Chicken Maternal Lineage Retained Long Historical Relationship Between Zanzibar and Oman

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Abstract

The aim of this study is to appreciate the long historical relationship between Zanzibar and Oman, through the investigation of maternal lineage of chickens found in Zanzibar and Oman. Earlier traders and explorer from Arabia, Persian Gulf, West India and China probably visited Zanzibar as earlier as the 1st Century AD. Oman in Southern Coast of the Arabian Peninsula at the Persian Gulf played a tense relationship between seafaring and commercial people in Indian Ocean. Furthermore, the history of Zanzibar is directly linked to Oman, after Oman Empire expelled and ended the Portuguese dominance of the Indian Ocean trade routes. In 1650 Oman becomes one of the main maritime and mercantile powers in the Persian Gulf and in the Indian Ocean. The sultans of Oman ruled over a substantial part of the Swahili Coast along the Indian Ocean from 1689-1856, controlling elaborate trade routes and cash crop plantations in East Africa. In the mid-1800s, they moved their seat of power from Muscat, Oman, to Stone Town, Zanzibar, and ruled as a constitutional monarchy. This historical relationship can be traced from maternal lineage of chickens that currently exist in Zanzibar and Oman. The mitochondrial genome has been the most widely used system for the investigation of the evolutionary history of species. The high rate of sequence divergence and its uniparental, maternal inheritance can retain evolution relationship as genetic fossils. The Phylogenetic network and Medial-Joining network analysis revealed strong association of evolution relationship between chicken ecotypes from Zanzibar and Oman. The prominent ancestral haplogroups indicated strong association of these chicken populations that were descended from the common ancestry. The Maritime trade interactions and consequences of Oman sultanate regimes in Zanzibar could significantly contributed to the ancestral relationship that existing today between Zanzibar and Oman Chickens.

Keywords: Zanzibar, Oman, Mitochondrial DNA, Phylogenetic network, Medial-Joining network

Introduction

Zanzibar is an autonomous part of the United Republic of Tanzania that counted with famous tropical Spice Islands and a world heritage site, located merely twenty miles off the east coast of Africa. Zanzibar is a popular archipelago in the Indian Ocean that consists of two main islands of Unguja and Pemba, together with many other smaller islands. Zanzibar played significant roles in the pre-historical activities on east coast of Africa due to its access to traders and several adventurers comes to exploring Africa continent. The islands of Zanzibar were prime gate way to East Africa and strong base for traders voyaging between the African Great Lakes, the Somali Peninsula, the Arabian Peninsula, Iran, and the Indian subcontinent (Shariff, 2018). Occupants of coastal and islands of Swahili coast in East Africa were involved in long distance trade with the Indian Ocean world during the later first millennium CE (Prendergast *et al.,* 2017a). Throughout this time, they used monsoon winds to sail across the Indian Ocean to land at the sheltered harbour located on the site of Zanzibar. The first mention of Zanzibar comes from the Greek mariner's guide, Periplus of the Erythraean Sea, which was written around 50 AD. Referred to as Menouthais, the island described as being 'low and wooded, in where there are rivers and many kinds of bird and the mountain tortoise'. According to the 16th

Century explorers, Zanzibar (Zanjibār in Arab and Zang-bār in Persian) was referred by Arabs and Persians as East African Coast that running from Kenya to Mozambique.

The long historical relationship between Zanzibar and Oman, currently can evidently be observed in islands of Zanzibar from old artefacts', historical architectural, vintage furniture and crafts, food, agricultural, language, cultural practices and affinity relationship among Zanzibarian and Omanian people. Oman is a country located on the Southern Coast of the Arabian Peninsula at the confluence of the Persian Gulf and Arabian Sea in Western Asia. It shares land borders with Saud Arabia, the United Arab Emirates and Yemen, and it shares maritime borders with Iran and Pakistan. Oman is the oldest independent state in the Arab world that has been ruled by the Al-Said family since 1744 and form Al Busaidi dynasty in the Sultanate of Oman until to date. The Omani Sultanate was a major maritime force from 17th to 19th centuries, which established its dominance in the Indian Ocean sea routes after defeating the Portuguese. Muscat and Oman extended its conquests to Zanzibar, other parts of the eastern coast of Africa and portions of the southern Arabian Peninsula.

