



TRACING THE ANCESTORS OF MPONDO CLANS ALONG THE WILD COAST OF THE EASTERN CAPE

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Declaration

I, David de Veredicis, declare that this dissertation is my own work, unless otherwise stated. It is being submitted for the degree of Master of Science in Medicine in the branch of Human Genetics, in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

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.....day of....., 2016.

Abstract

Oral history and anthropological data indicate that several Xhosa clans in the mPondoland region of the Eastern Cape (formerly the Transkei) were established by individuals of non-African ancestry. Several oral and few written accounts state that circa 1730, survivors from trade- and slave-bearing vessels shipwrecked along the Wild coast of the Eastern Cape. Castaways who had survived the shipwrecking events had assimilated with the indigenous people of the area, married local women, and established clans of their own. The group of clans, which claim their ancestors to be of European and/or Eurasian descent, are known as the *abeLungu*, meaning “the Whites”. These clans are discerned from other local groups by variations in the practice of rituals from that of traditional Xhosa rituals, as these clans retain an affiliation with the European culture to which their ancestors belonged. Nowadays they still retain subtle phenotypic features like blue eyes, which are seen in several clan members. The identity of these clans has, to date, been shrouded in myth due to conflicting versions in the oral history and anthropological data, which leave the picture of the cultural identity of the *abeLungu* people unresolved.

With the advent of molecular biology, it has been shown that DNA may be used as a tool to trace population ancestry. The non-recombining region of the Y chromosome (NRY) serves as a marker for patrilineal ancestry and similarly mitochondrial DNA, which is inherited from mother to progeny, serves as a record for the matrilineal human history.

This study aims at exploring the degree of agreement between culture and genetics by investigating the genetic variation of the *abeLungu* - a culturally and geographically defined group. Focus is placed on their patrilineal history, since their oral history indicates clan progenitors to be predominantly male, but also due to the patriarchal social structure with regards to marriage and kinship of the *abeLungu*.

Buccal swabs were taken from which extracted DNA was used to perform Y chromosome microsatellite short-tandem repeat (STR) and SNP minisequencing using a total of 60 SNPs and 19 STRs taken from 146 *abeLungu* clan-affiliated individuals and 42 non-clan members from the greater region of mPondoland. Mitochondrial DNA SNP determination and sequencing analyses were also performed on 188 males and 10 females (the wives/ direct relatives of primary male clan elders), so as to trace the matrilineal origins and examine the congruence between the molecular and anthropological data.

The frequency of European and Eurasian haplogroups in the male samples was 69.86%, which are delineated predominantly by European haplogroups R1b, and West Asian haplogroup R1a1a. Haplogroups G, I and Q which occur at high frequencies in Europe and Eurasia were observed as well. It has also been observed (which was as expected) that culturally defined groups with a unique (or a limited number of) common origins whose membership is inherited only through the male line showed a relatively low intragroup variation for genetic markers similarly transmitted. The maternal lineages of the abeLungu clan members segregate with ancient and deeply-rooted African haplogroup L lineages, with increased diversity on account of migration due to their exogamous marriage practices.

This study affirms the non-African paternal origin of the abeLungu clans of lineages originating from few distinct founders, and elucidates the previously unresolved oral accounts of genealogical information, which has been transferred across generations with considerable accuracy, despite its propensity for change over time.

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List of abbreviations

AIMs - Ancestry Informative Markers

CMH - Cohen Modal Haplotype

D-loop - Displacement loop

DNA – Deoxyribonucleic Acid

ddNTPs - Dideoxynucleotide-triphosphates

GWAS - Genome-wide association studies

HCV - Hepatitis C virus

HVR - Hypervariable Regions

ISOGG - International Society of Genetic Genealogy

minHt - Minimal haplotype

mtDNA - Mitochondrial DNA

NJ - Neighbour-Joining

NPTs - Non-patrilineal transmissions

NRV - Non-Recombining region of the Y chromosome

RMJ - Reduced-median-joining

rCRS - Revised Cambridge Reference Sequence

SWGAM - Scientific Working Group on DNA Analysis Methods

STR - Short Tandem Repeat

SBE - Single Base Extension

SNPs - Single Nucleotide Polymorphism

TMRCA - Time to the Most Recent Common Ancestor

YHRD - Y-STR Haplotype Reference Database

VOC - Vereenigde Oost-Indische Compagnie (United East-India Company in Dutch)

CHAPTER 1

Introduction

1.1 Background and history of the abeLungu clans

1.1.1 The Wild Coast, shipwrecks and the clans of their castaways

mPondoland is a region on South Africa's Wild Coast which forms part of the Transkei republic in the Eastern Cape. The mPondo are one of 12 Xhosa speaking tribes who had settled in mPondoland between 500 and 1200 years ago (Soga, 1930). At the time the cultural territories were divided, with Khoi pastoralists dominating the area around Port Elizabeth, San hunter-gatherers lived in the Drakensberg foothills and Nguni mixed-farmers lived throughout the Transkei, while the mPondo resided closer to the coast (Soga, 1930). Today the Transkei and its Wild Coast are still in a developmental backwater and, even though South Africa has been in a state of developmental transition since 1994, service delivery in this region has been so slow that for many people things have not improved much. The history of the abeLungu exemplifies a point in human history when foreigners from very distant shores harmoniously integrated with indigenous populations, in strong contrast to some of the more recent political history of racial prejudice and segregation in South Africa (Soga, 1930; Crampton, 2004).

The coastline of the Eastern Cape is notoriously harsh, and estimates are that there are famous accounts for at least 20 shipwrecks which have occurred in the period c.1500-1800 along the Wild Coast alone, and numerous others which have gone unaccounted for, since Vasco de Gama first rounded the Cape of Good Hope in 1498 (Crampton, 2004). Some of the more well-known accounts of the wrecks and their castaways include the *São João Baptista*, which ran aground east of the Kei River in 1622, the *Stavenisse* of 1686, the *Bennebroek* of 1713, the *Doddington* in 1755 and the famous *Grosvenor* which met its fate in 1782 (Soga, 1930; Crampton, 2004). During the course of history, however, a number of castaways, with no option of returning to their homes, had harmoniously integrated with local communities of mPondo people, even marrying into them, living out their days not far from where their ships went down. Regarding whites who assimilated, often times the extent of

integration was marked, for example Stephen Taylor accounts that “...even more curious perhaps are those white men and women who have felt called to enter initiation, train as diviners and establish homesteads and followings in rural areas and beyond” (Taylor, 2005; Kalis, 2010).

Near Mthatha, the capital of the Transkei, at the Xora River Mouth, exists a clan family known as the *abeLungu*, who proclaim that they are descendants of European (white) castaways. Theories of the clan’s origins are linked to the story of the arrival of a young girl named Bessie on the Wild Coast, however details of her arrival remain unclear (Soga, 1930; Crampton, 2004; online reference 1 and 2).

1.1.2 The origins of the *abeLungu*

The Xhosa have a saying which states that “*If you want the truth, get it from the original, rather than from one who has heard it second hand*” (Crampton, 2004). With this in mind, key resources by authorities on the *abeLungu* include Xhosa-Scot historian John Henderson Soga in his account of *The South-East Bantu* (Soga, 1930) and Hazel Crampton in her novel *The Sunburnt Queen* (Crampton, 2004), as well as first-hand interviews with extant relatives of *abeLungu* clans of the Wild Coast which were performed in the field by my collaborator, anthropologist Janet Hayward Kalis (Kalis, 2006-2010, personal communications).

The *abeLungu*, or the “*Whites*”, are a black, Xhosa-speaking clan whose origins can be traced to three white, English castaways, who were named Jekwa, Hatu and Badi. While Jekwa, Hatu, and Badi are cited as the progenitors of the *abeLungu*, they were not the only foreigners from whom the clan descends. Crampton (2004) learned that the name *abeLungu* comes from the isiXhosa word meaning *white foam*, referring to the frothy sea foam from where their ancestors had emerged and had originally been encountered by the mPondo people. The *abeLungu* take pride in their unusual history, but, as time goes by, it is slowly being forgotten (Crampton, 2004). Clan members have become more confused about their history and through time, with names and the sequence of their progenitors beginning to blend into a potpourri of castaways, dates and wrecks. Upon interviewing contemporary members, the situation had declined further, exacerbated by the fragmentation of traditional life due to the migrant labour system and westernisation. Oral history serves as a tool for passing on a cultural

identity and a system of values, and in the telling and retelling the tale of the *abeLungu*, filtered down through several generations, different patterns have been woven into the whole, but the basic fabric holds true. A famous narrative about foreign shipwreck survivors becoming integrated into the local population is that of Bessie. The ship in which Bessie was a passenger remains unconfirmed to date, but it is suspected that she may have been aboard one of the Dutch East India (VOC) vessels which became wrecked sometime around 1737. Bessie was just one of thousands of people of various nationalities who were castaway on the shores of the Eastern Cape over the centuries. Survivors who did not wish to return to their homelands and their previous lives, sought to take refuge with the local clans of their new found emplacement. Many of these stories may have been lost forever, but some like that of Bessie remain as part of the South African oral narrative. This is mostly because Bessie, a white woman, most probably of British descent, came to marry into the *amaTshomane* royal family, thus she is remembered in the oral histories of her people. This was further enabled as two of her children were still living when the first English missionaries visited the area in the 1880s, and so, her story had been recorded in written history and was not lost (Crampton, 2004).

From an excerpt of Crampton's novel: "It was on this notorious coast, at about the same time that a Dutch fleet was destroyed in Table Bay in (1737), huddled against a rock lay a little, white, English girl named Bessie, who was cast ashore from her ship, at a remote spot known as Lambasi (the Bay of Mussels)..." (Crampton, 2004, p.12). In time Bessie acquired a Xhosa name, Gquma which means "*the roar of the sea*". Even though Gquma became more frequently used, she never forgot her real name and later, even named one of her children Bessy (Crampton, 2004).

Furthermore, "...legend has it that she was not alone, but the theory as to who her companions were still remains a mystery" (Soga, 1930, p.379). From Soga's account we understand that there were "...four in number; three males and one female child", who were named Jekwa, Hatu, Badi, and Gquma, respectively. The first two were thought to have been brothers, and the young girl was believed to be the daughter of Badi. According to the mPondos, they were relatives of one another "as they came from the same 'house', (viz. the ship) ..." (Soga, 1930, p.379). However, this need not be accepted as true since the mPondo are a polygamous society and family relations

operate differently, so it is important to note that the terms '*brother*' may have a wider application and meaning in isiXhosa than in English, which may not necessarily reflect biological relations. It is in this context that the relationships of Bessie and her fellow castaways should be understood (Crampton, 2004). The several accounts that have survived the intervening centuries, are fragmented and contradictory, falling largely into two camps: one in which the girl's companions are said to have been white men, and the other in which her companions are said to have been black, which may be interpreted that her companions could have also been Indian or Arabic. "Several slaves were with her...and were 'black' with long hair..." (Crampton, 2004). Soga (1930) contrastingly claims that all of Bessie's accompanying castaways were white.

From the independent expeditions of Dutch traders Hubner in 1736 and van Reenen, in 1790, several clues contribute to better understanding the mystery of Bessie and her Englishmen's origins (Crampton, 2004; Soga, 1930). Hermanus Hubner who headed an ivory-trade expedition in 1736, discovered a clan where three European shipwreck survivors (named Miller, Clerk and Billyert) resided with "numerous wives and offspring who had been shipwrecked many years before" (Crampton, 2004). It is about 50 years later where van Reenen on his expedition had also discovered a place with about 400 persons of mixed race, and three elderly women who had survived a wreck sometime around 1730, who were presumably of the same party as that encountered by Hubner (Crampton, 2004). Judging by the date, one of the women that van Reenen came in contact with was Gquma's (Bessie's) daughter, Bessy, and so it is evident that both Hubner and van Reenen are referring to having met the same woman, and that it is fairly clear that this group of mixed race existed some time before 1730 (Soga, 1930).

Since the Dutch butchered English names as badly as they did isiXhosa, and the names of the three English men who were encountered by Hubner in 1736 have a great resemblance to those of Bessie's three fellow castaways, it is therefore possible, that through examining their conversion into English, Hendrik Clercq becomes *Henry Clarke*; Tomas Willer becomes *Thomas Miller*, and Wellem Billyert may have been (*Bill*) *Elliot* or perhaps even *Billy Hart*. When juxtaposed with the names of Bessie's white men, the result is striking. 'Hatu' resembles 'Henry' and 'Badi' 'Billy' enough to suggest that the former are simply *Xhosa-ised* (corrupted) versions of the latter.

Finally, if Henry and Billy were Hatu and Badi, Thomas Willer (or Miller) must have been the man known as Jekwa. It was, and in fact still is, customary for a man to take on a new name when he became chief, and his Xhosa name, Jekwa, was probably bestowed on him when his mentor, Chief Matayi, appointed him as Chief of the abeLungu (Soga, 1930; Crampton, 2004).

Bessie grew to be an extremely attractive adolescent, who eventually caught the eye of Tshomane, Great Son of the Tshomane chief Matayi, who would eventually marry her and make her his Great Wife. Mysteriously, Tshomane died soon after their marriage, without an heir, and so the chieftainship was taken up by a close relative, Xwebisa (also known as Sango). In time Gquma (Bessie) married Sango, who became the Paramount Chief of mPondoland. This was initially met with great shock and disapproval as it implied the breaking of strict incest taboos. A very strong 'exogamy' law exists among the Xhosa where it is considered incestuous to have any sexual relations, let alone marry a person belonging to the same clan (Soga, 1930; Crampton, 2004). Gquma and Sango had three sons, and a daughter, Bessy, who were physically markedly different to the other clans-children: 'Several children were "yellow" in colour, having long hair and blue eyes' (Soga, 1930; Crampton, 2004). It is understood that Bessie had died sometime around 1810, aged about 80. Crampton, quoting Scully (1984), accords Bessie a very romantic end: "On the day she died she was, at her own request, carried down to the cleft in the reef where she partly lifted up herself, and pointed across the sea, turned, and gave out her life with a long drawn sigh". "In the night a terrible storm arose, and the shore was found strewn with myriads of dead fish" (Crampton, 2004).

1.1.3 The amaMolo

Although castaways were predominantly European, a large number of people were of other races and cultures, including black, Japanese, Javanese, and South-Indian Lascars. Just as the abeLungu identify their progenitors as having been white castaways, the amaMolo identify theirs as 'black' castaways – it is thought that they might have been Malagasy or Malay, but the general consensus is that they were Indian (Crampton, 2004). Crampton (2004) chronicles an mPondo legend, which describes the arrival of "long strange ships which anchored off shore, and at night had sent a number of small boats with musket bearing men, in white headdresses and long

flowing robes... much before the first Europeans.” The clan progenitors, Bhayi (the son of Jafiliti), and Pita, were said to have had “an Arab look about them, whose hair was straight, long and black.” Soga agrees with Kirby (1953) in that the name “amaMolo” probably a derivation, brought about by the corruption of the word *Moor* (pertaining to natives of North Africa). In Kalis’ interviews with extant amaMolo members however, it was reported that the name comes from the traditional isiXhosa greeting, *Mholo*, which was the only isiXhosa word that the castaways knew and which they would repeat when asked ‘*from which clan do they originate?*’ (Kalis, 2010, personal communication with permission).

Another possible theory to their origins is that some Malabar slaves who had survived the wreck of the *Bennebroek*, in 1713, had remained with the mPondo, instead of trying to return to their own country or to search for civilization (Soga, 1930; Crampton, 2004). The story of the origin of the amaMolos, as retold to Soga by the Great Son of the amaMolo Chief Mxhaka, is as follows: “Bhayi and his wife *Nosali*, Pita (his brother) and another man, *Mera* were captured by white men and taken aboard a ship which became wrecked, where they were washed ashore the coast of mPondoland.” As Bhayi’s wife was barren, he settled down at Brazen-Head, Mganzana; three kilometres from where Bessie resided, and married an mPondo woman with whom he had five children named Poto, Falteni, Mnyuri, Mngcolwana, Nyango and lastly Mgareni (some of which are indicated in the partial pedigree - Figure 1.1).

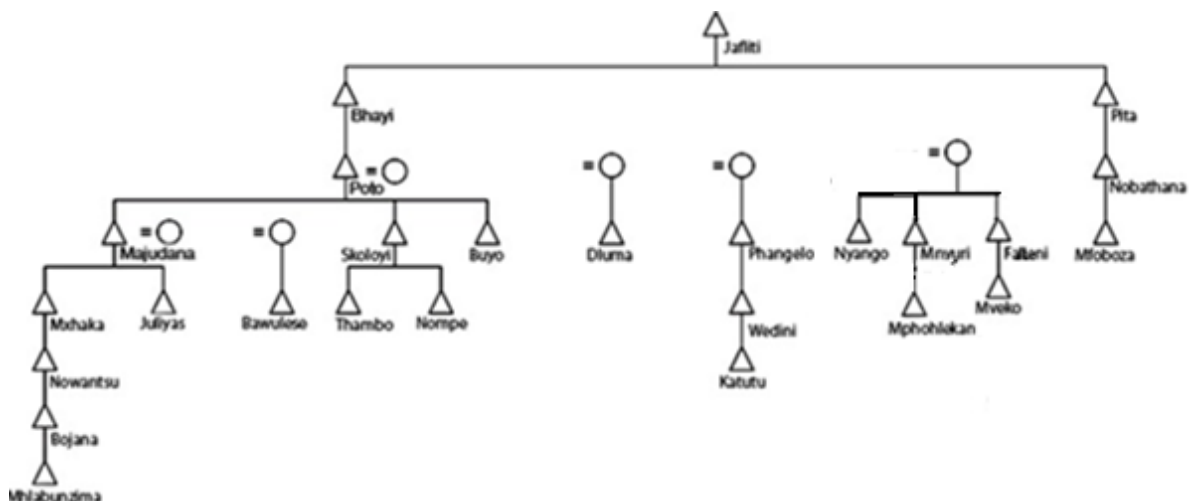


Figure 1.1. Partial pedigree of the amaMolo clan based on interviews with Chief of the amaMolo clan, Mhlabunzima Mxhaka, by Janet Kalis, as part of her research (Kalis, 2009; personal communication). Two primary branches indicate the relations of Bhayi, and his “brother”, Pita, who are the alleged sons of Jafiliti (Soga, 1930; Crampton, 2004). Bhayi’s sons Poto, Falteni, Mnyuri, and Nyango also have been indicated. Males are designated by triangle symbols and females are indicated by circles. The “=” indicates that these are multiple wives of specific males.

The well documented story of the wreck of the Grosvenor, tells of an English East India Company (EIC) vessel, wrecked in 1782; this date corresponds well with the presumed date of Bhayi's arrival in mPondoland. Among its survivors were 25 Indian seamen, including an Indian maidservant called Mary (possibly a version of Bhayi's fellow castaway *Mera*), accompanied by another Indian woman, Sally, whose name is similar to Bhayi's wife's name, *Nosali*. The mystery of the Indian origins of the amaMolo was seemingly resolved by a friend of Crampton, who, born and raised in India, immediately recognized that the names were of Hindi origin. 'Bhayi', she said comes from 'bhay' or 'brother' in Hindi. 'Pita' – with whom Bhayi was captured – means 'father', and 'Poto', the name of Bhayi's eldest son is a corruption of 'pota', meaning grandson.

Makuliwe, who has conducted research during the 1990s on Southern-Nguni clans, states of the amaMolo clan that it "...can be traced back to white people that were shipwrecked in the Indian Ocean and then married to Pondos". This was echoed by Chief Mxhaka and other contemporary clan members, interviewed by Kalis in 2010, who proclaim that amaMolo clan forebears were white (Kalis, personal communication, 2010). Thus, 80 years ago when Soga did his research, amaMolo were considered to be of Asian descent, but their recent cultural association is with white or European forebears. Kalis has learned that contemporary members of the amaMolo clan consider the clan name 'abeLungu' to be synonymous with 'amaMolo' and members of the abeLungu clan recognise those of amaMolo as patrilineal kin and both account for one another in their oral histories. Exactly when and from where the arrival of the amaMolo and abeLungu forebears on this continent had occurred, and to what extent their origins are bound up with one another has not yet been ascertained. Earlier accounts suggest that the amaMolo and abeLungu forebears had survived the same wreck, and that Asian and European survivors subsequently went their own ways, both assimilating into their own local communities, founding the amaMolo and abeLungu clans, respectively (Soga, 1930; Kirby, 1953).

