indigenous	MSEA	Cambodia	Cam58	OM240424	D1 (SEA subclade)	Hap_11	this study
Indigenous	MSEA	Cambodia	Cam44	OM240299	D1 (SEA subclade)	Hap_57	this study
Indigenous	MSEA	Cambodia	Cam48	OM240301	D1 (SEA subclade)	Hap_57	this study
Indigenous	MSEA	Cambodia	Cam81	OM240326	D1 (SEA subclade)	Hap_60	this study
Indigenous	MSEA	Cambodia	Cam88	OM240332	D1 (SEA subclade)	Hap_62	this study
Indigenous	MSEA	Cambodia	Cam94	OM240335	D1 (SEA subclade)	Hap_63	this study
Indigenous	MSEA	Cambodia	Cam105	OM240344	D1 (SEA subclade)	Hap_66	this study
Indigenous	MSEA	Cambodia	Cam112	OM240348	D1 (SEA subclade)	Hap_68	this study
Indigenous	MSEA	Cambodia	Cam137	OM240356	D1 (SEA subclade)	Hap_71	this study
Indigenous	MSEA	Cambodia	Cam147	OM240361	D1 (SEA subclade)	Hap_71	this study
Indigenous	MSEA	Cambodia	Cam119	OM240432	D1 (SEA subclade)	Hap_124	this study
Indigenous	MSEA	Cambodia	Cam122	OM240434	D1 (SEA subclade)	Hap_125	this study
Indigenous	MSEA	Cambodia	Cam133	OM240438	D1 (SEA subclade)	Hap_126	this study
Indigenous	MSEA	Cambodia	Cam79	OM240324	D2	Hap_07	this study
Indigenous	MSEA	Cambodia	Cam82	OM240327	D2	Hap_61	this study
Indigenous	MSEA	Cambodia	Cam84	OM240328	D2	Hap_61	this study
Indigenous	MSEA	Cambodia	Cam101	OM240340	D2	Hap_61	this study
Indigenous	MSEA	Cambodia	Cam125	OM240350	D2	Hap_61	this study
Indigenous	MSEA	Cambodia	Cam175	OM240399	D2	Hap_61	this study
Indigenous	MSEA	Cambodia	Cam90	OM240428	D2	Hap_122	this study
Indigenous	MSEA	Cambodia	Cam28	OM240285	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam34	OM240290	D2	Hap_19	this study

Indigenous	MSEA	Cambodia	Cam37	OM240293	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam50	OM240303	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam55	OM240306	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam56	OM240307	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam62	OM240310	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam67	OM240314	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam68	OM240315	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam70	OM240316	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam71	OM240317	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam72	OM240318	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam80	OM240325	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam85	OM240329	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam86	OM240330	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam87	OM240331	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam89	OM240333	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam95	OM240336	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam109	OM240347	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam127	OM240351	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam129	OM240353	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam144	OM240358	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam145	OM240359	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam57	OM240372	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam116	OM240378	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam117	OM240379	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam139	OM240383	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam163	OM240385	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam166	OM240386	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam168	OM240387	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam170	OM240388	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam178	OM240389	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam204	OM240391	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam278	OM240393	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam282	OM240394	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam172	OM240397	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam188	OM240405	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam195	OM240408	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Can228	OM240417	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam253	OM240421	D2	Hap_19	this study
Indigenous	MSEA	Cambodia	Cam30	OM240287	D2	Hap_20	this study
Indigenous	MSEA	Cambodia	Cam36	OM240292	D2	Hap_20	this study
Indigenous	MSEA	Cambodia	Cam149	OM240363	D2	Hap_20	this study
Indigenous	MSEA	Cambodia	Cam198	OM240410	D2	Hap_20	this study
Indigenous	MSEA	Cambodia	Cam202	OM240411	D2	Hap_20	this study
Indigenous	MSEA	Cambodia	Cam206	OM240414	D2	Hap_20	this study
Indigenous	MSEA	Cambodia	Cam74	OM240320	D2	Hap_59	this study
Indigenous	MSEA	Cambodia	Cam75	OM240321	D2	Hap_59	this study
Indigenous	MSEA	Cambodia	Cam153	OM240365	D2	Hap_59	this study
Indigenous	MSEA	Cambodia	Cam93	OM240375	D2	Hap_73	this study
Indigenous	MSEA	Cambodia	Cam199	OM240390	D2	Hap_73	this study
Indigenous	MSEA	Cambodia	Cam264	OM240423	D2	Hap_73	this study
Indigenous	MSEA	Cambodia	Cam203	OM240412	D2	Hap_79	this study
Indigenous	MSEA	Cambodia	Cam65	OM240425	D2	Hap_121	this study
Indigenous	MSEA	Cambodia	Cam69	OM240426	D2	Hap_121	this study
Indigenous	MSEA	Cambodia	Cam78	OM240427	D2	Hap_121	this study
Indigenous	MSEA	Cambodia	Cam131	OM240436	D2	Hap_121	this study
Indigenous	MSEA	Cambodia	Cam132	OM240437	D2	Hap_121	this study
Indigenous	MSEA	Cambodia	Cam171	OM240439	D2	Hap_121	this study
Indigenous	MSEA	Cambodia	Cam196	OM240409	D2	Hap_78	this study
Indigenous	MSEA	Cambodia	Cam8	OM240271	D3	Hap_31	this study
Indigenous	MSEA	Cambodia	Cam64	OM240312	D3	Hap_31	this study
Indigenous	MSEA	Cambodia	Cam73	OM240319	D3	Hap_31	this study
Indigenous	MSEA	Cambodia	Cam106	OM240345	D3	Hap_47	this study
Indigenous	MSEA	Cambodia	Cam128	OM240352	D3	Hap_47	this study
Indigenous	MSEA	Cambodia	Cam2	OM240268	E	Hap_05	this study
Indigenous	MSEA	Cambodia	Cam6	OM240270	E	Hap_05	this study
Indigenous	MSEA	Cambodia	Cam9	OM240272	E	Hap_05	this study
Indigenous	MSEA	Cambodia	Cam97	OM240376	E	Hap_05	this study
Indigenous	MSEA	Cambodia	Cam4	OM240269	E	Hap_37	this study
Indigenous	MSEA	Cambodia	Cam10	OM240273	E	Hap_37	this study
Indigenous	MSEA	Cambodia	Cam12	OM240274	E	Hap_37	this study
Indigenous	MSEA	Cambodia	Cam16	OM240275	E	Hap_37	this study
Indigenous	MSEA	Cambodia	Cam17	OM240276	E	Hap_37	this study
Indigenous	MSEA	Cambodia	Cam18	OM240277	E	Hap_37	this study
Indigenous	MSEA	Cambodia	Cam20	OM240278	E	Hap_37	this study
Indigenous	MSEA	Cambodia	Cam21	OM240279	E	Hap_37	this study

Indigenous	MSEA	Cambodia	Cam22	OM240280	E	Hap_37	this study
Indigenous	MSEA	Cambodia	Cam24	OM240282	E	Hap_37	this study
Indigenous	MSEA	Cambodia	Cam25	OM240283	E	Hap_37	this study
Indigenous	MSEA	Cambodia	Cam27	OM240284	E	Hap_37	this study
Indigenous	MSEA	Cambodia	Cam23	OM240281	E	Hap_54	this study
Indigenous	MSEA	Cambodia	Cam260	OM240422	F	Hap_74	this study
Indigenous	MSEA	Cambodia	Cam261	OM240392	F	Hap_74	this study
Indigenous	MSEA	Cambodia	Cam41	OM240297	V2	Hap_48	this study
Indigenous	MSEA	Cambodia	Cam60	OM240309	V2	Hap_48	this study
Indigenous	MSEA	Cambodia	Cam76	OM240322	V2	Hap_48	this study
Indigenous	MSEA	Cambodia	Cam77	OM240323	V2	Hap_48	this study
Indigenous	MSEA	Cambodia	Cam148	OM240362	V2	Hap_48	this study
Indigenous	MSEA	Cambodia	Cam113	OM240377	V2	Hap_48	this study
Indigenous	MSEA	Cambodia	Cam164	OM240396	V2	Hap_48	this study
Indigenous	MSEA	Cambodia	Cam179	OM240400	V2	Hap_48	this study
Indigenous	MSEA	Cambodia	Cam180	OM240401	V2	Hap_48	this study
Indigenous	MSEA	Cambodia	Cam183	OM240402	V2	Hap_48	this study
Indigenous	MSEA	Cambodia	Cam29	OM240286	V2	Hap_55	this study
Indigenous	MSEA	Cambodia	Cam99	OM240338	V2	Hap_65	this study
Indigenous	MSEA	Cambodia	Cam108	OM240346	V2	Hap_67	this study
Indigenous	MSEA	Cambodia	Cam115	OM240349	V2	Hap_67	this study
Indigenous	MSEA	Cambodia	Cam130	OM240354	V2	Hap_69	this study
Indigenous	MSEA	Cambodia	Cam136	OM240355	V2	Hap_70	this study
Indigenous	MSEA	Cambodia	Cam138	OM240382	V2	Hap_70	this study
Indigenous	MSEA	Cambodia	Cam186	OM240404	V2	Hap_76	this study
Indigenous	MSEA	Cambodia	Cam225	OM240415	V2	Hap_80	this study
Indigenous	MSEA	Cambodia	Cam227	OM240416	V2	Hap_80	this study
Indigenous	MSEA	Cambodia	Cam229	OM240418	V2	Hap_80	this study
Indigenous	MSEA	Cambodia	Cam107	OM240429	V2	Hap_123	this study
Indigenous	MSEA	Cambodia	Cam123	OM240435	V2	Hap_123	this study
Indigenous	MSEA	Cambodia	Cam226	OM240440	V2	Hap_127	this study
Indigenous	MSEA	Cambodia	Cam39	OM240295	I	Hap_56	this study
Indigenous	MSEA	Myanmar	Mya59	OM240461	A	Hap_22	this study
Indigenous	MSEA	Myanmar	Mya63	OM240463	A	Hap_22	this study
Indigenous	MSEA	Myanmar	Mya71	OM240468	A	Hap_22	this study
Indigenous	MSEA	Myanmar	Mya83	OM240472	A	Hap_22	this study
Indigenous	MSEA	Myanmar	Mya99	OM240479	A	Hap_22	this study
Indigenous	MSEA	Myanmar	Mya116	OM240491	A	Hap_22	this study
Indigenous	MSEA	Myanmar	Mya131	OM240501	A	Hap_22	this study
Indigenous	MSEA	Myanmar	Mya58	OM240460	A	Hap_75	this study
Indigenous	MSEA	Myanmar	Mya61	OM240462	A	Hap_75	this study
Indigenous	MSEA	Myanmar	Mya42	OM240453	A	Hap_88	this study
Indigenous	MSEA	Myanmar	Mya48	OM240457	A	Hap_90	this study
Indigenous	MSEA	Myanmar	Mya81	OM240470	A	Hap_91	this study
Indigenous	MSEA	Myanmar	Mya123	OM240505	A	Hap_114	this study
Indigenous	MSEA	Myanmar	MyaFx1	OM240507	A	Hap_114	this study
Indigenous	MSEA	Myanmar	MyaFx2	OM240508	A	Hap_114	this study
Indigenous	MSEA	Myanmar	MyaFx3	OM240509	A	Hap_114	this study
Indigenous	MSEA	Myanmar	MyaFx4	OM240510	A	Hap_114	this study
Indigenous	MSEA	Myanmar	MyaFx5	OM240511	A	Hap_114	this study
Indigenous	MSEA	Myanmar	MyaFx6	OM240512	A	Hap_114	this study
Indigenous	MSEA	Myanmar	MyaFx7	OM240513	A	Hap_114	this study
Indigenous	MSEA	Myanmar	Mya76	OM240469	B	Hap_08	this study
Indigenous	MSEA	Myanmar	Mya70	OM240467	B	Hap_28	this study
Indigenous	MSEA	Myanmar	Mya125	OM240496	B	Hap_28	this study
Indigenous	MSEA	Myanmar	MyaFx8	OM240514	B	Hap_28	this study
Indigenous	MSEA	Myanmar	Mya43	OM240454	B	Hap_40	this study
Indigenous	MSEA	Myanmar	Mya64	OM240464	B	Hap_40	this study
Indigenous	MSEA	Myanmar	Mya65	OM240465	B	Hap_40	this study
Indigenous	MSEA	Myanmar	Mya66	OM240466	B	Hap_40	this study
Indigenous	MSEA	Myanmar	Mya82	OM240471	B	Hap_40	this study
Indigenous	MSEA	Myanmar	Mya84	OM240473	B	Hap_40	this study
Indigenous	MSEA	Myanmar	Mya120	OM240492	B	Hap_40	this study
Indigenous	MSEA	Myanmar	Mya124	OM240495	B	Hap_40	this study
Indigenous	MSEA	Myanmar	Mya128	OM240499	B	Hap_40	this study
Indigenous	MSEA	Myanmar	Mya130	OM240500	B	Hap_40	this study
Indigenous	MSEA	Myanmar	Mya32	OM240450	B	Hap_50	this study
Indigenous	MSEA	Myanmar	Mya114	OM240489	B	Hap_50	this study
Indigenous	MSEA	Myanmar	Mya135	OM240502	B	Hap_102	this study
Indigenous	MSEA	Myanmar	Mya133	OM240506	B	Hap_118	this study
Indigenous	MSEA	Myanmar	Mya103	OM240481	D1 (SEA subclade)	Hap_96	this study
Indigenous	MSEA	Myanmar	Mya100	OM240480	E	Hap_06	this study
Indigenous	MSEA	Myanmar	Mya1	OM240441	E	Hap_37	this study
Indigenous	MSEA	Myanmar	Mya6	OM240443	E	Hap_37	this study

Indigenous	MSEA	Myanmar	Mya9	OM240444	E (E3)	Hap_84	this study
Indigenous	MSEA	Myanmar	Mya40	OM240452	E (E3)	Hap_84	this study
Indigenous	MSEA	Myanmar	Mya54	OM240459	E (E3)	Hap_84	this study
Indigenous	MSEA	Myanmar	Mya23	OM240446	E	Hap_85	this study
Indigenous	MSEA	Myanmar	Mya28	OM240448	E	Hap_86	this study
Indigenous	MSEA	Myanmar	Mya121	OM240493	E	Hap_100	this study
Indigenous	MSEA	Myanmar	Mya126	OM240497	E (E3)	Hap_101	this study
Indigenous	MSEA	Myanmar	Mya30	OM240503	E (E3)	Hap_116	this study
Indigenous	MSEA	Myanmar	Mya16	OM240445	F	Hap_74	this study
Indigenous	MSEA	Myanmar	Mya52	OM240458	F	Hap_74	this study
Indigenous	MSEA	Myanmar	Mya94	OM240478	F	Hap_74	this study
Indigenous	MSEA	Myanmar	Mya104	OM240482	F	Hap_74	this study
Indigenous	MSEA	Myanmar	Mya106	OM240483	F	Hap_74	this study
Indigenous	MSEA	Myanmar	Mya113	OM240488	F	Hap_74	this study
Indigenous	MSEA	Myanmar	Mya122	OM240494	F	Hap_74	this study
Indigenous	MSEA	Myanmar	Mya127	OM240498	F	Hap_74	this study
Indigenous	MSEA	Myanmar	Mya2	OM240442	F	Hap_83	this study
Indigenous	MSEA	Myanmar	Mya27	OM240447	F	Hap_83	this study
Indigenous	MSEA	Myanmar	Mya29	OM240449	F	Hap_87	this study
Indigenous	MSEA	Myanmar	Mya36	OM240451	F	Hap_87	this study
Indigenous	MSEA	Myanmar	Mya45	OM240455	F	Hap_89	this study
Indigenous	MSEA	Myanmar	Mya46	OM240456	F	Hap_89	this study
Indigenous	MSEA	Myanmar	Mya86	OM240474	F	Hap_92	this study
Indigenous	MSEA	Myanmar	Mya89	OM240475	F	Hap_93	this study
Indigenous	MSEA	Myanmar	Mya91	OM240476	F	Hap_94	this study
Indigenous	MSEA	Myanmar	Mya93	OM240477	F	Hap_95	this study
Indigenous	MSEA	Myanmar	Mya107	OM240484	F	Hap_95	this study
Indigenous	MSEA	Myanmar	Mya109	OM240485	F	Hap_97	this study
Indigenous	MSEA	Myanmar	Mya112	OM240487	F	Hap_97	this study
Indigenous	MSEA	Myanmar	Mya111	OM240486	F	Hap_98	this study
Indigenous	MSEA	Myanmar	Mya115	OM240490	F	Hap_99	this study
Indigenous	MSEA	Myanmar	Mya50	OM240504	F	Hap_117	this study
Indigenous	MSEA	Myanmar	MyaFx9	OM240515	F	Hap_119	this study
Indigenous	MSEA	Thailand	Thai_chicken24	OM240529	A	Hap_22	this study
Indigenous	MSEA	Thailand	Thai_chicken23	OM240528	A	Hap_75	this study
Indigenous	MSEA	Thailand	Thai_chicken21	OM240526	A	Hap_106	this study
Indigenous	MSEA	Thailand	Thai_chicken25	OM240530	B	Hap_28	this study
Indigenous	MSEA	Thailand	Thai_chicken28	OM240531	B	Hap_81	this study
Indigenous	MSEA	Thailand	Thai_chicken22	OM240527	E	Hap_06	this study
Indigenous	MSEA	Thailand	Thai_chicken30	OM240532	F	Hap_74	this study
Red junglefowl	MSEA	Myanmar	MyaJF2	OM240517	F	Hap_120	this study
Red junglefowl	MSEA	Myanmar	MyaJF3	OM240518	F	Hap_120	this study
Red junglefowl	MSEA	Myanmar	MyaJF1	OM240516	B	Hap_40	this study
Red junglefowl	MSEA	Thailand	Thai_chonburi11	OM240522	A	Hap_104	this study
Red junglefowl	MSEA	Thailand	Thai_chonburi14	OM240523	A	Hap_105	this study
Red junglefowl	MSEA	Thailand	Thai_chonburi20	OM240525	A	Hap_105	this study
Red junglefowl	MSEA	Thailand	Thai_chonburi8	OM240520	B	Hap_40	this study
Red junglefowl	MSEA	Thailand	Thai_chonburi9	OM240521	D2	Hap_19	this study
Red junglefowl	MSEA	Thailand	Thai_northern45	OM240542	D2	Hap_20	this study
Red junglefowl	MSEA	Thailand	Thai_sakonakon37	OM240539	E	Hap_109	this study
Red junglefowl	MSEA	Thailand	Thai_sakonakon34	OM240536	F	Hap_95	this study
Red junglefowl	MSEA	Thailand	Thai_northern46	OM240543	F	Hap_111	this study
Red junglefowl	MSEA	Thailand	Thai_sakonakon31	OM240533	V1	Hap_107	this study
Red junglefowl	MSEA	Thailand	Thai_sakonakon32	OM240534	V1	Hap_107	this study

Red junglefowl	MSEA	Thailand	Thai_sakonakon33	OM240535	V1	Hap_107		this study
Red junglefowl	MSEA	Thailand	Thai_sakonakon36	OM240538	V1	Hap_107		this study
Red junglefowl	MSEA	Thailand	Thai_sakonakon40	OM240540	V1	Hap_107		this study
Red junglefowl	MSEA	Thailand	Thai_sakonakon35	OM240537	V1	Hap_108		this study
Red junglefowl	MSEA	Thailand	Thai_northern41	OM240541	V1	Hap_110		this study
Red junglefowl	MSEA	Thailand	Thai_chonburi4	OM240519	V2	Hap_103		this study
Red junglefowl	MSEA	Thailand	Thai_chonburi15	OM240524	V2	Hap_103		this study
Indigenous	ISEA	Philippines	LNC1		D1 (SEA subclade)	Hap_01	H_69 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC26		D1 (SEA subclade)	Hap_01	H_69 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC11		D1 (SEA subclade)	Hap_02	H_49 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC57		D1 (SEA subclade)	Hap_02	H_49 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC67		D1 (SEA subclade)	Hap_02	H_49 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC73		D1 (SEA subclade)	Hap_02	H_49 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC80		D1 (SEA subclade)	Hap_02	H_49 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC86		D1 (SEA subclade)	Hap_02	H_49 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC88		D1 (SEA subclade)	Hap_02	H_49 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC31		D1 (SEA subclade)	Hap_11	H_49 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC33		D1 (SEA subclade)	Hap_11	H_49 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC34		D1 (SEA subclade)	Hap_11	H_49 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC36		D1 (SEA subclade)	Hap_11	H_49 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC37		D1 (SEA subclade)	Hap_11	H_49 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC39		D1 (SEA subclade)	Hap_11	H_49 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC40		D1 (SEA subclade)	Hap_11	H_49 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC80		D1 (SEA subclade)	Hap_11	H_49 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC9		D1 (SEA subclade)	Hap_11	H_49 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SamNC12		D1 (SEA subclade)	Hap_11	H_49 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC45		D1 (SEA subclade)	Hap_11	H_49 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC93		D1 (SEA subclade)	Hap_11	H_49 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC94		D1 (SEA subclade)	Hap_11	H_49 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC97		D1 (SEA subclade)	Hap_11	H_49 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC4		D1 (Phil-Pac subclade)	Hap_14	H_65 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC5		D1 (Phil-Pac subclade)	Hap_14	H_65 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC68		D1 (Phil-Pac subclade)	Hap_14	H_65 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC72		D1 (Phil-Pac subclade)	Hap_14	H_65 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC12		D1 (Phil-Pac subclade)	Hap_03	H_74 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC14		D1 (Phil-Pac subclade)	Hap_03	H_74 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC17		D1 (Phil-Pac subclade)	Hap_03	H_74 (D1)	Godinez et al., 2021