From the late 17th century, the Oman Sultanate was a powerful empire which has much influence in the Persian Gulf and Indian Ocean. Oman Empire becomes a powerful state that take control on maritime trade all along from vast strips of southwest Iran, most of the north-eastern and southern parts of the Arabian Peninsula, and much of the Horn of Africa coast and all the way down till Northern Mozambique (Vernet, 2009; Shariff, 2018). In the mid of 17th century Zanzibar becomes part of Oman territory and started to be fully controlled by sultanate regimes from Oman until mid of 20th Century. From 1698, Zanzibar was under the control of the Sultanate of Oman Saif bin Sultan, the Imam of Oman who started to strengthen and developed an economy of trade, infrastructures and cash crops in the Zanzibar islands. The clove, originating from the Moluccan Islands (Indonesia), was introduced in Zanzibar by the Omani sultans. The plantations of spices were later intensified and hence moniker of the Spice Island name.

Sayyid Said Bin Sultan Al-Bu Said was the last ruler of the united Omani Empire from 1806 to June 1856 (Srinivasan, 1998). He appreciably turned his great attention to East Africa for the purpose of establishing political and economic power in the region. By the year 1828, Said bin Sultan succeeded to create a flourishing commercial empire along the East African coast, in which made Zanzibar the principal power in East Africa and the commercial capital of the western Indian Ocean (Rhodes *et al.,* 2015). In 1840 he officially moved his capital from Muscat to Stone Town in Zanzibar Island, which also allows him to reinforce the security and administration of his territories on the East African coast (Al-Farsi, 1989). Said bin Sultan allows this archipelago to become the principal port on the East African coast and economic centre of the Arab Trades that favoured from its geographical location (Srinivasan, 1998). During his ruling time, Zanzibar received a lot of influence and interactions from Oman. His regime emphasized on the massive growth of spices in the Islands and made Zanzibar to be the centre of the profitable spice trade between countries and regions bordering the Indian Ocean. Said bin Sultan ordered the landowners of the islands of Unguja and Pemba to cultivate cloves, which eventually became an economic mainstay of Zanzibar (Al-Farsi, 1989). By the 1860s, Zanzibar had become the world's major producer of cloves (Fage and Roland, 1976).

Said bin Sultan introduced liberal customs policies to attract foreign merchants at Swahili coast in East Africa. He also allowed Indians to trade in Zanzibar, who brought the administrative and financial skills that were essential in developing a trading centre (Sheriff, 1971). The Island emerged as an inter-port for international trade for having deep and wider harbour, with well suited to monsoon winds directions that facilitate the linkage of maritime routes between East African coast, Persian Gulf and India (Sheriff, 1987). Furthermore, Zanzibar offered a protected and defensible harbour, conducive environment for trade, favourable climate, and fertile soil. These features would enhance Zanzibar's attractiveness as a trading centre and farming, thereby significantly contributing to Said's wealth (Al-Farsi, 1989). After the death of Said bin Sultan in 1856, the empire was divided between his sons into two sultanates, where Sultanate of Zanzibar that occupied African section was ruled by Majid bin Said, and Sultanate of Muscat and Oman on the Asian section ruled by Thuwaini bin Said.