1.1.4 Secondary clans and multiple castaway settlements

The *abeLungu* constitutes a broader super-clan family, incorporating numerous lineages of clans which claim affiliation to non-African ancestry. The 'primary' clans initiated by the original European and Asian castaways are the *abeLungu* Jekwa, *abeLungu* Hatu, *abeLungu* Buku as well as the *amaMolo* clans. However, numerous other clans exist within the *abeLungu* clan family. Multiple settlements and more recent establishments of clans are believed to have originated from later shipwreck incidents along the Eastern Cape's Wild Coast, with survivors also having assimilated with local Xhosa clans. As these founders were of non-African descent as well, they too had founded their own independent *abeLungu* clans. This is supported by oral history as well as the genealogies reconstructed thereof. The time-depths of the primary *abeLungu* clan genealogies extend for ten generations on average until the common non-African clan founder, while those of the more recently established clans go back five generations on average (Kalis, 2009 – personal communication). A brief description of clan families and subclans with hypothetical geographical regions of origin is as follows: The founders of the original *abeLungu* clans Jekwa, Hatu and Buku presumably came from Western Europe (Britain and/or Ireland). The founders of secondary *abeLungu* clans France, Horner, Irish, Caine, Ogle, Hastoni, Fuzwayo, Sukwini and Thaka are believed to have also come from Western Europe (England/Ireland) as well as Eurasia, while the *amaMolo* are believed to be of Eurasian descent.

With regards to names of clans and clan-name prefixes used, Soga states "Etymology as a science is unknown to the Bantu, and there are no phonetic rules laid down by them. As a general rule the prefix is not a matter of choice, but it is subject to what we may call dialectic phonetics. What, then, governs the selection of a clan or tribal prefix? The answer is that the selection is governed purely by phonetic requirements. There is no rule determining the use of any prefix attached to the tribal name but that which suits the tongue. "It would be phonetically awkward to say for instance, *aba-Xosa*, or *aba-Huhu*" (Soga, 1930). For all practical purposes, the naming of the descendants of the clan progenitors, Jekwa, Buku and Hatu as well as secondary *abeLungu* clans retain the clan family prefix 'abeLungu' while the descendants of Bhayi and Pita, are referred to as the 'amaMolo'.

1.1.5 The clan system

The dynamics through which an individual is recognised as a member of a group are a critical part of the mechanism to defining the identity of a person (Montinaro, 2016). The clan, here defined as a group of households reporting a shared ancestry, refers to an intermediate level distributed between lineages within the hierarchical structuring of a given society (Montinaro, 2016). Clan membership signifies descent from a common ancestor after whom the clan itself has been named (Preston-Whyte, 1974). Clan membership in agnatic societies like that of the *abeLungu*, is determined by the principles of patrilineal descent - meaning clan name passes exclusively through the male line and is infringed upon in the case of illegitimacy. The *abeLungu* are a patrilocal society, where historically, migration has been limited from these clan nodes (Soga, 1930). Clan *lalis* (homesteads) are geographically situated for the most part, where clan forebears had originated their clans. The *abeLungu* observe strict clan-exogamy practices and so it is customary not to marry inside one's own clan. Polygyny is widespread and the degree of which depends on the wealth of the husband (Soga, 1930; Chaix, 2007; Sanchez-Faddev, 2013).

Although the Xhosa clans in the sample live in deeply rural contexts, amongst traditional Xhosa people, and have adopted their customs and religious practices, they retain an affiliation with the European culture to which their ancestors belonged, which is expressed in various ways (Kalis' interviews, 2009). All of Kalis' male informants were able to name their male antecedents right back to the man who gave his name to their clan, even when this went much further back than three generations. This genealogical information is publicly recited on ritual occasions. Through the recitation of clan names (*iziduko*) as well as praise names and poetry (*izibongo*), the presence of deceased patrilineal ancestors is invoked at important occasions which are often organised with the primary intention of appealing to ancestral spirits and seeking their appeasement. Maguliwe recites the *nqulo* (praise) of the *amaMolo* (Kalis, field interviews, 2009), listing the main forefathers of the clan (Figure 1.2).

However, as has been witnessed, *abeLungu* clans claim a degree of independence in terms of how both rituals and praises to ancestors are performed. Our translator Qaqambile had asked an *abeLungu* Horner clan member of these discrepancies:

Qaqambile: “You are of this nation with mixed blood living among indigenous people who have their own ways of living. How do you do it?”

Mlungisa: “Well that’s easy. We were born in Xhosaland and we are living among Xhosas. We have customs and traditions but we don’t do our rituals like the Xhosas. For example, when Xhosa people kill a goat they use a spear but we just slaughter it with a knife and enjoy the meat, that’s it. We are white people so we don’t perform rituals. We can even perform rituals with a chicken rather than a goat. We are not governed by the strong traditions of the true Xhosa people (2009-11-05 Mlungisi Horner).”

1.2 Molecular Anthropology

This study was initiated as a collaboration with Janet Hayward Kalis (University of Mthatha), who had been conducting anthropological and genealogical research on 13 different clans in the Transkei-mPondoland region of the Eastern Cape. Kalis has documented details of genealogical relations and ritualistic practices of sacrifice and praise (*izinqula*) to clan ancestors of contemporary abeLungu clans, which differ from traditional Xhosa people’s as they retain an affiliation with European and Eurasian culture to which their ancestors belonged (Janet Kalis, 2010, personal communication with permission).

The recent genealogical history of human populations is a complex mosaic formed by individual migration, large-scale population movements, and other demographic events. Reconstructing human history requires the collection of various narratives from disciplines such as anthropology, archaeology, history, linguistic studies, paleontology and climatology. In the absence of written history, oral history has recorded the transmission of biographical and historical information, but it is proven that it is subject to changes and distortion over time. Genealogical oral histories, however, can now be tested through the application of genetic markers present in Y chromosome and mitochondrial DNA (mtDNA), which shed light on anthropological questions by documenting the similarities and differences between people in terms of molecular characteristics which parallel anthropological historical events, thereby providing a clearer understanding of the abeLungu’s origins, and ultimately contributing to understanding humanity’s past history.

<i>Linkomo ZikaMolo,</i>	Cattle of Molo,
<i>ZikaMlungu</i>	Of Mlungu
<i>Mpondo</i>	Mpondo
<i>Khoboko</i>	Khoboko
<i>Gxagxa</i>	Gxagxa
<i>Lawu</i>	Lawu
<i>NgabakwaSilanda yinaliti</i>	Thin as a needle,
<i>BakwaMkhonto yimesi</i>	Where they come from a spear is a knife,
<i>Nkonjane emnyama yaphesheya kolwandle,</i>	Black swallow from overseas,
<i>Inyok'emnyama ecandl'ulwandle,</i>	Black snake that crosses the sea,
<i>Nkomo zoMlung'omnyama nenwele zakhe,</i>	Cattle of white people who are black, even their hair (is black).
<i>Mlambo omkhulo ongwelwa nangamehlo</i>	A river so wide that you cannot see the other side, that cannot be crossed,
<i>Wona awel'umlambo ugcwele,</i>	They cross such a river even when it is at its fullest!
<i>ZikaBhayi,</i>	Of Bhayi,
<i>ZikaSomlungwana,</i>	Of Somlungwana,
<i>ZikaMerha,</i>	Of Merha,
<i>ZikaTulwana,</i>	Of Tulwana,
<i>ZikaPita,</i>	Of Pita,
<i>ZikaNgcolwane,</i>	Of Ngcolwane,
<i>ZikaMagarheni,</i>	Of Magarheni,
<i>ZikaFalteni,</i>	Of Falteni,
<i>ZikaNyango,</i>	Of Nyango,
<i>ZikaNywantsu,</i>	Of Nywantsu,
<i>ZikaMxhaka.</i>	Of Mxhaka.

Figure 1. 2. Nqulo (praise) to the ancestors of the amaMolo

1.2.1 Patrilineal descent and Y chromosome DNA

As unique organisms, most of humanity carry a cultural marker of coancestry, a surname (and similarly with clan names), which is a counterpart to the biological marker of coancestry common to all organisms - DNA (King and Jobling, 2009 [a]). Surnames (and clan names) have been shown to be specific to particular indigenous populations and to show geographical specificity within regions. This property means that they find wide application as convenient proxies for ethnic origin in healthcare as well as epidemiological studies (Shriver and Kittles, 2004; King and Jobling, 2009 [a]). However, analysis where surnames are combined with Y chromosomes has also enabled them to be used in genetic studies of historical migrations and admixture (King and Jobling, 2009 [b]). We may expect that a clan name should correlate with a type of Y chromosome, which has been inherited from a shared paternal ancestor – possibly even the clan name’s original founder.

Several features of Y chromosome DNA make it a suitable marker for investigating population histories. The Y chromosome is inherited paternally, which coincides well with the fact that clan name is also paternally inherited, making it a suitable marker for delineating patrilineal ancestral lineages (Jobling, 2001; Shriver and Kittles, 2004). Very little of the Y chromosome is made up of coding-DNA and as a result, markers in the Non-Recombining region of the Y chromosome (NRY) are examined for insight into patrilineal population history (Cann et al., 1987; Jobling and Tyler-Smith, 2003; Ralph and Coop, 2013).

Formally, any combination of polymorphic markers along a non-recombining molecule, and that tend to be inherited together constitute a haplotype [Jobling and Tyler-Smith, 2000; Y Chromosome Consortium (YCC), 2002]. Combinations of the biallelic markers define stable lineages of Y chromosomes that we refer to as ‘haplogroups’; a haplogroup describes haplotype groups which coalesce to a point where certain coding-region Single Nucleotide Polymorphism (SNPs) are found in common, and thus define a common ancestor, by having the same SNP in all haplotypes (Jobling and Tyler-Smith, 2000; YCC, 2002). Hammer and Zegura (2002) define the term haplogroup as ‘NRY lineages defined by binary polymorphisms’, whereas the term haplotype is reserved ‘for all sub-lineages of haplogroups that are defined by variation at STRs on the NRY’.

The estimated average Y chromosome SNP mutation rate is approximately 10^{-7} to 10^{-8} per generation (Jorde et al., 1998; Gray et al., 2000). Thus the low mutation rate of SNPs allows us to investigate pre-history of humans, but these polymorphisms are relatively uninformative about recent history (Jorde et al., 1998; Gray et al., 2000). Microsatellite short tandem repeats (STRs) can provide better information about recent evolutionary events than that of slowly evolving SNPs, due to their high mutation rate. The mutation rates of STRs are on average about 4 to 5 orders of magnitude higher than that of SNPs, and approach 10^{-3} per generation which is high enough to be directly determined in pedigree studies, spanning only a few generations (Jorde et al., 1998). We can hope to identify genetic evidence of more recent relatedness, and so obtain insight into the population history of the past tens of generations ago (Forster et al., 2000; Zhivotovsky et al., 2004; Ralph and Coop, 2013).

1.2.2 Y chromosome haplogroups and phylogeographic variation

Lineage based ancestry tests are popular because NRY haplotypes can provide information that is regionally specific. Y chromosome haplotypes act as barcodes or profiles of individuals sharing common ancestry. These profiles constitute haplogroups that phylogenetically represent charted human lineages, which are consistent with the movement of modern-day humans out of Africa (Jorde et al., 2000; Quintana-Murci et al., 2004; Barik et al., 2008). The Y Chromosome Consortium (YCC) and the International Society of Genetic Genealogy (ISOGG) have published updated versions of the maximum parsimonious phylogenetic tree of human Y chromosomes accompanied with proposed universal nomenclature. The most recent phylogenetic tree from consists of 311 haplogroups, which are defined by 600 SNPs (Geppert and Roewer, 2012; ISOGG, 2016). The phylogeny maps marker SNPs which correlate to the current global phylogeographic diversity of the Y chromosome (Hammer and Zegura, 2002; YCC, 2002; ISOGG 2016; Jobling and Tyler-Smith, 2003). The major clades (haplogroups) are labeled with a capital letter (e.g., R) and sub-haplogroups are designated alternately with numbers and lower-case letters (e.g., R1b). Usually the terminal SNP is included to determine the branch unequivocally (R-M343 alias R1b) (Hammer and Zegura, 2002; Geppert and Roewer, 2012). For the purpose of clarity, the combined SNP-marker/haplogroup-name nomenclature will be used when discussing haplogroups (for example, R1b (R-M343)).

Since Y chromosome haplogroup frequencies are highly structured by geography, it is possible to distinguish between African and non-African Y chromosomes, and in most instances, the combined haplogroup-haplotype information can reveal a judicious indication of the location of the broader geographic region of the origin of the Y chromosome (Hammer and Zegura, 2002; Shriver and Kittles, 2004; Naidoo et al., 2010). Figure 1.3 has been adapted from Chiaroni et al., (2009) and illustrates the prevalence and frequency distribution of Y chromosome macro-haplogroups globally.

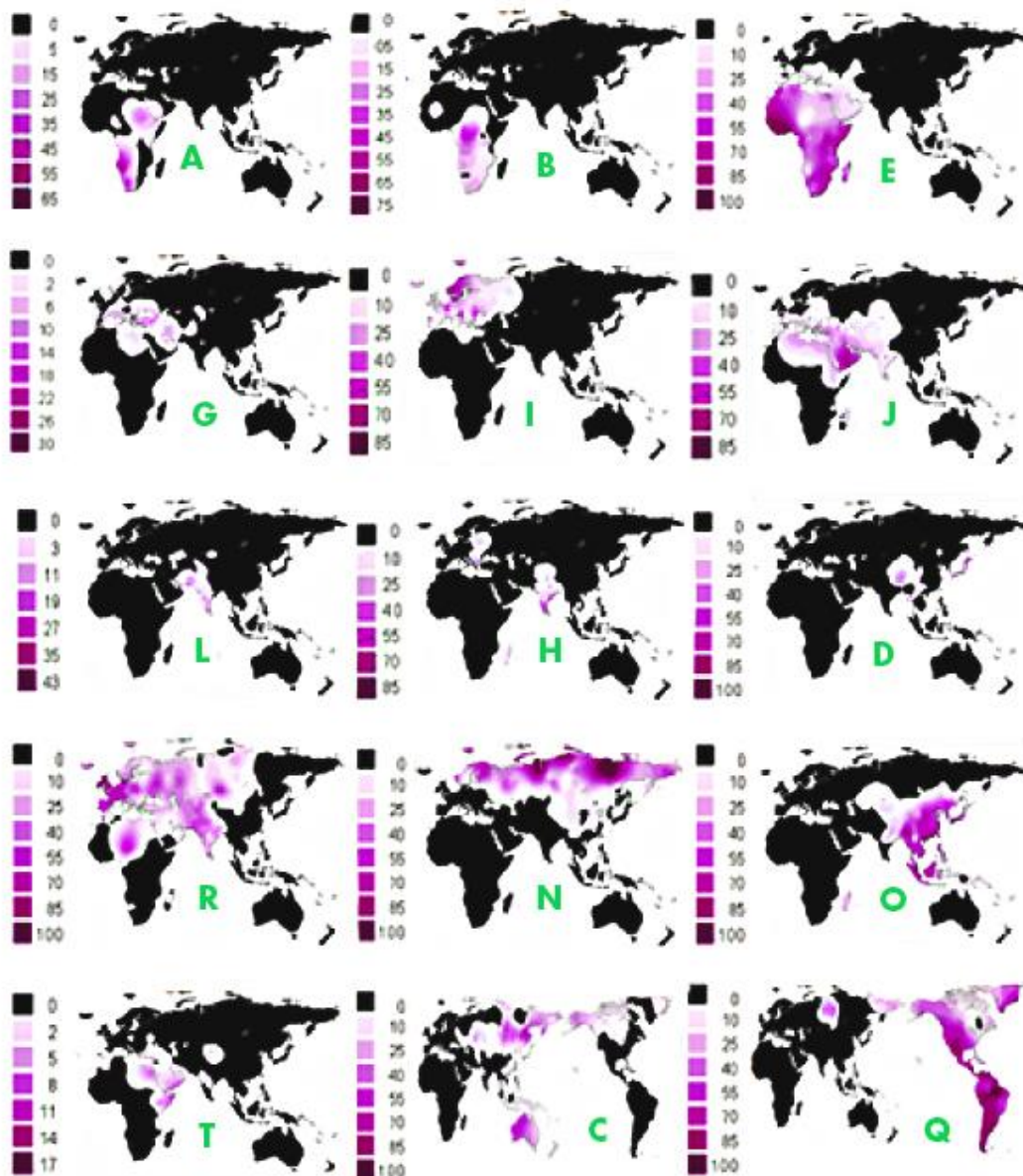


Figure 1.3. Geographic distribution map of Y chromosome macro-haplogroups - adapted from Chiaroni et al., (2009).

Based on the anthropological and oral histories it is expected to observe predominantly European Y chromosome haplogroups in the abeLungu clans and Eurasian ancestral haplogroups in the amaMolo. The majority of lineages observed in contemporary European and Eurasian populations fall into the following main haplogroups, namely R, G, I, J and Q, and are defined by SNP markers M198 and M343, M201, M170, M172 and M242 respectively (Jobling and Tyler-Smith, 2003; Karafet et al., 2008; Chiaroni et al., 2009; Myres et al., 2011). The prevalence of macro-haplogroups A, B and E which originate in, and are largely restricted to the African continent, will invariably be observed in the sample on account of gene-flow, admixture and non-patrilineal events (Jobling and Tyler-Smith, 2003; Karafet et al., 2008; Chiaroni et al., 2009; Ralph and Coop, 2013).

1.2.2.1 European and Eurasian haplogroups

Typically, greater than 50% of men in Europe are affiliated with haplogroup R (Chiaroni et al., 2009; Myres et al., 2011). Macro-haplogroup R is defined by marker M207 and is the most common clade throughout north-western Eurasia, and the majority of European Y chromosomes segregate under this haplogroup (Jobling and Tyler-Smith, 2003; Karafet et al., 2008; Chiaroni et al., 2009; Myres et al., 2011; Geppert and Roewer, 2012). Haplogroup R accounts for more than one-third of Indian Y chromosomes, and its daughter clades R1 and R2 are both found in tribal and caste groups (Sahoo et al., 2006). Clade R1 splits into R1a and R1b, which are similarly variable in Indians and western Asians but are less so in Estonians, Czechs, and central Asians (Kivisild et al., 2002). Lacau et al., (2012) showed that majority of Afghan individuals (67.4%), segregate under R-M207 with sub-haplogroup R1a1a-M198 variants present in both the North and South.

Haplogroup R1a1a (with its alternate name R-M198) is the dominant Y chromosome lineage found in modern Eurasia, having originated in the Eurasian Steppes north of the Black and Caspian Seas (Jobling and Tyler-Smith, 2003; Klyosov and Rozhanskii, 2012). R1a1a is particularly common in the large region extending from South Asia and Southern Siberia, across India to Central and Eastern Europe (Slavic populations) and Scandinavia (Sengupta et al., 2006; Underhill, 2010; Klyosov and Rozhanskii, 2012). Even though haplogroup R1a occurs as the most frequent Y chromosome haplogroup among populations such as Slavic, Indo-Iranian, Dravidian,

Turkic and Finno-Ugric, many authors have interest in the link between R1a and the Indo-European language family (Sengupta et al., 2006; Underhill, 2010). Haplogroup R1a1 (R-M198) haplotypes were brought to India around 3500 years ago (Sengupta et al., 2006). The present understanding is that R1a1 bearers, known later as the Aryans, brought to India not only their haplotypes and the haplogroup, but also their language, thereby building the linguistic and cultural bridge between India (and Iran) and Europe, possibly creating the Indo-European family of languages (Sengupta et al., 2006). This has relevance for the possible Indian roots of the Lascar slaves believed to be the forefathers of the amaMolo clan, in that through discovering Eurasian haplogroups it would support the oral history with regards to their non-African, and possibly Indian origins.