Indigenous	ISEA	Philippines	LNC41	D1 (Phil-Pac subclade)	Hap_03	H_74 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC45	D1 (Phil-Pac subclade)	Hap_03	H_74 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC53	D1 (Phil-Pac subclade)	Hap_03	H_74 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC54	D1 (Phil-Pac subclade)	Hap_03	H_74 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC55	D1 (Phil-Pac subclade)	Hap_03	H_74 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC58	D1 (Phil-Pac subclade)	Hap_03	H_74 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC59	D1 (Phil-Pac subclade)	Hap_03	H_74 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC62	D1 (Phil-Pac subclade)	Hap_03	H_74 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC63	D1 (Phil-Pac subclade)	Hap_03	H_74 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC74	D1 (Phil-Pac subclade)	Hap_03	H_74 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC75	D1 (Phil-Pac subclade)	Hap_03	H_74 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC83	D1 (Phil-Pac subclade)	Hap_03	H_74 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SamNC6	D1 (Phil-Pac subclade)	Hap_03	H_74 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC3	D1 (Phil-Pac subclade)	Hap_03	H_74 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC35	D1 (Phil-Pac subclade)	Hap_03	H_74 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC4	D1 (Phil-Pac subclade)	Hap_03	H_74 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC6	D1 (Phil-Pac subclade)	Hap_03	H_74 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC24	D1 (Phil-Pac subclade)	Hap_09	H_68 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC28	D1 (Phil-Pac subclade)	Hap_09	H_68 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC32	D1 (Phil-Pac subclade)	Hap_12	H_68 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC35	D1 (Phil-Pac subclade)	Hap_12	H_68 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC79	D1 (Phil-Pac subclade)	Hap_12	H_68 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC82	D1 (Phil-Pac subclade)	Hap_12	H_68 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SamNC7	D1 (Phil-Pac subclade)	Hap_12	H_68 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SamNC8	D1 (Phil-Pac subclade)	Hap_12	H_68 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC11	D1 (Phil-Pac subclade)	Hap_12	H_68 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC34	D1 (Phil-Pac subclade)	Hap_12	H_68 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC89	D1 (Phil-Pac subclade)	Hap_12	H_68 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC64	D1 (Phil-Pac subclade)	Hap_15	H_77 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC77	D1 (Phil-Pac subclade)	Hap_17	H_74 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC84	D1 (Phil-Pac subclade)	Hap_17	H_74 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC88	D1 (Phil-Pac subclade)	Hap_18	H_78 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SamNC10	D1 (Phil-Pac subclade)	Hap_23	H_57 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC99	D1 (Phil-Pac subclade)	Hap_23	H_57 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SamNC11	D1 (Phil-Pac subclade)	Hap_24	H_85 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC14	D1 (Phil-Pac subclade)	Hap_24	H_85 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SamNC9	D1 (Phil-Pac subclade)	Hap_30	H_90 (D1)	Godinez et al., 2021

Indigenous	ISEA	Philippines	SNC25	D1 (Phil-Pac subclade)	Hap_30	H_90 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC27	D1 (Phil-Pac subclade)	Hap_30	H_90 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC31	D1 (Phil-Pac subclade)	Hap_30	H_90 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC39	D1 (Phil-Pac subclade)	Hap_34	H_94 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SamNC1	A	Hap_22	H_14 (A)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC100	A	Hap_22	H_14 (A)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC87	A	Hap_22	H_14 (A)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC95	A	Hap_22	H_14 (A)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SamNC2	A	Hap_26	H_87 (A)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC23	B	Hap_08	H_73 (B)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SamNC3	B	Hap_27	H_88 (B)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC1	B	Hap_27	H_88 (B)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC85	B	Hap_27	H_88 (B)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SamNC4	B	Hap_28	H_51 (B)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC56	B	Hap_28	H_51 (B)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SamNC5	B	Hap_29	H_89 (B)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC16	E	Hap_04	H_75 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC19	E	Hap_05	H_1 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC20	E	Hap_05	H_1 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC56	E	Hap_05	H_1 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC70	E	Hap_05	H_1 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC76	E	Hap_05	H_1 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC78	E	Hap_05	H_1 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC81	E	Hap_05	H_1 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC87	E	Hap_05	H_1 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC29	E	Hap_05	H_1 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC50	E	Hap_05	H_1 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC79	E	Hap_05	H_1 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC2	E	Hap_06	H_1 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC6	E	Hap_06	H_1 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC71	E	Hap_06	H_1 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC85	E	Hap_06	H_1 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC86	E	Hap_06	H_1 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SamNC13	E	Hap_06	H_1 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SamNC15	E	Hap_06	H_1 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC36	E	Hap_06	H_1 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC47	E	Hap_06	H_1 (E1)	Godinez et al., 2021

Indigenous	ISEA	Philippines	SNC61		E	Hap_06	H_1 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC83		E	Hap_06	H_1 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC91		E	Hap_06	H_1 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SNC92		E	Hap_06	H_1 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC3		E	Hap_10	H_29 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SamNC14		E	Hap_10	H_29 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC38		E	Hap_13	H_76 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SamNC17		E	Hap_13	H_76 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC66		E	Hap_16	H_29 (E1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	SamNC16		E	Hap_25	H_86 (E1)	Godinez et al., 2021
Red junglefowl	ISEA	Philippines	LeytePHRJF01	OM240544	D1 (SEA subclade)	Hap_112		this study
Red junglefowl	ISEA	Philippines	LeyteRJF15		D1 (Phil-Pac subclade)	Hap_12	H_68 (D1)	Godinez et al., 2021
Red junglefowl	ISEA	Philippines	LeyteRJF19	OM240548	D1 (Phil-Pac subclade)	Hap_17		this study
Red junglefowl	ISEA	Philippines	PhilRJF_4	MK085036	D1 (Phil-Pac subclade)	Hap_24		Godinez et al., 2019
Red junglefowl	ISEA	Philippines	PhilRJF_2	MK085034	D1 (Phil-Pac subclade)	Hap_32		Godinez et al., 2019
Red junglefowl	ISEA	Philippines	PhilRJF_3	MK085035	D1 (Phil-Pac subclade)	Hap_33		Godinez et al., 2019
Red junglefowl	ISEA	Philippines	LeytePHRJF05	OM240545	D1 (Phil-Pac subclade)	Hap_113		this study
Red junglefowl	ISEA	Philippines	LeyteRJF17	OM240547	D1 (Phil-Pac subclade)	Hap_113		this study
Indigenous	ISEA	Philippines	LNC22		D2	Hap_07	H_50 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC27		D2	Hap_07	H_50 (D1)	Godinez et al., 2021
Indigenous	ISEA	Philippines	LNC29		D2	Hap_07	H_50 (D1)	Godinez et al., 2021
Red junglefowl	ISEA	Philippines	PhilRJF_5	MK085037	D2	Hap_07		Godinez et al., 2019
Red junglefowl	ISEA	Philippines	LeyteRJF6	MN986403	D2	Hap_19	H_25 (D2)	Godinez et al., 2021
Red junglefowl	ISEA	Philippines	LeyteRJF2	MN986399	D2	Hap_20	H_25 (D2)	Godinez et al., 2021
Red junglefowl	ISEA	Philippines	LeyteRJF3	MN986400	D2	Hap_20	H_25 (D2)	Godinez et al., 2021
Red junglefowl	ISEA	Philippines	LeyteRJF1	MN986398	D2	Hap_20	H_25 (D2)	Godinez et al., 2021
Red junglefowl	ISEA	Philippines	LeyteRJF14	OM240546	D2	Hap_20		this study
Red junglefowl	ISEA	Philippines	LeyteRJF22	OM240549	D2	Hap_20		this study
Red junglefowl	ISEA	Philippines	LeyteRJF4	MN986401	D3	Hap_21	H_80 (D3)	Godinez et al., 2021
Red junglefowl	ISEA	Philippines	LeyteRJF5	MN986402	D3	Hap_21	H_80 (D3)	Godinez et al., 2021
Red junglefowl	ISEA	Philippines	PhilRJF_1	MK085033	D3	Hap_31		Godinez et al., 2019
Indigenous	Oceania	Fiji (Pacific)	Fiji1	OM240181	D1 (Phil-Pac subclade)	Hap_128		this study
Indigenous	Oceania	Fiji (Pacific)	Fiji3	OM240182	D1 (Phil-Pac subclade)	Hap_128		this study
Indigenous	Oceania	Fiji (Pacific)	Fiji4	OM240183	D1 (Phil-Pac subclade)	Hap_128		this study
Indigenous	Oceania	Fiji (Pacific)	Fiji5	OM240184	D1 (Phil-Pac subclade)	Hap_128		this study
Indigenous	Oceania	Fiji (Pacific)	Fiji6	OM240185	D1 (Phil-Pac subclade)	Hap_128		this study
Indigenous	Oceania	Fiji (Pacific)	Fiji7	OM240186	D1 (Phil-Pac subclade)	Hap_128		this study

Indigenous	Oceania	Fiji (Pacific)	Fiji9	OM240187	D1 (Phil-Pac subclade)	Hap_128	this study
Indigenous	Oceania	Fiji (Pacific)	Fiji11	OM240188	D1 (Phil-Pac subclade)	Hap_128	this study
Indigenous	Oceania	Fiji (Pacific)	Fiji14	OM240190	D1 (Phil-Pac subclade)	Hap_128	this study
Indigenous	Oceania	Fiji (Pacific)	Fiji22	OM240191	D1 (Phil-Pac subclade)	Hap_128	this study
Indigenous	Oceania	Fiji (Pacific)	Fiji25	OM240193	D1 (Phil-Pac subclade)	Hap_128	this study
Indigenous	Oceania	Fiji (Pacific)	Fiji26	OM240194	D1 (Phil-Pac subclade)	Hap_128	this study
Indigenous	Oceania	Fiji (Pacific)	Fiji12	OM240189	D1 (Phil-Pac subclade)	Hap_129	this study
Indigenous	Oceania	Fiji (Pacific)	Fiji24	OM240192	D1 (Phil-Pac subclade)	Hap_130	this study
Indigenous	Oceania	Fiji (Pacific)	Fiji8	OM240195	E	Hap_05	this study
Indigenous	Oceania	Fiji (Pacific)	Fiji10	OM240196	E	Hap_05	this study
Indigenous	Oceania	Fiji (Pacific)	Fiji18	OM240197	E	Hap_131	this study
Indigenous	Oceania	Fiji (Pacific)	Fiji19	OM240198	E	Hap_131	this study
Indigenous	Oceania	Fiji (Pacific)	Fiji20	OM240199	E	Hap_131	this study
Indigenous	Oceania	Fiji (Pacific)	Fiji21	OM240200	E	Hap_132	this study
Indigenous	Oceania	Fiji (Pacific)	Fiji27	OM240201	E	Hap_133	this study
Indigenous	Oceania	Fiji (Pacific)	Fiji28	OM240202	E	Hap_133	this study
Indigenous	Oceania	Fiji (Pacific)	Fiji29	OM240203	E	Hap_133	this study
Indigenous	Oceania	Fiji (Pacific)	Fiji30	OM240204	E	Hap_133	this study

* previously assigned haplotypes (or haplogroups) by Godinez et al., 2021. Accession number for domestic chicken samples was assigned by haplotype (Godinez et al., 2021).

Appendix Table S3.3. Genetic diversity and neutrality tests of identified haplogroups of Southeast Asian and Pacific chickens

Population	Molecular diversity indices				Neutrality tests	
	N	Ht	*Hd*	π	Tajima's *D*	Fu's *Fs*
Haplogroup A	67	18	0.669 ± 0.064	0.00079 ± 0.00061	-2.1228**	-18.3924**
Haplogroup B	65	14	0.833 ± 0.030	0.00129 ± 0.00086	-1.0401	-6.6381**
Haplogroup D	229	54	0.920 ± 0.011	0.00223 ± 0.00131	-1.7036*	-26.2787**
Haplogroup E	86	20	0.849 ± 0.021	0.00178 ± 0.00110	-1.6074*	-10.0045**
Haplogroup F	32	14	0.851 ± 0.057	0.00176 ± 0.00112	-1.3764	-7.3856**
Haplogroup V	39	12	0.768 ± 0.064	0.00315 ± 0.00180	-0.2356	-1.1948

N – number of sequences; Ht – number of haplotypes; *Hd* – haplotype (gene) diversity; π - nucleotide diversity; * *p*-value <0.05; ** *p*-value <0.01

Appendix Table S3.4. Domestic chicken and red junglefowl mitochondrial control region information used to reconstruct the matrilineal phylogeny

Sample Name	Source	Accession No.	(Sub-) Haplogroup	Variation [RefSeq: AP003321]	Location	Reference
	domestic chicken	AB086102	A	167 212 225 246 315 355 686 859d 1215	Japan: Hiroshima	Wada et al. 2014
B	domestic chicken		A	167 212 223 225 246 315 1215	China: Anhui	Huang et al. 2018
yp20085	domestic chicken	GU261684	A	167 212 225 246 315 859d 1215	China: Yunnan	Miao et al. 2013
YP19289 (H5)	wild fowl	GU261695	A	167 212 225 246 315 396 859d 1215	China: Yunnan	Miao et al. 2013
Man1	wild fowl	GU261700	A	167 212 225 246 256 315 391A 859d 1215	Myanmar	Miao et al. 2013
	domestic chicken	KF981434	A	167 212 225 240 246 315 859d 1215	China: Hunan	Liu et al. 2016
	domestic chicken	KJ778617	A	167 212 225 246 315 859d 1215	China: Jiangxi	Wang et al. 2016
	domestic chicken	KM886936	A	167 212 225 246 315 399 686 859d 1058 1108 1215	China: Hunan	Lin et al. 2014. Direct submission
G	domestic chicken		B	same as RefSeq	China: Henan	Huang et al. 2018
YP20190 (W56)	domestic chicken	GU261699	B	256 792	China: Yunnan	Miao et al. 2013
YP19902 (H8)	wild fowl	GU261704	B	223 296 859d	China: Yunnan	Miao et al. 2013
yp20081	domestic chicken	GU261705	B	686 792	China: Yunnan	Miao et al. 2013
J37	domestic chicken	GU261714	B	296 792	China: Yunnan	Miao et al. 2013
	domestic chicken	KM096864	B	306 792 859d	China: Guangdong	Huang et al. 2016
	domestic chicken	KM433666	B	792	China: Guangxi	Xie et al. 2016
	domestic chicken	KM886937	B	399	China: Hunan	Lin et al. 2014. Direct submission
	domestic chicken	KP681581	B	792	China: Guangxi	Zhang et al. 2015. Direct submission
	domestic chicken	KP742951	B	219 261 859+C	China: Jiangsu	Zhao & Fan. 2015 Direct submission
	domestic chicken	AP003317	E	212 217 243 246 256 261 310 315 446 859d 1214 1215	Commercial Line	Nishibori et al. 2003
	domestic chicken	AP003318	E	212 217 243 246 256 261 310 315 446 859d 1214 1215	Commercial Line	Nishibori et al. 2003
	domestic chicken	AP003319	E	212 217 243 246 256 261 310 315 342 446 686 859d 1214 1215	Laos: Vientiane	Nishibori et al. 2003
	domestic chicken	AP003580	E	212 217 243 246 256 261 310 315 446 1214 1215	Commercial Line	Nishibori et al. 2003
	domestic chicken	AY235570	E	212 217 243 246 256 261 310 315 446 1214 1215	Commercial Line	Froman & Kirby 2005
	domestic chicken	AY235571	E	212 217 222 243 246 256 261 310 315 330 446 859d 1214 1215	Commercial Line	Froman & Kirby 2005
yp20488	domestic chicken	GU261686	E	212 217 243 256 261 310 315 446 859d 1214 1215	China: Henan	Miao et al. 2013
YP20648 (CD60)	domestic chicken	GU261694	E	212 217 246 256 261 310 315 446 686 1214 1215	China: Hebei	Miao et al. 2013
GGM4	wild fowl	GU261708	E	212 217 243 249 256 261 310 315 344 355 446 859d 1214 1215	India	Miao et al. 2013
ggm1	domestic chicken	GU261709	E	212 217 243 246 256 261 310 315 446 859d 1214 1215	India	Miao et al. 2013
YP20320	domestic chicken	GU261712	E	212 217 243 246 256 261 310 315 446 859d 1214 1215	China: Yunnan	Miao et al. 2013
J41	domestic chicken	GU261713	E	199 212 217 243 246 256 261 310 315 446 859d 1214 1215	China: Yunnan	Miao et al. 2013
yin100	domestic chicken	HQ857209	E	212 217 224 243 256 261 310 315 446 859d 1174 1214 1215	Northeast India	Miao et al. 2013
yin109	domestic chicken	HQ857210	E	207 212 217 238 243 256 261 310 315 330 446 859d 1214 1215	Northeast India	Miao et al. 2013
yin125	domestic chicken	HQ857211	E	212 217 222 243 246 249 256 261 281 310 315 355 446 686 1214 1215	Northeast India	Miao et al. 2013
yin46	domestic chicken	HQ857212	E	212 217 222 243 246 249 256 261 310 315 355 446 1214 1215	Northeast India	Miao et al. 2013

	domestic chicken	KF826490	E	212 217 222 243 246 256 261 310 315 330 446 859d 1214 1215	China: Hunan	Liu et al. 2016
	domestic chicken	KF954727	E	212 217 243 246 256 261 310 315 446 859d 1214 1215	China: Hunan	Yu et al. 2016
	domestic chicken	KP244335	E	212 217 243 246 256 261 310 315 446 1214 1215	China: Hunan	He JH. 2014. Direct submission
	domestic chicken	DQ648776	F	212 234 236 243 254 256 261 310 317C 446 686 859d 904 1214 1215	China: Yunnan	Tong et al. 2006
YP20069 (TC48)	domestic chicken	GU261688	F	212 234 236 243 246 254 256 261 265 310 317C 446 859d 904 1214 1215	China: Yunnan	Miao et al. 2013
YP20051 (TC30)	domestic chicken	GU261689	F	212 234 236 243 246 254 256 261 310 317C 342 446 859d 904 1214 1215	China: Yunnan	Miao et al. 2013
MAN2	wild fowl	GU261691	F	212 234 236 243 246 254 256 261 310 317C 396 446 859d 904 1214 1215	Myanmar	Miao et al. 2013
YP19915 (H20)	wild fowl	GU261702	F	212 234 236 243 246 254 256 261 310 317C 347 396 446 904 1214 1215	China: Yunnan	Miao et al. 2013
YP19906 (H11)	wild fowl	GU261703	F	212 234 236 243 246 254 256 261 310 317C 446 859d 904 1214 1215	Myanmar	Miao et al. 2013
YP20266 (N10)	domestic chicken	GU261711	F	212 234 236 243 246 254 256 261 310 317C 446 859d 904 1214 1215	China: Yunnan	Miao et al. 2013
YP20123	domestic chicken	GU261717	F	212 234 236 243 254 256 261 310 317C 446 686 859d 904 1214 1215	China: Yunnan	Miao et al. 2013
YP18844	domestic chicken	GU261676	G	243 246 256 261 296A 302 310 446 1214 1215	China: Yunnan	Miao et al. 2013
YP19315	domestic chicken	GU261678	G	243 246 256 261 296A 302 310 322 347 446 859d 1214 1215	China: Henan	Miao et al. 2013
DH40 (nD40)	wild fowl	GU261690	G	243 246 256 261 296A 302 310 322 362 446 1214 1215	China: Yunnan	Miao et al. 2013
YP20307 (N51)	domestic chicken	GU261710	G	243 246 256 261 281 296A 302 310 317C 391A 446 686 1214 1215	China: Yunnan	Miao et al. 2013
YP20092	domestic chicken	GU261719	G	243 246 256 261 296A 302 310 322 446 859d 1214 1215	China: Yunnan	Miao et al. 2013
YP20112 (D32)	domestic chicken	GU261715	H	198 199 212 243 246 256 261 281 306 310 315 363 391 446 1215	China: Yunnan	Miao et al. 2013
yin21	domestic chicken	GU261698	I	171 207 221 229 243 256 261 310 315 322A 354 362 363 391 446 521 686 859d 1215	Northeast India	Miao et al. 2013
YP19297 (H6)	wild fowl	GU261706	W	133 167 212 243 246 256 261 315 363 391 417 446 521 859d 1214 1215	China: Yunnan	Miao et al. 2013
DHZ2	wild fowl	GU261692	X	212 242 243 246 256 261 310 315 326 363 417 446 859d 1214 1215	China: Yunnan	Miao et al. 2013
DHZ1	wild fowl	GU261693	Y	197 212 243 246 256 261 270 281 310 315 391A 1215	China: Yunnan	Miao et al. 2013
1210-1	wild fowl	GU261674	Z	212 246 256 306 310 315 391A 859d 889 1215	China: Hainan	Miao et al. 2013
YP20736	wild fowl	GU261696	Z	199 212 246 256 291 306 310 315 889 1215	China: Hainan	Miao et al. 2013
	wild fowl	AP003321	B	reference sequence	Laos	Nishibori et at. 2005
HXH53	wild fowl		V2	243 246 256 261 281 299 310 315 355 363 1215	Thailand	Huang et al. 2018
HXH52	wild fowl		V1a	233 243 256 261 281 299 310 315 355 359Y 363 711R 1215	Thailand	Huang et al. 2018
HXH48	wild fowl		V1a	233 243 246 256 261 268 281 299 310 315 355 359 363 711 1215	Thailand	Huang et al. 2018
HXH41	wild fowl		V1	168 233 243 246 256 261 281 299Y 310 315 355 363 391A 975W 1215	Thailand	Huang et al. 2018
	domestic chicken	KF939304	D1	212 243 246 256 261 281 306 310 315 1215	China: Yunnan	Yan et al. 2016
yp20463	domestic chicken	GU261682	D1	212 220 243 246 256 261 281 306 310 315 344 859d 1215	Laos	Miao et al. 2013
yp20477	domestic chicken	GU261687	D1a2	212 243 246 256 261 281 306 310 315 342 859d 1215	Laos	Miao et al. 2013
	wild fowl	NC_007237	D1a1	212 243 246 256 261 281 306 310 315 342 1215	Indonesia	Nishibori et at. 2005
	wild fowl	NC_007236	D1a1	212 243 246 256 261 281 296 306 310 315 342 686 1215	Philippine	Nishibori et at. 2005
YP20697	domestic chicken	GU261683	D2	212 243 246 256 261 281 306 310 315 859d 1215	China: Xinjiang	Miao et al. 2013
HXH29	wild fowl		D3a	212 243 246 256 261 281 291 306 310 315 1215	China: Guangxi	Huang et al. 2018
YP66	domestic chicken	GU261697	D3a	212 243 246 256 261 306 310 315 1215	Southern India	Miao et al. 2013
yin12	domestic chicken	GU261685	D3a	210 212 221 243 246 256 261 281 306 310 315 342 859d 1215	Northeast India	Miao et al. 2013
yp19263	domestic chicken	GU261677	D3b	212 220 243 246 256 261 302 306 310 315 391A 859d 1215	China: Zhejiang	Miao et al. 2013