The existed long relationship between Oman and Zanzibar might allow the exchange of domestic animals. Zooarchaeological studies in Zanzibar tracing earlier introduction of Zebu cattle (*Bos indicus*) and domestic chickens (Gallus gallus) with Asian taxa (Horton, 1996; Chami 2001; Lyimo *et. al.,* 2013; Boivin *et. al.,* 2013). Several studies shows the spread of chickens around the world are intimately linked to the movement of people, since chickens are not migratory birds (West and Zhou, 1998; Tixier-Boichard *et al.,* 2011; Storey *et al.,* 2012; Thomson *et al.,* 2014, Lyimo *et al.,* 2015). Interestingly, several genetic studies using mitochondrial DNA (mtDNA) that accessed the dispersal pattern of chickens from their centres of origin, have shed light on prehistoric human migration, trade routes, and cross cultural diffusion (Gongora *et al.,* 2008; Muchadeyi *et al.,* 2008; Razafindraibe *et al.,* 2008; Dana *et al.,* 2010; Storey *et al.,* 2012; Peters *et al.,* 2016).

Earlier traders and explorer from Arabia, Persian Gulf, West India and China probably visited Zanzibar as earlier as the 1st Century AD. The Arabian Peninsula has played an important role in the dispersal of commodities, plants, crops and animals between India and Africa (Boivin *et al.,* 2010; Groucutt and Petraglia, 2012). They used monsoon winds to sail across the Indian Ocean and landed at the sheltered harbour in Zanzibar. Different scholars reported that domestic chicken in Zanzibar, were introduced during maritime exchanges (Boivin *et al.,* 2014; Prendergast *et al.,* 2017b; Crowther *et al.,* 2018). The aim of this study is to examine the relationship of maternal lineage between Zanzibar and Oman chickens that could be associated with the long interaction between these two countries. Mitochondrial DNA sequences can be used to estimate phylogenetic relationships among individual taxa and perform molecular phylogenetic evolution analysis (Vogel *et al.,* 2011; Zhang and Zhang, 2013). The evolution relationship of individual could be traced from the control region of mitochondrial DNA (D-loop region), which has higher evolutionary rate compared to genomic DNA. The mutations that accumulate sequentially along maternal lineages allows associating them with populations from different geographical regions of the world (Ingman *et al,.* 2000; Herrnstadt *et al.,* 2002).

Methodology

A total of 138 mtDNA sequences of eight ecotypes from Zanzibar and Oman chicken were studied. Two ecotypes of Pemba (PEMB) and Unguja (UNGJ) were named after their main islands of Zanzibar (Msoffe *et al.,* 2004; Lyimo, *et al.,* 2013), and six ecotypes of Oman chickens, that is Musadam (MU), Batinah (BT), North Hajar (NH), East Hajar (EH), East Coast (EC) and Dhofar (DF) were sampled and named after major agroecological zones existed in Oman (Al-Qamashoui *et al.,* 2014a).

Mitochondrial DNA Sequences

For the six populations of Oman chickens, DNA was extracted using standard silica-column based commercial kits (D-Neasy Blood & Tissue Kit, Qiagen, UK). A 550 bp fragment from mtDNA D-loop region was amplified by PCR using two primers L16750 (5'-AGGACTACGGCTTGAAAAGC-3) and H522 (5'-ATGTGCCTGACCGAGGAACCAG-3'). PCR were performed in a 25 µl volume [1x reaction buffer, 75mM MgCl2, 5mM of each dNTP, 10pM of each primer, and 1U of Taq polymerase (SABC Inc.)] following 35 cycles of 1min at 94°C, 1min at 63°C, and 1min at 72°C. PCR products were then purified and sequenced in both directions (forward and reverse) using the Big DyeTM Terminator v.3.1 Cycle Sequencing Ready Reaction on an ABI PRISM 3100 sequencer (Applied Biosystems, Warrington, UK). Forward and reverse sequences were edited manually and aligned to get the consensus sequences using BioEdit 7.0.9.0 program (Hall and Carlsbad, 2011).