Haplogroup R1b is believed to have originated and expanded as humans began to re-colonize Europe after the last glacial maximum, approximately 10 to 12,000 years ago (Myres et al., 2011). R1b is the most common haplogroup found in Western Europe and is also found in Eastern European and West-Asian populations at lower frequencies, and is also prevalent in the vast majority of the British Isles (Kivisild et al., 2002; Campbell, 2007; Karafet et al., 2008; Chiaroni et al., 2009; Myres et al., 2011; Raghavan et al., 2014), and also in parts of sub-Saharan Central Africa, for example around Chad and Cameroon (Balaresque et al., 2009). About one in five males sampled in northwestern Ireland stems from an R1b-delineated haplogroup, R1b3, and is linked via the patriline which descends from the most important dynasty of early medieval Ireland, the Uí Néill (Moore et al., 2006; King and Jobling, 2009 [b]).

Other haplogroups which segregate under the Eurasian subcontinent include haplogroup G-M201 which originated around 30,000 years ago, in either the Middle East or South Asia (Cruciani et al., 2002; Cinnioglu et al., 2004; Karafet et al., 2008). While haplogroup G (G-M201) occurs at its highest levels in the Caucasus region (e.g. 74% in Ossetians from Digora), it is widespread; occurring at low to moderate levels from Northwest Europe to South and East Asia (Cinnioglu et al., 2004). Around 1 in 10 Ashkenazi Jewish males fall into haplogroup G (G-M201), and it is found at an average frequency of 7.9% in the Afghan gene pool, established during the Neolithic expansion throughout the region (Behar et al., 2004; Sengupta et al., 2006; Lacau 2012).

Haplogroup I (I-M170) is considered as the only native European Haplogroup, and appeared in Europe from the Middle East roughly 20,000 years ago and, alongside haplogroup R, it is considered as the second major European haplogroup (Semino et al., 2000; Hammer and Zegura, 2002). Haplogroup I (I-M170) Y chromosomes occur in nearly 20% of the European male population, and has also been found among some populations of the Near East, the Caucasus, Northeast Africa and Central Siberia (Hammer and Zegura, 2002; Karafet et al., 2008).

Haplogroup J lineages are found at high frequencies in the Middle East, North Africa, Europe, Central Asia, Pakistan, and India (Underhill et al., 2001; Semino et al., 2002; Behar et al., 2004; Sengupta et al., 2006). Haplogroup J-M172 is the most common J sub-haplogroup in Europe, which emerged 30,000 years ago in the Middle East and has been carried by Middle Eastern traders into Europe, central Asia, India, and Pakistan (Di Giacomo et al., 2004; Karafet et al., 2008). Haplogroup J-M267 predominates in the Middle East, North Africa, and Ethiopia (Semino et al., 2004), which contains the Cohen Modal Haplotype (CMH). The CMH is found exclusive to a lineage believed to have originated from the Cohanim (Jewish high priests) in the northern portion of the Fertile Crescent, where it later spread throughout central Asia, the Mediterranean, and south into India around 10,000 years ago (Hammer et al., 2009; Soodyall, et al., 2013). The lineage eventually migrated south, back into Africa and a variant of the modal haplotype features in the Lemba people. Soodyall, et al., (2013) publicised revised haplotype data on the Lemba peoples' origins, which through carrying the CMH, feature semitic origins in majority of haplogroups. The name "Lemba" may originate from chilemba, a Swahili word for turbans worn by Bantu peoples, or lembi, a Bantu word meaning "non-African" or "respected foreigner" (Shimona, 2003).

Haplogroup Q is defined by marker Q-M242, and is the lineage that links Asia and the Americas (Jobling and Tyler-Smith, 2003; Zegura et al., 2004). This lineage is believed to have originated in southern/central Siberia and central Asia, migrated through the Altai / Baikal region of northern Eurasia and across the Bering straits eventually into the Americas, thereby characterising a novel founder Native American haplogroup (Jobling and Tyler-Smith 2003; Bortolini et al., 2004; Zegura et al., 2004; Karafet et al., 2008).

1.2.2.2 African Y haplogroups

The frequently observed clinal pattern of reduced genetic diversity away from Africa is seen as strong evidence for the out-of-Africa movement(s) of anatomically modern humans approximately 35,000 and 89,000 years ago, where a minority of contemporary East Africans and Khoisan represent the descendants of these most ancient ancestral patriline (Underhill et al., 2000; Soares et al., 2011; Sanchez-Faddev, 2013). Haplogroups A and B are the deepest branches in the Y chromosome phylogeny and are essentially restricted to Africa, providing the evidence that modern humans first arose there (Underhill et al., 2001; Jobling and Tyler-Smith, 2003; Karafet et al., 2008; Chiaroni et al., 2009). Macro-haplogroup A is not mono-phyletic and contains many sub-clades (Karafet et al., 2008). It is mainly restricted to the Rift Valley from the Cape up to Ethiopia, to mostly, but not exclusively some of the oldest hunter-gatherers who still survive and speak Khoikhoi and San languages, which are believed to be the oldest human languages represented by haplogroup A00 (Underhill et al., 2001; Cruciani et al., 2002; Salas et al., 2002; Karafet et al., 2008). The interruption of its distribution in the middle of the Rift Valley is possibly due to replacement by Bantu-speaking farmers who settled the region starting in the first millennium of the Christian era (Chiaroni et al., 2009).

Haplogroup B is found mainly among African Pygmies of the central African forest who are still predominantly hunters-gatherers but speak Bantu languages borrowed from farmers who arrived in the area between 2,000 and 3,000 years ago (Underhill et al., 2001; Karafet et al., 2008). Haplogroup B (B-M152) occurs at low to moderate frequencies in most sub-Saharan African populations, including populations from Cameroon and East Africa, and among Southern Bantu-speakers (Cruciani et al., 2002; Underhill et al., 2001).

Haplogroup E1b1a (E-M2) is the most common haplogroup in sub-Saharan Africa which originated in Northeast Africa between 30,000 to 40,000 years ago (Hammer and Zegura, 2002; Cruciani et al., 2002). Today its lineages are found occurring in the Mediterranean and the Near East (Cruciani et al., 2002; Karafet et al., 2008). Settlement outside of Africa by haplogroup E members involves the later subhaplogroup E-M35 varieties like M78, M81, and M123 that extended to Arabia and the northern Mediterranean coast (Cruciani et al., 2002; Chiaroni et al., 2009).

Haplogroup E2b1 (E-M85) is seen throughout sub-Saharan Africa at moderate levels and is a haplogroup that diversified some time afterward other haplogroup sublineages, probably having descended from the East African population that generated the Out-of-Africa expansion (Cruciani et al., 2002). Haplogroup E1b1a1a1c1a (E-M191) most likely spread throughout sub-Saharan Africa as a result of migrations associated with the Bantu Expansion. It is now the most common haplogroup in sub-Saharan Africa, although, its highest levels are still seen in West Africa (Cruciani et al., 2002).

1.2.3 Y chromosome Short Tandem Repeats (Y-STRs) and Y-haplotypes

Previous population-ancestry type studies have shown the utility of STR haplotypes in pedigree analyses, which include King and Jobling (2009 [b]), Wu et al., (2010), Balanovsky et al., (2011), Reguiero et al., (2012), Soodyall (2013) and Westen et al., (2015).

Y chromosome data is organized such that haplogroups provide an indication of geographic clustering and haplotypes further refine variation within haplogroups. Y-STR haplotypes are used to predict haplogroups which directs the sequence of multiplexes for Single Base Extension (SBE) - SNP affirmation. The use of SNP haplogroup data in conjunction with STR haplotype data creates extended haplotypes which allows for the measure of unique transmissions in the pedigree, and permits the examination of relationships between haplotypes in haplogroup-specific Y-haplotype networks.

1.3 Matrilineal ancestry

1.3.1 Mitochondrial DNA

Mitochondrial DNA (mtDNA) has proved to be a powerful tool in reconstructing population history and diversity studies (Richards et al., 2000; Finnila et al., 2001; Fadhlou-Zid et al., 2004; Ralph and Coop, 2013). MtDNA is present in the mitochondrion organelle of the cell which are usually numerous and polymorphic in morphology. The mitochondria are involved with energy manufacture and processing of the cell, where most mitochondrial genomes encode for 13 subunits of the oxidative phosphorylation system, two ribosomal RNAs (rRNAs), and 22 transfer RNAs (tRNAs) (Figure 1.4) (Scheffler, 2000; Iborra et al., 2004; Doosti and Dehkordi, 2011). Genetic

analysis of mtDNA has been an important tool in understanding human evolution due to the characteristics of mtDNA, such as its high copy number, near-absence of recombination, high substitution rate, as well as its maternal mode of inheritance (Cann et al., 1987; Scheffler, 2000; Destro-Bisol et al., 2004; Iborra et al., 2004; Behar et al., 2007; Gonder et al., 2007). Knowledge of mtDNA sequence variation is rapidly accumulating, and the field of anthropological genetics, which initially made use of only the first hypervariable segment (HVS-I) of mtDNA, is advancing to the point where complete mtDNA genome analysis will be the common genotyping practice (Salas et al., 2002; Kivisild et al., 2004; Behar et al., 2007; Gonder et al., 2007). Most studies of human evolution include mtDNA sequences from the 1kb, non-coding control region known as the displacement loop or 'd-loop', which occupies less than 7% of the mtDNA genome (Scheffler, 2000; Iborra et al., 2004; Gonder et al., 2007) (Figure 1.4; adapted from "the Mito Blog" online blog). Mutations within the hypervariable regions I and II (situated within this 1kb non-coding region) act as highly informative marker loci for delineating mitochondrial ancestry (Scheffler, 2000; Gonder et al., 2007; Schlebusch et al., 2009). Clusters of HVR mutations delineate haplogroups, which are represented as the major branch points on the mitochondrial phylogenetic tree. The Phylotree mtDNA tree (Build_16) provides a phylogenetic tree of global human mitochondrial DNA variation, based on both coding- and control-region mutations, and includes haplogroup nomenclature as defined by its developers, van Oven and Kayser (2009). The phylogenetic tree is updated regularly to incorporate information from novel mitochondrial genome sequences and was last updated on the 19 February of 2014.

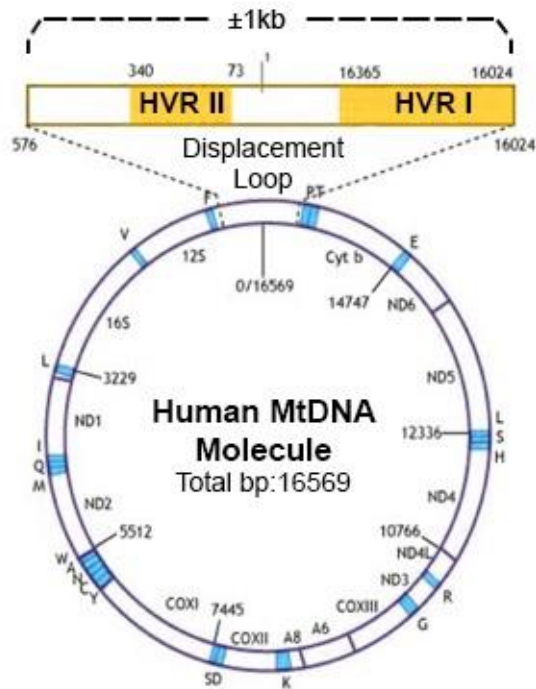


Figure 1.4. Schematic overview of the mitochondrial DNA molecule. HVRI & HVRII as situated in the D-Loop or control region of the mitochondrial DNA molecule, amidst other primary mitochondrial coding genes. Numbers indicate positions of DNA base pairs. Adapted from the “MitoBlog” online blog.

1.3.2 mtDNA phylogeographic variation and inferring matriline

Without a cultural marker such as clan name which can be used to confer the patriline, the strict patrilineal inheritance of the amaXhosa clan name means that it would be difficult to trace the lineages of abeLungu women. However, mitochondrial genotyping also shows strong geographic structuring and will allow us to infer the phylogeographic landscape of the maternal lines. It will reveal traces of non-African ancestry found in the matriline, having possibly derived from Bessie (whose maternal legacy is the most historically renowned), or of any other female surviving members.

Given the historic nature of the abeLungu, descendants of non-African female survivors may harbour possible European and/or Eurasian origins which could be observed in the mtDNA of these lineages. Several historians including Soga (1930), Crampton (2004) and Kirby (1953) are in agreement that the oral history states that the non-African survivors from shipwrecks were predominantly male individuals, who had integrated into Xhosa communities and married local Xhosa women with whom

they began clan families of mixed ethnicities. From this tenet we may expect to observe a majority of African haplogroups in the maternal lineages, if not entirely.

The study of the geographic distribution and diversity of genetic variation is known as the “phylogeographic approach” (King and Jobling, 2009). The global distribution of mitochondrial haplogroups is such that Eurasia, Asia, Europe and the Americas all retain haplogroup diversity signatures which reflect the migration of anatomically modern humans out of Africa into the Near East, approximately 100 to 130 000 years ago (Behar et al., 2008; Soares et al., 2011). Mitochondrial DNA diversity, in Africa, can be assigned into seven macro-haplogroups (L0 to L6), with haplogroups L0–L3 and L5 as the primary mtDNA haplogroups whose spread is restricted mainly to sub-Saharan Africa (Kivilsild et al., 2004; Loogvali et al., 2004; Behar et al., 2008) with the rest of the worlds’ lineages classified as subgroups of macrohaplogroups M, N and R (Behar et al., 2008; Soodyall and Schlebusch, 2010). A world map illustrating the global distribution of mtDNA haplogroups has been adapted from that found on the J.D MacDonald family name reference database (Figure 1.5).

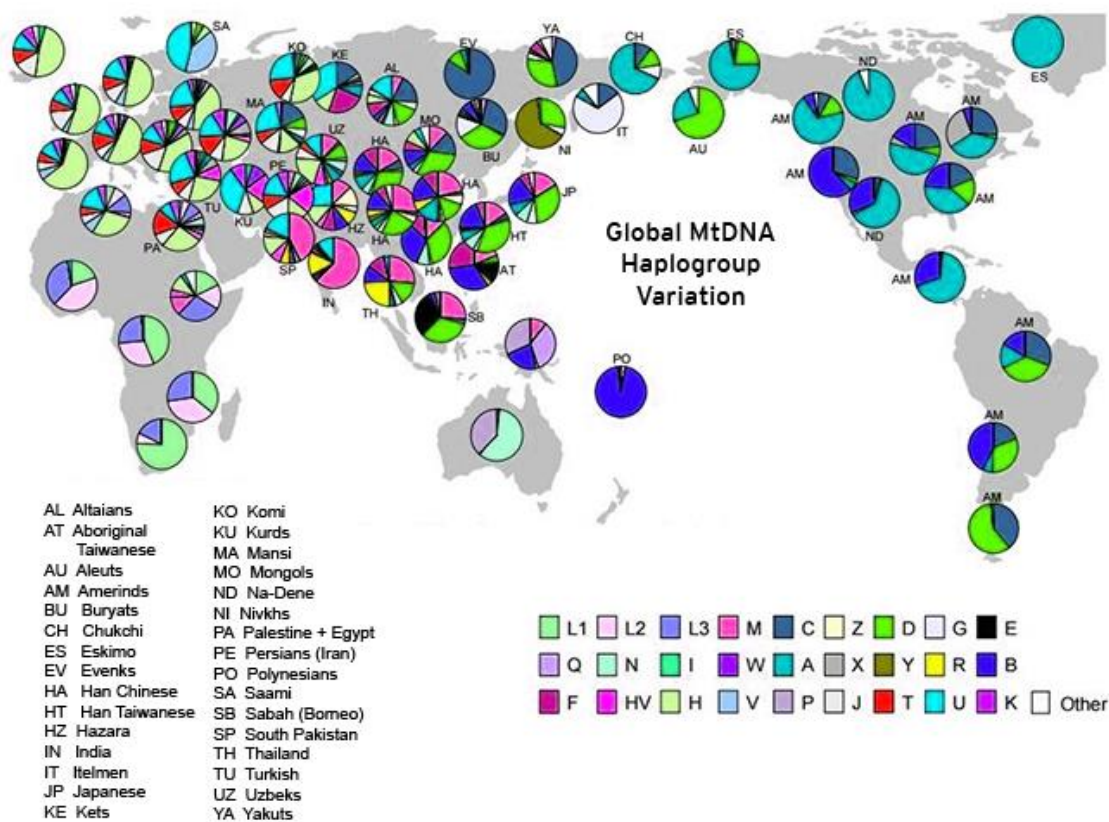


Figure 1.5. Global distribution map of mtDNA haplogroups, adapted from MacDonald (2005). The map illustrates the global distribution of mtDNA haplogroups partitioned by ethnic groups across the globe

1.3.2.1 African mitochondrial haplogroups

Macro-haplogroup L is geographically restricted to sub-Saharan Africa and has been divided into haplogroups L0–L6 (Salas et al., 2002; Behar et al., 2008). Haplogroup L0 is divided into sub-haplogroups L0a, L0d, L0f, and L0k, and the time to the most recent common ancestor (TMRCA) of L0k, L0f, and L0a is 139.8 ± 24.6 kya (Gonder et al., 2007). L0a is believed to have originated in eastern Africa and is the largest, most diverse and widespread haplogroup of the L0's. L0a common in eastern, central, and southeastern Africa, but is almost absent in northern, western, and southern Africa. Haplogroup L0a was probably brought to south-eastern Africa by the eastern movement of the Bantu Expansion (Salas et al., 2002; Plaza et al., 2004). L0d is thought to be the oldest of the L0 clans. The distribution of L0d and L0k strongly point to an origin of these haplogroups among Khoe-San ancestors, which occurred prior to the arrival of Bantu-speaking populations in southern Africa. The frequencies of these clades are of up to 40% in different south-eastern Bantu-speaking tribes (Schlebusch et al., 2009). Haplogroup L0d is present in the !Xun and Khwe peoples at frequencies of 51% and 16%, respectively, while L0k was found at frequencies of 26% in the !Xun and 23% in the Khwe (Salas et al., 2002; Schlebusch et al., 2009). Haplogroup L0f is a rare group, scattered throughout populations from East Africa to South Africa, and is most common in Kenya, Sudan, Tanzania, and Uganda (Gonder et al., 2007).

Macro-haplogroup L1 encompasses 52% of the haplogroup L haplotypes and 29% of all African mtDNAs according to a study by Wallace et al., (1999), and is comprised of sub-haplogroups L1a, L1b and L1c (Gonder et al., 2007). Salas et al., (2002) stipulate that haplogroup L1a was most likely to have been brought to south-eastern Africa by the eastern stream of the Bantu expansion, after having been picked up in East Africa. Haplogroup L1b is concentrated in western Africa, but it also occurs in central and northern Africa (particularly in areas adjacent, geographically, connected by the West African coastal pathway) but prevalent little in East, southeastern, or southern Africa (Salas et al., 2002; Gonder et al., 2007). Haplogroup L1c is the largest and most diverse group in the L1 clan, and most likely arose in Central Africa, around 20,000 years ago. Haplogroup L1c is seen at high levels in Central Africa, and is also found commonly in African Americans and central African Bantu speakers (Salas et al., 2002). The origin of L1c can be placed somewhere in Central Africa towards the

Atlantic west coast, in the uncharacterized areas of Angola and the Congo delta, to the south of the putative Bantu home-land, on the route of the “western stream” of the Bantu expansion (Salas et al., 2002). Both L1b and L1c are nearly absent in eastern and southern Africa (Gonder et al., 2007).

Haplogroup L2 is commonly subdivided into four main subclades, L2a through L2d with haplogroup L2a as the most frequent and widespread haplogroup in Africa (Salas et al., 2002). L2a appears to have arisen in West Africa around 33,000 years ago before drastically increasing in number in south-eastern Africa, with the distribution of haplogroup L2a possibly being a signature of the Bantu Expansion (Torroni et al., 2001; Salas et al., 2002). Haplogroups L2b, L2c, and L2d appear to be largely confined to West and western Central Africa Haplogroup L2c is frequent in western Africa, and is rarely found in other parts of Africa (Salas et al., 2002). L2d being the oldest of the L2 haplogroups is thought to have originated in West Africa and is found in most western and central African populations, declining in frequency toward the south (Salas et al., 2002).

Haplogroup L3e is the most widespread, frequent, and ancient of the African L3 clades, comprising approximately one-third of all L3 types in sub-Saharan Africa and possibly arose in Central Africa near Sudan around 35,000 years ago (Bandelt et al., 2001; Soares et al., 2011). Haplogroup L3d is found mainly in West Africa and was found at high frequencies among southwestern Bantu speakers (Schlebusch et al., 2009). It is said to have been brought into southern Africa with the western movement of the Bantu Expansion (Bandelt et al., 2001; Soares et al., 2011).