HXH28	domestic chicken		D3b	212 220 243 246 256 261 306 310 315 391A 975W 1215	China: Jiangxi	Huang et al. 2018
J	domestic chicken		D3b	212 220 243 246 256 261 302 306 310 315 391A 1215	China: Henan	Huang et al. 2018
HXH45	wild fowl		D3	212 243 246 256 261 281 306 310 315 1215	Thailand	Huang et al. 2018
HXH23	wild fowl		D4	212 243 246 256 261 281 306 310 315 367 1153 1215	China: Guangxi	Huang et al. 2018
HXH43	wild fowl		D5	171 212 243 256 261 281 306 310 315 1215	Thailand	Huang et al. 2018
GGM7	wild fowl	GU261707	C3	177T 212 243 246 256 261 265 281 306 310 315 363 859d 1215	India	Miao et al. 2013
	domestic chicken	KT283576	C3	177T 212 243 246 256 261 265 281 306 310 315 363 859d 1215	China: Liaoning	Gu J, Li S. 2015. Direct Submission.
YP19903 (sp4)	wild fowl	GU261716	C3	177T 212 243 246 256 261 265 281 306 310 315 342 363 686 859d 1215	Myanmar	Miao et al. 2013
	domestic chicken	KT283576	C3	177T 212 243 246 256 261 265 281 306 310 315 342 363 686 859d 1215	China: Liaoning	Gu J, Li S. 2015. Direct Submission.
YP63	domestic chicken	GU261680	C2	212 243 246 256 261 306 310 315 342 362 363 1215	Southern India	Miao et al. 2013
yp18793	domestic chicken	GU261675	C1a	212 242 243 246 256 281 310 315 363 367 859d 1215	China: Hunan	Miao et al. 2013
	domestic chicken	KP681580	C1b	212 242 243 246 256 281 310 315 342 363 367 1215	China: Guangxi	Zhang et al. 2014. Direct Submission
HXH11	domestic chicken		C1b	212 242 243 246 256 281 310 315 342 363 367 1215	China: Jiangxi	Huang et al. 2018
HXH3	domestic chicken		C1b	212 242 243 246 256 281 310 315 342 363 367 1215	China: Guangdong	Huang et al. 2018
yp20332	domestic chicken	GU261718	C1b	212 242 243 246 256 281 310 315 342 363 367 1215	China: Yunnan	Miao et al. 2013
	domestic chicken	KP269069	C1b	212 242 243 246 256 281 310 315 342 363 367 1215	China: Guangxi	Zhang et al. 2014. Direct Submission
Q	domestic chicken		C1b	212 242 243 246 256 281 310 315 342 363 367 1215	China: Shandong	Huang et al. 2018
HXH37	domestic chicken		C1b	212 242 243 246 256 281 310 315 342 363 367 1215	China: Guangxi	Huang et al. 2018
YP20523	domestic chicken	GU261679	C1b	212 242 243 246 256 281 310 315 342 363 367 391 1215	China: Henan	Miao et al. 2013
HXH9	domestic chicken		C1b	212 242 243 246 256 281 310 315 342 363 367 391 1215	China: Hunan	Huang et al. 2018
HXH26	domestic chicken		C1b	212 242 243 246 256 281 310 315 342 363 367 391 686 1215	China: Guangdong	Huang et al. 2018
HXH16	domestic chicken		C1b	212 242 243 246 256 281 310 315 330 342 363 367 391 1215	China: Hunan	Huang et al. 2018
HXH17	domestic chicken		C1b	212 242 243 246 256 281 310 315 342 363 367 391 1215	China: Hunan	Huang et al. 2018
HXH10	domestic chicken		C1b	212 242 243 246 256 281 310 315 363 367 391 1215	China: Jiangxi	Huang et al. 2018
HXH12	domestic chicken		C1b	212 242 243 246 252 256 310 315 342 363 367 1215	China: Shandong	Huang et al. 2018
GS03	domestic chicken		C1b	212 242 243 246 252 256 310 315 342 363 367 1215	China: Henan	Huang et al. 2018
HXH6	domestic chicken		C1b	212 242 243 246 256 310 315 342 363 367 1215	China: Shandong	Huang et al. 2018
HH131	domestic chicken	GU261681	C1b	212 242 243 246 248 256 310 315 342 363 367 859d 1215	China: Hunan	Miao et al. 2013
HXH22	domestic chicken		C1b	212 242 243 246 256 310 315 342 363 367 1215	China: Jiangxi	Huang et al. 2018
HXH5	domestic chicken		C1b	212 242 243 246 256 310 315 342 363 367 975W 1215	China: Jiangxi	Huang et al. 2018
HXH40	domestic chicken		C1b	212 242 243 246 256 281 310 342 363 367 1215	China: Guangxi	Huang et al. 2018
K	domestic chicken		C1b	212 242 243 246 256 281 310 315 342 363 367 1215	China: Anhui	Huang et al. 2018
O	domestic chicken		C1b	212 242 243 246 256 281 310 315 342 363 367 1215	China: Zhejiang	Huang et al. 2018
HXH33	domestic chicken		C1b	212 242 243 246 256 281 310 315 342 363 367 686 1215	China: Hunan	Huang et al. 2018
L	domestic chicken		C1b	212 242 243 246 256 281 310 315 342 363 367 686 1215	China: Anhui	Huang et al. 2018
HXH13	domestic chicken		C1b	212 242 243 246 256 261 281 310 315 342 363 367 1215	China: Jiangxi	Huang et al. 2018
HXH14	domestic chicken		C1b	212 242 243 246 256 261 281 310 315 342 363 367 1215	China: Shandong	Huang et al. 2018

HXH36	domestic chicken		C1b	212 242 243 246 248 256 281 310 315 342 363 367 1215	China: Guangxi	Huang et al. 2018
HXH8	domestic chicken		C1b	212 242 243 246 256 281 310 315 342 360 363 367 1215	China: Shandong	Huang et al. 2018
ZY08	domestic chicken		C1b	212 242 243 246 256 281 310 315 342 363 367 975R 1215	China: Henan	Huang et al. 2018
HXH20	domestic chicken		C1b	212 242 243 246 256 281 310 315 342 363 367 1215	China: Henan	Huang et al. 2018
HXH18	domestic chicken		C1b	212 242 243 246 256 281 310 315 342 363 367 686R 1215	China: Jiangxi	Huang et al. 2018
M	domestic chicken		C1b	212 242 243 246 256 281 310 315 342 363 367 1215	China: Hubei	Huang et al. 2018
N	domestic chicken		C1b	212 242 243 246 256 281 310 315 342 363 367 859d 1215	China: Hubei	Huang et al. 2018
HXH39	domestic chicken		C1b	212 242 243 246 256 281 310 315 342 363 367 686 1215	China: Guangxi	Huang et al. 2018
HXH31	domestic chicken		C1b	212 242 243 246 256 261Y 281 310 315 342 363 367 686 1215	China: Guangdong	Huang et al. 2018
P	domestic chicken		C1b	212 242 243 246 256 281 310 315 342 363 367 686 1215	China: Guangxi	Huang et al. 2018
HXH24	domestic chicken		C1b	212 242 243 246 256 281 310 315 342 363 367 711 975W 1215	China: Liaoning	Huang et al. 2018
HXH25	domestic chicken		C1b	212 242 243 246 256 281 306Y 310 315 342 363 367 711 1215	China: Liaoning	Huang et al. 2018
HD38	domestic chicken	GU261701	C1c	242 243 246 256 281 310 315 363 367 859d 1215	China: Henan	Miao et al. 2013
E	domestic chicken		C1c	242 243 246 256 281 310 315 363 367 859d 1215	China: Anhui	Huang et al. 2018
F	domestic chicken		C1c	242 243 246 256 281 310 315 363 367 1215	China: Anhui	Huang et al. 2018
C	domestic chicken		C1d	212 242 243 246 256 281 310 315 363 367 859d 1215	China: Anhui	Huang et al. 2018
ZY06	domestic chicken		C1d	212 242 243 246 256 281 308 310 315 363 367 391 1215	China: Henan	Huang et al. 2018
HXH4	domestic chicken		C1d	212 242 243 246 256 261 281 310 315 363 367 859d 1215	China: Shandong	Huang et al. 2018
HXH19	domestic chicken		C1d	212 242 243 246 256 261 281 310 315 363 367 1215	China: Shandong	Huang et al. 2018
ZY27	domestic chicken		C1d	212 242 243 246 256 261 281 310 315 363 367 975W 1215	China: Henan	Huang et al. 2018
H	domestic chicken		C1d	212 242 243 246 256 261 281 310 315 363 367 859d 1215	China: Hubei	Huang et al. 2018
I	domestic chicken		C1d	212 242 243 246 256 261 281 310 315 363 367 1215	China: Zhejiang	Huang et al. 2018
A	domestic chicken		C1e	212 242 243 246 256 281 310 315 363 367 1215	China: Hunan	Huang et al. 2018
D	domestic chicken		C1f	212 242 243 246 256 281 310 315 363 367 859d 1215	China: Anhui	Huang et al. 2018
Tosa-jidori	domestic chicken	AB268522	D2		Japan: Kochi	Oka et al. 2007
Tosa-jidori_3	domestic chicken	LC507814	D2		Japan: Kochi	Osman et al. 2021
Tosa-jidori_5	domestic chicken	LC507816	D2		Japan: Kochi	Osman et al. 2021
CamRJF11	wild fowl	LC146461	V		Cambodia: Takeo Province	Osman et al. 2014
CamRJF16	wild fowl	LC146464	V		Cambodia: Takeo Province	Osman et al. 2014

Appendix Table S3.5. Included complete mtDNA D-loop sequences of East Asia, South Asia, Middle East, and Kenya chickens used for the multivariate PCoA

Sample name	Source	Breed	Accession No.	Region	Location	Reference
	domestic chicken	Baier Yellow	KY307966	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307967	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307968	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307969	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307970	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307971	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307972	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307973	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307974	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307975	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307976	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307977	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307978	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307979	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307980	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307981	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307982	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307983	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307984	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307985	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307986	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307987	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307988	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307989	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307990	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307991	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307992	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307993	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307994	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Baier Yellow	KY307995	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY307996	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY307997	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY307998	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY307999	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308000	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308001	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308002	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308003	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308004	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308005	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308006	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308007	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308008	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308009	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308010	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308011	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308012	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308013	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308014	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308015	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308016	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308017	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308018	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308019	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308020	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308021	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308022	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308023	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308024	East Asia	China: Jiangxi	Gao et al. 2017
	domestic chicken	Chongren Partridge	KY308025	East Asia	China: Jiangxi	Gao et al. 2017

domestic chicken	Dongxiang Blue-eggshell	KY308026	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308027	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308028	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308029	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308030	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308031	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308032	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308033	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308034	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308035	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308036	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308037	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308038	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308039	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308040	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308041	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308042	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308043	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308044	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308045	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308046	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308047	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308048	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308049	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308050	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308051	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308052	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308053	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308054	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Dongxiang Blue-eggshell	KY308055	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Silkie	KY308056	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Silkie	KY308057	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Silkie	KY308058	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Silkie	KY308059	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Silkie	KY308060	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Silkie	KY308061	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Silkie	KY308062	East Asia	China: Jiangxi	Gao et al. 2017

domestic chicken	Silkie	KY308063	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Silkie	KY308064	East Asia	China: Jiangxi	Gao et al. 2017
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domestic chicken	Anyi Gray	KY308087	East Asia	China: Jiangxi	Gao et al. 2017
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domestic chicken	Anyi Gray	KY308094	East Asia	China: Jiangxi	Gao et al. 2017
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domestic chicken	Anyi Gray	KY308096	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Anyi Gray	KY308097	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Anyi Gray	KY308098	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Anyi Gray	KY308099	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Anyi Gray	KY308100	East Asia	China: Jiangxi	Gao et al. 2017
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domestic chicken	Anyi Gray	KY308103	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Anyi Gray	KY308104	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Anyi Gray	KY308105	East Asia	China: Jiangxi	Gao et al. 2017
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domestic chicken	Anyi Gray	KY308107	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Anyi Gray	KY308108	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Anyi Gray	KY308109	East Asia	China: Jiangxi	Gao et al. 2017
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domestic chicken	Anyi Gray	KY308113	East Asia	China: Jiangxi	Gao et al. 2017
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domestic chicken	Anyi Gray	KY308115	East Asia	China: Jiangxi	Gao et al. 2017
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domestic chicken	XianJu	KY308117	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308118	East Asia	China: Jiangxi	Gao et al. 2017
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domestic chicken	XianJu	KY308120	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308121	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308122	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308123	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308124	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308125	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308126	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308127	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308128	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308129	East Asia	China: Jiangxi	Gao et al. 2017

domestic chicken	XianJu	KY308130	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308131	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308132	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308133	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308134	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308135	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308136	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308137	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308138	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308139	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308140	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308141	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308142	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308143	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308144	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	XianJu	KY308145	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308146	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308147	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308148	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308149	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308150	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308151	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308152	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308153	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308154	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308155	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308156	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308157	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308158	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308159	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308160	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308161	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308162	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308163	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308164	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308165	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308166	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308167	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308168	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308169	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308170	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308171	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308172	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308173	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Jinhu Black-bone	KY308174	East Asia	China: Jiangxi	Gao et al. 2017
domestic chicken	Huainan Partridge	MT705249	East Asia	China: Anhui	Zang et al., 2020
domestic chicken	Huainan Partridge	MT705250	East Asia	China: Anhui	Zang et al., 2020
domestic chicken	Huainan Partridge	MT705251	East Asia	China: Anhui	Zang et al., 2020
domestic chicken	Huainan Partridge	MT705252	East Asia	China: Anhui	Zang et al., 2020
domestic chicken	Huainan Partridge	MT705253	East Asia	China: Anhui	Zang et al., 2020
domestic chicken	Huainan Partridge	MT705254	East Asia	China: Anhui	Zang et al., 2020
domestic chicken	Huainan Partridge	MT705255	East Asia	China: Anhui	Zang et al., 2020
domestic chicken	Huainan Partridge	MT705256	East Asia	China: Anhui	Zang et al., 2020
domestic chicken	Huainan Partridge	MT705257	East Asia	China: Anhui	Zang et al., 2020
domestic chicken	Huainan Partridge	MT705258	East Asia	China: Anhui	Zang et al., 2020
domestic chicken	Huainan Partridge	MT705259	East Asia	China: Anhui	Zang et al., 2020

domestic chicken	Huainan Partridge	MT705260	East Asia	China: Anhui	Zang et al., 2020
domestic chicken	Huainan Partridge	MT705261	East Asia	China: Anhui	Zang et al., 2020
domestic chicken	Huainan Partridge	MT705262	East Asia	China: Anhui	Zang et al., 2020
domestic chicken	Huainan Partridge	MT705263	East Asia	China: Anhui	Zang et al., 2020
domestic chicken	Huainan Partridge	MT705264	East Asia	China: Anhui	Zang et al., 2020
domestic chicken	Huainan Partridge	MT705265	East Asia	China: Anhui	Zang et al., 2020
domestic chicken	Huainan Partridge	MT705266	East Asia	China: Anhui	Zang et al., 2020
domestic chicken	Huainan Partridge	MT705267	East Asia	China: Anhui	Zang et al., 2020
domestic chicken	Huainan Partridge	MT705268	East Asia	China: Anhui	Zang et al., 2020
domestic chicken	Huainan Partridge	MT705269	East Asia	China: Anhui	Zang et al., 2020
domestic chicken	Huainan Partridge	MT705270	East Asia	China: Anhui	Zang et al., 2020
domestic chicken	Huainan Partridge	MT705271	East Asia	China: Anhui	Zang et al., 2020
domestic chicken	Huaibei native	MT773645	East Asia	China: Anhui	Jin, 2020
domestic chicken	Huaibei native	MT773646	East Asia	China: Anhui	Jin, 2020
domestic chicken	Huaibei native	MT773647	East Asia	China: Anhui	Jin, 2020
domestic chicken	Huaibei native	MT773648	East Asia	China: Anhui	Jin, 2020
domestic chicken	Huaibei native	MT773649	East Asia	China: Anhui	Jin, 2020
domestic chicken	Huaibei native	MT773650	East Asia	China: Anhui	Jin, 2020
domestic chicken	Huaibei native	MT773651	East Asia	China: Anhui	Jin, 2020
domestic chicken	Huaibei native	MT773652	East Asia	China: Anhui	Jin, 2020
domestic chicken	Huaibei native	MT773653	East Asia	China: Anhui	Jin, 2020
domestic chicken	Huaibei native	MT773655	East Asia	China: Anhui	Jin, 2020
domestic chicken	Huaibei native	MT773656	East Asia	China: Anhui	Jin, 2020
domestic chicken	Huaibei native	MT773657	East Asia	China: Anhui	Jin, 2020
domestic chicken	Huaibei native	MT773658	East Asia	China: Anhui	Jin, 2020
domestic chicken	Huaibei native	MT773659	East Asia	China: Anhui	Jin, 2020
domestic chicken	Huaibei native	MT773660	East Asia	China: Anhui	Jin, 2020
domestic chicken	Huaibei native	MT773661	East Asia	China: Anhui	Jin, 2020
domestic chicken	Huaibei native	MT773662	East Asia	China: Anhui	Jin, 2020
domestic chicken	Huaibei native	MT773663	East Asia	China: Anhui	Jin, 2020
domestic chicken	Wannan Yellow	MT780920	East Asia	China: Anhui	Jin, 2020
domestic chicken	Wannan Yellow	MT780922	East Asia	China: Anhui	Jin, 2020
domestic chicken	Wannan Yellow	MT780923	East Asia	China: Anhui	Jin, 2020
domestic chicken	Wannan Yellow	MT780924	East Asia	China: Anhui	Jin, 2020
domestic chicken	Wannan Yellow	MT780925	East Asia	China: Anhui	Jin, 2020
domestic chicken	Wannan Yellow	MT780926	East Asia	China: Anhui	Jin, 2020
domestic chicken	Wannan Yellow	MT780927	East Asia	China: Anhui	Jin, 2020
domestic chicken	Wannan Yellow	MT780928	East Asia	China: Anhui	Jin, 2020
domestic chicken	Wannan Yellow	MT780929	East Asia	China: Anhui	Jin, 2020
domestic chicken	Wannan Yellow	MT780930	East Asia	China: Anhui	Jin, 2020
domestic chicken	Wannan Yellow	MT780931	East Asia	China: Anhui	Jin, 2020
domestic chicken	Wannan Yellow	MT780932	East Asia	China: Anhui	Jin, 2020
domestic chicken	Wannan Yellow	MT780933	East Asia	China: Anhui	Jin, 2020
domestic chicken	Wannan Yellow	MT780934	East Asia	China: Anhui	Jin, 2020
domestic chicken	Wannan Yellow	MT780935	East Asia	China: Anhui	Jin, 2020
domestic chicken	Lueyang	KY091859	East Asia	China: Shaanxi	Zhang et al., 2018
domestic chicken	Lueyang	KY091860	East Asia	China: Shaanxi	Zhang et al., 2018
domestic chicken	Lueyang	KY091861	East Asia	China: Shaanxi	Zhang et al., 2018
domestic chicken	Lueyang	KY091862	East Asia	China: Shaanxi	Zhang et al., 2018
domestic chicken	Lueyang	KY091863	East Asia	China: Shaanxi	Zhang et al., 2018

	domestic chicken	Lueyang	KY091864	East Asia	China: Shaanxi	Zhang et al., 2018
	domestic chicken	Lueyang	KY091865	East Asia	China: Shaanxi	Zhang et al., 2018
	domestic chicken	Lueyang	KY091866	East Asia	China: Shaanxi	Zhang et al., 2018
	domestic chicken	Lueyang	KY091867	East Asia	China: Shaanxi	Zhang et al., 2018
	domestic chicken	Lueyang	KY091868	East Asia	China: Shaanxi	Zhang et al., 2018
	domestic chicken		GU261684	East Asia	China: Yunnan	Miao et al., 2013
	wild fowl		GU261695	East Asia	China: Yunnan	Miao et al., 2013
	domestic chicken		GU261699	East Asia	China: Yunnan	Miao et al., 2013
	wild fowl		GU261704	East Asia	China: Yunnan	Miao et al., 2013
	domestic chicken		GU261705	East Asia	China: Yunnan	Miao et al., 2013
	domestic chicken		GU261714	East Asia	China: Yunnan	Miao et al., 2013
	domestic chicken		GU261712	East Asia	China: Yunnan	Miao et al., 2013
	domestic chicken		GU261713	East Asia	China: Yunnan	Miao et al., 2013
	domestic chicken		DQ648776	East Asia	China: Yunnan	Miao et al., 2013
	domestic chicken		GU261688	East Asia	China: Yunnan	Miao et al., 2013
	domestic chicken		GU261689	East Asia	China: Yunnan	Miao et al., 2013
	wild fowl		GU261702	East Asia	China: Yunnan	Miao et al., 2013
	domestic chicken		GU261711	East Asia	China: Yunnan	Miao et al., 2013
	domestic chicken		GU261717	East Asia	China: Yunnan	Miao et al., 2013
	domestic chicken		GU261676	East Asia	China: Yunnan	Miao et al., 2013
	wild fowl		GU261690	East Asia	China: Yunnan	Miao et al., 2013
	domestic chicken		GU261710	East Asia	China: Yunnan	Miao et al., 2013
	domestic chicken		GU261719	East Asia	China: Yunnan	Miao et al., 2013
	domestic chicken		GU261718	East Asia	China: Yunnan	Miao et al., 2013
B	domestic chicken			East Asia	China: Anhui	Huang et al., 2018
G	domestic chicken			East Asia	China: Henan	Huang et al., 2018
HXH29	wild fowl			East Asia	China: Guangxi	Huang et al., 2018
HXH28	domestic chicken			East Asia	China: Jiangxi	Huang et al., 2018
J	domestic chicken			East Asia	China: Henan	Huang et al., 2018
HXH11	domestic chicken			East Asia	China: Jiangxi	Huang et al., 2018
HXH3	domestic chicken			East Asia	China: Guangdong	Huang et al., 2018
Q	domestic chicken			East Asia	China: Shandong	Huang et al., 2018
HXH37	domestic chicken			East Asia	China: Guangxi	Huang et al., 2018