The generated raw sequences were edited and aligned with additional sequences using software package DNASTAR v.7.1 (DNASTAR

Inc., Madison, WI, USA) and aligned using MEGA software (Tamura *et al.* 2011). In addition, 40 mtDNA sequences of chickens from Zanzibar were retrieved from GeneBank (http://www.ncbi.nlm.nih.gov/genbank). Zanzibar chickens include 20 mtDNA sequences of Unguja ecotype and the other 20 mtDNA sequences from Pemba ecotype (Lyimo *et. al.,* 2013). The accession number in the NCBI Gene Bank of Unguja and Pemba ecotypes are given in Appendix 1 table.

Data analyses

All the Zanzibar and Oman chicken mtDNA sequences were aligned using the AlignIR software (LI-COR Inc.). Extra nucleotide bases were trimmed from all sequences to make a homogeneous length of 350 bp. Extra nucleotide sequences that were outside the nucleotide sequences of the D-loop region were excluded from analysis. Number of haplotypes between Zanzibar and Oman chickens were estimated by DnaSP 6.12.03 (Rozas *et al.,* 2017). Median-joining networks were constructed to determine the evolutionary relationships of mtDNA chicken haplotypes following the algorithms of Bandelt *et al.* (1995), using Network 4.6.1.0 software (http:// https://www.fluxus-engineering.com/sharenet.htm). Phylogenetic networks based on the Neighbour-Joining (NJ) among mitochondrial sequences of Zanzibar and Oman chickens was inferred using SPLITSTREE4 4.16.2 software (Huson and Bryant, 2006).

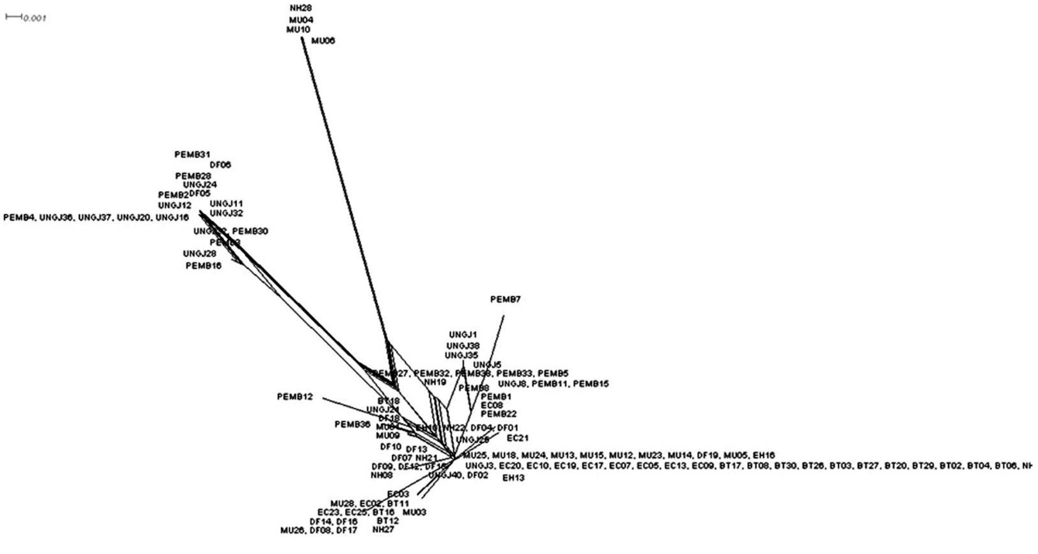

Figure 1: Neighbour-joining network showing the maternal evolution relationships between Zanzibar and Oman chickens

Results

The Phylogenetic analysis and haplotypes distributions revealed three distinct clusters among Zanzibar and Oman chickens. The Neighbour Joining network represents two main clusters that have mixed chicken ecotypes from Zanzibar and Oman, while the distant isolated third cluster has only Oman chickens of Musadan and North Hajar ecotypes (Fig. 1). The most prominent cluster in the phylogenetic network contains greater number of chickens, which includes all chicken ecotypes from Zanzibar and Oman.