Haplogroup L4 is common in East Africa and the Horn of Africa, and is prevalent in North-eastern African populations, while less prevalent in central Africa (Batai et al., 2013). It is found at low frequency or almost absent in southern African populations. The highest frequencies are in Tanzania among the Hadza at 60-83%, and in the Sandawe at 48% (Tishkoff et al., 2007).

Haplogroup L5 (previously referred to as L1e) has been observed at low frequency in eastern Africa (Salas et al., 2002; Kivilsid et al., 2004), Egypt, and among the Mbuti Pygmies (Kivisild et al., 2004; Gonder et al., 2007). The geographic spread of

haplogroup L5b lineages is more southern, extending to the Sukuma from Tanzania (Knight et al., 2003; Kivisild et al., 2004; Gonder et al., 2007).

An East African origin of haplogroup L6 seems most likely, because of its presence in Ethiopians and the fact that its sister haplogroups L2, L3, and L4 are all diverse and frequent there (Kivisild et al., 2004). This is confirmed in a study on African origins in the Arabian Peninsula, where haplogroup L6 had been observed most frequently in populations of Yemen and Ethiopia (Abu-amero et al., 2007). Due to a lack of an exact match from the African database for Southern-Arabian L6 samples, and the relatively deep time-depth of its variation in Ethiopians and Yemenis—taken together, at approximately 36,600 years ago, it is possible that this haplogroup has been preserved in isolation in the Ethiopian Highlands and southern Arabia for tens of thousands of years (Kivisild et al., 2004; Abu-amero et al., 2007). However, the most frequent haplotype of L6 in Yemenis does not bear any descendant lineages, which suggests that its carriers coalesce to a common ancestor which occurs within only a couple of thousands of years (Kivisild et al., 2004; Abu-amero et al., 2007).

1.3.2.2 Non-African mtDNA haplogroups

Possible non-African mtDNA haplogroups which may be observed are those which the oral history accounts for and would presumably be those found in Western Europe. Analysis of diversity in European mtDNA reveals a relatively homogeneous landscape comprised of approximately 10 haplogroups (Torrioni et al., 1996; Rosser et al., 2000; Loogvali et al., 2004).

Bryan Sykes in his seminal work, entitled “The seven daughters of Eve”, assigned haplogroup names which classify the seven major mitochondrial lineages for modern Europeans which trace back along the maternal lineages, to seven prehistoric women, each stemming from the African Mitochondrial Eve, the most recent common maternal ancestor (Sykes, 2001). Loogvali et al., (2004) re-mapped European and western Eurasian haplogroups as those including haplogroups H, J, K, N1, T, U4, U5, V, X and W. The study by Torrioni et al., (1994[a]) and then that of Finnila et al., (2001), had identified four European clusters (H, I, J, and K) individuals of European ancestry. Torrioni et al., (1996) applied the same methodology to two Scandinavian population samples which identified five additional clusters (T, U, V, W, and X), which, together

with the previous four clusters, appeared to encompass virtually all examined European mtDNAs (Torroni et al., 1996; Macaulay et al., 1999; Simoni et al., 2000; Fu et al., 2012). Haplogroup H alone constitutes about one half of the European mtDNA pool and is widespread also in western Asia (Simoni et al., 2000; Loogvali et al., 2004; Brotherton et al., 2013). The United Kingdom is comprised of 44.7% Eurasian haplogroup H, which is predominant in Western Europe, and is found distributed amidst the Iberian Peninsula, found in Spain at 27.8%, Morocco (19.2%) and Sardinia in 17.9% of mtDNAs (Achilli et al., 2004). Haplogroup U is represented by its subclades U1a, U3, U5, U5a1a, U7a, and K which are predominant in the Near East and Europe (Macaulay et al., 1999; Richards et al., 2000; Fadhlouli-Zid et al., 2004). Haplogroup V, which is largely distributed in Western Mediterranean populations most likely originated within Europe and spread eastward (Richards et al., 2000; Fadhlouli-Zid et al., 2004).

1.4 Aims and objectives of the study

It has always been the case that unlike much written history, the oral transmission of biographical and historical information cannot be verified as it is subject to change and elaboration over time. In this study we address the issue of markers of identity from a genetic perspective. The main focus of this research is to use molecular markers to shed light on the history of the ancestry of the abeLungu clans (and amaMolo) from the Wild Coast region to the Eastern Cape. More specifically,

- i) Y chromosome DNA data will be used to trace the geographic region(s) of origin(s) of the male founders of the clans; male subjects are the focus of the study because both matrilineal (mtDNA) and patrilineal (Y-DNA) studies can be conducted on DNA from a single individual, and more fundamentally because Y-DNA is inherited in tandem with the cultural marker of clan name
- ii) MtDNA will be used to assess the maternal genetic contribution of females in these groups. A corollary would be to determine any links to Bessie and her maternal legacy
- iii) The genetic data will be used in conjunction with genealogical information to test/refine the oral history of the abeLungu and the amaMolo, which claims European/Eurasian paternal ancestry. In particular, I hope to see whether, and to what extent, oral histories and genealogies that link contemporary isiXhosa clans to non-African forebears have survived.

Given that Y chromosomes are transmitted from father to sons, like surnames (clans in this instance), the Y chromosomes found in living people from the group ought to coalesce within clans, to the founding father of the clan. Since the patterns of Y chromosome variation show strong correlation with geography, this study will make use of the global Y chromosome phylogeny to assess the geographic region of origin of the founding fathers of the abeLungu and amaMolo clans. Also, Y chromosome data would be used in conjunction with the revised genealogy would enable an examination of the fidelity of Y chromosome transmission within clans.

Similarly, the mtDNA data would resolve the ancestry of females who have contributed to shaping the gene-pool of these clans. MtDNA data are also highly structured by geography and African haplogroups can be distinguished from non-African ones.

2.1 Subjects and sampling

2.1.1 Ethics approval for study

This research has been approved by the Human Research Ethics Committee at the University of the Witwatersrand under protocol numbers M090576 (previously M980553) for Professor Himla Soodyall, and protocol number M120364 for David de Veredicis (Appendix A). DNA was collected from research subjects with their informed consent, following the appropriate ethical guidelines for research on human subjects by the NHLS.

2.1.2 Sampling and research area

The sample set comprised of 198 subjects which consisted of 188 males and ten females. Of the 188 males, 146 self-identified into one of 13 abeLungu clans. The remaining 42 individuals were not affiliated with any of the abeLungu clans, but were included in the study to represent males from regions co-habited with the abeLungu. These subjects self-identified as 'Xhosa', 'mixed' race, or were unsure of their ancestral background. Since the primary focus of the study revolves around the patrilineal heritage of the abeLungu, the ten females (who were relatives of principal male subjects), have been included for mtDNA studies. The inclusion criterion was to sample at least one male from each compound in the village, two males were preferred where available, and closely related individuals such as father-son pairs were randomly included. Present-day abeLungu subjects resemble their Xhosa relatives phenotypically for the most part, however some have retained recessive features that they inherited from their European/Eurasian roots. Three subjects, including an individual named "Lord Nicholas Beresford" of the clan Irish, were found to still retain blue eyes (Figure 2.2).



Figure 2.2. Photographs of three male subjects featuring blue eyes which illustrate the phenotypic links to their non-African patrilineal ancestry. Photographs have been taken with the individuals' permission; Mamolweni, Eastern Cape, 2009, 2010.

The research area spanned about 150 km of coastal region closely associated with locations of shipwrecks which were found between the Mzimvubu and Xhora rivers (Figure 2.3). In her fieldwork, Kalis had interviewed 13 *abeLungu* clans located in 24 homesteads or *lalis* (Table 2.1), all of which fall under the greater 'abeLungu' clan superfamily. However, it is only the *amaMolo*, *abeLungu Jekwa*, *abeLungu Hatu* and *abeLungu Buku* clans whose genealogies trace descent from their forebears of the original shipwreck survivors of the 16th and 17th century, as documented by Soga in 1930, and are considered as the *primary* *abeLungu* clans. The secondary, more recently established *abeLungu* clans include the clans Caine, France, Horner, Irish, Ogle, Hastoni, Sukwini, Fuzwayo, and Thakha (Table 2.1) which have from more recent origins from non-African shipwreck survivors having assimilated into the *mPondo* people. Individuals from these clans were genotyped for the purpose of elucidating their Y chromosomes origins as well, and were sampled from homesteads near those of the three primary *abeLungu* clans (*Buku*, *Hatu* and *Jekwa*) and the *amaMolo*. The geographic sampling locations of both primary and secondary *abeLungu* clans as well as the *amaMolo* can be found summarized in Table 2.1 and indicated in Figure 2.3.

Community engagement began by first visiting the amaMolo Chief Mhlabunzima Mxhaka residing at ‘the Great Place at Mamolweni’ in mPondoland, so as to gain endorsement for the study and introduction to people in this region. The Chief also pointed out that even though his people identify through the *amaMolo* clan name they are also known as *abeLungu*. This was the first indication that what had been read about in documented history about the amaMolo being an independent clan was not necessarily reflected in the field. Having obtained approval from the Chief, other members of the *abeLungu* were engaged with from whom clan family histories were collected. The research was facilitated by our interpreter Mr. Qaqambile Godlo, who enabled translations between English and isiXhosa. Most subjects were able to name their male antecedents back to their clan progenitor. The genealogies of clan representatives were constructed from oral histories and kinship data collected by Kalis, and are located in Appendix C, under Figures S1 – S6.

Table 2.1. Geographic sampling regions of abeLungu clans

Sampling region / Lali	Clans													
	Primary abeLungu clans							Secondary abeLungu clans						
	Jekwa	Hatu	Buku	Molo	Caine	France	Homer	Irish	Ogle	Hastoni	Sukwini	Fuzwayo	Thaka	
Ngwanguba														
Lusikisiki			3		6	1			2					
Mithatha					1				1		4			
Lambasi						4								
Tshani												4		
Mapuzi, Coffee Bay							10						1	
Mjikweni													1	
Madakeni										2				
Lucingweni				23						2	1			
Thombo										1				
Qatywa, Xhora		10												
Zwelitsha, Xhora		4												
Ntshilini								1						
Gogogo									1					
Mtalala									1					
Hobeni		4									1			
Sundwana, Xhora		29												
Xhora Mouth		3												
Mamolweni				14										
Bisho												5		
Mendwana												2		
Tyutyu												2		
Zwelitsha												1		
Port St. Johns													1	
Number of subjects (n=146)	36	14	3	37	7	5	11	2	3	5	16	6	1	

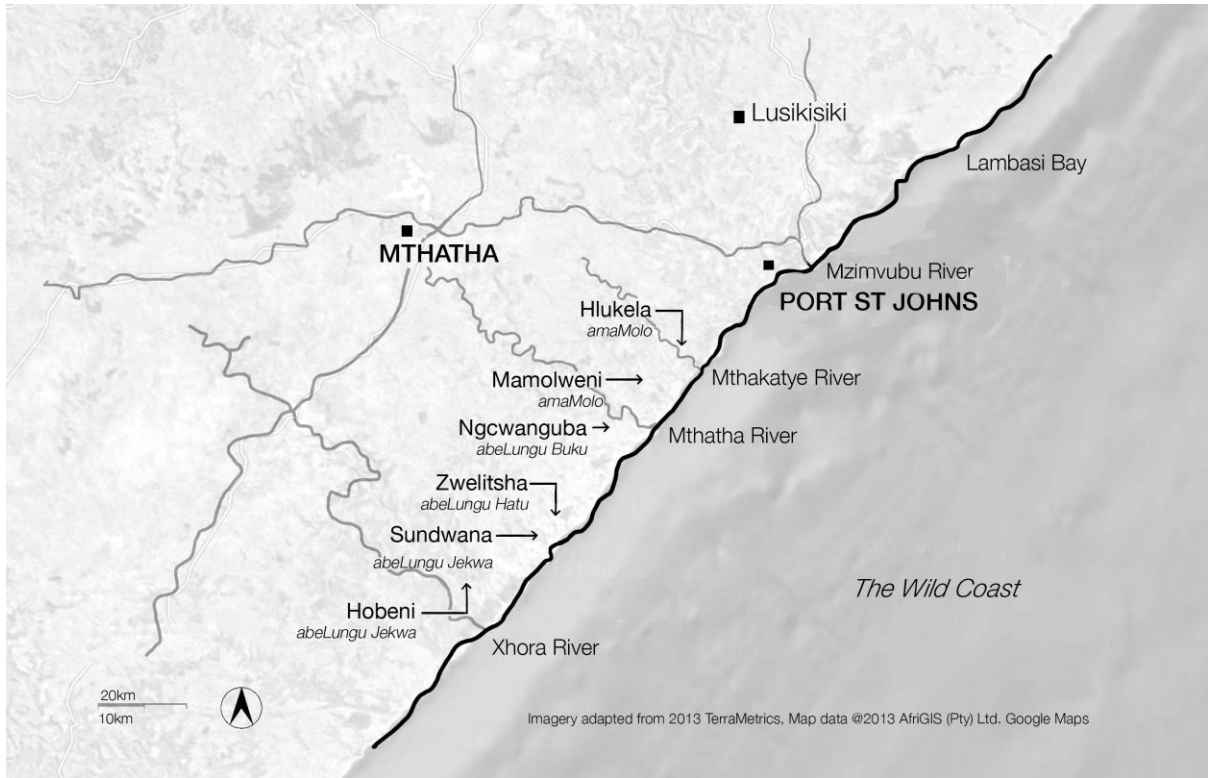


Figure 2.3. Research area of the study. Depicted are the locations of *lalis*, or homesteads of the three primary abeLungu clans, namely abeLungu Jekwa, Buku, Hatu as well as the amaMolo. The research area is located between the Xhora and Mzimvubu rivers, as situated along the Wild Coast, in the Eastern Cape of South Africa. This distribution can be found under Table 1. This map has been adapted from Google Maps online service: Imagery @2013 TerraMetrics, Map data @2013 AfriGis (Pty) Ltd. Google Maps

2.2. Laboratory Methods

2.2.1 DNA extraction and quantification

Buccal swabs were taken using sterile cytology brushes from Gentra Puregene® Buccal Cell Kits (Qiagen, Germany). Swab heads were placed in labelled 2.0 ml Eppendorf tubes containing 300µl Cell Lysis Solution (Puregene® Kit). Two brush swabs (an A and B sample) were taken per individual, in the event of the contamination the first sample or simply if there was not enough DNA of the first sample to fully resolve the subject's ancestry genotype. DNA extraction was performed on cheek cells collected by using Puregene® DNA purification kits (Qiagen, Germany) according to the product manufacturer's instructions. The basic principles of this method of DNA extraction involve the lysis of the cell membrane, degradation of proteins and the final precipitation of DNA out of solution. Nucleic acid concentrations were quantified using a NanoDrop® ND-1000 Spectrophotometer and ND-1000 v2.2.0 software (Thermo-Fisher Scientific Inc.). Absorbance values were measured at a wavelength of 260nm. Once extracted, stock DNA was diluted with ddH₂O into the required working concentrations for the various genotyping processes (20ng/µl, 5ng/µl and 1 ng/µl).

2.2.2 Molecular methods for Y chromosome DNA studies

All methods for mtDNA and Y chromosome DNA screening have been developed and optimised within the Human Genomic Disease and Diversity Research Laboratory (HGDDRL). A combination SNP-STR method of genotyping Y chromosome DNA had been employed for the purpose of examining Y chromosomes both at the haplogroup level, and at the more resolved haplotype level. These include resolving haplogroup-defining Y chromosome SNPs by using several Single Base Extension (SBE) assays, as described by Naidoo et al., (2010), and secondly through examining STR variation using microsatellite loci. When using combination SNP-STR systems, one acquires an improved resolution of the landscape of geographic origins as well as improved precision and accuracy of divergence time estimates (Zhivotovsky et al., 2004; Ramakrishnan and Mountain, 2004; Klyosov, 2009; Naidoo et al., 2010).

2.2.2.1 Y-STR genotyping

STR genotyping was performed using the AmpF λ STR $\text{\textcircled{R}}$ Yfiler TM PCR Amplification Kit (Life Technologies) according to the manufacturer protocol. The AmpF λ STR $\text{\textcircled{R}}$ Yfiler TM assay is a multiplex which permits the simultaneous analysis of 17 STR loci in a single PCR amplification, allowing for increased discrimination capacity of haplotype analysis. The kit contains markers for the Y-STR 'minimal haplotype' (minHt) which encompasses the marker panel recommended by the Scientific Working Group on DNA Analysis Methods (SWGDM). The panel includes the Y-STR markers: DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, and DYS393. In addition to the panel, markers for the highly polymorphic loci DYS438, DYS439, DYS427, DYS448, DYS456, DYS458, DYS625 (Y GATA C4), and Y GATA H4 are included so as to increase the resolution capacity to that of a suite of 17 microsatellite loci (Ayub et al., 2000; Redd et al., 2002, Mulero et al., 2006; AmpF λ STR $\text{\textcircled{R}}$ YFiler TM user guide). STR screening using the STR Yfiler Kit System begins with the initial PCR amplification step, with the reagents used, listed in Table 2.2.

Table 2.2. Amplification of Y-STR loci

PCR reagents	Final Volume (μ l)
DNA sample (1ng/ μ l)	1.00
Ampf λ STR Yfiler PCR reaction mix	2.30
Ampf λ STR Yfiler Primer set	1.25
AmpliTaq Gold $\text{\textcircled{R}}$ DNA polymerase	0.20
ddH $_2$ O	1.50

The Y-STR amplification PCR Thermal Cycler (9700) conditions were: An initial denaturation step of 11 minutes at 95 $^{\circ}$ C, followed by 30 cycles of denaturation at 94 $^{\circ}$ C for one minute, annealing at 61 $^{\circ}$ C and a ligation and extension at 72 $^{\circ}$ C for one minute. Lastly a final extension step at 60 $^{\circ}$ C for 80 minutes occurs preceding the final cooling and holding step which brings the temperature to rest at 4 $^{\circ}$ C. PCR products were visualized by suspending one microliter of PCR product together with 0.3 μ l internal lane standard (GS500 LIZ) (Life Technologies) in 8.7 μ l Hi-Di $\text{\textcircled{R}}$ Formamide, before

being analyzed on a 3130xl Genetic Analyzer (Life Technologies), and subsequently visualized using Genemapper® ID Software v3.2 (Life technologies).

2.2.2.2 Y chromosome binary marker screening

Single base extension (SBE), is a fluorescence-based multiplex PCR system that allows for numerous ancestry informative SNPs to be typed in a single reaction (Gray, 2000; Schlebusch et al., 2009). The principle of the SBE method lies behind the extension of a “detection” primer (which vary through the attachment of varying sized polynucleotide tail) and has annealed immediately upstream to the 5’ of the mutation site, using one of four fluorescently-labelled dideoxynucleotide-triphosphates (ddNTPs), with a fifth colour (LIZ 120) used as the internal lane standard. Naidoo et al., (2010), developed seven SBE assay multiplex panels which resolve Y chromosomes to one of 61 terminal haplogroup branches, where six panels focus on resolving markers delineating subclades of African haplogroups A, B and E. All samples were initially run with the YSNP1 panel (as they had presumably European or Eurasian ancestry) and subsequent SBE multiplexes were performed hierarchically, following the phylogenetic placement and allelic states of resolved polymorphisms on the Y chromosome SNP phylogeny [Figure 2.4(a)] (YCC, 2002; Jobling and Tyler-Smith, 2003; Karafet et al., 2008; Schlebusch et al., 2009; Geppert and Roewer, 2012). Haplogroup nomenclature is in accordance with the most current International Society of Genetic Genealogy (ISOGG) standard; when last accessed in November, 2016 and topology of the phylogeny is based on that of Karafet et al., (2008). The YSNP1 marker panel was used for resolving European and Eurasian haplogroups, and included primers for the binary markers SRY1083.1, M168, M89, M201, M69, M170, M172, M9, M207, M198 and M343 [Figure 2.4(b)]. Samples which were found to be ancestral at marker SRY 1083.1 and M91 were run using the using HG-A and HG-B SBE assays. The SNPs used in the various mini-sequencing panels are listed under Appendix B, Table S1, where haplogroup nomenclature is also in accordance with the most current ISOGG, 2016 nomenclature. The ancestral and derived states of markers as they appear on electropherogram profiles of YSNP1 assays are illustrated in Figure 2.4(b). The ABI PRISM® SNaPshot™ Multiplex Kit (Life Technologies) was used for all SBE assays, according to the manufacturer’s protocol

with modifications on methods established by Naidoo et al., (2010). PCRs were conducted using GeneAmp® PCR System 9700 Thermal Cyclers (Life Technologies).