HXH9	domestic chicken		East Asia	China: Hunan	Huang et al., 2018
HXH26	domestic chicken		East Asia	China: Guangdong	Huang et al., 2018
HXH16	domestic chicken		East Asia	China: Hunan	Huang et al., 2018
HXH17	domestic chicken		East Asia	China: Hunan	Huang et al., 2018
HXH10	domestic chicken		East Asia	China: Jiangxi	Huang et al., 2018
HXH12	domestic chicken		East Asia	China: Shandong	Huang et al., 2018
GS03	domestic chicken		East Asia	China: Henan	Huang et al., 2018
HXH6	domestic chicken		East Asia	China: Shandong	Huang et al., 2018
HXH22	domestic chicken		East Asia	China: Jiangxi	Huang et al., 2018
HXH5	domestic chicken		East Asia	China: Jiangxi	Huang et al., 2018
HXH40	domestic chicken		East Asia	China: Guangxi	Huang et al., 2018
K	domestic chicken		East Asia	China: Anhui	Huang et al., 2018
O	domestic chicken		East Asia	China: Zhejiang	Huang et al., 2018
HXH33	domestic chicken		East Asia	China: Hunan	Huang et al., 2018
L	domestic chicken		East Asia	China: Anhui	Huang et al., 2018
HXH13	domestic chicken		East Asia	China: Jiangxi	Huang et al., 2018
HXH14	domestic chicken		East Asia	China: Shandong	Huang et al., 2018
HXH36	domestic chicken		East Asia	China: Guangxi	Huang et al., 2018
HXH8	domestic chicken		East Asia	China: Shandong	Huang et al., 2018
HXH20	domestic chicken		East Asia	China: Henan	Huang et al., 2018
ZY08	domestic chicken		East Asia	China: Henan	Huang et al., 2018
HXH18	domestic chicken		East Asia	China: Jiangxi	Huang et al., 2018
M	domestic chicken		East Asia	China: Hubei	Huang et al., 2018
N	domestic chicken		East Asia	China: Hubei	Huang et al., 2018
HXH39	domestic chicken		East Asia	China: Guangxi	Huang et al., 2018
HXH31	domestic chicken		East Asia	China: Guangdong	Huang et al., 2018
P	domestic chicken		East Asia	China: Guangxi	Huang et al., 2018
HXH24	domestic chicken		East Asia	China: Liaoning	Huang et al., 2018
HXH25	domestic chicken		East Asia	China: Liaoning	Huang et al., 2018
F	domestic chicken		East Asia	China: Anhui	Huang et al., 2018
A	domestic chicken		East Asia	China: Hunan	Huang et al., 2018
	domestic chicken	GU261678	East Asia	China: Henan	Miao et al., 2013
	wild fowl	GU261674	East Asia	China: Hainan	Miao et al., 2013

wild fowl		GU261696	East Asia	China: Hainan	Miao et al., 2013
domestic chicken		GU261675	East Asia	China: Hunan	Miao et al., 2013
domestic chicken		GU261679	East Asia	China: Henan	Miao et al., 2013
domestic chicken		GU261681	East Asia	China: Hunan	Miao et al., 2013
domestic chicken		GU261701	East Asia	China: Henan	Miao et al., 2013
domestic chicken		AB268506	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268507	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268508	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268509	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268510	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268511	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268512	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268513	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268515	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268516	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268517	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268518	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268519	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268520	East Asia	Japan	Oka et al., 2007
domestic chicken		AB294232	East Asia	Japan	Oka et al., 2007
domestic chicken		AB294233	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268522	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268523	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268524	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268529	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268530	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268531	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268532	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268533	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268534	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268535	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268536	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268537	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268538	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268539	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268541	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268542	East Asia	Japan	Oka et al., 2007
domestic chicken		AB268522	East Asia	Japan	Oka et al., 2007
domestic chicken		LC507814	East Asia	Japan	Osman et al. 2021
domestic chicken		LC507816	East Asia	Japan	Osman et al. 2021
domestic chicken	Korean native black	HQ836346	East Asia	Korea	Cho et al., 2011
domestic chicken	Korean native black	HQ836347	East Asia	Korea	Cho et al., 2011
domestic chicken	Korean native black	HQ836350	East Asia	Korea	Cho et al., 2011
domestic chicken	Korean native black	HQ836356	East Asia	Korea	Cho et al., 2011
domestic chicken	Korean native black	HQ836359	East Asia	Korea	Cho et al., 2011
domestic chicken	Korean native red	HQ836351	East Asia	Korea	Cho et al., 2011
domestic chicken	Korean native red	HQ836358	East Asia	Korea	Cho et al., 2011
domestic chicken	Korean native red	HQ836362	East Asia	Korea	Cho et al., 2011
domestic chicken	Korean native yellow	HQ836343	East Asia	Korea	Cho et al., 2011
domestic chicken	Korean native yellow	HQ836348	East Asia	Korea	Cho et al., 2011
domestic chicken	Korean native yellow	HQ836349	East Asia	Korea	Cho et al., 2011
domestic chicken	Korean native yellow	HQ836355	East Asia	Korea	Cho et al., 2011
domestic chicken	Korean native yellow	HQ836357	East Asia	Korea	Cho et al., 2011
domestic chicken	Korean native yellow	HQ836360	East Asia	Korea	Cho et al., 2011
domestic chicken		LC147035	South Asia	Bangladesh	Islam *et al,* 2019
domestic chicken		LC147036	South Asia	Bangladesh	Islam *et al,* 2019
domestic chicken		LC147037	South Asia	Bangladesh	Islam *et al,* 2019

domestic chicken	LC147038	South Asia	Bangladesh	Islam *et al,* 2019
domestic chicken	LC147039	South Asia	Bangladesh	Islam *et al,* 2019
domestic chicken	LC147040	South Asia	Bangladesh	Islam *et al,* 2019
domestic chicken	LC147041	South Asia	Bangladesh	Islam *et al,* 2019
domestic chicken	LC147042	South Asia	Bangladesh	Islam *et al,* 2019
domestic chicken	LC147043	South Asia	Bangladesh	Islam *et al,* 2019
domestic chicken	LC147044	South Asia	Bangladesh	Islam *et al,* 2019
domestic chicken	LC147045	South Asia	Bangladesh	Islam *et al,* 2019
domestic chicken	LC147046	South Asia	Bangladesh	Islam *et al,* 2019
domestic chicken	LC147047	South Asia	Bangladesh	Islam *et al,* 2019
domestic chicken	LC147048	South Asia	Bangladesh	Islam *et al,* 2019
domestic chicken	LC147049	South Asia	Bangladesh	Islam *et al,* 2019
domestic chicken	LC147050	South Asia	Bangladesh	Islam *et al,* 2019
domestic chicken	LC147051	South Asia	Bangladesh	Islam *et al,* 2019
domestic chicken	LC147052	South Asia	Bangladesh	Islam *et al,* 2019
domestic chicken	LC147053	South Asia	Bangladesh	Islam *et al,* 2019
domestic chicken	LC147054	South Asia	Bangladesh	Islam *et al,* 2019
domestic chicken	LC147055	South Asia	Bangladesh	Islam *et al,* 2019
domestic chicken	MH094578	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094579	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094580	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094581	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094582	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094583	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094584	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094585	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094586	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094587	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094588	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094589	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094590	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094591	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094592	South Asia	Pakistan	Nisar et al., 2019

domestic chicken	MH094593	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094594	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094595	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094596	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094597	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094598	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094599	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094600	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094601	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094602	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094603	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094604	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094605	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094606	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094607	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094608	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094609	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094610	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094611	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094612	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094613	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094614	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094615	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MH094616	South Asia	Pakistan	Nisar et al., 2019
domestic chicken	MT470265	South Asia	India	Suja et al., 2020
domestic chicken	MT470266	South Asia	India	Suja et al., 2020
domestic chicken	MT470267	South Asia	India	Suja et al., 2020
domestic chicken	MT470268	South Asia	India	Suja et al., 2020
domestic chicken	MT488420	South Asia	India	Suja et al., 2020
domestic chicken	MT488421	South Asia	India	Suja et al., 2020
wild fowl	GU261708	South Asia	India	Miao et al., 2013
domestic chicken	GU261709	South Asia	India	Miao et al., 2013
domestic chicken	HQ857209	South Asia	India	Miao et al., 2013

domestic chicken	HQ857210	South Asia	India	Miao et al., 2013
domestic chicken	HQ857211	South Asia	India	Miao et al., 2013
domestic chicken	HQ857212	South Asia	India	Miao et al., 2013
domestic chicken	KJ399461	Middle East	Iran	Ahmadian *et al*., 2014
domestic chicken	KJ399462	Middle East	Iran	Ahmadian *et al*., 2014
domestic chicken	KJ399463	Middle East	Iran	Ahmadian *et al*., 2014
domestic chicken	KJ399464	Middle East	Iran	Ahmadian *et al*., 2014
domestic chicken	KJ399465	Middle East	Iran	Ahmadian *et al*., 2014
domestic chicken	KJ399466	Middle East	Iran	Ahmadian *et al*., 2014
domestic chicken	KJ399467	Middle East	Iran	Ahmadian *et al*., 2014
domestic chicken	KJ399468	Middle East	Iran	Ahmadian *et al*., 2014
domestic chicken	KJ399469	Middle East	Iran	Ahmadian *et al*., 2014
domestic chicken	KJ399470	Middle East	Iran	Ahmadian *et al*., 2014
domestic chicken	KJ399471	Middle East	Iran	Ahmadian *et al*., 2014
domestic chicken	KJ399472	Middle East	Iran	Ahmadian *et al*., 2014
domestic chicken	KJ399473	Middle East	Iran	Ahmadian *et al*., 2014
domestic chicken	KJ399474	Middle East	Iran	Ahmadian *et al*., 2014
domestic chicken	KJ399475	Middle East	Iran	Ahmadian *et al*., 2014
domestic chicken	KJ399476	Middle East	Iran	Ahmadian *et al*., 2014
domestic chicken	AB829473	Middle East	Egypt	Osman *et.al*, 2016
domestic chicken	AB829474	Middle East	Egypt	Osman *et.al*, 2016
domestic chicken	AB829475	Middle East	Egypt	Osman *et.al*, 2016
domestic chicken	AB829476	Middle East	Egypt	Osman *et.al*, 2016
domestic chicken	AB829477	Middle East	Egypt	Osman *et.al*, 2016
domestic chicken	AB829478	Middle East	Egypt	Osman *et.al*, 2016
domestic chicken	AB829479	Middle East	Egypt	Osman *et.al*, 2016
domestic chicken	AB829480	Middle East	Egypt	Osman *et.al*, 2016
domestic chicken	AB829481	Middle East	Egypt	Osman *et.al*, 2016
domestic chicken	AB829482	Middle East	Egypt	Osman *et.al*, 2016
domestic chicken	AB829483	Middle East	Egypt	Osman *et.al*, 2016
domestic chicken	AB829484	Middle East	Egypt	Osman *et.al*, 2016
domestic chicken	AB829485	Middle East	Egypt	Osman *et.al*, 2016
domestic chicken	AB829486	Middle East	Egypt	Osman *et.al*, 2016

domestic chicken	AB829487	Middle East	Egypt	Osman *et.al*, 2016
domestic chicken	AB829488	Middle East	Egypt	Osman *et.al*, 2016
domestic chicken	AB829490	Middle East	Egypt	Osman *et.al*, 2016
domestic chicken	AB829491	Middle East	Egypt	Osman *et.al*, 2016
domestic chicken	EU095039	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095040	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095041	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095042	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095043	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095044	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095045	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095046	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095047	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095048	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095053	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095054	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095055	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095056	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095057	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095058	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095059	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095060	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095061	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095062	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095068	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095069	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095070	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095071	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095072	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095073	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095074	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095075	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095076	Africa	Kenya	Mwacharo et al., 2011

domestic chicken	EU095077	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095082	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095083	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095084	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095085	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095086	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095087	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095088	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095089	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095090	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095091	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095097	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095098	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095099	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095100	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095101	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095103	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095104	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095102	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095105	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095111	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095112	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095113	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095114	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095115	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095116	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095117	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095118	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095119	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095120	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095126	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095127	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095128	Africa	Kenya	Mwacharo et al., 2011

domestic chicken	EU095129	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095130	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095131	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095132	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095133	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095134	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095135	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095141	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095142	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095143	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095144	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095145	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095146	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095147	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095148	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095149	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095154	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095155	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095156	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095157	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095158	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095159	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095160	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095161	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095162	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095163	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095168	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095169	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095170	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095171	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095172	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095173	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095174	Africa	Kenya	Mwacharo et al., 2011

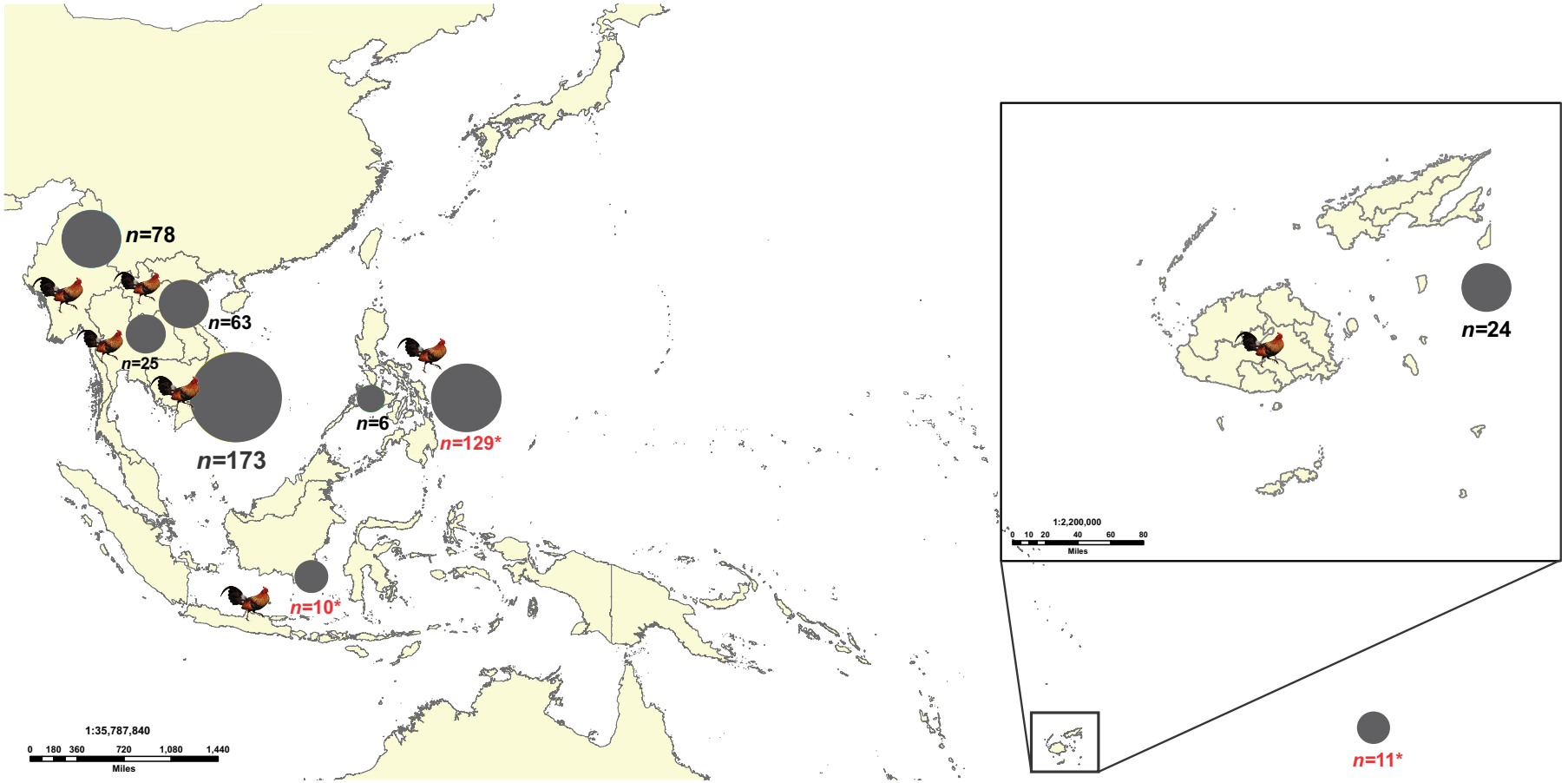
domestic chicken	EU095175	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095176	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095177	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095183	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095184	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095185	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095186	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095187	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095188	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095189	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095190	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095191	Africa	Kenya	Mwacharo et al., 2011
domestic chicken	EU095192	Africa	Kenya	Mwacharo et al., 2011

Appendix Table S3.6. Population differentiation (Pairwise *Fst)* of Asian chickens

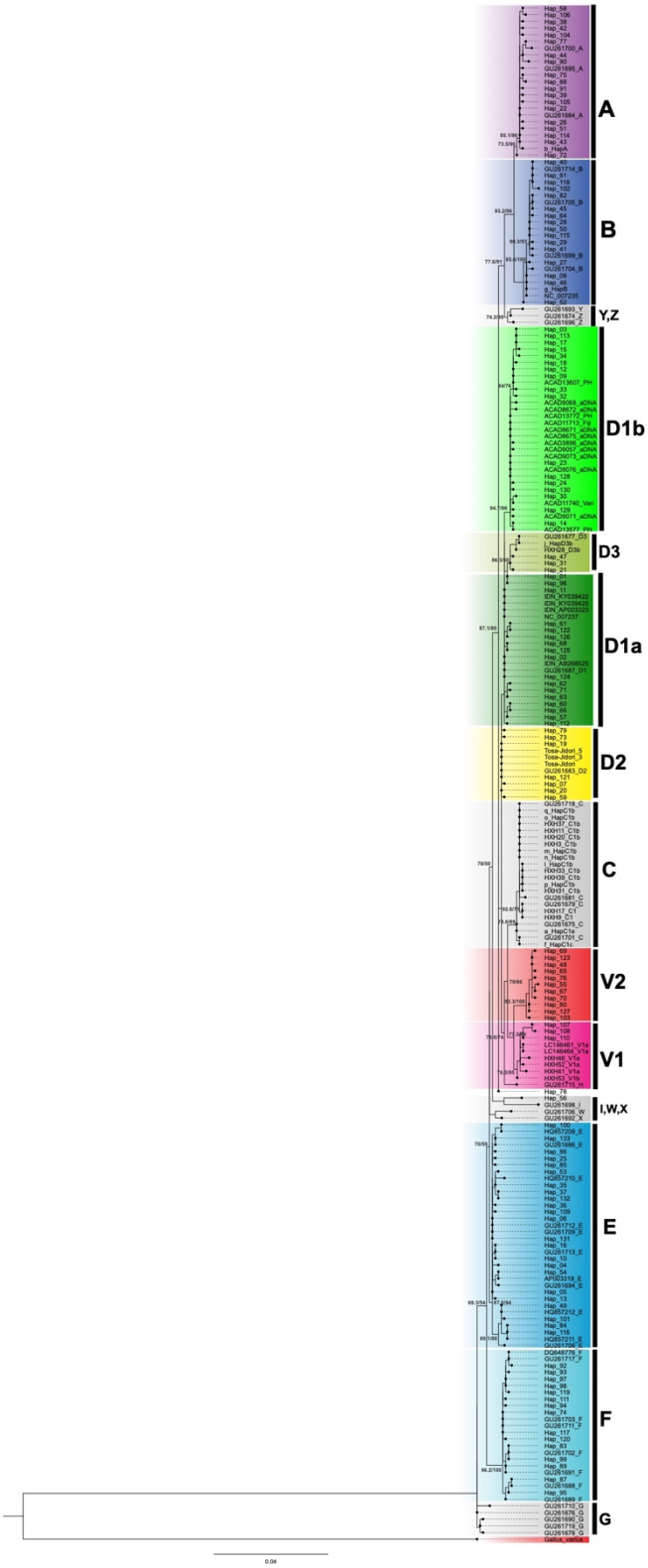
Population pairwise F_{ST} Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
(1) Philippine chickens	0.00000																								
(2) Cambodian chickens	0.10009**	0.00000																							
(3) Laotian chickens	0.26312**	0.11207**	0.00000																						
(4) Myanmar chickens	0.27329**	0.19202**	0.0811**	0.00000																					
(5) Thailand chickens	0.25012**	0.1023**	0.06895*	0.10347**	0.00000																				
(6) Pacific chickens	0.06936*	0.21542**	0.35028**	0.31758**	0.31943**	0.00000																			
(7) Egyptian chickens	0.29661**	0.27967**	0.23861**	0.19175**	0.25587**	0.39307**	0.00000																		
(8) Kenyan chickens	0.28899**	0.3019**	0.34567**	0.3185**	0.35616**	0.35753**	0.16012**	0.00000																	
(9) Iran chickens	0.38556**	0.35511**	0.37136**	0.29852**	0.41742**	0.55104**	0.0437*	0.22309**	0.00000																
(10) Pakistan chickens	0.27053**	0.25821**	0.23672**	0.20249**	0.25539**	0.339**	*0.04344*	0.22989**	0.13843**	0.00000															
(11) Bangladesh chickens	0.14518**	0.15718**	0.22952**	0.18386**	0.1749**	0.18575**	0.10914**	0.17752**	0.23282**	0.12561**	0.00000														
(12) Indian chickens	0.31816**	0.2574**	0.21774**	0.18215**	0.19286**	0.39421**	0.10166*	0.28737**	0.24736**	*0.03914*	0.11843**	0.00000													
(13) Korean chickens	0.23041**	0.13003**	0.10933**	0.16581**	0.14205**	0.32153**	0.22186**	0.30159**	0.32292**	0.21164**	0.20395**	0.20707**	0.00000												
(14) Japanese chickens	0.19738**	0.16194**	0.13235**	0.18031**	0.16447**	0.26415**	0.19516**	0.18642**	0.30383**	0.2052**	0.19927**	0.23504**	0.10633**	0.00000											
(15) Baier Yellow chicken	0.54281**	0.41066**	0.21994**	0.26294**	0.30907**	0.62463**	0.57812**	0.61799**	0.73051**	0.5438**	0.53456**	0.54433**	0.41929**	0.37031**	0.00000										
(16) Chongren Partridge chicken	0.45676**	0.32123**	0.1494**	0.1947**	0.22694**	0.55815**	0.48802**	0.53046**	0.66234**	0.46034**	0.4422**	0.46624**	0.35705**	0.28238**	0.19201**	0.00000									

(17) Silkies	0.522 2**	0.349 76**	0.160 97**	0.236 77**	0.302 16**	0.665 19**	0.621 97**	0.616 06**	0.771 15**	0.549 61**	0.561 97**	0.570 15**	0.268 09**	0.311 31**	0.383 21**	0.402 11**	0.000 00								
(18) Anyi Gray chicken	0.366 24**	0.206 56**	0.118 43**	0.193 26**	0.128 33**	0.448 49**	0.418 18**	0.457 74**	0.586 42**	0.403 36**	0.340 09**	0.381 6**	0.185 02**	0.178 99**	0.277 75**	0.202 29**	0.318 23**	0.000 00							
(19) Xianju chicken	0.356 61**	0.232 92**	0.204 73**	0.253 54**	0.201 **	0.436 29**	0.451 64**	0.422 64**	0.619 53**	0.428 54**	0.351 82**	0.423 41**	0.166 61**	0.125 08**	0.431 **	0.376 59**	0.414 98**	0.076 28*	0.000 00						
(20) Huainan Partridge	0.350 2**	0.219 64**	0.151 76**	0.215 31**	0.159 66**	0.417 03**	0.422 54**	0.453 81**	0.612 36**	0.404 69**	0.337 19**	0.383 26**	0.172 38**	0.155 69**	0.353 19**	0.273 28**	0.352 43**	*0.044 66*	*0.037 17*	0.000 00					
(21) Huaibei native chicken	0.506 8**	0.347 92**	0.152 98**	0.215 14**	0.237 82**	0.598 31**	0.519 53**	0.596 95**	0.706 36**	0.484 71**	0.470 46**	0.450 65**	0.283 68**	0.328 45**	0.288 49**	0.308 5**	0.127 46**	0.265 64**	0.376 13**	0.290 44**	0.000 00				
(22) Wannan Yellow chicken	0.359 6**	0.191 71**	0.034 63	0.122 13**	0.112 07**	0.469 87**	0.378 58**	0.461 **	0.602 83**	0.356 29**	0.325 43**	0.316 81**	0.097 96**	0.140 06**	0.263 24**	0.238 43**	0.077 01*	0.100 73*	0.158 39*	0.106 39*	0.078 86**	0.000 00			
(23) Lueyang chicken	0.275 21**	0.144 54**	0.046 91	0.115 21**	0.099 7**	0.379 82**	0.286 95**	0.315 22**	0.554 51**	0.286 86**	0.218 66**	0.245 52**	0.078 41*	*0.007 72*	0.328 42**	0.237 34**	0.285 77**	*0.071 59*	*0.048 45*	*0.051 16*	0.230 42**	*0.018 69*	0.000 00		
(24) Yunnan chickens	0.288 83**	0.219 47**	0.133 8**	*0.008 16*	0.107 65**	0.343 7**	0.188 17**	0.318 61**	0.368 69**	0.217 86**	0.149 38**	0.182 **	0.216 76**	0.199 69**	0.351 08**	0.244 94**	0.414 59**	0.221 94**	0.300 68**	0.247 73**	0.315 67**	0.199 78**	0.143 28*	0.000 00	
(25) Indonesian chickens	0.110 92**	0.116 31**	0.286 26**	0.294 98**	0.224 7**	0.206 58**	0.456 29**	0.370 2**	0.646 56**	0.412 88**	0.227 35**	0.418 76**	0.298 93**	0.221 02**	0.583 74**	0.509 11**	0.644 16**	0.355 7**	0.349 76**	0.350 56**	0.548 11**	0.389 39**	0.277 27**	0.314 93**	0.00 000