Twenty four polymorphic sites that lead to nineteen haplotypes definition were observed in Zanzibar and Oman chicken ecotypes. The median-joining (MJ) network analysis of the mtDNA D-loop haplotypes observed in Zanzibar and Oman chickens were clustered into three distinct clades, which were identified as A, B and C (Fig. 2). Clade B and C, which includes Zanzibar and Oman chickens were found to be most ancestral that contained torso structure with ancient haplotypes, which are dominated with higher appearance (79.7%)

from all haplotypes (Appendix 2). Clade A is the smallest (2.9%) haplogroup and the most recent cluster with two chicken ecotyapes Musadam and North Hajar from Oman. Clade B is the most prominent cluster (78.94) with greater number of haplotypes and all chicken ecotypes. The three prominent haplogroups (68.1%) with similar haplotypes that share common ancestor with a single nucleotide polymorphism mutation, accommodated chicken haplotypes from Zanzibar and Oman (Appendix 3). The largest haplogroup (46.4%), which found in Clade B, contained all chicken ecotypes from Zanzibar and Oman. The second largest haplogroup (11.6%) found in Clade C, comprised Unguja and Pemba ecotypes from Zanzibar, and Dhofar ecotype from Oman. The third largest haplogroup (10.2%) located in Clade B consisted of Unguja and Pemba ecotypes from Zanzibar, and North Hajar, East Hajar, East Coast and Dhofar ecotypes from Oman. In this study, the phylogenetic network gives comparable insight to the similarities in mtDNA as in Median-Joining Network, where very similar individuals with the same evolution history cluster together.

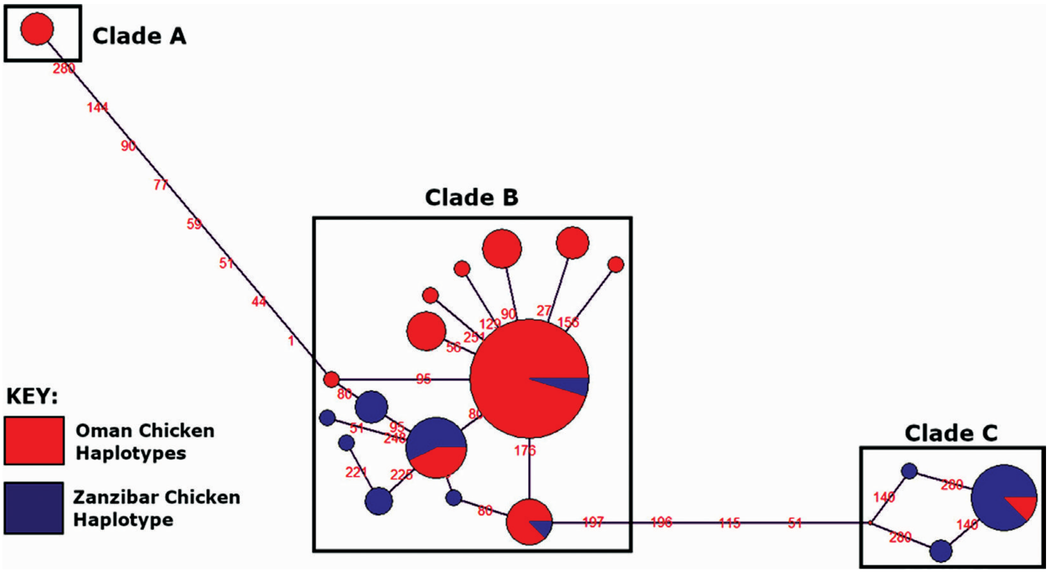

Figure 2: Median-joining network profiles of 19 haplotypes observed in Zanzibar and Oman Chickens. Note that the circle size corresponds to haplotype frequency and the number between the haplotype nodes refers to the position of nucleotide mutations