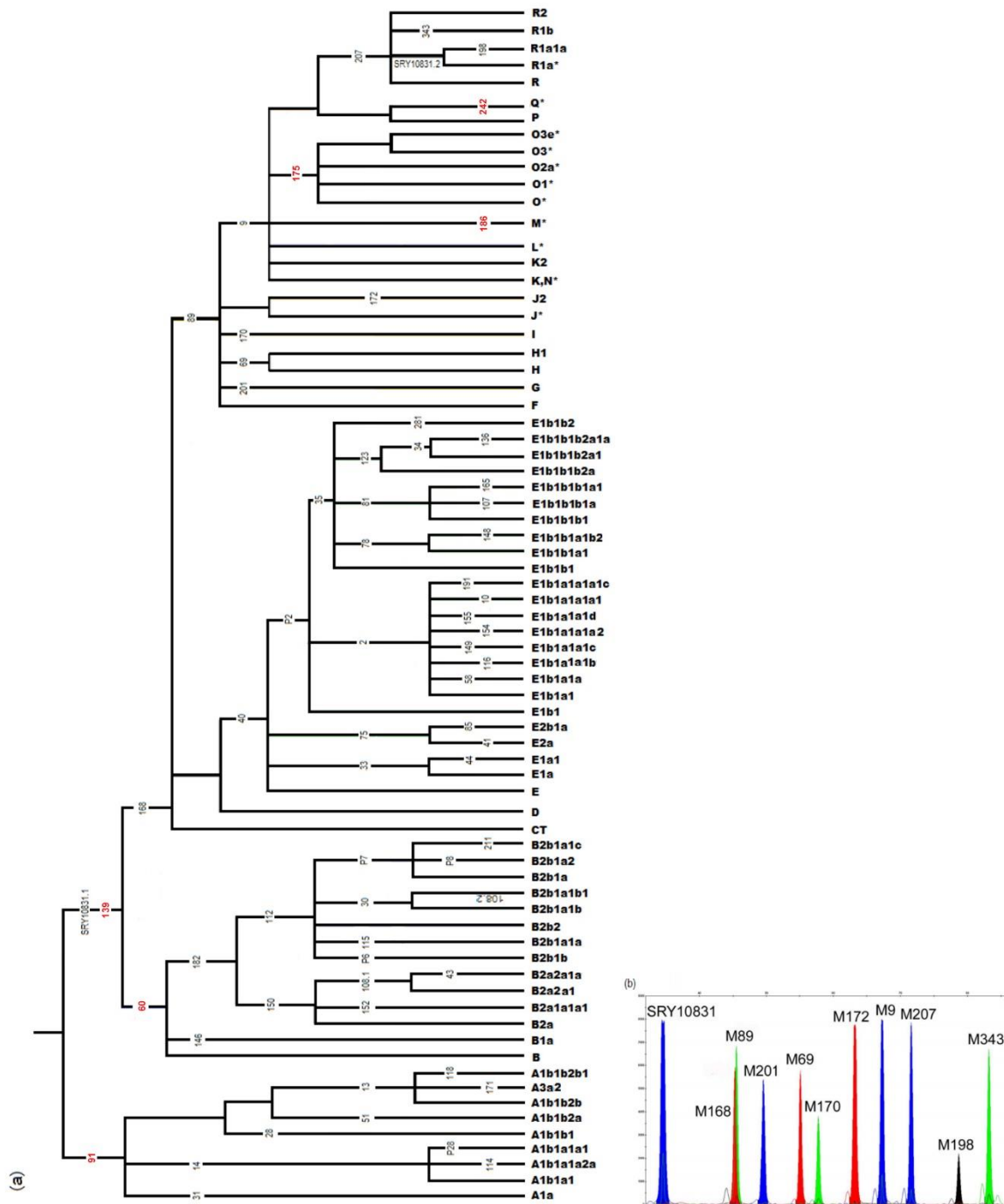


Figure 2.4 (a) The Y chromosome SNP phylogeny with topology adapted from Karafet et al., (2008) and nomenclature based on the ISOGG Y-DNA Haplogroup Tree (2016), illustrating the Y haplogroups which are designated by specific SNP markers screened for using the SNaPshot™ SBE method, as well as the multiplex II assay. Newer branches of haplogroup A (A0 and A00), more recently defined by ISOGG (2015) have not been indicated on the phylogeny (b) An electropherogram showing the relative peak height, position and colour of peaks illustrating the derived states of the Y-STR markers screened for when using the YSNP1 SBE marker panel

Products were separated on an ABI PRISM® 3130xl Genetic Analyzer (Life technologies) and data was visualized using GeneMapperID v3.2 software (Life technologies). The reagents used for the YSNP1 multiplex PCR are listed in Table 2.3.

Table 2.3. YSNP1 SBE multiplex PCR reagents

PCR reagents	Final Volume (µl)
FastStart 10x Buffer (with MgCl ₂)	2.5
MgCl ₂ (25 mM) [2.5 mM]	2
dNTPs	3
YSNP1 Forward Primer Mix	1
YSNP1 Reverse Primer Mix	1
ddH ₂ O	14.3
FastStart Taq	0.2
Total	24.0

The PCR conditions used for the YSNP1 multiplex PCR are listed in Table 2.4.

Table 2.4. SBE PCR thermal cycler conditions

Temperature	Phase	Time (minutes)	
95°C	Initial Denaturation	6:00	
95°C	Denaturation	00:30	35 cycles
54°C	Annealing	00:30	
72°C	Ligation and extension	00:30	
72°C	Final extension	10:00	
25°C	Hold	∞	

Excess PCR primers and dNTPs were removed via enzymatic purification. For every 5µl PCR product, 2µl of purification mix (see constituents in Table 2.5) was added which was incubated at 37°C for 1 hour. Enzyme inactivation was at 75°C for 15min.

Table 2.5. Post-PCR purification reaction reagents

Reagents	Volumes per reaction (µl)
Shrimp Alkaline Phosphatase (1U/µl)	1.4
Exonuclease I (20U/µl)	0.2
ddH ₂ O	2.4

Once PCR-product had been purified, the SBE reaction proceeded. In this step the “detection” primers were extended by ddNTPs that are complementary to the SNPs of interest, using the ABI Prism SNaPshot™ Multiplex kit (ABI Life Technologies). A positive control and a negative control template were included in the assay. The PCR protocol is made of 35 cycles of a 10 second denaturation step at 96°C, followed by annealing which occurs at 50°C for 5 seconds and lastly the ligation and extension at 60°C for 30 seconds. The reagents and their proportions as used in the SBE reaction are listed below in Table 2.6.

Table 2.6. YSNP1 Multiplex SBE reaction

SBE mix	Volumes per reaction (µl)	
SNaPshot™ Multiplex Ready Reaction Mix	1	
YSNP1 SBE primer mix	1	
ddH ₂ O	1.5	
Total	3.5	
	Positive control	Negative control
Control DNA Template	1.5	~
SNaPshot™ Multiplex Ready Reaction Mix	1	1
Control Primer mix	1	1
ddH ₂ O	1.5	3
Total	5	5

Post-Extension enzymatic purification was then performed so as to remove excess dNTPs and reagents. For every 5µl of SBE product 2µl of post-extension mix (comprising of 0.5µl SAP (1U/µl, 0.7µl 10X SAP buffer and 0.8µl ddH₂O) was added. The mix was incubated at 37°C for 1 hour, and inactivation of the enzymes was done at 75°C for 15 minutes. Detection of SNPs was performed by suspending 2.0µl of cleaned SBE product in 7.5µl Hi-Di® Formamide (Life Technologies), together with 0.5µl of internal lane standard (GS120 LIZ), prior to running on the on a 3130x/ Genetic Analyzer (Life Technologies).

2.2.2.3 Additional marker screening

Additional ancestry informative SNPs and STR markers not covered in the Yfiler™ and SNaPshot™ systems were screened for using the Multiplex-II assay developed in the HGDDRL. The additional SNP markers included in the Multiplex II assay were M139, M17, M175, M186, M60 and M91, which screen for haplogroup clades A, B, R1a1 as well as internal nodes within the Y chromosome phylogeny and can aid to direct genotyping by indicating whether to exclude or include certain SNaPshot™ systems. The additional highly polymorphic Y-STRs included in the multiplex II assay were DYS426 and DYS388 which also increased the resolution of haplotype profiles to that of 19 STR markers.

The procedure for the Multiplex II PCR amplification entails suspending 1.0µl DNA template at a concentration of 1ng/µL in 9.0µl True-Allele PCR Premix, together with 5.0µl primer mix, totaling to a 15.0µl reagent mix. The PCR protocol for the Multiplex II PCR amplification is as follows: An initial denaturation step is performed at 95°C for 11 minutes. Thirty cycles of a three step process follow according to: a denaturation step at 94°C for one minute; annealing at 61°C for one minute and ligation and extension occur at 72°C for one minute. Lastly a final extension step occurred at 60°C for 80 minutes followed by a holding step at 4°C. PCR products were subsequently prepared for analysis by suspending 1.0µl product in 8.5µl Hi-Di® Formamide (Life Technologies), together with 0.5µl of internal lane standard (GS500 LIZ), prior to running on the on a 3130x/ Genetic Analyzer (Life Technologies). SNP and STR profiles were visualized using GeneMapperID v3.2 software (Life technologies).

The ABI Taqman® assay (Life Technologies) was used to screen for SNP marker M242 in order to confirm the presence of haplogroup Q samples (predicted using YSTR haplogroup prediction software), according to the manufacturer’s protocol, on a 7900HT Fast Real-Time PCR System (Life Technologies). The PCR to amplify the haplogroup Q-M242 SNP loci was performed by suspending 5.0µl of DNA (5ng) in 2.5µl Taqman® Universal Master Mix together with 0.25µl primer mix, and finally adding 1.25µl of ddH₂O, totaling the reagent mix at 5.0µl per reaction. The conditions for the Taqman® assay consisted of an initial denaturation step at 95°C for 11 minutes followed by 30 cycles of denaturation at 94°C for one minute, annealing at 61°C for one minute and extension and ligation at 72°C for one minute. A final extension occurred at 60°C for 80 minutes prior to the end hold at 4°C.

2.2.3 Mitochondrial DNA molecular methods

Two approaches have been used for examining mitochondrial DNA (mtDNA) variation. Firstly, sequencing of mitochondrial hypervariable region (HVR) d-loop variant sites was performed and secondly to confirm haplogroups, minisequencing of mtDNA control region SNPs was performed according to the methods described in Schlebusch et al., (2009).

2.2.3.1 Mitochondrial D-loop HVR sequencing

Mitochondrial D-loop HVR sequencing was performed according to ABI Prism Dye Terminator cycle-sequencing protocols developed by Life technologies, in conjunction with methods previously published by Vigilant et al., (1989) and Behar et al., (2007). Determining sequence variation of the mtDNA hypervariable regions was accomplished by firstly amplifying the 1kb D-loop segment containing both the HVRs using primers 15876F and 639R (Table 2.7).

Table 2.7. Primer sequences for the mtDNA1kb D-loop PCR amplification

Primer name	Primer sequence (5' – 3')
15876F (forward)	TCA AAT GGG CCT GTC CTT GTA G
639R (reverse)	GGG TGA TGT GAG CCC GTC TA

The 50µl reagent mix needed per reaction, for the mtDNA1kb D-loop PCR amplification reaction included 3.0µl of DNA template (5ng/µl), 2.0µl of dNTPs (2.5 mM), 2.0µl of each primer (15876F and 639R), 0.2µl FastStart® Taq, and made up to 50.0µl with 35.8 µl ddH₂O. Cycle sequencing of HVR I & II was performed both in the forward and reverse direction to confirm sequence information with primers used listed in Table 2.8. The conditions for the template fragment amplification consisted of an initial denaturation step at 95°C, 35 cycles of a three-step process: i) cyclical denaturation at 95°C for 30 seconds, ii) cyclical annealing at 55°C for 30 seconds, and iii) two minutes of cyclical extension at 72°C, followed by a final extension step at 72°C for 10 minutes and the final resting phase at 25°C.

The amplified fragment was resolved on a 2.0 % agarose gel with ethidium bromide staining to check the presence and quality of PCR product using 1 X TBE buffer, Bromophenol blue, Ficoll dye loading buffer and 1kb+ DNA ladder size standard (Thermo Fisher Scientific). Each gel run included a negative and positive control as well. Gels were run at 120V for 30 min and samples were visualized using the *G-Box* and GeneSnap software (SynGene). After confirming the presence of DNA bands and PCR product quality, products were then purified via enzymatic digestion to eliminate excess ddNTPs and reagents, using 0.2µl exonuclease I (at a concentration of 20U/µl) and 1.4µl shrimp alkaline phosphate (at a concentration of 1U/ µl), made up to 5.0µl with ddH₂O.

Table 2.8. Primer sequences for HVR I & II cycle sequencing

Region	Primer	Sequence
HVR I	15946 Forward	CAA GGA CAA ATC AGA GAA AA
	132 Reverse	GAC AGA TAC TGC GAC ATA GG
HVR II	L29 Forward	GGT CTA TCA CCC TAT TAA CCA C
	H408 Reverse	CTG TTA AAA GTG CAT ACC GCC A

The volumes per reaction of cycle sequencing reagents included 2.0µl amplified 1kb-PCR template, 1.0µl Big Dye Primer Mix (containing ddNTPs, Mg²⁺, *Taq* polymerase), 1.0µl of each of the four primers Primer L15946 / L29 / H132 / H408 (at a concentration of 3.3µM), lastly made up to 10µl with ddH₂O. The cycle-sequencing conditions consisted of an initial denaturation step at 96°C for one minute, 25 cycles of {cyclical denaturation at 96°C for 10 seconds, cyclical annealing at 50°C for five seconds, and four minutes of cyclical extension at 60°C}, followed by the final resting phase at 4°C. Prior to the final sequencing analysis sequencing products were purified using vacuum purification and filtration using Montage SEQ₉₆ sequencing reaction cleanup plates (Millipore). After purification sequencing products were resuspended in Hi-Di™ formamide and were resolved on an ABI PRISM® 3130xl Genetic Analyzer (Life technologies). POP_7 polymer was used instead of the supplier's recommended POP_4 polymer as recommended by a Life Technologies product bulletin (Life Technologies, P/N: 4267258). Sequence data was visualized using Sequencing Analysis Software v5.2 (Life technologies) which converted raw sequence data into base-sequence and electropherograms.

2.2.3.2 Mitochondrial SNaPshot™ Sequencing (MTSS)

MTSS is an SBE based method which acts to target one of 14 SNPs in the mitochondrial genome, which resolves to one of ten major macro-haplogroups corresponding to global mitochondrial variation (Schlebusch et al., 2009), represented in the mtDNA SNP phylogeny (Figure 2.5). The minisequencing protocol allows for distinction between seven African L mitochondrial macro-haplogroups and three non-African macro-haplogroups, namely M, N and R. MTSS assays were performed using the ABI PRISM® SNaPshot™ Multiplex Kit (Life Technologies) according to methods established by Schlebusch et al., (2009). The methods are based on those employed in Y chromosome SNP SBE screening using the ABI PRISM® SNaPshot™ Multiplex system (see section 2.2.2.2 - *Y chromosome binary marker screening*).

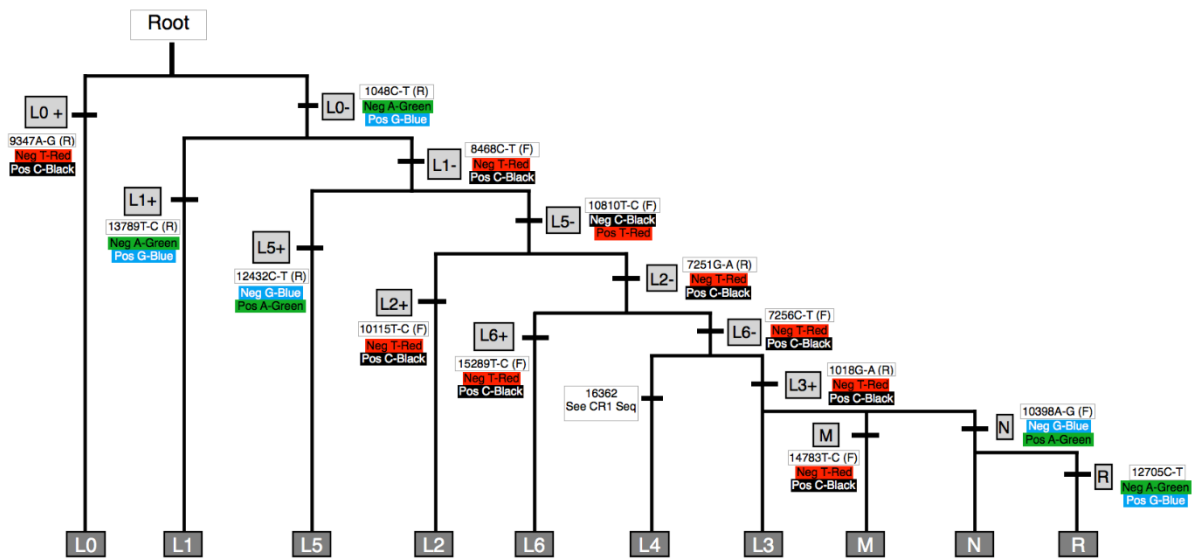


Figure 2.5. MtDNA SNP phylogeny, adapted from Schlebusch et al., (2009) with nomenclature established by Behar et al., (2008) and van Oven and Kayser (2008). Positions of SNPs are indicated and correspond to where they are found within the mtDNA molecule as illustrated in Figure 1.4 above in section 1.3.1. Colours are as they appear as peaks on electropherograms, which define ancestral or derived allelic states of the terminal SNP.

2.3 Data analysis

2.3.1 Y chromosome data analyses

Genemapper® *ID* Software v3.2 (ABI Life technologies) was used for visualisation of STR allele peaks. Allelic variation was determined by measuring the size of STR peaks which indicate the number of repeat copies of a specific locus. It is to be noted that the DYS389 locus is composite in nature and contains phylogenetically informative as well as fast-evolving regions that may obscure structure. To account for this, the peak height value at locus DYS389I has been subtracted from DYS389II, to give the derived value DYS389IIc which was further used in analyses (Moore et al., 2006; Roewer, 2009). The Whit Athey haplogroup predictor was used to determine haplogroups, using Y-STR haplotype loci peak heights as input data. An ‘equal priors’ search criterion was used so as to avoid geographic bias in predictions (Athey, 2005). Haplogroups were confirmed using informative SNPs resolved in Y chromosome SNaPshot™ SBE typing.

Extended haplotypes were generated when haplogroup SNPs were used in conjunction with STR haplotype data. Modal haplotypes are those which appeared

most frequently, and in most instances in the genealogies can be presumed to be the profiles tracing back to the original founding haplotype. Alternatively, modal haplotypes acquired the higher frequencies due to drift or rapid expansion. Throughout the haplotype analysis, for haplotype names a nomenclature system was implemented to retain the anonymity of research subjects. For example, the haplotype name “R343_2” represents the second (“_2”) unique haplotype found in haplogroup R1b (R-M343) samples. Extended haplotype names were superimposed onto clan genealogies to examine the patrilineal transmission of Y chromosomes in the abeLungu. Genealogies were constructed using information collated from interviews with clan elders and clan members, as well as subjects’ consent form genealogical information (Appendix C, Figures S1 – S6).

Additional data from ongoing projects in the HGDDRL, as well as published Y chromosome data from Eurasian, sub-Saharan African populations, including South African samples as well as samples from Near-African islands including the Maldives, Zanzibar and Madagascar were included in the comparative data analyses. Data from publications which have been used as comparative data include Cadenas et al., (2008), Varzari et al., (2013), Roewer et al., (2008) and Jarve et al., (2009). For haplogroup R1a1a segregating haplotypes comparative data was obtained from Nebel, (2001), Qamar et al., (2002), Capelli et al., (2007), Sengupta et al., (2006), Zalloua et al., (2008), Cadenas et al., (2008), Di Gaetano, (2009), Thanseem et al., (2006), and Msaidie et al., (2011). Depending on the comparative data, certain studies had typed profiles using fewer STR marker loci, data was subject to truncation on the number of useable loci from 19-markers to 12-marker haplotypes. A comprehensive breakdown of comparative data exhibiting number of samples, population groups, publication and/or in-house project and number of STR loci compared is included as Appendix E. See Appendix E table S2.