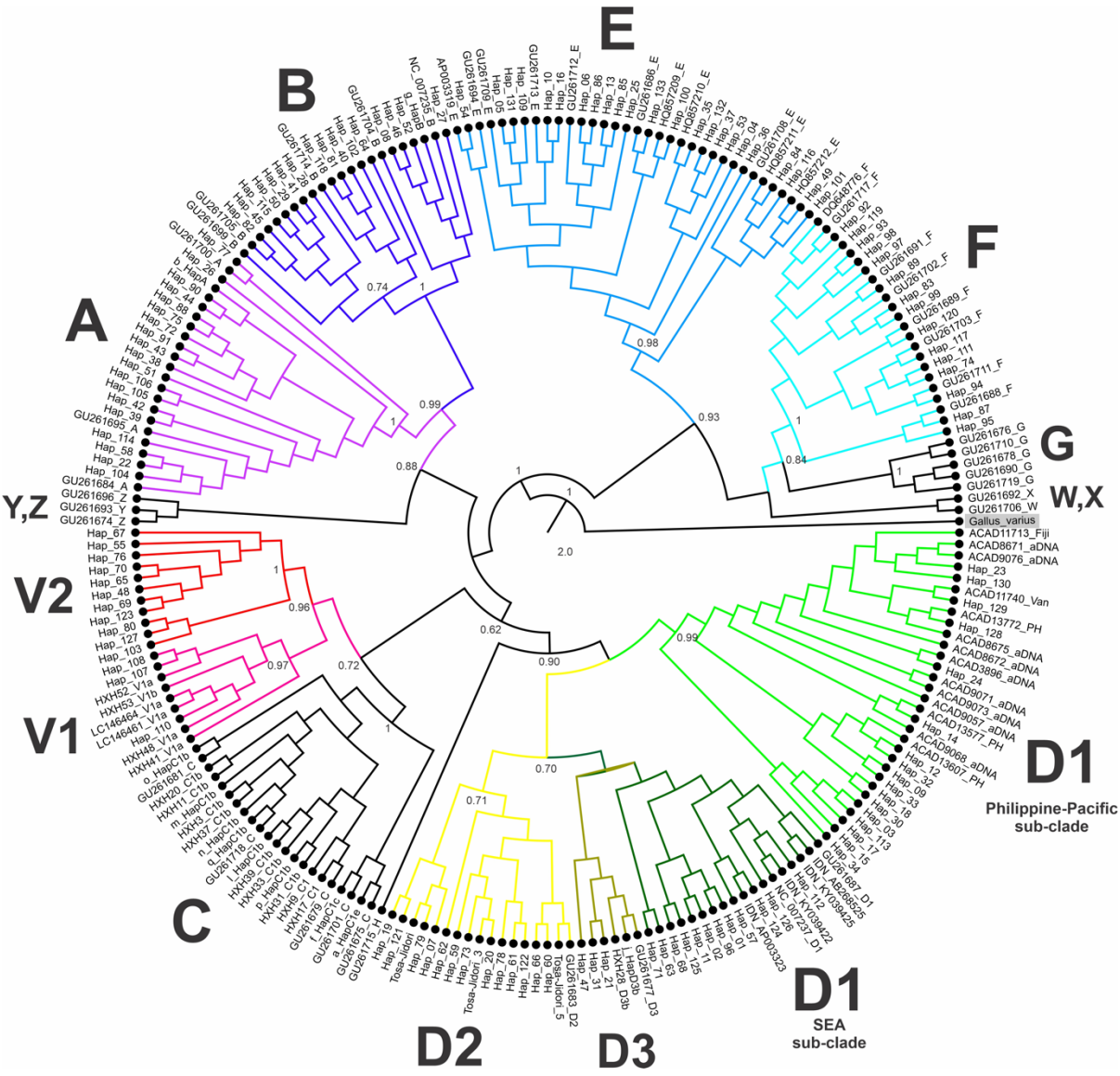
Appendix Figure S3.1. Sampling locations and distribution of SEA and Pacific chickens used in this study. Red labelled complementary samples were retrieved from GenBank.



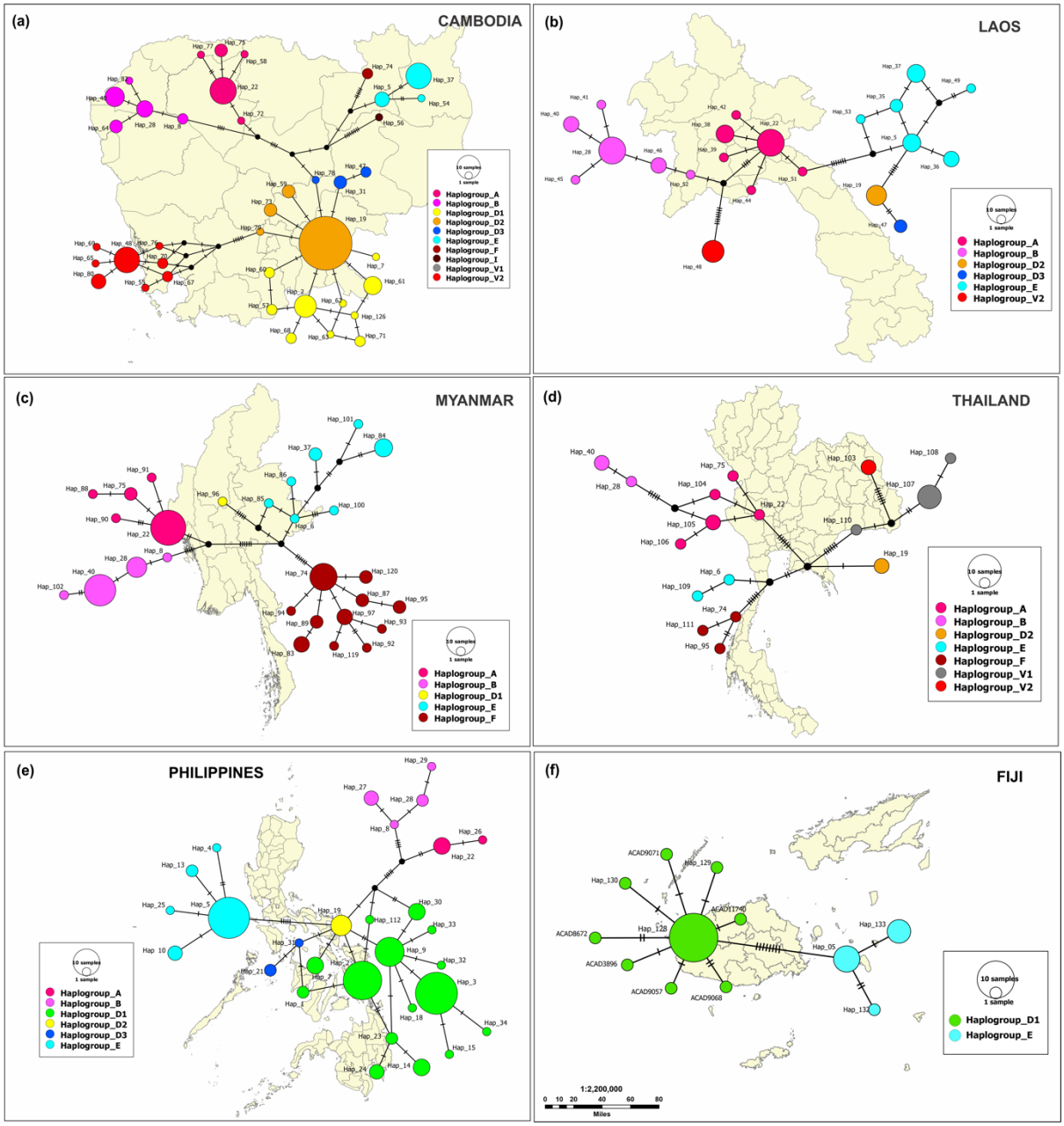
Appendix Figure S3.2. Maximum likelihood phylogenetic tree for complete mtDNA D-loop nucleotide sequences of SEA and Pacific chickens. Node labels correspond to SH-aLRT/UFBoot support values evaluated with 1,000 ultrafast bootstrap replicates in IQ-TREE. The scale bar (0.04) indicates the genetic distance (substitution per site). Bootstrap values under 50% are not shown.



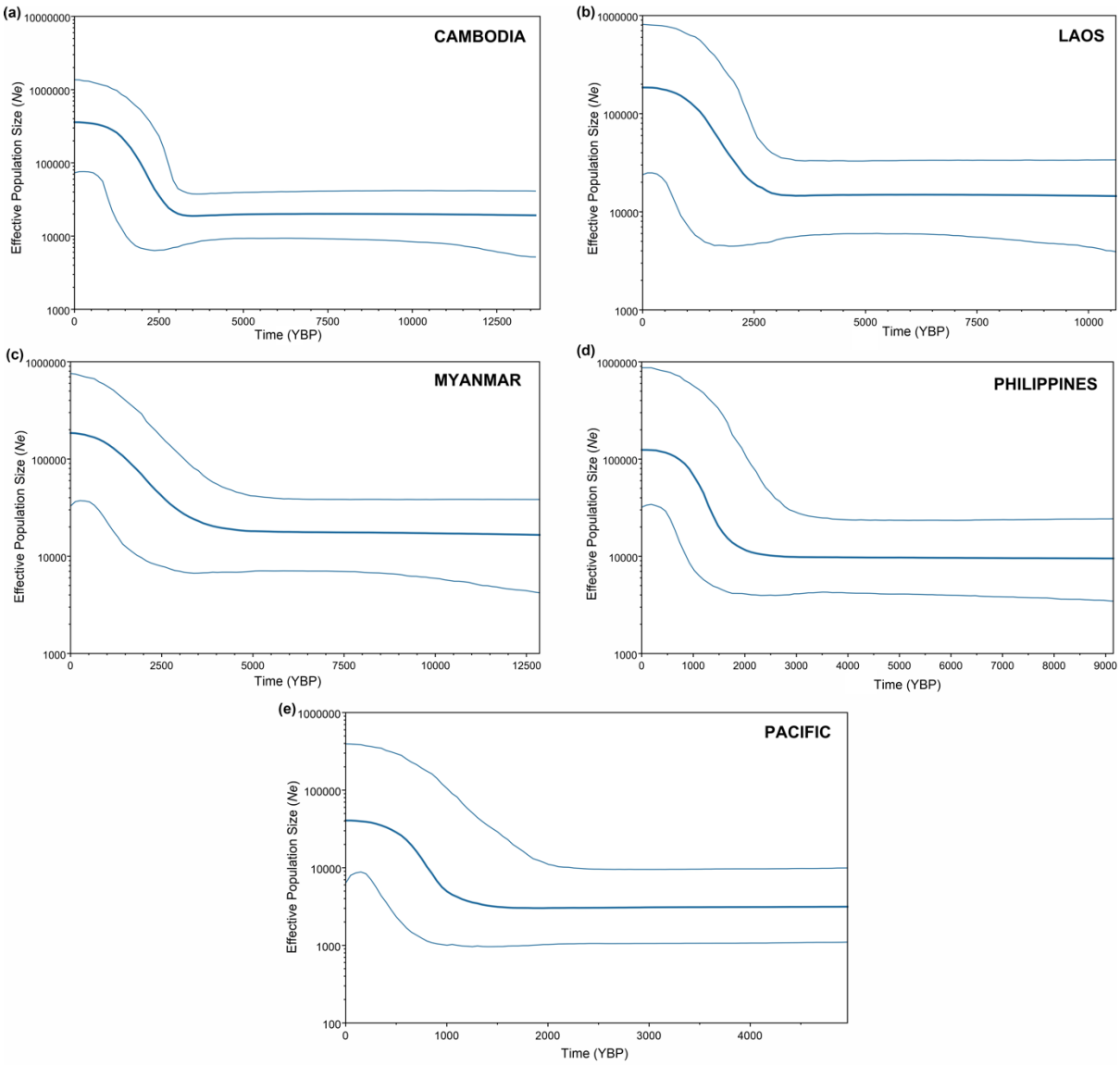
Appendix Figure S3.3. Bayesian phylogenetic tree of complete mtDNA D-loop nucleotide sequences of Southeast Asian and Pacific chickens showing position of terminals. The tree was constructed together with database sequences defined by Huang et al. (2018) (Appendix Table S4). Node labels correspond to posterior probability support values. *Gallus varius* was used as outgroup.



Appendix Figure S3.4. Geographic-specific median-joining network of the complete mtDNA D-loop region (1,232 bp) depicting within population evolutionary relationship. The area of each circle is proportional to the frequency of the corresponding haplotypes. The length of branch connecting to other haplotypes corresponds to mutational positions.



Appendix Figure S3.5. Geographic-specific Bayesian coalescent skyline plot showing estimated within population demographic history. The central blue line is the median estimate effective population size. The shaded area shows the upper and lower estimates of 95% credibility interval. The x-axis is the time (in years before present) and y-axis indicates population size (as the product of N_e and the generation length in years).



CHAPTER IV

Time-calibrated phylogeny of Southeast Asia and Pacific chickens based on Bayesian molecular clock analysis

Abstract

The earlier domestication of chickens in mainland Southeast Asia and consequent translocation to the island archipelago are deliberately linked with human movement. However, little is known about the evolutionary links and temporal divergence between continental and island chickens, especially since archaeological records of chicken bones in the region are scarce. With the increasing genetic data and resolving power of sequence data in recent years, coalescence simulation methods have been shown efficient at testing different evolutionary and demographic models of expanding and migrating populations. This study simulated 303 haplotypes and estimated the lineage-specific divergence of MSEA, ISEA, and Pacific chickens using the Bayesian molecular clock method. Initially, the bayesian phylogenetic inference returned a concordant and well-supported phylogeny. It revealed the separation of haplogroup D from haplogroups CV, likely corresponding to their exclusive evolutionary and demographic history. The red junglefowl from Palawan and Mindoro clustered with the ancestral sub-haplogroup D2 lineage, supporting the hypothesis of admixed ancestry of island chickens. The coalescence node age of macrohaplogroup CDV was estimated to be 6.67 kya with credibility intervals of 4,235–7,996 years (95% HPD). Additionally, the coalescence time estimates of haplogroups D and V are consistent with their demographic evolution and expansion in the region, around 3,700-4,000 years BP and 3,800-4,400 years BP, respectively. Likewise, the most recent common ancestor (TMRCA) of the latterly expanded Philippine-Pacific sub-haplogroup D1b, including the ancient Pacific sequences, dates to 2.1 kya (95% HPD 1,467–2,815 years). The two root age calibration points gave slightly different average node age estimates but provided overlapping HPD intervals. Conclusively, this study suggests that geographical isolation, colonization, and independent admixture have significantly shaped the genetic architectures and diversity of the present-day chickens in the region. This study accounts for uncertainty in primary calibration constraints, models of molecular evolution, and

tree topology, but this also means that if additional fossil calibration data is available in the future, deep-level calibration points will likely bring important information.

Keywords: Bayesian molecular clock, divergence age estimate, *Gallus gallus*, Southeast Asia, Time tree

4.1 Introduction

The beginning of animal domestication allowed the expansion of agriculture and herding, involving a protracted and continuous relationship between humans and animal domesticates driven by diverse environmental and ecological gradients (Larson et al., 2014). The migration of earlier agricultural societies taking domesticates away from the domestication centers, led to the emergence of domestic populations becoming more genetically divergent (Larson & Burger, 2013). The multistage process of animal domestication exemplifies ways in which animals respond to anthropogenic niches. Recognizing that not all animals were introduced into a domestic relationship with humans in the same trajectory or possibility of differential stages before wild turned into domesticated, Zeder et al. (2012) recognized three separate domestication pathways: a commensal pathway, a prey pathway, and a directed pathway. These perspectives allow for a deeper perception of the domestication process and set off hypotheses and population models that can be tested using genetic data and statistical inference (Larson & Burger, 2013).

The earlier domestication of chickens in mainland Southeast Asia and consequent translocation to the island archipelago are deliberately linked with human movement. This establishes a commensal relationship or habituation of wild fowls to a human niche, likely through a process of hybridization (Eriksson et al., 2008; Larson & Fuller, 2014; Zeder, 2012). However, little is known about the antiquity of chickens in Southeast Asia and there are only

few archaeological records of chicken bone traces in SEA (Storey et al., 2012). Such evolutionary links would likely provide a better understanding of the evolutionary history and population dynamics of the world's most common farm animal.

Although mtDNA-based studies are unlikely to completely reflect the complex past demographies, mtDNA has several advantages. With increasing worldwide data set and availability of explicit hypothesis-testing modeling approaches, nonrecombining marker remains valuable and has been extensively used in investigating the chicken populations, types, evolutionary relationships, and domestication history. For example, previous work used spatially explicit coalescent simulations to demonstrate the asymmetrical introgression patterns when an expanding population colonized an area occupied by another population (Currat et al., 2008). This is particularly true for organelle DNA markers, such as mtDNA, where the direction of introgression is always from the founding population that first colonized an empty territory into the expanding one after the pioneer population has disappeared (Currat et al., 2008; Larson & Burger, 2013).

Computer simulation methods (e.g., coalescence simulation) have been shown to be efficient at testing different evolutionary and demographic models (Larson & Burger, 2013), thus allowing for novel insights into the reoccurring questions of the domestication process. Time trees or phylogenies with absolute divergence times provide incomparably richer information than a species phylogeny without a temporal clue, making it possible for species divergence or coalescence events to be calibrated to time (dos Reis et al., 2016). The increased availability of DNA sequences to date enables evolutionary biologists to use the molecular clock as a simple but powerful tool to estimate species divergence times (e.g., lineage-specific divergence times).

This chapter utilizes complete mitochondrial DNA (mtDNA) D-loop sequences of chickens from mainland SEA (Cambodia, Laos, Thailand, and Myanmar), the Philippines, and

the Pacific, combined with published genetic data of ISEA domestic chickens and RJFs (the Philippines and Indonesia), Pacific chickens, and other chicken populations in Asia to estimate their lineage-specific divergence and genetic similarities and differentiation within and between continental and island populations using Bayesian molecular clock dating method.

4.2 Materials and Methods

4.2.1 Sampling and DNA extraction

Sampling procedure was the same as discussed in Chapter III. Genomic DNA was extracted from stored whole blood samples using the phenol-chloroform method (Green & Sambrook, 2012). This study pooled available sequences of Philippine red junglefowl (*n*=55), and Indonesian red junglefowl (*n*=25) (Compendio, 2022; Compendio et al., 2022) archived from the GenBank database and complemented in the final dataset (Appendix Table S3.1 in Chapter III; Appendix Table S4.1).

4.2.2 PCR amplification and sequencing

The target complete mitochondrial control region (1,232 bp) was amplified in two procedures. First, about 5.0 kbp mtDNA D-loop fragments were amplified using a long and accurate – PCR (LA-PCR) kit (KOD-FX Neo Polymerase, TOYOBO, Osaka, Japan) with chicken DNA as a template and LA-PCR primer sets: *Cytb-Forward*: 5′-TACACGAATCAGGCTCAAACAACCCCCTAGGCATC-3′, *16S-Reverse*: 5′-TGCACCATTAGGTTGTCCTGATCCAACATCGAGGT-3′ recommended by Nishibori et al., (2003). The reaction began with a preliminary denaturation at 94 °C for 2 min, followed by 30 cycles of DNA denaturation at 98 °C for 10 sec, annealing of primers at 57 °C for 30 sec, and primer extension at 68 °C for 2 min and 30 sec, using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, USA). Second, the amplified fragments were used for segmental amplification of the complete mtDNA D-loop region (1.3 kbp) following the primer

sets: *Gal1F* 5′-AGGACTACGGCTTGAAAAGCCATTG-3′ and *Gal1R* 5′-GCTGAGTACCCGTGGGGGTGTGGCT-3′ in 20 µl reaction volume containing 2x PCR buffer, 0.4 mM dNTPs, 0.3 µM concentrations of each primer, 0.4 U of KOD-FX Neo DNA Polymerase, and 15-25 ng of amplified fragment DNA as template. The PCR cycling condition began with a preliminary denaturation at 94 °C for 2 min, followed by 30 cycles of DNA denaturation at 98 °C for 10 sec, annealing of primers at 59 °C for 30 sec, and primer extension at 68 °C for 30 sec, using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, USA). The DNA fragments obtained from the segmental amplification were cleaned and purified using Exonuclease I (ExoI) and Shrimp Alkaline Phosphatase (SAP) to degrade the residual PCR primers and dephosphorylate the remaining dNTPs, respectively. The two PCR primers and one internal primer: *Gal1-2F* 5′ -TCCACCTCACGAGAGATCAGCAACCC-3′ (Nishibori et al., 2001) were used for the sequencing reaction. Subsequently, the mtDNA D-loop fragments were directly sequenced using 3130/3130xl Genetic Analyzers (Applied Biosystems, Foster City, USA).

4.2.3 Sequencing alignment

Three hundred sixty-nine complete mtDNA control region sequences generated in this study were initially edited using GeneStudio Pro tool (GeneStudio, Inc., http://genestudio.com/). Ambiguous sites were trimmed and cleaned sequences were aligned in MEGAX (Kumar et al., 2018) with ClustalW (Thompson et al., 1994). Aligned nucleotide sequences were viewed using BioEdit 7.2.5 software (Hall, 1999). All newly generated sequences were deposited in the GenBank database with accession numbers OM240181-OM240549 (Appendix Table S3.1).