Discussion

Mitochondrial DNA (mtDNA) analysis has been greatly considered an essential tool for studying origins, migration patterns, and demographic history of a given population (Torroni *et al.,* 1996). The variation in mtDNA sequences can be used to construct phylogenetic networks that potentially displaying the relationships among sequences and estimating the time of appearance of mutations associated with each haplotype (Bandelt *et al.,* 1999). In this study, Zanzibar and Oman chickens have indicated a closer evolution relationship from both phylogenetic and Median-Joining network of the mitochondria DNA haplotypes. The chicken haplotypes from both Zanzibar and Oman were assigned into three most prominent haplogroups. These specific haplogroups indicate a single line of descendant from the maternal lineage (Malhi *et al.,* 2002; Starikovskaya *et al.,* 2005; Sandoval *et al.,* 2018).

The existence of most common ancestral haplotypes from Zanzibar and Oman could be associated with the long historical interactions from maritime trading network and the influence of sultanate regimes along the Swahili coast. In the history of chickens' dispersal, it has been revealed that coastal maritime trading networks around the Indian Ocean were the main responsible means for the introduction of chicken into Eastern Africa (Williamson 2000; Blench 2003; Muchadeyi *et al.,* 2008; Mtileni *et al.,* 2011; Mwacharo *et al.,* 2011). The Arabian Peninsula is thought to have played a major role in the diffusion of livestock across the Indian Ocean. The history of Oman is the history of the tense relationship between seafaring and commercial people. For centuries the Arabs sailed with the Monsoon winds from Oman to trade at Swahili coast. Although Zanzibar had few resources of interest to the traders, but it offered a good location from which to make contact and trade with the towns of the Swahili Coast. These movements might substantially contribute to early introduction of chicken to Zanzibar.

Dhorfa and Musadan chicken ecotypes from Oman, were found to be most distributed Median-Joining clades and most sharing haplotypes with Unguja and Pemba chicken ecotypes from Zanzibar. Previous study reported by Al-Qamashoui *et al.,* (2014b), verified Dhofar and Musandan ecotypes represented largest contributions of diversity in Oman chicken populations using microsatellite markers. These chicken ecotypes reflect more or less similar heterozygosity to Unguja and Pemba chicken ecotypes from Zanzibar, with the same set of microsatellite markers (Lyimo *et al.,* 2013). The expected heterozygosity in Dhofar and Musadan were 0.67 ± 0.027 and 0.65 ± 0.028 respectively, while for Unguja and Pemba were 0.63 ± 0.016 and 0.67 ± 0.016 respectively.

Conclusion

The interactions and close associations between Oman and Zanzibar are highly reflected in ancient maternal lineage of chicken ecotypes currently existing in Zanzibar and Oman. This could be greatly contributed from the maritime trade influence and the long persistence of sultan regimes operated in Zanzibar.

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Appendices

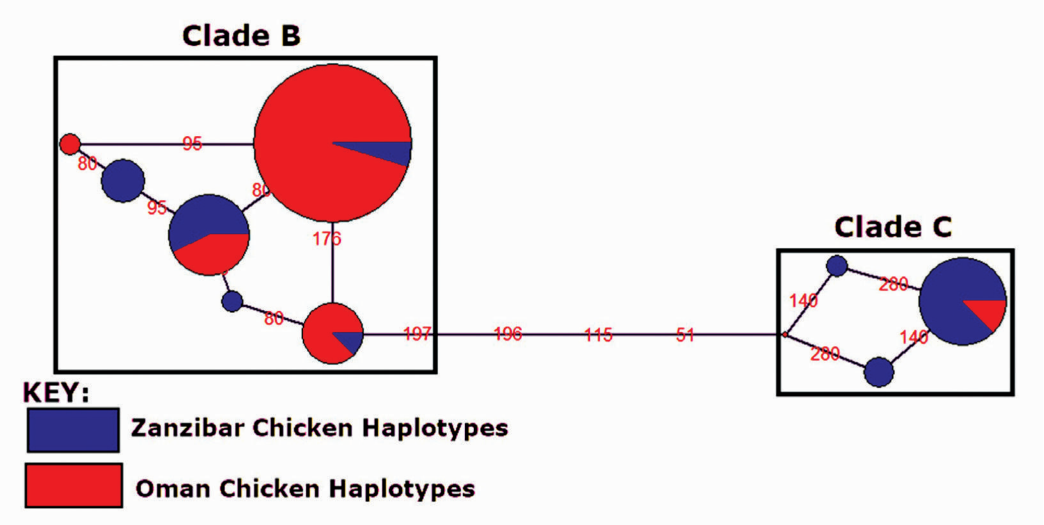
Appendix 1: Mitochondrial DNA Sequence accession number of Zanzibar chickens from National Centre for Biotechnology Information (NCBI) Gene Bank