Regarding matches to databases and published literature, the primary database queried was the Y Haplotype Reference Database, the YHRD, which currently has data compiled from 49781 haplotypes defined by seven major populations (which are further divided into 20 subgroups). These include Eurasian, African, Afro-Eurasian, East Asian, Amerindian, Australian Aboriginal, Eskimo Aleut, as well as an admixed

population which has equal contributions from different ancestral populations (Willuweit and Roewer, 2007).

2.3.1.1 Y chromosome haplotype Networks

The association of haplotypes Y-haplogroups was examined within clans using reduced-median-joining (RMJ) networks constructed using the program Network version 4.6.1.1 (Fluxus-engineering) (Bandelt et al., 1999). Reduced-median-joining (RMJ) networks were done by calculating reduced-median (RM) and subsequently median-joining (MJ) trees. The combined RM-MJ technique was used to reduce network complexity as an RMJ network is often simpler than a pure MJ network, because implausible parallelisms have been avoided, where additional star contraction preprocessing has been used (Bandelt et al., 1999). For each haplogroup modal, ancestral haplotypes were listed with variant haplotypes and submitted as related sequences for processing using a reduction threshold ($r = 2$, by default) (Bandelt et al., 1999). STR markers were weighted proportionally to the inverse of STR allelic variance (Cruciani et al., 2004). Data was cleaned and readied as an input file for the RMJ network calculations using Microsoft Excel as the tab-delimited text-editor. During analyses the epsilon parameter criteria (which increases reticulation possibilities) was set to zero (Network 4.6.1 user guide; Bandelt et al., 1999).

2.3.1.2 Database Search queries

The Y-STR Haplotype Reference Database (YHRD) international forensic STR reference database allows for the assessment of male population stratification among world-wide populations as far as reflected by Y-STR haplotype frequency distributions (Roewer et al., 2001). Y haplotypes were blasted to the YHRD database in search for global haplotypic matches at the 17-loci level of resolution as well as at the “minimal haplotype” eight-locus level of resolution. In addition, the Genographic Consortium Y chromosome database and the HGDDRL Y-STR comparative dataset, as well as STR profiles from unpublished data collected in the HGDDRL were blasted and queried for haplotype matches against profiles from European, Asian, Eurasian, as well as sub-Saharan-African populations (which include South African samples of a number of different ethnic backgrounds as well as samples from the Maldives, Zanzibar and Madagascar).

2.3.2 Mitochondrial DNA analyses

The mtDNA haplogroups of all 198 subjects including the ten females sampled were generated by using the online software prediction tool, Haplogrep, which operates by comparing HVRI and HVRII variant sites to the mtDNA phylogeny available through the PhyloTree platform (Build 16) (van Oven and Kayser, 2009). In addition, mtDNA SNaPshot™ minisequencing was used for typing 17 coding-region SNPs to confirm haplogroup identity. The regions of HVRI and HVRII which were sequenced ranged from positions 15997-407 of the mtDNA molecule.

HVR sequences were aligned in BioEdit v 7.9, using the Clustal W algorithm (Hall, 1999) and variant sites were compared with the revised Cambridge Reference Sequence (rCRS) (Andrews et al., 1999). The software program *S-compare* (Ronnie Nelson, University of Pretoria) was used to identify and extract variant sites from the alignment files. The accepted nomenclature used for mtDNA haplogroups is based on that proposed by Behar et al., (2008), and van Oven and Kayser (2009) with recent modifications as found on the Phylotree database (<http://www.phyotree.org/>).

Out of the 198 individuals originally sampled, 21 sibling pairs were observed by directly counting them in the clan genealogies (Appendix C, Figures S1-S6). Duplicates from these sib pairs were excluded from mtDNA analysis so as to avoid a bias in the data, while the 51 individuals not affiliated with clans (including the ten females which were sampled) were retained. This resulted in a subset of 176 individuals' sequences which were used in the subsequent analyses.

MtDNA haplotypes using HVR I and HVR II variant site data were used to construct a phylogenetic tree using the Neighbour-Joining (NJ) method in the phylogenetic software MEGA v5 (Tamura et al., 2007). The bootstrap consensus tree is taken to represent the evolutionary history of the haplotypes analyzed, with the percentage of replicate trees in which the associated taxa clustered together is shown next to the branches. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of substitutions per site. All positions containing

gaps and missing data were eliminated. Phylogenetic analyses of Neandertal mtDNA suggests that it diverged from the extant human mtDNA lineage on the order of 660,000 years ago, and that Neandertal mtDNA falls outside the variation of modern human mtDNA (Green et al., 2008). Since the mtDNA genome is maternally inherited without recombination, these results indicate that Neandertals made no lasting contribution to the modern human mtDNA gene pool, thereby making it a suitable sequence to include as an evolutionary out-group for phylogenetic analyses. The Neanderthal mtDNA genome sequence made available through the NCBI, under GenBank accession number: *NC_011137.1* was included as the out-group in the alignment (Green et al., 2008).

CHAPTER 3

Results

3.1 Y chromosome DNA studies

The Y chromosome results are presented three-fold: Firstly results are presented at the haplogroup/SNP level indicating possible geographic locations of origin. The results then proceed to discuss the associations of further resolved results at the haplotype level in networks within haplogroups. Lastly, haplogroup/haplotype data is consolidated with anthropological data of clan-genealogies and findings are presented as well.

3.1.1 Y chromosome haplogroups

From the sample of 188 males including non-clan affiliated samples, 55.79% of Y chromosomes segregate with non-African ancestry (Figure 3.1). When examining the *abeLungu* and *amaMolo* clans only, the frequency of non-African Y chromosome lineages escalates to 69.86% (Figure 3.1).

Individuals found to have Eurasian and European ancestry, had Y chromosomes associated with haplogroups R1b (R-M343), R1a1a (R-M198), Q (Q-M242), G (G-M201), I (I-M170) and J (J-M172). Haplogroup R1b was the most common haplogroup, found at a frequency of 41.10% (Figure 3.1). This is the dominant haplogroup in Western Europe and is also found in Eastern Europe and Western Asia (Kivilsild et al., 2002; Campbell, 2007; Karafet et al., 2008; Chiaroni et al., 2009; Myres et al., 2011; Raghavan et al., 2014). The second most frequently observed haplogroup, R1a1a (R-M198), was found in 14.38% of the sample, is the dominant Y chromosome lineage found in modern Eurasia, and is a prevalent in India and Eastern Europe and the Caucasus region (Jobling and Tyler-Smith, 2003; Sengupta et al., 2006; Klyosov and Rozhanskii, 2012). Haplogroup Q (Q-M242) is another haplogroup prevalent in the Eurasian subcontinent, which was observed in 5.48% of clan-affiliated samples. Haplogroup G (G-M201) is typically located in the Caucasus, the Middle East and Southern Asia was found in two *abeLungu* *Buku* individuals (Cruciani et al., 2002; Cinnioglu et al., 2004; Karafet et al., 2008). Haplogroup I (I-M170), appears in 8.65%

of samples and depicts Western European origins mostly from Britain. It is the haplogroup which occurs in nearly 20% of the European male population, but has also been found among populations of the Near East, the Caucasus, Central Siberia and Northeast Africa (Hammer and Zegura, 2002; Karafet et al., 2008). Haplogroup J (J-M172) was found in one individual; this haplogroup is found at high frequencies in the Middle East, North Africa, Europe, Central Asia, Pakistan, and India (Underhill et al., 2001; Semino et al., 2002; Behar et al., 2004; Sengupta et al., 2006).

Several haplogroups with origins found in the sub-Saharan-African region were observed at relatively low frequencies in clans and at higher frequencies (some greater than 20%) in non-clan affiliates (Figure 3.1; Table 3.1). These included haplogroups A1b1b2a (A-M51), B2a1a1a1 (B-M152), E1b1a1 (E-M2), E1b1a1a1c1a (E-M191), E2b1a (E-M85), and E1b1b1 (E-M35). The most frequently observed haplogroup in these samples was haplogroup E-M85, which was observed in 20 of the non-clan affiliated individuals. Only one non-clan affiliated individual featured non-African haplogroup R1a1a (R-M198) and one featured haplogroup I (I-M170) (Table 3.1).

Table 3.1. Y chromosome haplogroup distribution for non-clan affiliated samples

Haplogroup	(<i>n</i> =42)	Frequency
B2a1a1a1 (B-M152)	2	4.7%
E1b1a1a1c1a (E-M191)	9	21.4%
E1b1a1 (E-M2)	8	19%
E1b1b1 (E-M35)	1	2.38%
E2b1a (E-M85)	20	47.6%
I (I-M170)	1	2.38%
R1a1a (R-M198)	1	2.38%

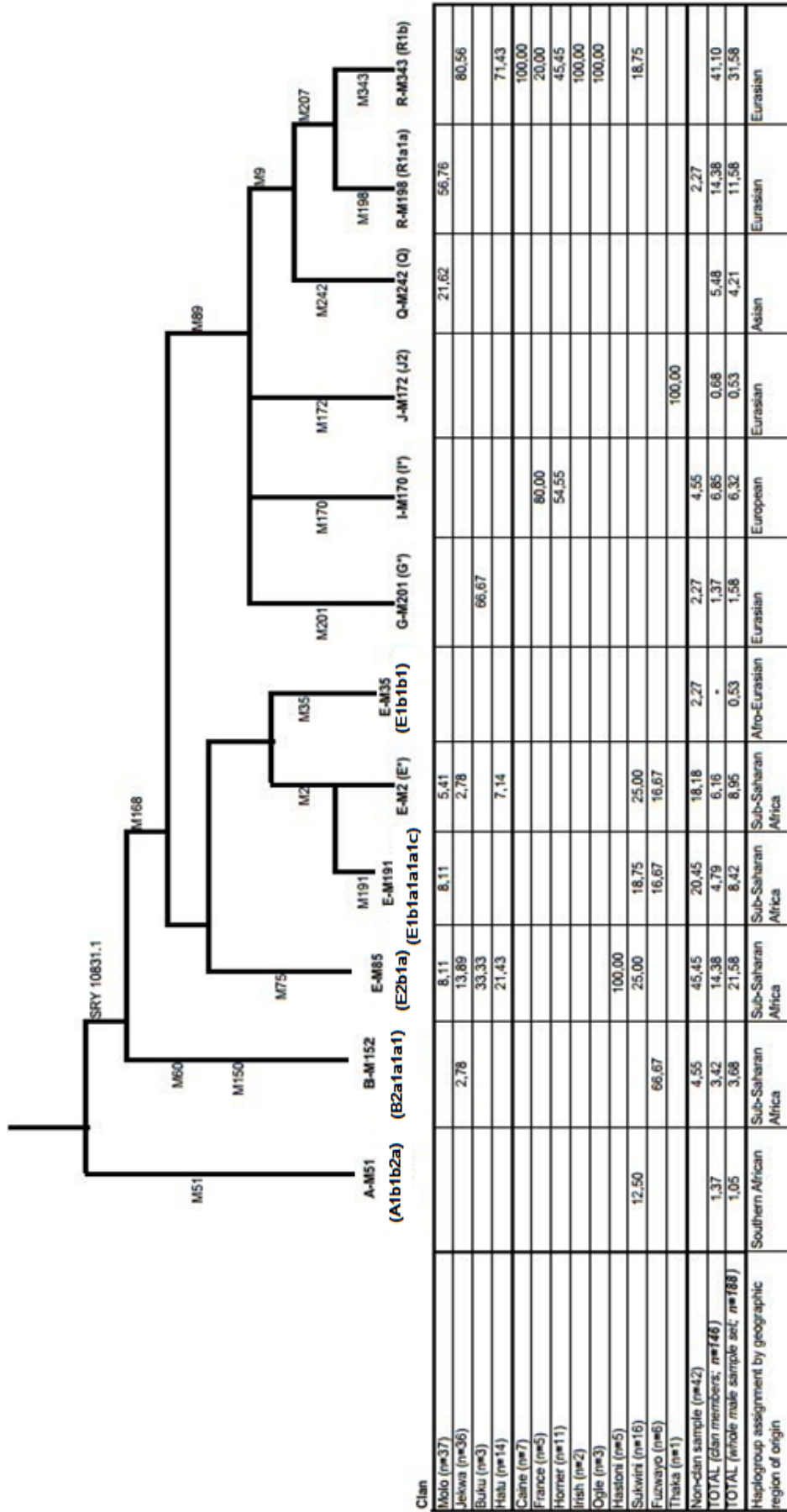


Figure 3.1. Phylogeny and frequency distribution of Y chromosome haplogroups observed i) in the abeLungu clans-people, as well as ii) in the whole sample set, including non-clan affiliated samples. Nomenclature based on ISOGG (2016)

3.1.2 Y chromosome DNA haplotype variation

Altogether 78 unique haplotypes were derived from the sample of 188 male individuals using the 19 Y-STR loci system (in the order DYS19, DYS385A, DYS385B, DYS388, DYS389I, DYS389IIc, DYS390, DYS391, DYS392, DYS393, DYS426, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635, GATA H4), which are summarised in Table 3.2(a) (non-African ancestry) and Table 3.2(b) (African ancestry). To maximise the use of space for Tables 3.2(a) and 3.2(b), an abbreviated haplotype-name format was used. For example, unique haplotype_4a within haplogroup R1b (R-M343), is named "R343_4a". Throughout the remainder of the text however, haplotypes are named with the alphanumeric haplogroup name, followed by the unique haplotype number, for example, 'R1b_3'.

Network analysis permitted the examination of the relationships of haplotypes within haplogroups R1b (R-M343), R1a1a (R-M198), I (I-M170), B2a1a1a1 (B-M152), E1b1a1 (E-M2), E1b1a1a1c1a (E-M191) and E2b1a (E-M85) that had at least three different haplotypes within them (Figures 3.2 - 3.8). Those haplogroups which did not meet this criteria, like haplogroups G (G-M201), J (J-M172), Q (Q-M242), E1b1b1 (E-M35) and A1b1b2a (A-M51), were excluded from network analyses. Note, in the networks, branch lengths are proportional to the number of mutational steps between haplotypes and the locus at which mutations are observed are shown. The size of the circle is proportional to the number of individuals that have a particular haplotype and nodes (positions in the network not found in the sample but which connects haplotypes in the network) are shown as solid dots.

In addition to the haplotype networks, haplotype distribution within clans was also examined in conjunction with genealogical information. In these genealogies constructed from oral histories by Ms Janet Kalis, the genealogical information linking extant males to their clan forefather, as well as their female relatives, including (multiple) wives where applicable, are shown. Extended haplotype names were superimposed next to individuals tested (Appendix C, Figures S1-S6). In these pedigrees, triangles represent males, while circles are females, adopting the nomenclature used by genealogists (which differs from that used by geneticists where males are represented as squares). Filled shapes indicate sampled subjects. Individuals with strikes through are deceased, which have been included to provide

clarity with kinship. Suffixes (lower case) letters are necessary to differentiate between individual haplotypes under a modal haplotype.

There was no haplotype sharing observed among clan members with European and Eurasian derived haplogroups, however several African haplotypes are found shared between individuals among different clans, namely haplotypes E2b1a_2, E2b1a_4, E2b1a_6, E2b1a_9, E2b1a_16, E1b1a1a1c1a_10, and B2a1a1a1_2 (Table 3.2(b)). The presence of African haplotypes within clan patrilineages can be attributed to genetic input by males from other Xhosa clans of the neighbouring region.

Note: Figure 3.2(a) and (b) cover two pages each to ensure maximized resolution.

HG	Haplotype loci																							HT Name	Total
	DYS19	DYS285A	DYS285B	DYS288	DYS289I	DYS289IIc	DYS390	DYS391	DYS392	DYS393	DYS426	DYS437	DYS438	DYS439	DYS448	DYS456	DYS458	DYS635	GATA H4						
R-M343	14	10	14	12	12	12	12	10	13	13	12	12	12	11	19	16	17	23	13	1	1				
	14	11	14	12	13	16	25	11	14	13	12	12	12	12	18	17	17	23	12	2	5				
	14	11	14	12	13	16	25	10	13	13	11	15	12	11	19	16	17	23	13	3	1				
	14	11	14	12	12	16	25	10	13	13	12	15	12	11	19	15	17	23	12	4	2				
	14	11	14	12	12	16	25	10	13	13	12	15	12	11	19	16	17	23	13	5	19				
	14	11	14	12	12	16	25	10	13	13	12	15	12	11	19	16	18	23	13	6	2				
	14	11	14	12	12	17	25	10	13	13	12	15	12	11	19	16	17	23	13	7	3				
	14	11	14	12	13	16	22	11	13	13	12	15	12	12	12	19	17	23	12	8	1				
	14	11	14	12	13	16	23	11	13	13	12	15	12	13	19	16	19	23	12	9	2				
	14	11	14	12	13	16	24	11	13	13	12	14	12	12	19	15	17	23	11	10	1				
	14	11	14	12	13	16	24	11	13	13	12	15	12	13	19	15	17	23	12	11	1				
	14	11	14	12	13	16	24	12	13	13	12	15	12	13	19	15	17	23	12	12	1				
	14	11	15	12	12	16	25	10	13	13	12	15	12	11	19	16	17	23	13	13	1				
	14	11	15	12	13	16	23	10	13	13	12	14	12	12	19	16	17	24	11	14	2				
	14	13	14	12	13	14	23	11	13	13	12	15	12	11	19	16	18	23	13	15	2				
14	13	14	12	14	14	23	11	13	13	12	15	12	11	19	16	18	23	13	16	5					
15	11	13	14	13	16	24	11	13	13	12	14	12	11	19	15	16	23	11	17	1					
15	11	13	14	13	17	24	11	13	13	12	14	12	11	19	15	17	23	11	18	9					
16	11	15	12	13	17	25	10	11	11	13	12	14	11	11	21	15	17	23	13	1					
16	11	15	12	14	17	25	10	11	11	13	12	14	11	11	21	15	17	23	13	2					
16	11	15	12	14	17	25	10	11	11	13	12	14	11	11	21	15	18	23	13	3					
16	11	15	12	14	17	25	10	11	11	13	12	14	11	12	21	15	17	23	13	4					
17	18	18	12	13	18	21	10	11	11	15	12	14	11	11	20	15	17	21	11	5	1				
17	14	19	12	13	17	25	10	13	13	12	15	12	11	11	18	16	16	23	11	8					
J-M172	14	14	16	12	13	18	23	8	11	12	11	14	9	11	15	17	22	11	1	1					
	14	13	14	14	12	16	22	10	11	13	11	16	10	11	20	14	15	22	11	1	1				
I-M170	14	14	14	14	12	16	22	11	11	15	11	16	10	11	20	13	15	21	11	2	1				
	14	14	14	14	12	17	21	11	11	12	11	16	10	11	20	14	16	22	11	3	4				
	15	14	15	13	13	18	23	10	12	15	11	14	10	11	20	15	16	21	12	4	1				
	15	15	15	13	13	18	23	10	12	15	11	14	10	11	20	15	16	21	12	5	4				
	15	15	15	13	13	18	23	10	12	15	11	14	10	11	20	15	16	21	12	6	1				
G-M201	15	14	14	13	13	18	23	10	12	15	11	14	10	11	20	15	16	21	13	1	1				
	17	14	15	13	12	18	23	11	11	14	11	16	10	11	21	15	16	20	12	2	2				