4.2.4 Phylogenetic analyses

Phylogenetic analyses were inferred using a model-based approach Bayesian inference (BI). BI was performed using BEAST2 v2.6.6 (Bouckaert et al., 2019) under uncorrelated

relaxed clock log-normal distribution setting a clock rate, 3.13 x 10^{-7} mutations/site/year rate (Alexander et al., 2015). A general time reversible (GTR) nucleotide substitution site model was used with assumed rate heterogeneity among sites modeled under gamma distribution and a calibrated yule model as a tree prior. The posterior distributions of parameters were estimated via Markov chain Monte Carlo (MCMC) with duplicate runs of 50 million generations, sampling every 10,000 steps, and the initial 10% trees of each MCMC run were discarded as burn-in. Convergence of MCMC chains was assessed using Tracer v.1.7.1, and sufficient sampling was verified with all estimated parameters exceeding 200 ESS values. A Maximum Clade Credibility (MCC) tree (target tree) was obtained from a sample of trees using TreeAnnotator v2.6.3 (Bouckaert et al., 2019). Phylogenetic trees were visualized and edited in FigTree v1.4.4. (http://tree.bio.ed.ac.uk/software/figtree/).

4.2.5 Divergence time estimate

Bayesian analyses were performed to estimate divergence times using the program BEAST2 v2.6.6. In Bayesian molecular clock dating, calibration information is incorporated in the analysis through prior on times (dos Reis et al., 2016). A relaxed molecular clock model was used, allowing substitution rates to vary across branches setting with 3.13 x 10^{-7} mutations/site/year rate under uncorrelated lognormal distribution and GTR + G4 substitution model as determined by BIC in jModelTest v2.1.1. In addition, this study set a coalescent-based constant population to model the tree prior, as it appears to be a better fit when data sets are predominantly intraspecific data (Ho et al., 2011). The ancient DNA records of Polynesian chickens were used to calibrate the crown node (node calibration) of the Philippine-Pacific subclade or sub-haplogroup D1b (Appendix Table S3.1). For this calibration point, a lognormal prior (mean: 2.5, SD: 0.20, offset: 0) was used with the maximum age of the archaeological record set as the minimum bound for the crown calibration (soft bounds). For the calibration of the root node of the tree, the divergence time between red junglefowl and domestic chickens

(8,093 years CI: 7014–8768) was used as a secondary calibration (Lawal et al., 2020). A lognormal prior (mean: 8.09, SD: 0.05, offset: 0) was employed, covering the range of the confidence interval of the divergence time estimate. For comparison, this study also simulated the suggested divergence time between *G. g. spadiceus* sampled in Thailand and domestic chickens (~9,500 ± 3,300 years ago) reported by Wang et al. (2020). A lognormal prior (mean: 9.5, SD: 0.20, offset: 0) was employed, covering the range of the confidence interval of the divergence time estimate. Time tree analysis was run for 50 million generations, sampling every 5,000 generations, and the initial 10% trees of each MCMC run were discarded as burn-in. The resulting log files were examined in Tracer v1.7.1 software to confirm acceptable mixing and convergence of all parameters in the independent runs and adequate effective sample sizes (ESS>200). The maximum clade credibility (MCC) tree was created from the tree file using TreeAnnotator v2.6.3 with the posterior probability set to 0.5 and common ancestor node heights summarized. These results were visualized as a single tree in FigTree v1.4.4. (http://tree.bio.ed.ac.uk/software/figtree/).

Running the same simulation without data was done to test whether the priors dominated the posterior distribution. The results from these runs were compared with those obtained when the actual data were analyzed.

4.3 Results

4.3.1 Phylogenetic inference

Bayesian phylogenetic inference returned a concordant and well-supported phylogeny with posterior probability (PP) ≥90%, except macrohaplogroup CDV which is moderately supported (Figure 4.1; Appendix Figure S4.1). The Bayesian phylogeny reveals a clear separation of major haplogroup D from haplogroups CV, which likely correspond exclusive evolutionary and demographic history of this clade. Meanwhile, within haplogroup D, island chicken populations formed a separate subclade from their potential founding ancestors in

MSEA. This identified Philippine-Pacific sub-haplogroup D1b is strongly supported (PP=0.9 to PP=1.0) and consists of ancient DNA samples of Polynesian chickens. Likewise, there is a likelihood of ancestral founding lineage of haplogroup D (i.e., sub-haplogroup D2) mostly detected in Cambodian chickens and partly represented in Laotian and Thai chickens (Figure 4.1-4.2; Appendix Figure S4.1; Appendix Tables S3.1-S3.2 in Chapter III).

A robust phylogenetic tree, including the Philippine and Indonesian red junglefowl sequences (Compendio, 2022), validated the dispersal event of the Philippine-Pacific D1b lineage to the Pacific chicken population (Figure 4.2). This Philippine-Pacific D1b lineage experienced *in situ* diversification with their founding population (herein the Philippines subclade D1) in the Philippines before expanding eastward. Most of the red junglefowls from Palawan and Mindoro clustered with the ancestral Hap-D lineage (Haplogroup D2), suggesting that the Philippine red junglefowl descended from this founding population in mainland SEA and went exoferal or admixed (a mixture of domesticated ancestors from MSEA that went feral and the original wild red junglefowl reservoir matriline that was already inhabited the archipelago) after colonization. Likewise, no red junglefowl sampled in Sulawesi and indigenous chickens from Maluku, Kalimantan, and Java, were detected with genetic affinity to the Pacific chickens (Figure 4.2). Instead, the Indonesian red junglefowl formed a subgroup (SEA subclade D1) with the *G. g. bankiva* (NC_007237) and indigenous chickens from Cambodia and the Philippines, suggesting that the Javan red junglefowl likely the founding population of this matriline.

Within macrohaplogroup CDV, the Bayesian phylogenetic tree classified haplogroup V as a sister clade to haplogroup C. This result is discordant with the schematic classification tree described by Huang et al. (2018). Haplogroup V was divided into two subclades (i.e., sub-haplogroup V1 and sub-haplogroup V2), showing unique mutational motifs. Most red junglefowls sampled in Thailand are restricted to sub-haplogroup V1, defined by diagnostic

mutational motifs 197-233-299-300-301. On the contrary, sub-haplogroup V2, defined by diagnostic mutational motifs 228-237-391, is widely distributed in Cambodia and partly detected in Laos, but it is rare in Thailand (Appendix Table S3.1 in Chapter III).

4.3.2 Divergence dates

The maximum clade credibility (MCC) trees estimating the divergence time using a calibration method under an uncorrelated lognormal relaxed clock model revealed age estimates for biogeographically important nodes of haplogroups D and V (Figure 4.3-4.4; Table 4.1). This study collapsed the trees to highlight the most biogeographically critical nodes (i.e., haplogroups D and V) relevant to this study (Figure 4.3; 4.4). Despite having varying root node age calibration points, broadly similar topologies were recovered for each time-calibrated tree. The main differences observed in these phylogenies involve a rearrangement position of sub-haplogroup D3 with low posterior probability support (<50%), which perhaps warrants further investigations. The node age of macrohaplogroup CDV was estimated to be 6.67 thousand years ago (kya) with credibility intervals of 4,235–7,996 years (95% HPD). The coalescence age of the Philippine-Pacific subclade (PP=1) dates back to 2.1 kya (95% HPD 1,467–2,815 years) while diverging from the ancestral D-lineage approximately 3.7 kya (95% HPD 1,985–4,835 years). A similar age estimate is also observed even with the addition of ISEA red junglefowl sequences into the overall dataset (Appendix Figure S4.2). The hypothesized founding matriline within haplogroup D (i.e., sub-haplogroup D2) revealed an earlier coalescence age estimate in the D-lineage. Nonetheless, the SEA subclade D1, which consists of predominant Indonesian red junglefowl (*G. g. bankiva* subspecies) and some indigenous chickens from the Philippines and Cambodia, showed a later coalescence age estimate than the lineage classified for the Philippine and Pacific chickens (Appendix Figure S4.2).

Haplogroup CV (PP=0.80) diverged much earlier from macrohaplogroup CDV and coalesced around 5.5 kya (95% HPD 3,116–7,275 years) while succeeding divergence of

haplogroup V (PP=0.96) occurred around 3.9 kya (95% HPD 2,125–5,880 years). Newfound evidence of sub-haplogroup V2 (PP=1) has a more recent coalescence age dates back to 1.5 kya (95% HPD 690–2,788 years), while sub-haplogroup V1 (PP=0.97) diversified earlier (2.3 kya; 95% 1005–3815 years). Conversely, when using the older root age calibration point (~9,500 ± 3,300), coalescence ages among haplogroups showed slightly earlier age estimates but within the CI range of the previous estimate using the 8,093 (CI: 7014–8768) calibration point. Estimates for most major lineages are relatively congruent with overlapping HPD intervals. With the increased number of haplotypes in each haplogroup, estimated coalescence ages deviated from the previously reported study (Huang et al., 2018). However, this study acknowledges that the findings should be interpreted with caution because different data sources (i.e., taxon sampling), types of molecular data, and methods often based on different hypotheses (e.g., different calibration points) may introduced estimation biases that might compromise conclusion.

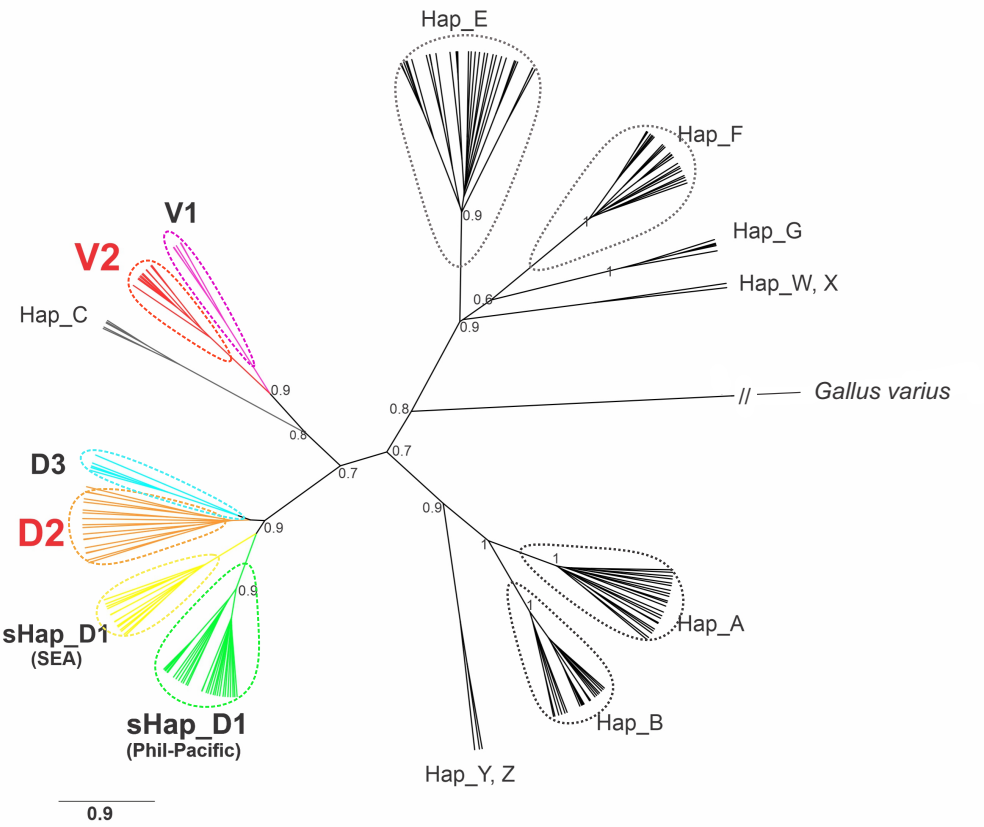


Figure 4.1. Rooted Bayesian phylogenetic tree of the complete mtDNA D-loop sequences of Southeast Asiana and Pacific chickens. Node labels correspond to posterior probability support. Posterior probability values under 50% are not shown. *Gallus varius* is used as an outgroup.

Table 4.1. Estimated coalescence ages of chicken mtDNA haplogroups calibrated based on uncorrelated lognormal relaxed molecular clock

Haplogroup	*n*	Relaxed clock rate [~8093 (CI 7014-8768)]		Relaxed clock rate [~9500 ± 3300]	
		Posterior	Node age (y)*	Posterior	Node age (y) *
ABYZ	48	0.86	5597 [3129; 7223]	0.86	6545 [3190; 9436]
AB	45	0.99	3848 [1934; 5676]	0.99	4502 [2074; 7149]
CDV	114	0.55	6668 [4188; 7963]	0.56	7712 [4047; 10225]
D	72	0.97	3725 [2190; 5370]	0.98	4112 [2224; 6280]
D1b	31	1	2107 [1463; 2819]	1	2191 [1492; 2990]
CV	42	0.80	5454 [3039; 7168]	0.79	6328 [3083; 8986]
V	20	0.96	3874 [2119; 5765]	0.97	4448 [2100; 6992]
V1	9	0.97	2346 [1008; 3814]	0.97	2675 [973; 4471]
V2	11	1	1540 [594; 2704]	1	1744 [579; 3197]
EFGIWX	64	0.96	5571 [3483; 7513]	0.96	6462 [3532; 9703]

n number of haplotypes; * 95% highest posterior density (HPD) interval

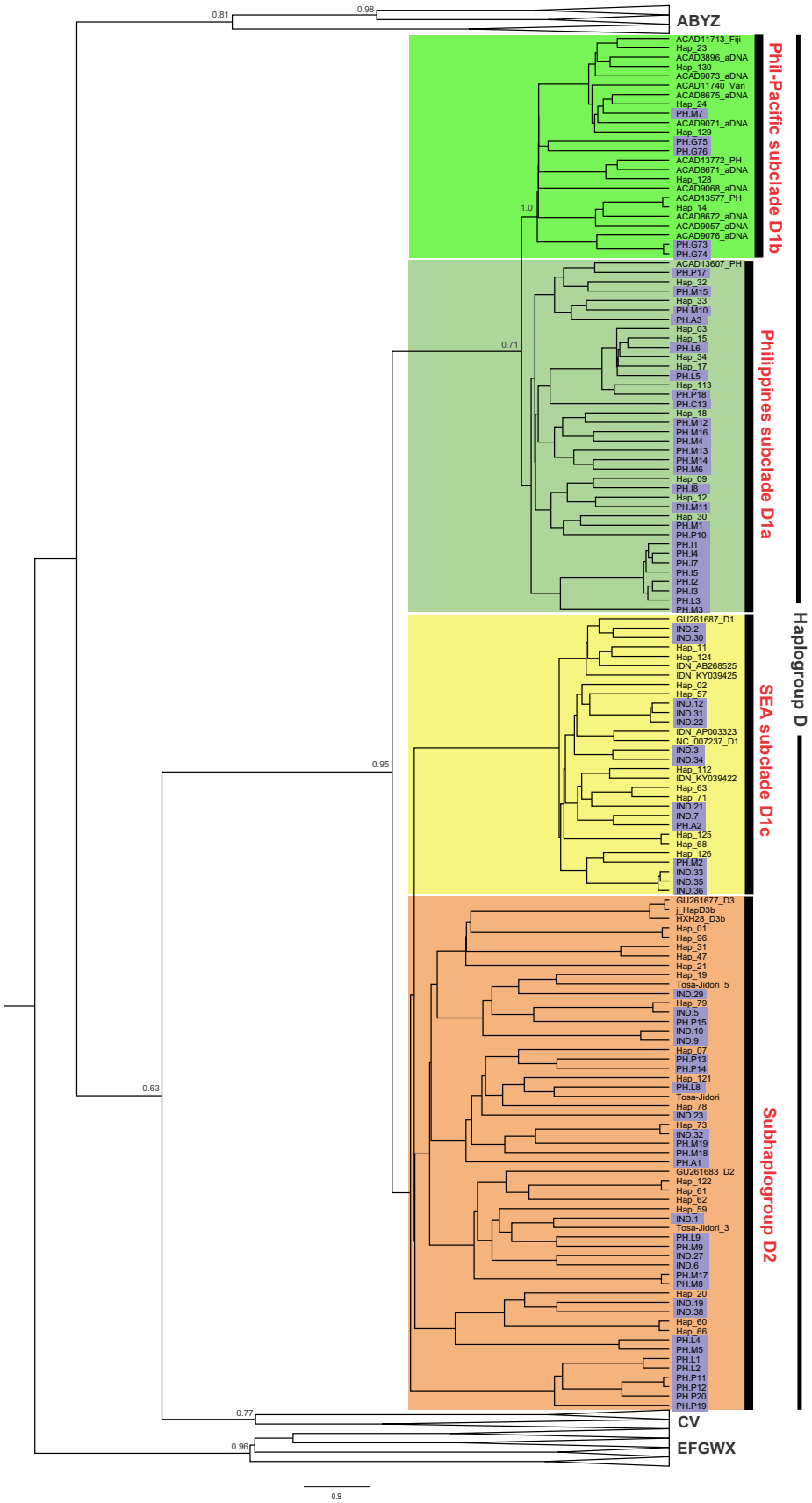


Figure 4.2. Bayesian phylogenetic tree of the complete mtDNA D-loop sequences of SEA, Pacific chickens, and ISEA red junglefowl. Node labels correspond to posterior probability support. Posterior probability values under 50% are not shown.

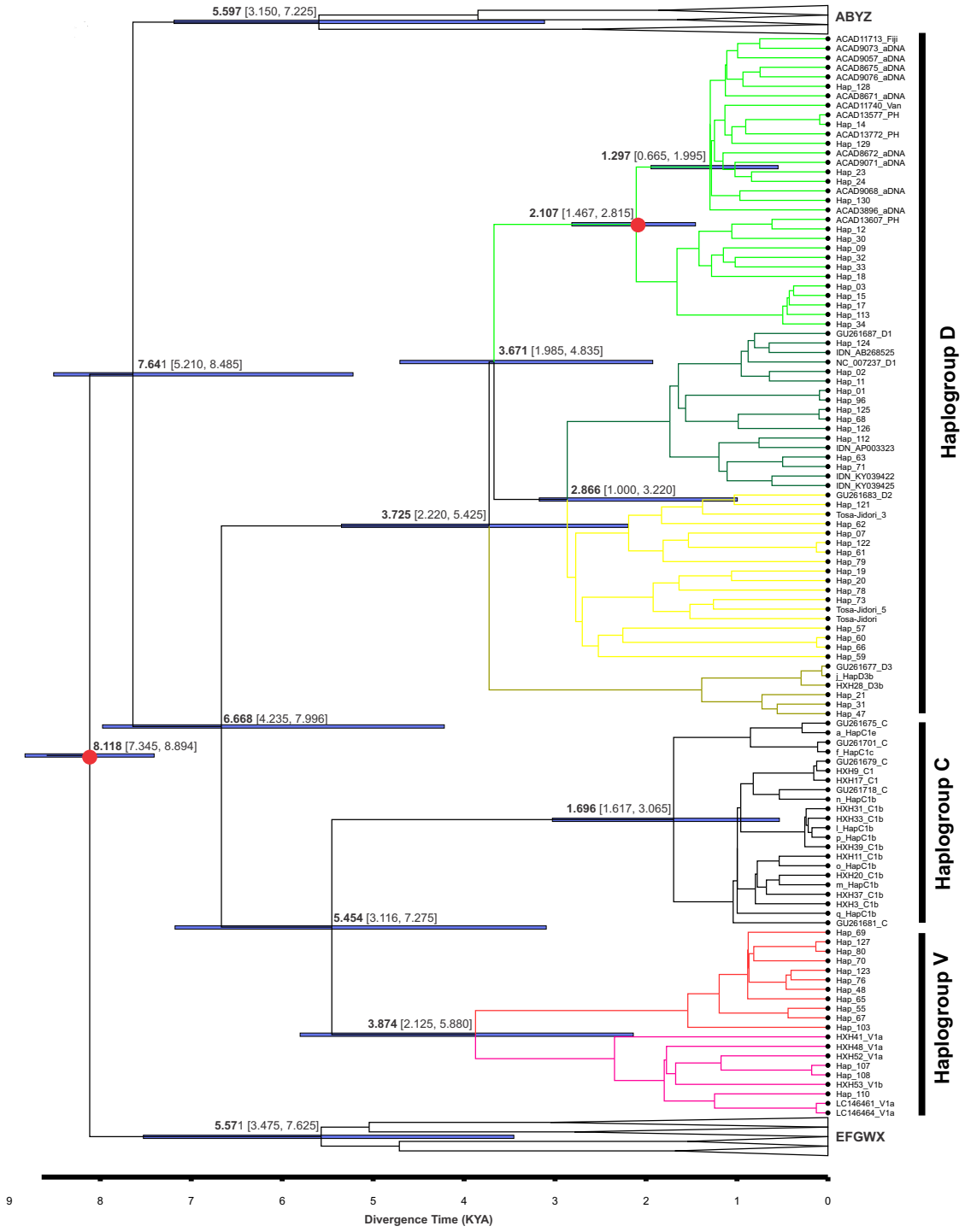


Figure 4.3. Divergence times estimate based on primary and secondary calibration [~8093 (CI 7014-8768)] using BEAST2 v2.6.6. Red dots indicate the nodes with calibrations. Node labels indicate the median estimated divergence time, blue bars indicate the 95% HPDs.

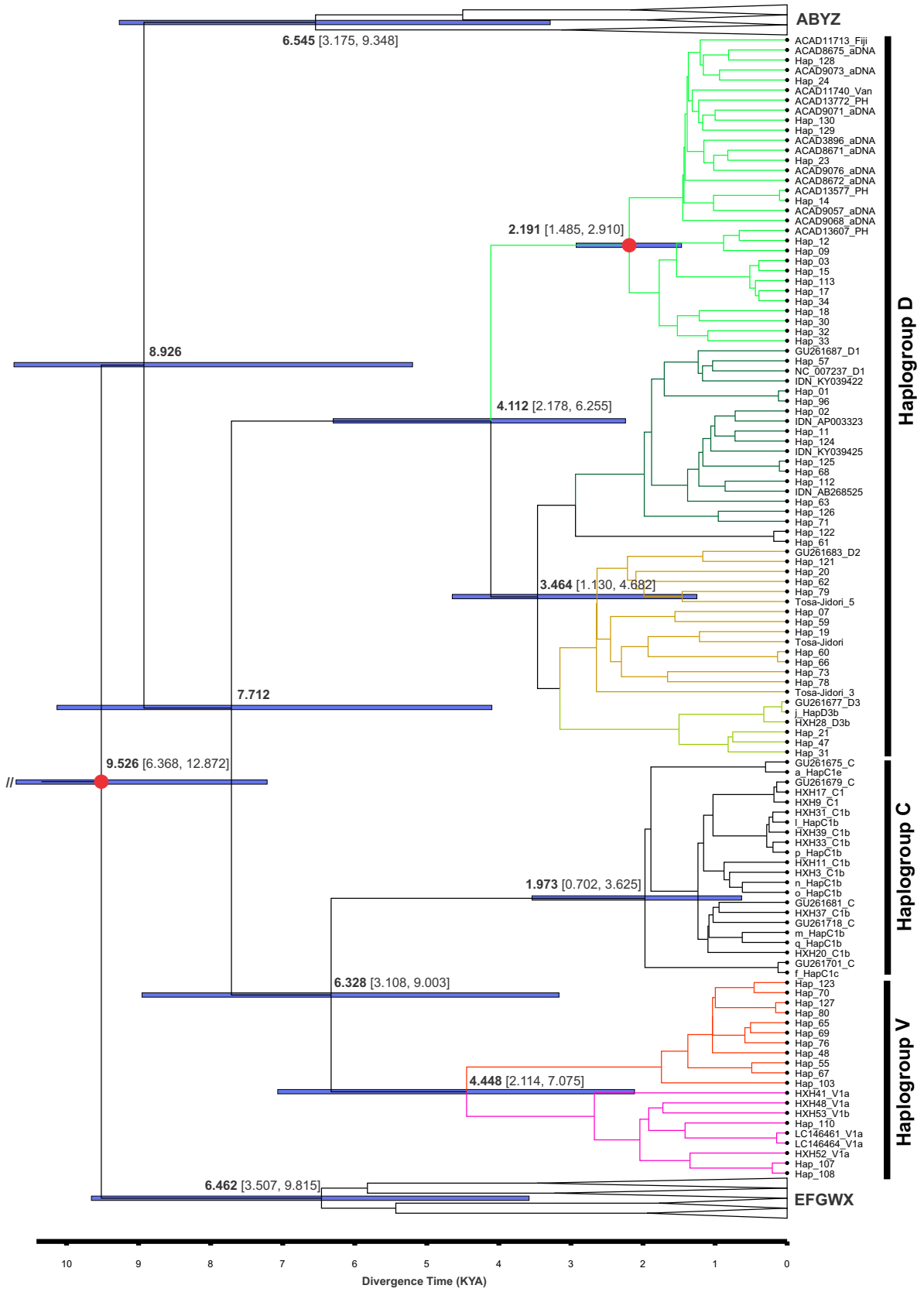


Figure 4.4. Divergence times estimate based on primary and secondary calibration [~9500 ± 3300] using BEAST2 v2.6.6. Red dots indicate the nodes with calibrations. Node labels indicate the median estimated divergence time, blue bars indicate the 95% HPDs.