SN	Abbreviation	Ecotype	Accession Number
1	UNGJ1	Unguja	KP067513
2	UNGJ11	Unguja	KP067514
3	UNGJ12	Unguja	KP067515
4	UNGJ21	Unguja	KP067516
5	UNGJ22	Unguja	KP067517
6	UNGJ24	Unguja	KP067518
7	UNGJ25	Unguja	KP067519
8	UNGJ28	Unguja	KP067520
9	UNGJ31	Unguja	KP067521
10	UNGJ32	Unguja	KP067522
11	UNGJ35	Unguja	KP067523
12	UNGJ36	Unguja	KP067524
13	UNGJ37	Unguja	KP067525
14	UNGJ38	Unguja	KP067526
15	UNGJ8	Unguja	KP067527
16	UNGJ16	Unguja	KP067535
17	UNGJ5	Unguja	KP067536
18	UNGJ3	Unguja	KP067542
19	UNGJ20	Unguja	KP067543
20	UNGJ40	Unguja	KP067544
21	PEMB1	Pemba	KP067497
22	PEMB11	Pemba	KP067498
23	PEMB15	Pemba	KP067499
24	PEMB16	Pemba	KP067500
25	PEMB2	Pemba	KP067501
26	PEMB22	Pemba	KP067502
27	PEMB27	Pemba	KP067503
28	PEMB28	Pemba	KP067504

SN	Abbreviation	Ecotype	Accession Number
29	PEMB30	Pemba	KP067505
30	PEMB31	Pemba	KP067506
31	PEMB32	Pemba	KP067507
32	PEMB36	Pemba	KP067508
33	PEMB38	Pemba	KP067509
34	PEMB4	Pemba	KP067510
35	PEMB5	Pemba	KP067511
36	PEMB8	Pemba	KP067512
37	PEMB12	Pemba	KP067531
38	PEMB3	Pemba	KP067532
39	PEMB33	Pemba	KP067533
40	PEMB7	Pemba	KP067534

Source: Lyimo *et al.,* (2013)

Appendix 2: Median-joining network of Zanzibar and Oman Chickens display the ancestry mtDNA haplotypes



Appendix 3: Haplotype frequency in each of the chicken ecotype from Zanzibar and Oman

Haplotype	Zanzibar Chickens		Oman chickens						Total
	UNGJ	PEMB	MU	BT	NH	EH	EC	DF	
1	3	1	10	11	11	15	8	5	64
2	9	5						2	16
3	4								4
4	1	1							2
5	1	8			1	1	1	2	14
6	1	1							2
7	1		2	1				4	8
8		1							1
9		1							1
10		1							1
11		1							1
12			2	1	3				6
13							1		1
14			1	1			2		4
15			1	1				4	6
16			3		1				4
17					1				1
18						1			1
19			1						1
	20	20	20	15	17	17	12	17	138

NB: *In the bracket below, define the abbreviations of the relevant chicken ecotypes: UNGJ (Unguja), PEMB (Pemba), MU (Musadam), BT (Batinah), NH (North Hajar), EH (East Hajar), EC (East Coast) and DF (Dhofar)*