Table 3.2(a). Y chromosome STR haplotypes for the 13 abeLungu clans segregating with European and Asian haplogroups R1b (R-M343), R1a1a (R-M198), Q (Q-M242), J (J-M172), I (I-M170) and G (G-M201)

		abelLungu clans														
HG	HT Name	Frequency	Jekwa	Hatu	Buku	Molo	Caine	Irish	Horner	France	Ogle	Hastoni	Fuzwayo	Sukwini	Thaka	Non-clan sample
	1	1	1													
	2	5							5							
	3	1	1													
	4	2	2													
	5	19	19													
	6	2	2													
	7	3	3													
	8	1														
R-M343	9	2									1			1		
	10	1						2						1		
	11	1												1		
	12	1												1		
	13	1	1													
	14	2									2					
	15	2					2									
	16	5					5									
	17	1		1												
	18	9		9												
	1	2				2										
R-M198	2	16				16										
	3	2				2										
	4	1														1
	5	1				1										
Q-M242	1	8				8										
J-M172	1	1													1	
	1	1														1
	2	1														1
I-M170	3	4								4						
	4	1							1							
	5	4							4							
	6	1							1							
G-M201	1	1														1
	2	2		2												
Total:		105	29	10	2	29	7	2	11	4	3	0	0	3	1	4

Table 3.2(a) continued .Frequencies of Y chromosome STR haplotypes for the 13 abelLungu clans segregating with European and Asian haplogroups R1b (R-M343), R1a1a (R-M198), Q (Q-M242), J (J-M172), I (I-M170) and G (G-M201)

HG	HT Name	abelungu clans													Non-clan sample				
		Frequenoy	Jekwa	Habu	Buku	Molo	Caine	Irtich	Homer	France	Ogle	Hastoni	Fuzwayo	Sukwini		Thaka			
E-M85	1	1		1														1	
	2	2			1													3	
	3	3																	
	4	2	1	1															
	5	1	1																
	6	7			1						6							1	
	7	1																1	
	8	1																1	
	9	11	1		1										1			7	
	10	1													1				
	11	2													2				
	12	1													1				
	13	1													1				
	14	1													1				
	15	1													1				
	16	4	2	1														1	
	17	1																1	
E-M35	1	1															1		
E-M2	1	1																1	
	2	2																2	
	3	1																1	
	4	1	1																
	5	1																	
	6	1																	
	7	1																	
	8	3																	
	9	1																	
	10	1																	
	11	1																	
	12	1																	
	13	1				1													
	14	1				1													
	E-M181	1	1		1														1
	2	1																	1
	3	1																	1
4	1																	1	
5	2																		
6	1																		
7	1																		
8	1																		
9	2																		
10	3																		
11	1																		
12	1																		
E-M152	1	1																1	
2	2	1																1	
3	4																		
A-M51	1	2																	
Total		84	7	4	1	3	0	0	0	0	0	0	5	8	17	0	0	38	

Table 3.2(b) continued. **Frequencies of Y chromosome STR haplotypes within the 13 abelungu clans segregating with African haplogroups E-M85, E-M35, E-M2, E-M191, B-M152 and A-M51**

3.1.2.1 Y chromosome variation linked with Eurasian origins: haplotypic variation within the amaMolo

Acknowledging that matches to haplotypes are only as good as the data within datasets and not representative of all Y chromosomes haplotypes found globally, these data do support a non-African origin of Y chromosome haplotypes in the amaMolo. Two distinct Eurasian haplogroups, namely haplogroup R1a1a (R-M198) and haplogroup Q (Q-M242), were observed within the amaMolo clan genealogy which spans 11 generations [Table 3.2(a) and Appendix C, (Figure S1)]. Five haplotypes are associated with haplogroup R1a1a (R-M198) (Table 3.2(a)) and their relationship with the modal haplotype R1a1a_2 are shown in Figure 3.2 below.

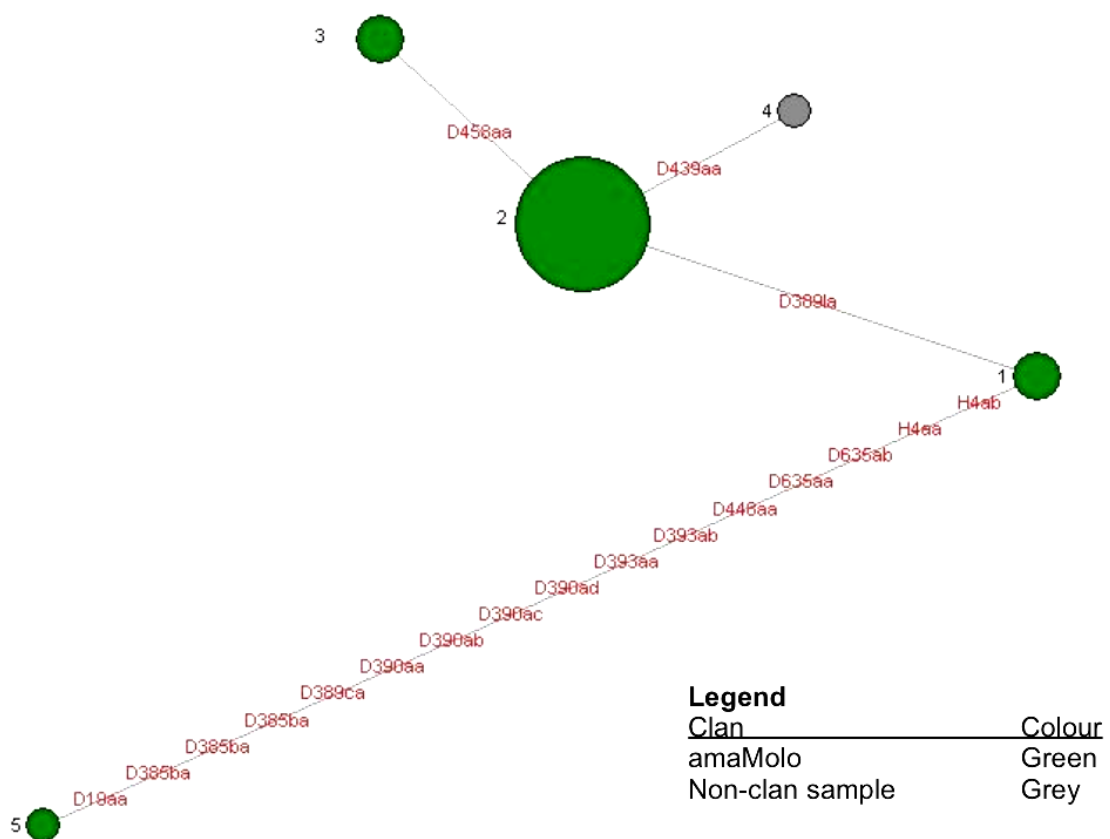


Figure 3.2. Haplogroup R1a1a (R-M198) RMJ network

Through comparison to the amaMolo clan genealogy we found that the R1a1a haplotype was successfully transmitted in 52 out of 74 transmissions, with the modal haplotype R1a1a_2 transmitted to 16 currently living amaMolo clan members

stemming from Bhayi. Similarly, the modal haplogroup Q (Q-M242) haplotype was transmitted successfully in 18 out of 28 transmissions from Pita (Appendix C, Figure S1). Using 19 STR marker haplotypes, the closest match to the modal haplotype R1a1a_2 was found in an individual of Hungarian descent, which differed with single mutations at three loci (Pamjav et al., 2011). When the YHRD minimal eight-STR marker set was queried, identical matches were found in 19 individuals from mostly Eastern European countries namely Croatia, Lithuania, Russia, Poland, Romania, Slovakia and Ukraine as well as to individuals from Belgium, Germany, Norway and the USA. In the amaMolo clan genealogy, haplotype R1a1a_5 is seen in an individual described as the brother to an individual who segregated with the R1a1a_2 modal haplotype (Appendix C, Figure S1). Although these both segregate under the R-M198 SNP marker the variation in the STRs between these two haplotypes is too different for the individuals to be considered biologically related. Thus the presence of the R1a1a_5 haplotype supports the theory of multiple contributions from several non-African founders.

3.1.2.2 Haplotypic variation within the primary abeLungu clans

The Y chromosome data reaffirms the historic and genealogical information regarding the multiple contributions of the male founders to the primary abeLungu clans as well as clans within the broader abeLungu clan family. The primary abeLungu clans are those which are believed to have first originated from non-African shipwreck survivors and include abeLungu Jekwa, Buku and Hatu.

Within clan Jekwa, 13 haplotypes were derived; seven of which were within haplogroup R1b (R-M343). Majority of the clan Jekwa members appear closely associated to the modal haplotype R1b_5 in a star-shaped phylogeny, suggestive of recent expansions (Figure 3.3, Table 3.2(a)). The modal haplotype, R1b_5 was successfully transmitted in 81 out of 100 transmissions in the abeLungu Jekwa clan genealogy, which spans 12 generations tracing back to the clan forefather, Jekwa (Appendix C, Figure S2).

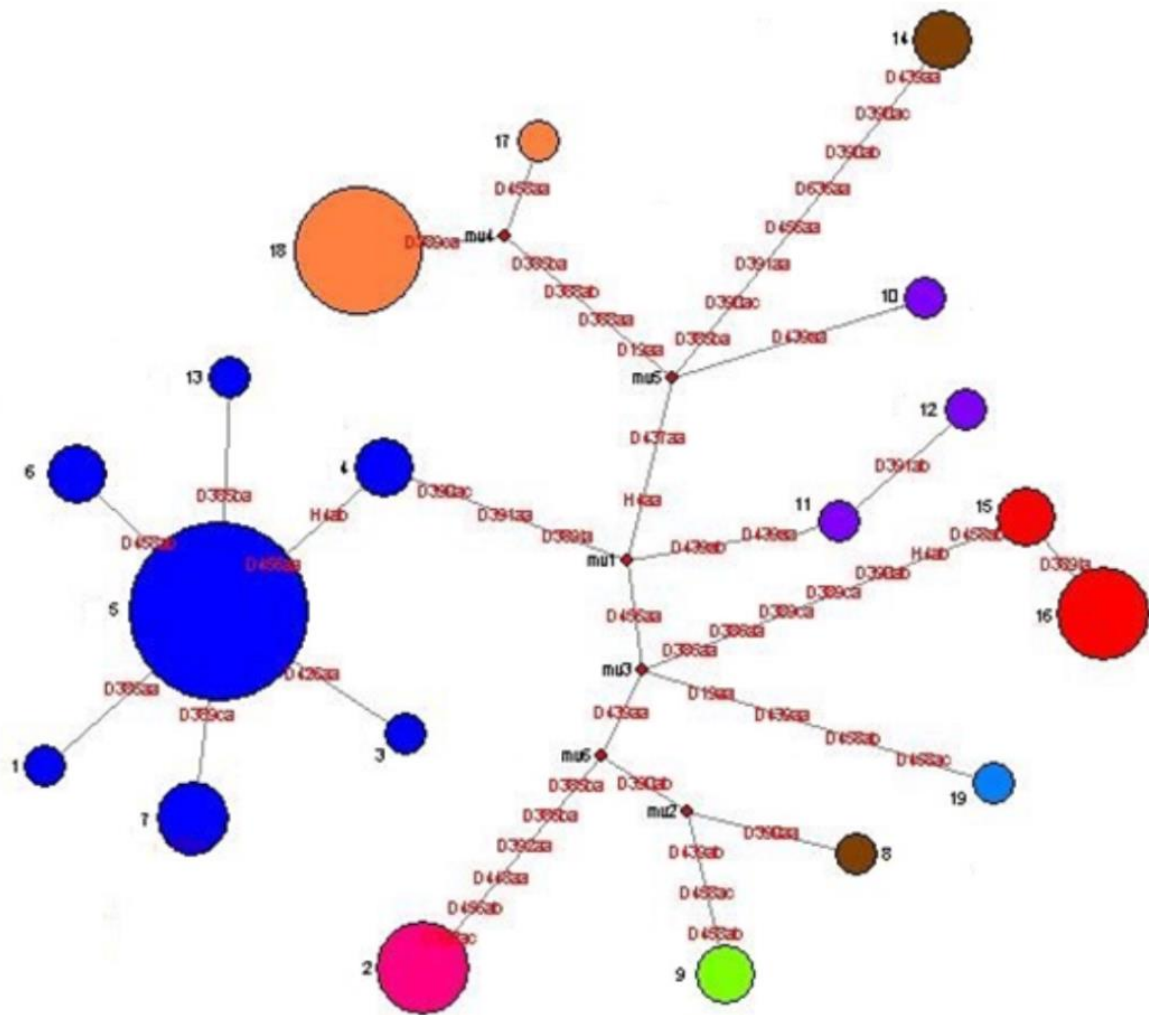
Two inconsistencies were observed in the transmission of Y chromosome lineages in the Jekwa clan genealogy (demarcated with red squares in Appendix C, Figure S2). In both instances, siblings segregated with African Y chromosome haplotypes

(E2b1a_9 and E2b1a_5) who were supposedly born from fathers segregating as European haplogroup R1b (R-M343). These are accounted for as non-patrilineal transmissions.

AbeLungu R1b haplotypes appear as uniquely segregating samples when examined against comparative haplotype data, as well as to the YHRD. Haplotype R1b_5 did not exactly match any of 33 Eastern Europeans (Jarve et al., 2009), nor any of 112 Hungarians genotyped in a study by Pamjav et al., (2011). When compared to data published from studies done by the HGDDRL using 17 Y-STR marker haplotypes, haplotype R1b_5 only partially matched to a single coloured-male sample from Uitenhage in the Eastern Cape, having differed by single-step mutations at seven loci. When the YHRD minimal eight-STR marker set was queried, identical matches were found in eight individuals who were found in Spain, Switzerland, Germany, the Czech Republic, the United Kingdom and the United States.

Descendants of the Hatu clan were linked with haplotype R1b_18 which features in 10 AbeLungu Hatu individuals, which was successfully transmitted in 33 out of 38 paternal transmissions in the Hatu clan genealogy which spans 12 generations (Appendix C, Figure S4). R1b_18 and its associated haplotype, R1b_17, (which deviates from the modal haplotype by a single-step mutation at Y-STR locus DYS389 II) make up the second most prominent cluster in the R1b network and are more closely related to each other than to the R1b haplotypes observed in other clans (Figure 3.3 and Appendix C, Figure S4).

Two AbeLungu Hatu individuals segregated with African Y chromosome haplotypes (one, a haplogroup E2b1a individual, and the other, a haplogroup E1b1a1 individual) were seen allegedly born from a haplogroup European R1b father. These too are regarded as non-patrilineal transmissions which may have been introduced from males of African origin from neighbouring clans (Table 3.2(b) and Appendix C, Figure S4).



Legend

Clan	Colour
Jekwa	Dark blue
Irish	Light green
Caine	Red
France	Light blue
Hatu	Orange
Horner	Pink
Ogle	Brown
Sukwini	Violet

Figure 3.3. Haplogroup R1b (R-M343) RMJ network

Two out of three *abeLungu Buku* clan members shared exactly the same Eurasian haplogroup G (G-M201) haplotype (Table 3.2(a)), which was transmitted successfully in six out of nine paternal transmissions in the *Buku* clan genealogy. It is unsure as to exactly how many generations there are before the *Buku* lineages trace to the original founding father, since genealogical information was incomplete for *abeLungu Buku*, but it is estimated to be at least four generations (Appendix C, Figure S5). Closest matches of *abeLungu Buku* haplogroup G (G-M201) haplotypes were to a Betsileo-Malagasy individual, a coloured-male sample from Uitenhage as well as a partial match to an Egyptian individual, differing at seven loci, found in HGDDRL in-house data from various projects (see Appendix E, Table S3: comparative data sources).

3.1.2.3 Haplotypic variation within the secondary *abeLungu* clans

Non-African ancestry was also observed in clans other than the three initial founder *abeLungu* clans and the *amaMolo*. According to the oral history, these *secondary* clans originated from founders who arrived in Pondoland at later points in time than the three original *abeLungu* founders *Jekwa*, *Buku*, *Hatu* and the founders of the *amaMolo*.

Three *Ogle* clan members originated from two R1b haplotypes, R343_8 and R343_14, which segregated as lineages distinct from each other and from the majority of R1b (R-M343) haplotypes, which may imply that these were two independent founders (Figure 3.3; Table 3.2(a); Appendix C, Figure S5).

The haplotypes R343_16 (and its related variant, R343_15, differing at one STR position) form the lineage tracing back six generations to *Kristjan Caine*, the founder of the *Caine* clan (Figure 3.3, Table 3.2(a) and Appendix C, Figure S3). The two Irish clan members featured a distinct R1b haplotype, R343_9, tracing back to its founder, Irish, who lived only two generations prior to current clan members, which supports the theory of multiple founding events having occurred at later points in time (Figure 3.3, Table 3.2(a) and Appendix C, Figure S3).

Three *Sukwini* samples emerge in the cluster of haplotypes including R343_10, R343_11 and R343_12 (Figure 3.3, Table 3.2(a) and Appendix C, Figure S6). Genealogical information for these three individuals is unknown so we cannot assert relationship of these.

Upon examining the Horner clan genealogy two distinct Eurasian lineages were observed (Appendix C, Figure S3). The R1b haplotype R343_2, stems from the son of Alfred Horner’s first marriage, Johnson (Appendix C, Figure S3 and Figure 3.3). The wives of his subsequent marriages bore the sons Ramsay, Charlie and Teddy, all of which who segregate with haplogroup I (I-M170) Y chromosomes, with the modal haplotype I170_5, and haplotypes I170_4 and I170_6 deviating by single mutations (Figure 3.4 and Appendix C, Figure S3). This may signify two lineages within the clan, introduced by separate founders. A second haplogroup I-M170 lineage, I170_3, distinct from those found in abeLungu Horner was found within the abeLungu France clan, tracing back five generations to the France clan founder, Tshali (Appendix C, Figure S5 and Figure 3.4).

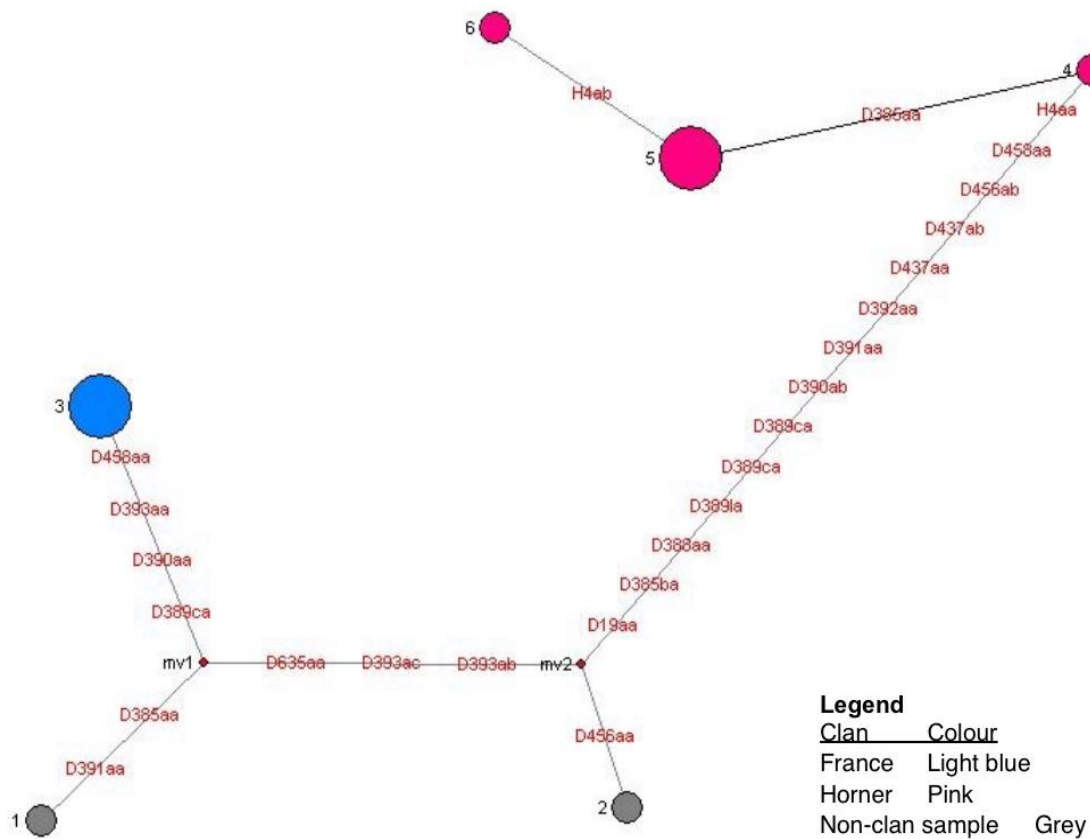


Figure 3.4. Haplogroup I (I-M170) RMJ network

When examined against comparative data the two haplogroup I (I-M170) modal haplotypes were found to have several matches. Haplotype I_3 found in four France clan members, was found to partially match two individuals from Archangelskaja and Vologodskaja respectively, found published in Roewer et al., (2008). Haplotype I_5, which was present in the one of the four Horner individuals was found to partially match individuals from Noworgodskaja and Vologodskaja respectively as well. Haplotype I_3 of clan Horner partially matched a coloured individual from Uitenhage in the Eastern cape, and haplotype I_5 (also of clan Horner) partially matched a Xhosa individual from the Eastern Cape, as well as to a Hungarian individual whose haplotype data was found published in Pamjav et al., (2011).