4.4 Discussion

The time tree phylogeny in the coalescent framework allows this study to estimate nodal ages of biogeographically important haplogroups in the context of SEA chickens. Combining primary calibration (i.e., fossil) from ancient Pacific chickens (Thomson et al., 2014) and secondary calibration from previous estimations by Lawal et al. (2020) and Wang et al. (2020). The latter calibration can provide close derived estimates from true time depending on the type of primary calibrations used (Ho & Phillips, 2009; Powell et al., 2020). Modeling the minimum-maximum constraints allows proximate measurement of uncertainties for estimated times and includes true time boundaries in the derived CI range (dos Reis et al., 2016; Ho & Phillips, 2009; Powell et al., 2020; Yang & Rannala, 2006). These calibrations and the robust phylogenetic trees allow estimating the divergence of major haplogroups and the coalescence ages of some lineage-specific matrilines that shaped the population demographics of Southeast Asian chickens. Trees resulting from two different root calibration points show slight variations in the node ages but have overlapping CI ranges, indicating that the choice of calibration points did affect the molecular clock dating. Likewise, the older calibration points (~9,500 ± 3,300) produced older node ages, and younger calibration points (8,093 years CI: 7014–8768) had younger estimates. A case that was also observed in calibrating age estimates of extant Bovidae (Bibi, 2013).

The coalescence time estimate for the node of macrohaplogroup CDV is 6.67 kya (95% HPD: 4,235-7,996 years). Huang et al. (2018) reported a similar age estimate under a relaxed molecular clock model using the same molecular rate. The coalescence of haplogroups C and V is consistent with their geographical distributions in East Asia (e.g., Southwestern China) and Southeast Asia. Evidence of recently classified haplogroup V remains a geographically restricted clade inhabited in mainland Southeast Asia. The age estimate of haplogroup V indicates an older coalescence age (3.9 kya; 95% HPD: 2,125–5,880 years) than the previous

estimate of this reclassified haplogroup (Huang et al., 2018). Meanwhile, the evidence of earlier coalescence age of the founding matriline sub-haplogroup D2 (3.5 kya; 1.2-4.8 HPD) from MSEA exemplified dispersal patterns to the ISEA, and thereafter island clade diversified as a distinct group, a phylogeographic scenario that was also documented in other avian taxa (Jones & Kennedy, 2008; Lohman et al., 2010, 2011). Earlier paleoenvironment and biogeographic evidence (Heaney, 1991) and more recent evidence on stable carbon isotope records from bat guano sequences (Wurster et al., 2019) suggest that seasonal forest or open vegetation existed in the continental landmass of Sundaland during the Last Glacial Period which likely facilitated early human dispersal through the region (Bird et al., 2005). Caution is warranted for this interpretation because the coalescence age estimate of gene copies in ancestral populations is not equivalent to a population split (Angelis & dos Reis, 2015; Nichols, 2001), nor does it represent the actual onset of domestication.

The zoogeographical argument against the timing of the introduction of continental chicken populations into the island archipelago steers hypotheses whether the Philippine chickens can be considered the same as the nominate race from MSEA or established as an indigenous race. Parkes (1962) initially suggested that the Philippine chickens were introduced birds from a subspecifically differentiated population somewhere on the mainland about 3,000 years ago. Considering that the prehistoric geographical range of RJF likely reached the Sunda shelf during periods of lowered sea level and subsequently spread onto the Philippines (Herrera, 2015). Nonetheless, the result of the time-calibrated tree indicates the coalescence age of haplogroup D is estimated to be around 3.8 to 4.1 kya. The most recent common ancestor (TMRCA) of modern Philippine-Pacific chickens (i.e., sub-haplogroup D1b) and the ancient Pacific samples date back to 2.1 kya (95% HPD 1,467–2,815 years), an earlier coalescence age estimates than the *G. g. bankiva* matriline in Indonesia (Appendix Figure S4.2). This estimate

also predates the sample ages of the recovered ancient Pacific chickens in Anatoloa site, Niue Island and Anakena site, Rapa Nui (Thomson et al., 2014).

The greater genetic variation observed within the haplogroup D likely represents a diverse admixed populations of both feral and descendants of wild endemic junglefowl that locally interbreed with once introduced populations. The genomic changes under feralization identified that feral island chicken shows adaptative traits associated with sexual selection and reproduction but are not simply selected along the domestication process (Johnsson et al., 2016). Moreover, possibilities of admixed ancestry of ISEA chickens based on morphological assessment, particularly in Philippine red junglefowl, unveiled the coexistence of *G. g. gallus* and *G. g. bankiva* subspecies in the archipelago (Compendio, 2022). Multiple maternal lineages and admixed populations were also documented in Indonesian chickens (Ulfah et al., 2017), consistent with the hypothesis that the descendants of ancestral RJF or the introduced domestics are interbreeding in the island archipelago. Conclusively, this present study suggests that geographical isolation, colonization, and independent admixture have significantly shaped the genetic architectures of the founding Philippine red junglefowl and the diversity of the present-day chickens in the archipelago.

Ultimately, prior probability distributions and soft bounds for calibration limit the effect of erroneous fossil calibrations (Ho & Phillips, 2009). Results from this study account for uncertainty in primary calibration constraints, models of molecular evolution, and tree topology, but this also suggests that deep-level calibration points (if available) likely bring important information.

4.5 Conclusion and Recommendation

In conclusion, this study provides a temporal evolutionary link between continental and island Southeast Asia chickens. The time-calibrated phylogenetic tree validated the presence of earlier ancestral matriline diversified in MSEA before island populations formed a distinct

subgroup. The coalescence time estimates of MSEA and ISEA chickens are consistent with their demographic evolution and expansion across the region, around 3,700-4,400 years BP and 2,000-2,500 years BP, respectively. Moreover, if additional fossil calibration data is available in the future, multi-calibrated molecular clock dating is recommended, to obtain a wider range of age estimates in chicken phylogeny.

4.6 Data availability

The compete mtDNA D-loop sequences are deposited and available in GenBank database (accession numbers: OM240181 - OM240549).

4.7 Acknowledgement

This work was supported by the Grant for Animal Research Overseas from the Institute of Animal Science and by the Monbukagakusho Scholarship (to C.J.P.G – No. 19372) of the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

4.8 Appendices

Other supplementary data for this study can be found in Chapter III.

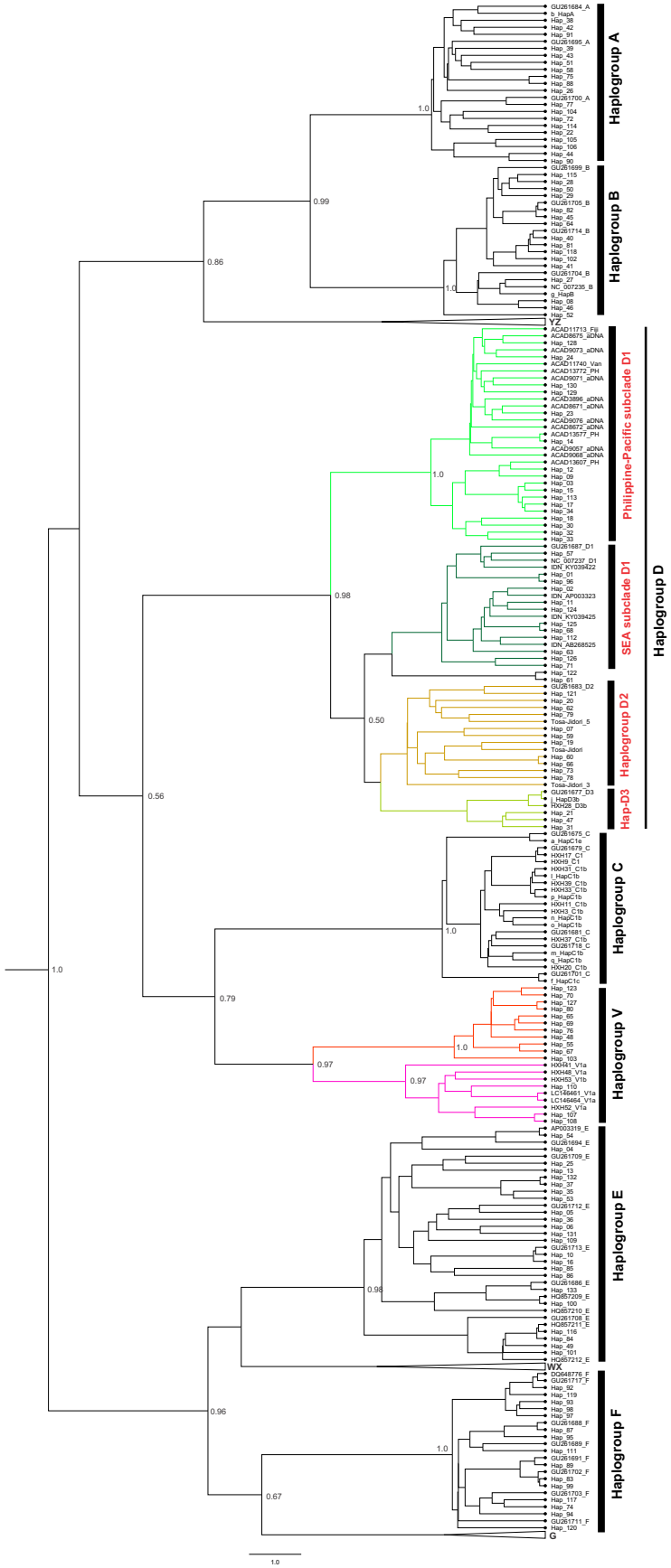
Appendix Table S4.1. Complete mtDNA control region sequences of Philippine and Indonesian red jungle fowl and their corresponding accession number (sequences adapted from Compendio, 2022)

Isolate name	Sampling site (region)	Country	Accession No.
PH.M1	Occidental Mindoro	Philippines	OL589006
PH.M2	Occidental Mindoro	Philippines	OL589007
PH.M3	Occidental Mindoro	Philippines	OL589008
PH.M4	Occidental Mindoro	Philippines	OL589009
PH.M5	Occidental Mindoro	Philippines	OL589010
PH.M6	Occidental Mindoro	Philippines	OL589011
PH.M7	Occidental Mindoro	Philippines	OL589012
PH.M8	Occidental Mindoro	Philippines	OL589013
PH.M9	Occidental Mindoro	Philippines	OL589014
PH.M10	Occidental Mindoro	Philippines	OL589015
PH.M11	Occidental Mindoro	Philippines	OL589016
PH.M12	Occidental Mindoro	Philippines	OL589017
PH.M13	Occidental Mindoro	Philippines	OL589018
PH.M14	Occidental Mindoro	Philippines	OL589019
PH.M15	Occidental Mindoro	Philippines	OL589020
PH.M16	Occidental Mindoro	Philippines	OL589021
PH.M17	Occidental Mindoro	Philippines	OL589022
PH.M18	Occidental Mindoro	Philippines	OL589023
PH.M19	Occidental Mindoro	Philippines	OL589024
PH.P10	Palawan	Philippines	OL589051
PH.P11	Palawan	Philippines	OL589052
PH.P12	Palawan	Philippines	OL589053
PH.P13	Palawan	Philippines	OL589054
PH.P14	Palawan	Philippines	OL589055
PH.P15	Palawan	Philippines	OL589056
PH.P17	Palawan	Philippines	OL589057
PH.P18	Palawan	Philippines	OL589058
PH.P19	Palawan	Philippines	OL589059
PH.P20	Palawan	Philippines	OL589060
PH.I1	Iloilo	Philippines	OL589029
PH.I2	Iloilo	Philippines	OL589030

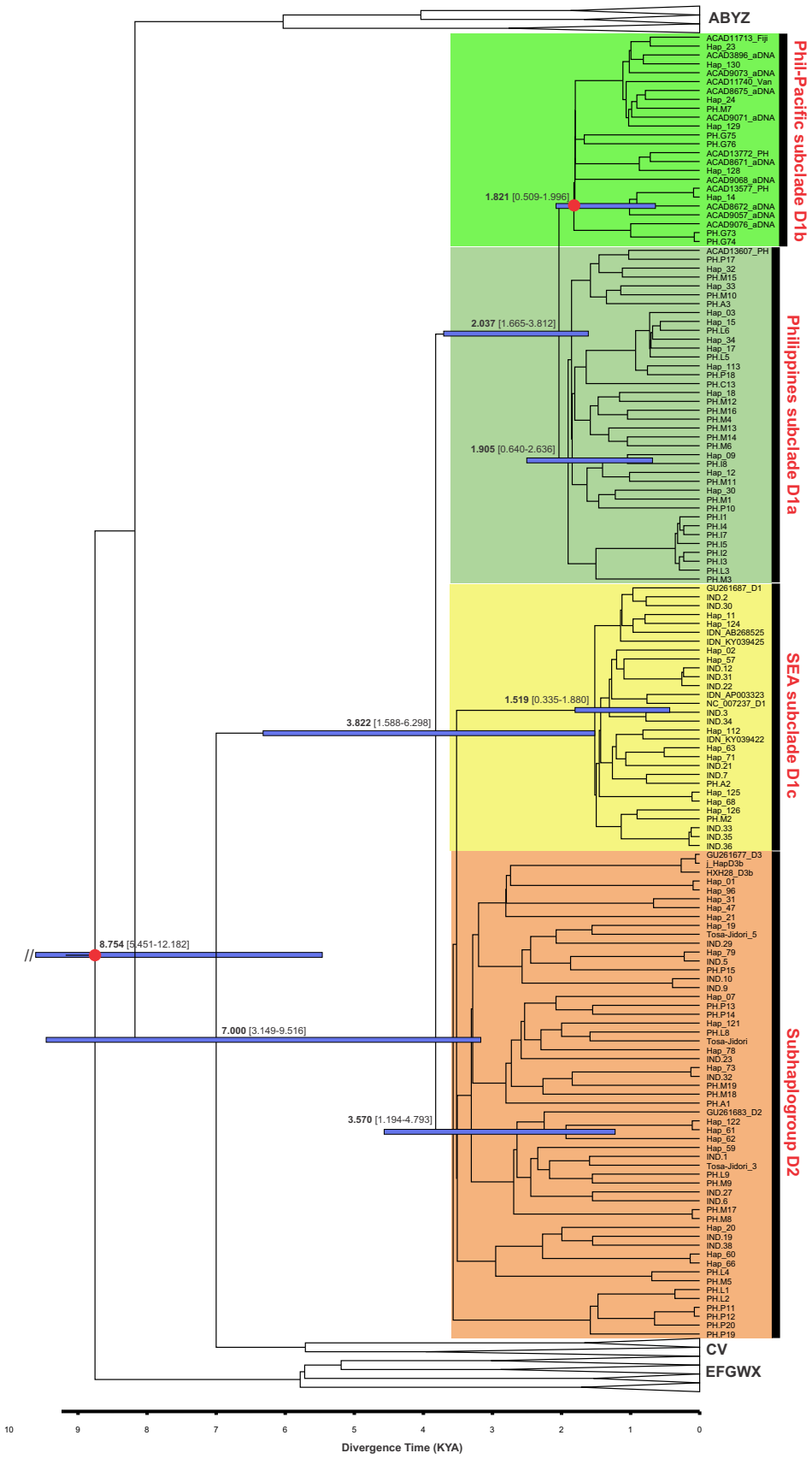
PH.I3	Iloilo	Philippines	OL589031
PH.I4	Iloilo	Philippines	OL589032
PH.I5	Iloilo	Philippines	OL589033
PH.I6	Iloilo	Philippines	OL589034
PH.I7	Iloilo	Philippines	OL589035
PH.I8	Iloilo	Philippines	OL589036
PH.C13	Capiz	Philippines	OL589037
PH.C14	Capiz	Philippines	OL589038
PH.L1	Leyte	Philippines	OL589043
PH.L2	Leyte	Philippines	OL589044
PH.L3	Leyte	Philippines	OL589045
PH.L4	Leyte	Philippines	OL589046
PH.L5	Leyte	Philippines	OL589047
PH.L6	Leyte	Philippines	OL589048
PH.L8	Leyte	Philippines	OL589049
PH.L9	Leyte	Philippines	OL589050
PH.G73	Guimaras	Philippines	OL589039
PH.G74	Guimaras	Philippines	OL589040
PH.G75	Guimaras	Philippines	OL589041
PH.G76	Guimaras	Philippines	OL589042
PH.A1	Agusan del Norte	Philippines	OL589025
PH.A2	Agusan del Norte	Philippines	OL589026
PH.A3	Agusan del Norte	Philippines	OL589027
PH.A4	Agusan del Norte	Philippines	OL589028
IND.1	Sulawesi	Indonesia	OM100841
IND.2	Sulawesi	Indonesia	OM100842
IND.3	Sulawesi	Indonesia	OM100843
IND.5	Sulawesi	Indonesia	OM100844
IND.6	Sulawesi	Indonesia	OM100845
IND.7	Sulawesi	Indonesia	OM100846
IND.9	Sulawesi	Indonesia	OM100847
IND.10	Sulawesi	Indonesia	OM100848
IND.12	Sulawesi	Indonesia	OM100849
IND.16	Sulawesi	Indonesia	OM100850
IND.19	Sulawesi	Indonesia	OM100851
IND.20	Sulawesi	Indonesia	OM100852
IND.21	Sulawesi	Indonesia	OM100853
IND.22	Sulawesi	Indonesia	OM100854
IND.23	Sulawesi	Indonesia	OM100855

IND.27	Sulawesi	Indonesia	OM100856
IND.29	Sulawesi	Indonesia	OM100857
IND.30	Sulawesi	Indonesia	OM100858
IND.31	Sulawesi	Indonesia	OM100859
IND.32	Sulawesi	Indonesia	OM100860
IND.33	Sulawesi	Indonesia	OM100861
IND.34	Sulawesi	Indonesia	OM100862
IND.35	Sulawesi	Indonesia	OM100863
IND.36	Sulawesi	Indonesia	OM100864
IND.38	Sulawesi	Indonesia	OM100865

Appendix Figure S4.1. Bayesian phylogenetic tree for complete mtDNA D-loop nucleotide sequences of SEA and Pacific chickens. Node labels correspond to posterior probability support. Posterior probability values under 50% are not shown.



Appendix Figure S4.2. Divergence times estimate of SEA chickens, Pacific chickens, and ISEA red junglefowl based on primary and secondary calibration using BEAST2 v2.6.6. Node labels indicate the median estimated divergence time and 95% HPDs.



CHAPTER V

GENERAL DISCUSSION

The development of agriculture over the past millennia was undeniably driven by several geological, ecological, biological factors, and human cultural exchanges (Bellwood, 2005; Larson et al., 2014). Such development provides almost all the world's food has occurred over the past thousand years and continues apace. Southeast Asia, the most geographically complex tropical region on Earth, has given rise to a diverse and highly endemic avifauna (Lohman et al., 2011; Myers et al., 2000). In particular, the Indo-Australian Archipelago (IAA) is an exceptional theatre for extraordinary species richness and endemism that exists in one of the most biologically rich and geologically dynamic regions in the world (Lohman et al., 2011). Two of the four leading biodiversity hotspots in the Malay Archipelago: Indo-Burma and the Philippines (Myers et al., 2000), are of biogeographically important for studying evolutionary history and phylogeography of chickens in the region.

The domestication of chickens seems to have a long history. The association of chickens to the agricultural societies from anthropophilic wildfowl attracted to kitchen scraps, animal dung, and crop-processing wastes, made this species a critical commensal domesticated animal (Larson & Fuller, 2014). Since their domestication, chickens have been dispersed globally across multiple cultural and religious boundaries. Several DNA sources and molecular strategies were used to resolve chicken phylogeny and their genetic expansion from their wild progenitors. Pioneering molecular studies based on the hypervariable region (Liu et al., 2006), complete mtDNA control region (Miao et al., 2013; Oka et al., 2007), mitogenome (Huang et al., 2018; Miao et al., 2013), and whole-genome data (Lawal et al., 2020; Mariadassou et al., 2021; Wang et al., 2020) provided important insights in resolving the chicken phylogeny. However, topological discrepancies have also been documented, often explained by differences in data sources and taxon sampling (Lawal et al., 2020; Mariadassou et al., 2021; Reddy et al., 2017).

Here, I summarize the significance of the findings based on the updated understanding of the evolutionary history of *Gallus gallus* in Southeast Asia and the South Pacific and the corresponding genetic insights into its phylogeography and population dynamics.

Aim 1 & 2: Characterize the genetic diversity, evolutionary history, and phylogeography of a finer mtDNA dataset for RJFs and domestic chickens in ISEA and South Pacific

The rich history of colonization in the ISEA provides interesting records of earlier agricultural populations in the Malay Archipelago (Bellwood, 2007; Piper, 2017). The Philippines presents essential models for understanding Southeast Asia's evolutionary processes and species diversification. This terrestrial island faunal laboratory is considered one of the most biologically rich regions globally in animal genetic resources and offers opportunities for elucidating evolutionary and ecological processes (Brown et al., 2013; Myers et al., 2000).

The two-wave hypothesis of peopling in the ISEA provides varying interpretations of the prehistoric evolution of the indigenous populations in the insular (Jinam et al., 2012). Human genetic data documented an earlier introduction from MSEA to the insular, predating the mid-Holocene Taiwan-centered Austronesian speakers migration model (Arenas et al., 2020; Jinam et al., 2012; Lipson et al., 2014; Soares et al., 2016). Evidence of diverse migration routes and dispersal events documented rapid human population expansion in the Philippines and the Pacific (Arenas et al., 2020; Bellwood, 2007). These human movement scenarios linking domestic animal translocation present broad interests in understanding the chicken domestication events in the ISEA.

Therefore, this study (Chapter II) extensively characterizes complete mtDNA D-loop sequences of native chickens from the Philippines and the South Pacific and Philippine RJFs to assess their matrilineal phylogeny and genetic diversity, and population genetic structure across ISEA and Oceania. In addition, existing complete mtDNA D-loop sequences archived

in the GenBank repository were also included in the analyses. This assembled mtDNA D-loop dataset, which contains wild junglefowl (RJF) sequences, provide finer resolution to the existing knowledge about the evolutionary history and phylogeography of ISEA chickens. The molecular resolution of this study outweighs the previous genetic characterization studies using partial sequences of the mtDNA control region.

The genetic information within the complete mtDNA D-loop region reveals the high genetic diversity of Philippine chickens across ISEA and Oceania. The high genetic diversity in Philippine chickens resulted from abundant haplotype signatures in the predominant haplogroup D, which points out that the population is large, expanding, and have undergone *in situ* diversification. A finding that was not fully explored using the complete information of the control region until recently. The phylogenetic analysis and median-joining network reveal a predominant haplogroup D throughout the population. This confirms previous genetic evidence that haplogroup D is the maternal lineage primarily concentrated in the ISEA-Pacific region (Liu et al., 2006; Miao et al., 2013) and distinctively traced as a specific signature for the Pacific sequence motif potentially found in the Philippines (Thomson et al., 2014). Even the analysis from the truncated 764 bp mtDNA D-loop region, purposely done to accommodate broadly available sequences of Indonesian chickens (Herrera et al., 2017), resulted in two divergent subclades within the ISEA sub-haplogroup D1. The divergence pattern of the Philippine-Pacific subclade harbored two mutational diagnostic motifs, C296T and G686A, while undetected in the Philippine-Indonesian subclade. Another interesting finding from this study shows the Philippine red junglefowls at the basal position of the tree (within Haplogroup D|), indicating an earlier introduction into the Philippines, potentially via mainland Southeast Asia.

Although an archaeological record of chicken bone in the Philippines is yet to be explored, mtDNA evidence of the Philippine-Pacific subclade provided strong inference for

the Philippine origin of Pacific chickens, especially ancient and modern Pacific haplotype D (Polynesian motif) chickens (Thomson et al., 2014) clustered along with the Philippine chickens forming a subclade. The result of multivariate ordination using all haplogroups reveals broad geographical population structure across Asia and Oceanic regions. Again, close genetic relatedness between Philippine and Pacific chickens is observed, while the latter is distantly linked to Indonesian and MSEA chickens. Meanwhile, there is no significant subgrouping among chicken populations in the Greater and Lesser Sunda Islands in all haplogroups. Low genetic differentiation among chicken populations in the Philippines and the Pacific is indicated by the analysis of molecular variance and populations pairwise F_{ST}, supporting genetic relatedness between two geographically isolated populations. The past population expansion signatures of Philippine chickens (haplogroup D) validated our previous findings, inferring the contributions of Philippine chickens to the genetic characteristics of the Pacific chickens. The negative and significant Fu's *Fs* statistical test (Fu, 1997) provided strong evidence for the past population growth of Philippine chickens (haplogroup D) in the ISEA.

Interestingly, the BSP analysis indicated demographic expansion of the Philippine chickens around 3,000-2,500 years BP, predating the recovered ancient DNA samples of Pacific chickens in the Anatoloa site, Niue Island and the Anakena site, Rapa Nui (~1,200-600 BP) (Thomson et al., 2014). This major finding corroborated the eastward expansion of the Austronesian speakers from the Philippines before reaching the Pacific region. Overall, the Philippine-Pacific subclade is congruent with the evidence of increased maternal effective population size of Philippine chickens while concordant with the demographic signals imprinted in DNA genealogies and timing of introduction brought by human dispersal.

Aim 3: Elucidate finer resolution of the matrilineal phylogeny and population dynamics of *Gallus gallus* in Southeast Asia

The course of chicken introduction into the ISEA continues to be a research area of interest, considering the rich history of human diaspora and colonization in the insular archipelago. The consensus from several molecular studies understanding the complex geographic and temporal origins of chicken domestication suggested that the domestication process started in Southeast Asia and was drawn to rice farming (Peters et al., 2022; Wang et al., 2020; West & Zhou, 1988). The distribution of maternal lineages signature brought by the movement of earlier agricultural societies led to the emergence of domestic populations becoming more genetically divergent (Larson & Burger, 2013). However, an important unresolved question remains whether the founding lineages of chickens introduced in the ISEA (particularly, the Philippines) arrived as wild or descendants of wild endemic populations that potentially entered the archipelago during lowered sea levels through the Sunda shelf. Alternatively, they are descendants of domestic chickens from MSEA that have undergone feralization.

To address this research question, Chapter III characterizes large-scale mtDNA sequences of chickens from MSEA (Cambodia, Laos, Thailand, and Myanmar), the Philippines, and the Pacific, spanning a geographical transect encompassing possible translocations of this domesticate in the region. This study combines the available mtDNA sequence data of ISEA chickens, Pacific chickens, and neighboring chicken populations in Asia. Furthermore, this study seeks to obtain an updated perspective of the matrilineal phylogeny and demographic events that shaped the genetic diversity of SEA and Pacific chickens.

Chapter III presented a comprehensive resolution of mitochondrial lineage diversity and phylogenetic analyses, population differentiation, and demographic inference of chickens in Southeast Asia and the Pacific region. Patterns of sequence variation indicate that chickens in the MSEA have higher intrapopulation genetic diversity than the island populations. The substantial diversity of SEA chickens reflects the high matrilineal genetic variation in the major

haplogroups, particularly haplogroup D with many divergent haplotypes, and haplogroup V which has been detected only in Thai, Cambodian, and Laotian chickens. Divergent sub-haplogroups that retained ancestral variations were also observed in these lineages, likely due to geographical proximity to the center of domestication. Strong topological supports from the phylogenetic trees consistently provide evidence for haplogroup D ancestral lineage (i.e., sub-haplogroup D2) from MSEA populations. A new matrilineage (i.e., sub-haplogroup V2) gave rise to the population of domestic chickens from Cambodia, Laos, and Thailand, with their ancestral lineage (i.e., sub-haplogroup V1) detected in Thai RJF, *G. g. gallus* subspecies.

Interestingly, this potential ancestral matriline sub-haplogroup D2 and newfound matriline haplogroup V were identified in sampling areas along the Lower Mekong subregion, for example, in Kampong Cham, Mondulkiri, Stung Treng, and Kratie provinces in Cambodia and Champasak and Bolikhamsai provinces in Laos. Within the native range of red junglefowl, Collias & Saichuae (1967) closely observed the bird's ecological habitat, which is drawn to the occurrence of primitive agriculture and converted primary forest. The bird also thrives and populates in the bamboo forest with lower elevation as well as near water holes or streams. The same observation was documented by Giles (1932), that the migratory junglefowl has been sighted in the areas closer to the Mekong River, apparently attempting to cross it: *"In crossing, the birds fly up as high as they can go, and then attempt to glide across… This movement does not seem to be caused by a lack of food as the birds are extraordinarily plump and in good condition. It is not easy to understand why it is taking place, as conditions on both sides of the Mekong seem the same"*. The favorable climatic conditions and vegetation in this area are suitable for the red junglefowl (and its earlier descendants) to diversify and expand their distribution within its native range (Higham, 1989; Peters et al., 2016; West & Zhou, 1988). It is evident from the molecular genetic characterization of these populations in Thailand and areas along the Lower Mekong subregion in Cambodia and Laos that divergent sub-

haplogroups and recently classified haplogroups retained ancestral mutational motifs indicating geographic proximity to the center of domestication.

The coalescent-based Bayesian demographic analyses detected earlier effective population size expansion in MSEA chickens, while island populations showed more recent demographic growth signatures. The timing of the demographic evolution of this hypothesized founding population can be explained by the cultural importance of stock-raising in the MSEA as early as 4,000 years BP (Higham, 1989). It was well documented that agriculture and animal-raising were among the subsistence activities of the domestic communities during prehistoric settlements in the broad valleys of the Lower Mekong (Higham, 1989). This study validated the unique population dynamics of Southeast Asian chickens, implying a large gene pool that has been conserved in the populations for a long time and that some were a subset of the RJF population involved in the domestication process. This suggests that island chickens are potentially descendants from populations domesticated in MSEA and diverged into distinct subgroups following colonization in the island archipelago. The phylogeographic dispersal of the Philippine-Pacific sub-haplogroup D1b, which diversified in the Philippine archipelago, likely corresponded to the initial introduction pattern of its ancestral matrilines (domesticated ancestors from MSEA that went feral) and admixed with wild RJF reservoir matriline that was already inhabited the archipelago after colonization. This translocation pattern may have been influenced by the numerous waves of human migration to the Philippines brought by the Manobo ancestry and the Sama ancestry into the southern Philippines and Palawan, as they showed high genetic relatedness to MSEA-affiliated populations (Larena et al., 2021). The genetic characterization of this large population study in SEA at the complete mtDNA D-loop scale has expanded our understanding of the demographic history and population dynamics of chickens in one of the hottest biodiversity hotspots on Earth.

Aim 4: Estimate the lineage-specific divergence time between the continental and island chicken populations

The zoogeographical argument against the timing of the introduction of continental chicken populations into the island archipelago steers hypotheses whether the Philippine chickens can be considered the same as the nominate race from MSEA or established as an indigenous race. Parkes (1962) initially suggested that the Philippine chickens were introduced birds from a subspecifically differentiated population somewhere on the mainland about 3,000 years ago. However, little is known about the evolutionary links and temporal divergence between continental and island chickens, especially since archaeological records of chicken bones in the region are scarce. Furthermore, the availability of presumed ancestral matrilineage from the genetic material of their closest wild RJF progenitor rather provides definitive clues to their domestication process since the antiquity of chickens in the archipelago is rare. With the increasing genetic data and resolving power of sequence data in recent years, computer simulation methods (e.g., coalescence simulation) have been shown efficient at testing different evolutionary and demographic models of expanding and migrating populations. Although mtDNA-based studies are unlikely to reflect the complex past demographies completely, the increasing worldwide mtDNA data set remains valuable and has been extensively used in investigating the chicken populations, types, evolutionary relationships, and domestication history.

Chapter IV estimates the lineage-specific divergence of MSEA, ISEA, and Pacific chickens using the Bayesian molecular clock method. The accuracy of inferences about the evolutionary process of chickens in Southeast Asia (particularly in ISEA) is significantly refined by comprehensive taxon sampling efforts and a broad sampling of characters in biogeographically important regions. The accessibility of important character samples continues positively impact phylogenetic analyses and the subsequent congruence among estimated trees (Reddy et al., 2017). The time tree phylogeny in the coalescent framework

estimates the nodal ages of biogeographically important haplogroups predominant in Southeast Asia. Phylogenies with absolute divergence times provide incomparably richer information than a species phylogeny without a temporal clue, as they make it possible for species divergence or coalescence events to be calibrated to time (dos Reis et al., 2016).

A robust phylogenetic tree of SEA and Pacific chickens and RJF individuals sampled from the Philippines and Indonesia (Compendio, 2022) validated the dispersal event of the Philippine-Pacific D1b lineage to the Pacific chicken population. This classified island matriline experiences *in situ* diversification with their founding population (herein the Philippines subclade D1) in the Philippines before expanding eastward. Most of the red junglefowls from Palawan and Mindoro clustered with the ancestral Hap-D lineage (Haplogroup D2), suggesting that the Philippine red junglefowl descended from this founding population in mainland SEA and went exoferal or admixed after colonization. Likewise, no Indonesian red junglefowls are detected with close genetic affinity to the Pacific chickens. Instead, the Indonesian red junglefowl formed a subgroup with the *G. g. bankiva* (NC_007237) and Cambodian indigenous chickens, suggesting that the Javan red junglefowl likely the founding population of the SEA subclade D1.

The coalescence time estimates of haplogroups D and V are consistent with their demographic evolution and regional expansion, around 3,700-4,000 years BP and 3,800-4,400 years BP, respectively. The hypothesized founding matriline within haplogroup D (i.e., sub-haplogroup D2) revealed an earlier coalescence age estimate in the D-lineage. Evidence of recently classified haplogroup V remains a geographically restricted clade inhabited in mainland Southeast Asia. Likewise, the most recent common ancestor of modern Philippine-Pacific chickens (i.e., sub-haplogroup D1b), including the ancient Pacific sequences, dates to 2.1 kya (95% HPD 1,467–2,815 years). However, this study acknowledges the findings should be interpreted with caution because different data sources (i.e., taxon sampling), types of

molecular data, and methods often based on different hypotheses (e.g., different calibration points) may introduce estimation biases which lead to a compromised conclusion. Furthermore, the coalescence age estimate of gene copies in ancestral populations is not equivalent to a population split, nor does it represent the actual onset of domestication.

The greater genetic variation observed within the haplogroup D likely represents a diverse admixed populations of both feral and descendants of wild endemic junglefowl that locally interbreed with once introduced populations. Morphological assessment posits possibilities of admixed ancestry of ISEA chickens, particularly in the Philippine red junglefowl, unveiling the coexistence of *G. g. gallus* and *G. g. bankiva* subspecies in the archipelago (Compendio, 2022). Meanwhile, Indonesian chickens also appeared to have multiple maternal lineages and admixed populations (Ulfah et al., 2017), consistent with the hypothesis that the descendants of ancestral RJF or the introduced domestics are interbreeding in the island archipelago. Conclusively, this present study suggests that geographical isolation, colonization, and independent admixture have significantly shaped the genetic architectures of the founding Philippine red junglefowl and the diversity of the present-day chickens in the archipelago.

All the genetic resources obtained in this study are archived in a publicly available data bank (i.e., GenBank) to ensure the accessibility and utilization of these sequences. In congruence to the Sustainable Development Goal (SDG) 15 of the United Nations (UN): Life on Land, Target 15.6, which promote fair and equitable sharing of the benefits arising from the utilization of genetic resources and promote appropriate access to such resources, as agreed internationally. All mtDNA D-loop sequences used in each study can be accessed in the following GenBank accession number: MN986370-MN986403; OM240181 - OM240549.

CHAPTER VI

Research Summary

Over the past millennia, the development of agriculture in Southeast Asia, ultimately brought by human forethought and activities, was undoubtedly driven by several geological, ecological, biological, and climatic factors and cultural exchanges. The multistage processes of animal domestication exemplify how animals respond to the anthropogenic niches. Decades of research on when, where, and how domestication took place have led to a better understanding of the complex past societies, though several important questions remain unresolved.

As one of the important commensal domesticates, chickens are the most widely domesticated animal species globally. Consequently, it plays a crucial role in human societies as the largest source of animal protein and as a significant biological factor in socio-cultural development. Since domestication, chickens have been distributed throughout various countries and continents inhabited by human migration and trade expansion. This led to the evolution of subpopulations of chickens in response to natural selection pressure and selective breeding for adaptation to a variety of agro-ecological conditions and subsequently resulted in a wide range of chicken breeds today.

The course of chicken introduction in the island of Southeast Asia (ISEA) and Oceania continues to be a research area of interest, considering the rich history of human diaspora and colonization in the insular archipelago. The Philippines presents essential models for understanding Southeast Asia's evolutionary processes and species diversification. This terrestrial island faunal laboratory is considered one of the most biologically rich regions globally in animal genetic resources and offers opportunities for elucidating evolutionary and ecological processes. However, insufficient evidence links the present-day chickens to their founding lineages due to an unclear timeline of translocations and routes of dispersal across the ISEA.

To address this research gap, Chapter II extensively characterized the complete mitochondrial DNA (mtDNA) D-loop sequences of native chickens (NCs) from the Philippines and the South Pacific and Philippine red junglefowl (RJF) to assess their matrilineal phylogeny and genetic diversity and population genetic structure across ISEA and Oceania. The phylogeny reconstruction and estimation of their population genetic structure were based on 107 newly generated mtDNA complete D-loop sequences, consisting of 34 haplotypes. This study found that the Philippine chickens showed high haplotypic diversity (*Hd*=0.915± 0.011) across Southeast Asia and Oceania. The phylogenetic analysis and median-joining network revealed predominant maternal lineage haplogroup D classified throughout the population. Concurrently, support for the Philippine-Pacific subclade was evident, suggesting a founding lineage of the Philippine chickens before diverging to the Pacific chicken populations. This study also significantly estimated the Philippine red junglefowl at the phylogenetic tree's basal position, suggesting an earlier introduction into the Philippines, potentially from mainland Southeast Asia. The extremely low genetic differentiation and high rate of gene introgression of the Philippine chickens into the Oceanic populations suggests an expansion signal. Furthermore, this study assessed their demographic signature based on Bayesian Skyline Plot analysis and demonstrated an increase in the maternal effective population size of the Philippine chickens around 3,000-2,500 years BP. This population expansion signal likely relates to the human settlement and expansion events of the Austronesian agricultural societies in the Philippines sometime in the past.

Subsequently, the unresolved question remains whether the founding lineages of chickens introduced in the Philippines arrived as wild or descendants of wild endemic populations that potentially entered the archipelago during lowered sea levels through the Sunda shelf. Alternatively, they are descendants of domestic chickens from mainland Southeast Asia (MSEA) that have undergone feralization. To provide context to this conjecture, Chapter

III characterized large-scale mtDNA sequences of chickens from MSEA (Cambodia, Laos, Thailand, and Myanmar), the Philippines, and the Pacific, spanning a geographical transect encompassing possible translocations of this species in the region. This study combined these newly generated sequence data with previously published data of ISEA chickens, Pacific chickens, and neighboring chicken populations in Asia. Furthermore, this study sought to obtain an updated perspective of the matrilineal phylogeny and demographic events that shaped the genetic diversity of SEA and the Pacific chickens.

The consensus from several molecular studies documented domestic chickens evolved from RJF somewhere in southwestern China and Southeast Asia. However, identifying their exact geographic center of origin and consequent translocation to the island archipelago has been challenging. Chapter III presented a comprehensive resolution of mitochondrial lineage diversity and phylogenetic analyses, population differentiation, and demographic inference of chickens in Southeast Asia and the Pacific region. Patterns of sequence variation indicated that chickens in the MSEA region have higher intrapopulation genetic diversity (Hd=0.963 ± 0.005; π=0.00782 ± 0.00398) than island populations (Hd=0.942 ± 0.009; π=0.00466 ± 0.00249). The substantial diversity of SEA chickens reflects the high matrilineal genetic variation documented in the major haplogroups, particularly haplogroup D with many divergent haplotypes and haplogroup V, which has been detected only in Thailand, Cambodia, and Laos. Divergent sub-haplogroups that retained ancestral mutational motifs were also observed in these lineages, likely due to the geographic proximity to the center of domestication. Strong topological supports from the phylogenetic trees consistently provide evidence for haplogroup D ancestral lineage (i.e., sub-haplogroup D2) from MSEA populations. A new matrilineage (i.e., sub-haplogroup V2) gave rise to the population of domestic chickens from Cambodia, Laos, and Thailand. Likewise, their ancestral lineage (i.e., sub-haplogroup V1) was represented in the Thai RJF (i.e., *G. g. gallus*).

Interestingly, this potential ancestral matriline sub-haplogroup D2 and newfound matriline haplogroup V were identified in sampling areas along the Lower Mekong subregion, for example, in Kampong Cham, Mondulkiri, Stung Treng, and Kratie provinces in Cambodia and Champasak and Bolikhamsai provinces in Laos. The coalescent-based Bayesian demographic analyses detected earlier effective population size expansion in MSEA chickens, while island populations showed more recent demographic growth signatures. The timing of the demographic evolution of this hypothesized founding population can be explained by the cultural importance of stock-raising in the MSEA as early as 4,000 years BP. It was well documented that agriculture and animal-raising were among the subsistence activities of domestic communities during prehistoric settlements in the broad valleys of the Lower Mekong. This study validated the unique population dynamics of Southeast Asian chickens, implying a large gene pool that has been conserved in the populations for a long time and that some were a subset of the RJF population involved in the domestication. This suggests that island chickens are potentially descendants from populations domesticated in MSEA and diverged into distinct subgroups following colonization in the island archipelago.

The earlier domestication of chickens in mainland Southeast Asia and consequent translocation to the island archipelago entering southern Philippines and Palawan are deliberately linked with human movement. However, little is known about the evolutionary links and temporal divergence between continental and island chickens, especially since archaeological records of chicken bones in the region are scarce. With the increasing genetic data and resolving power of sequence data in recent years, computer simulation methods (e.g., coalescence simulation) have been shown efficient at testing different evolutionary and demographic models of expanding and migrating populations. Furthermore, time trees or phylogenies with absolute divergence times provide incomparably richer information than a

species phylogeny without a temporal clue. It makes it possible for species divergence or coalescence events to be calibrated to time.

Chapter IV estimates the lineage-specific divergence of MSEA, ISEA, and Pacific chickens using Bayesian molecular clock method. The time tree phylogeny in the coalescent framework estimates the nodal ages of biogeographically important haplogroups predominant in Southeast Asia. The coalescence time estimates of haplogroups D and V are consistent with their demographic evolution and expansion in the region, around 3,700-4,000 years BP and 3,800-4,400 years BP, respectively. Likewise, the most recent common ancestor of modern Philippine-Pacific chickens (i.e., sub-haplogroup D1b), including the ancient Pacific sequences dates to 2.1 kya (95% HPD 1,467–2,815 years). Caution is warranted for this interpretation because the coalescence age estimate of gene copies in ancestral populations is not equivalent to a population split, nor does it represent the actual onset of domestication.

In conclusion, this study provides a comprehensive insight into the genetic diversity, phylogeography, and population dynamics of Southeast Asian chickens. High-resolution matrilineal phylogeny sheds new light on the evolutionary history of globally acknowledged haplogroups of SEA and Pacific chickens. It provides evidence of a new divergent matrilineage that is distributed across its native range in the Lower Mekong subregion. This study documented the presence of a distinct island chicken subgroup that represents a unique genetic uniformity between the Philippines and the Pacific despite their geographical isolation. This latterly expanded matriline is unique to the island archipelago, suggesting a human-mediated scenario on their translocation. Moreover, their phylogeographic signal corresponds to the initial introduction pattern of its founding matrilines (i.e., sub-haplogroup D2) from MSEA. The genetic information of this valuable animal resource is essential for conservation efforts, and these data serve as a baseline for monitoring to avoid further loss of genetic diversity.

Finally, this asserts excellent potential for genetic improvement and selection of traits for developing sustainable chicken production systems in Southeast Asia.

CHAPTER VII

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