To attempt to trace the clan name origins of the founders of the Irish, Horner and France clans, several publications and databases were referred to. These included the Type-III Irish Surname-Haplotype Reference Database, as well as the genealogical studies by McEvoy and Bradley (2006), McEvoy et al. (2008), Klyosov (2009) and King and Jobling (2009), who examined Y-chromosomal haplotype data of Irish and British surnames. The two abeLungu-Irish clan members possessed haplogroup R1b (R-M343) haplotypes which almost completely matched the ancestral Irish R1b haplotype identified in Klyosov (2009) and McEvoy et al., (2008). The haplotypes differed to the published sequence by only single mutations at Y-STR loci DYS390 and DYS439, thereby allowing us to infer that these individuals have Irish ancestral origins.

The Type-III Irish surname-haplotype reference database lists haplotypes for the whole haplogroup-I tree and includes all sub-clades, most of which are genealogically mapped to lineages of Irish surnames. The Jim Cullen sub-haplogroup I predictor, available through the site, was used to query the five haplogroup I (I-M170) haplotypes which had been resolved. Results showed that haplotypes I_1, I_2 and I_3 (found in clan France) fall into the I-M253 sub-clade, while haplotypes I_4 and I_5 (both found within clan Horner) segregate under the I-S24 sub-clade with 100% confidence. None of these haplotypes segregated under the Dalcassian R-L226 cluster of haplogroup I (I-M170) from Clare, Limerick and Tipperary - which is the predominant lineage of Ireland - however they did match haplogroup I (I-M170) haplotypes found at high frequencies in Ireland and Western Europe. Therefore, considering their Y

chromosome haplogroups of western European origins (haplogroups R1b (R-M343) and G (G-M201)), it is likely that the forebears of clans Hatu, Jekwa and Buku were from the British Isles. It is not possible to say definitively whether these forefathers were the three English men discovered with Bessie (Crampton, 2004), as there is no genealogical evidence or biological link to her maternal lineage, with which to verify a common geographical ancestry.

The sole haplogroup J (J-M172) haplotype observed in the sample set was of the single abeLungu Thaka clan member. The sample was found to partially match a Vologodskaja and Smolenskaja haplogroup J haplotype, differing by single-step mutations at eight Y-STR loci, published in Roewer et al., (2008). This haplotype also partially matched a Maldivian individual and a Malagasy individual genotyped in studies undergone by the HGDDRL. When examining truncated 12-loci haplotype data, the J-M172 haplotype was found to match one of 70 Malaysian-Indian haplotypes published in Pamjav et al., 2011.

Fuzwayo, Hastoni and Sukwini clan members featured predominantly African haplotypes (Figures 3.5, 3.6, 3.7 and 3.8, and Appendix C, Figure S6).

The judicious evaluation of genealogical history against molecular data has allowed for the determining of the ancestral haplotype present in each clan which could convincingly be traced to the clan progenitors documented in clan genealogies (Table 3.3).

Table 3.3: Haplotypes and presumed geographic origin of abeLungu clan male founders

Clan	Founder	Haplogroup	Ancestral haplotype	Presumed geographic origin
amaMolo	Bhayi	R1a1a	R1a1a_2	Eurasia *Most likely India
	Pita	Q	Q_1	Eurasia
Jekwa	Jekwa	R1b	R1b_5	western Europe *Most probably British Isles
Hatu	Hatu	R1b	R1b_18	western Europe *Most probably British Isles
Buku	Buku	G	G_2	Eurasia
Ogle	Ogle	R1b	R1b_14	western Europe *Most probably British Isles
Caine	Kristjan	R1b	R1b_16	western Europe *Most probably British Isles
Irish	Irish	R1b	R1b_9	western Europe *Most probably Ireland
Horner (Alfred)	Johnson	R1b	R1b_2	western Europe *Most probably British Isles
	Ramsay, Charlie and Teddy	I	I_5	Europe
France	Tshali	I	I_3	Europe

Thaka	Thaka	J2	J2_1	Eurasia *Middle-East
Fuzwayo	Fuzwayo	B2a1	B2a1a1_3	Southern African
Hastoni	Hecton (Hastoni)	E2b1	E2b1a_6	Southern African
Sukwini	Chwama	E2b1	E2b1a_9 & E2b1a_11	Southern African

3.1.2.4 Y chromosome variation linked with African origins

African haplotypes were found distributed predominantly among non-clan affiliated samples, but are observed sparsely throughout the clan-affiliated sample as well. African haplotypes were the only haplotypes to be found shared across clans, and non-clan affiliates alike. The complexity of the African haplogroup networks is attributed to the fact that haplotypes within African haplogroups are separated by many mutational steps, indicating multiple contributions. The RMJ networks of all non-African or Eurasian haplogroups do not feature reticulations and are all treelike forms based on star-contraction phylogenies of satellite variants emerging from an ancestral, founder, modal haplotype (Richards et al., 1998; Bandelt et al., 1999). However, the RMJ networks for the African haplogroups E1b1a1a1c1a (E-M191), E2b1a (E-M85) and E1b1a1 (E-M2) do feature reticulations which signify ambiguous connections/relations between haplotypes (Bandelt et al., 1999) (Figures 3.5, 3.6 and 3.7). This introduces doubt in the order of introduction of mutations, topology of the network and consequently the chronology of the introduction of haplotypes, and is indicative of more ancient, deeply-rooted haplotypes featuring greater diversity (Richards et al., 1998).

The remaining haplotypes found within the abeLungu Jekwa clan are of African descent, which included five E2b1a (E-M85) haplotypes. One haplogroup B2a1a1a1 (B-M152) and one haplogroup E1b1a1 (E-M2) haplotype was also observed in the abeLungu Jekwa pedigree (Table 3.2(b) and Figures 3.7 and 3.8). These were most likely due to non-patrilineal transmissions resulting in gene-flow and admixture from the indigenous Xhosa gene-pool.

Three amaMolo clan members, one Fuzwayo clan member as well as two Sukwini members segregate with haplogroup E1b1a1a1c1a (E-M191) haplotypes, while a larger proportion of haplogroup E-M191 chromosomes are present in non-clan affiliated samples, appearing as satellite haplotypes in the complex and diverse haplogroup E1b1a1a1c1a (E-M191) RMJ network (Figure 3.5). Haplotype E1b1a1a1c1a_10 was found to have matched the haplotype of a Khoisan individual from Upington in the Northern Cape (haplogroup/haplotype data from research done in the HGDDRL).

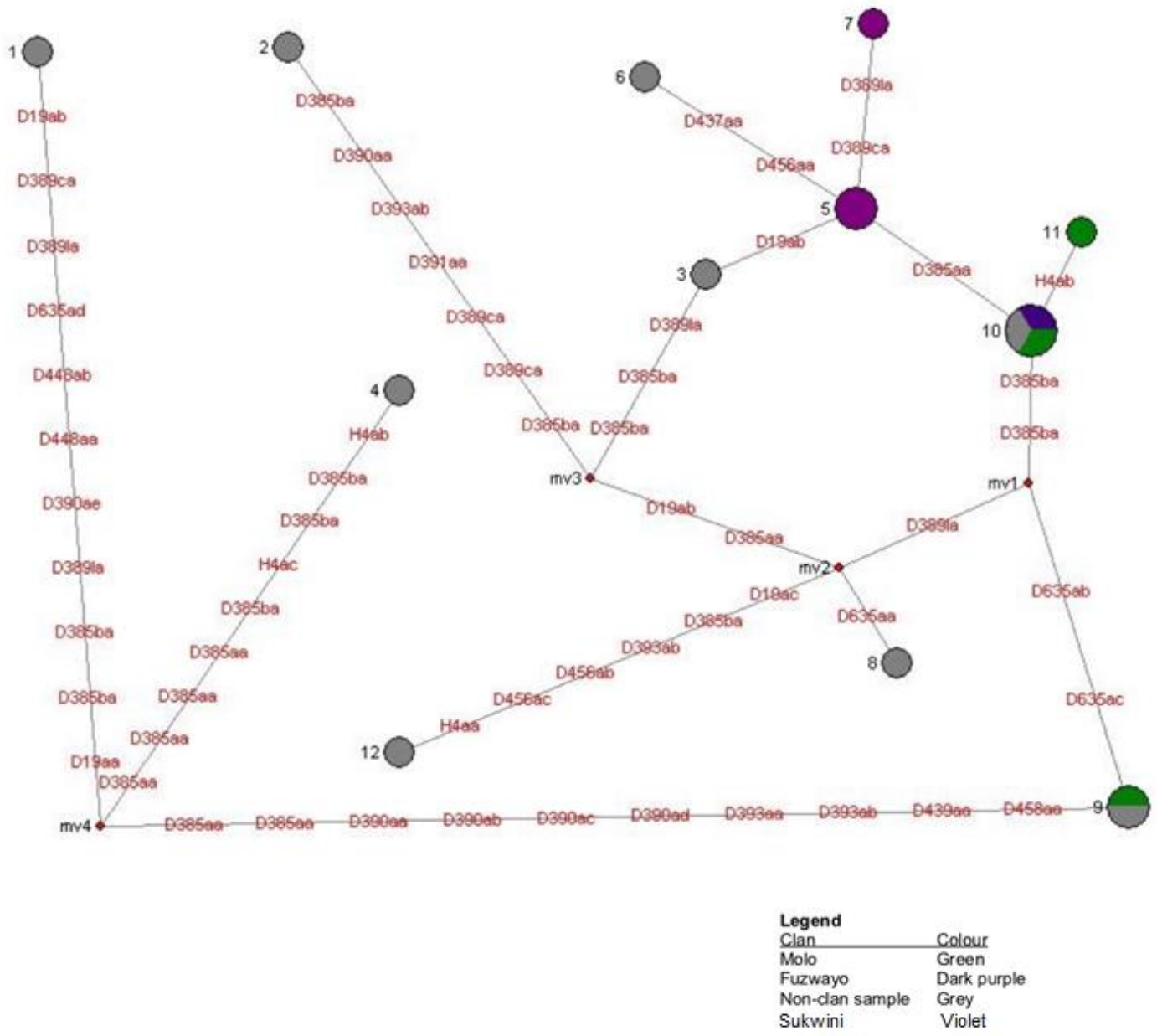


Figure 3.5. Haplogroup E1b1a1a1c1a (E-M191) RMJ network

Haplogroup E2b1a (E-M85) chromosomes appeared with more diversity. The largest proportion of which were present in non-clan affiliated samples (39.0%), with seven individuals identically sharing haplotype E2b1a_9 (Table 3.2(b) and Figure 3.6). Haplotype E2b1a_9 is a modal haplotype within haplogroup E2b1a (E-M85), was seen having given rise to 12 variant haplotypes, and pre-dates the arrival of non-African Y chromosomes (Figure 3.6). This haplotype was also exactly shared by one individual in the clan Buku, one in the amaMolo clan, one in abeLungu Jekwa and one in Sukwini (Figure 3.6). Haplotype E2b1a_6 is also found shared within the amaMolo clan and all of the members of the Hastoni clan (Figure 3.6). Haplotype E2b1a_9 also matched a coloured individual from Middleburg in the Eastern Cape, genotyped in a previous study by the HGDDRL unit. A contingent of haplogroup E2b1a (E-M85) haplotypes were also present in all five members of the Hastoni sample, and were also found in four Sukwini samples.

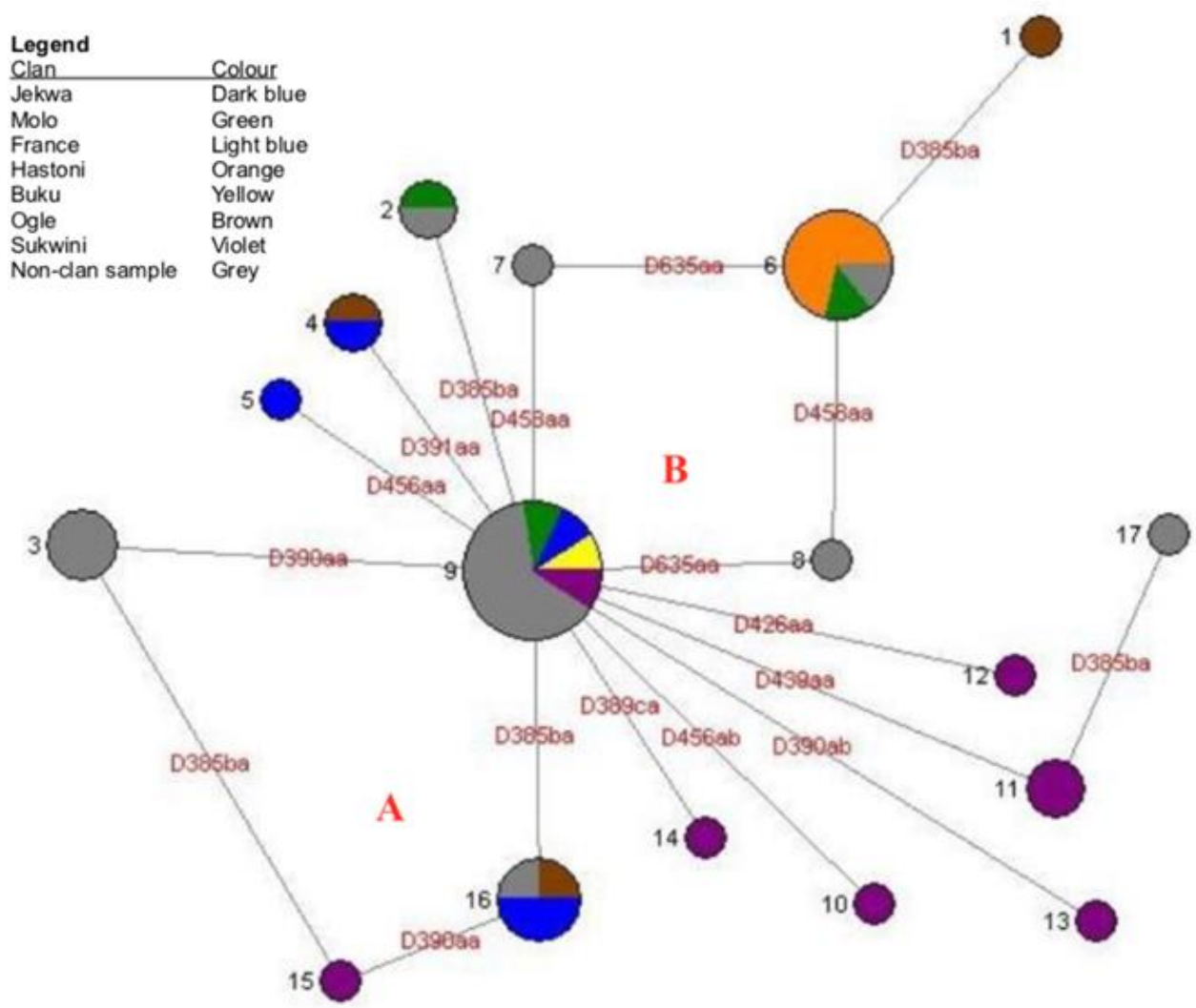


Figure 3.6. Haplogroup E2b1a (E-M85) RMJ network, featuring two reticulations marked A and B

Haplogroup E1b1a1 (E-M2) haplotypes featured predominantly in non-clan affiliates, but were observed sporadically in several clans as well (Figure 3.7). Haplotype E1b1a1_8, found in three Sukwini samples, was found to have matches to an individual from Northern Chad (unpublished data), as well as to two Malagasy individuals.

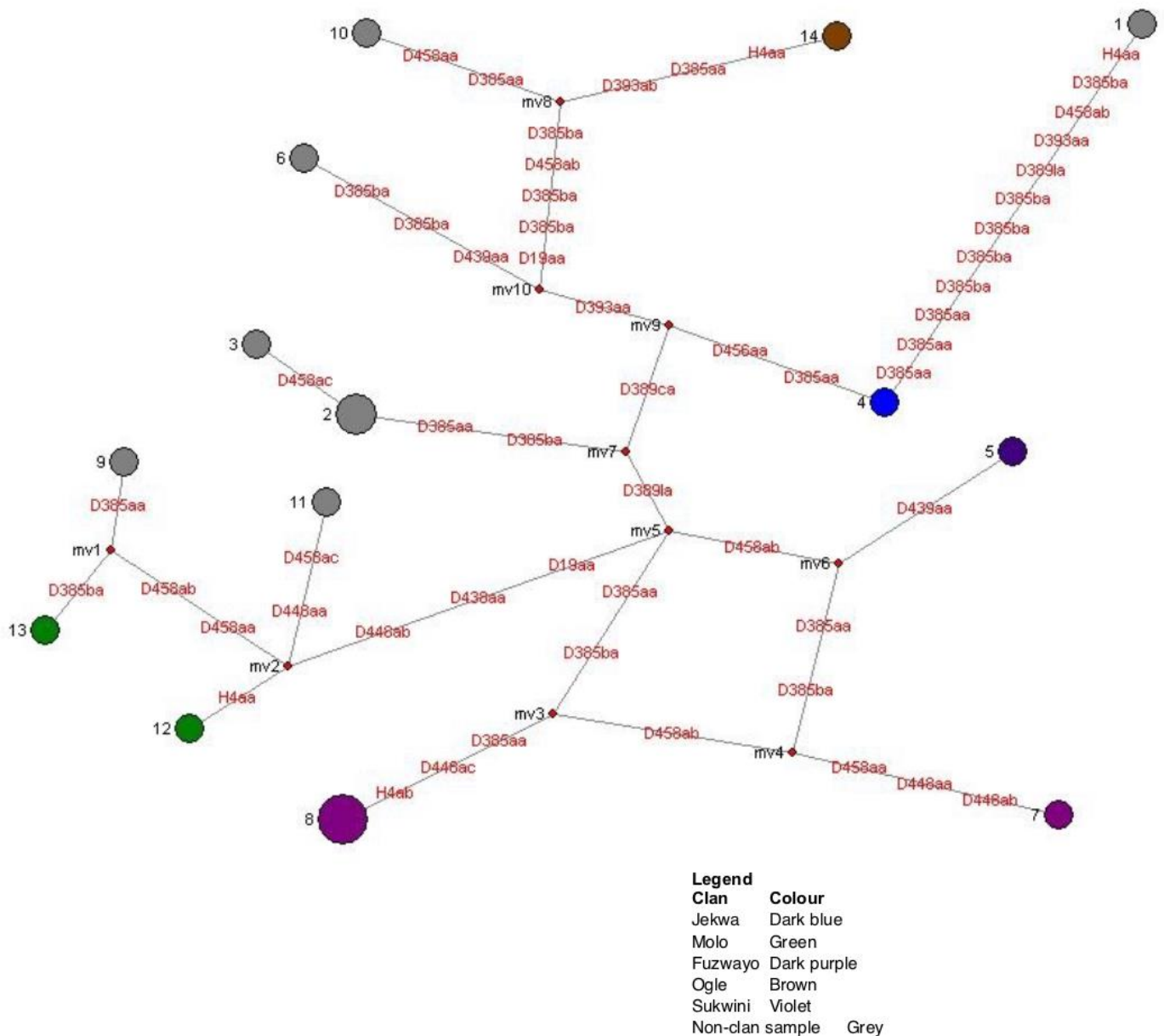


Figure 3.7. Haplogroup E1b1a1 (E-M2) RMJ network

Four of six clan members of the Fuzwayo clan possessed haplogroup B2a1a1a1 (B-M152) chromosomes (Appendix C, Figure S6 and Figure 3.8). Haplotype B2a1a1a1_3 matched a Khoisan sample, sampled in Riversdale in the Eastern Cape. Lastly, the haplogroup A1b1b2a (A-M51) haplotype, A1b1b2a_1, shared by two Sukwini subjects matched to a Northern Cape Khomani individual as well as a coloured male from Uitenhage in the Eastern Cape.

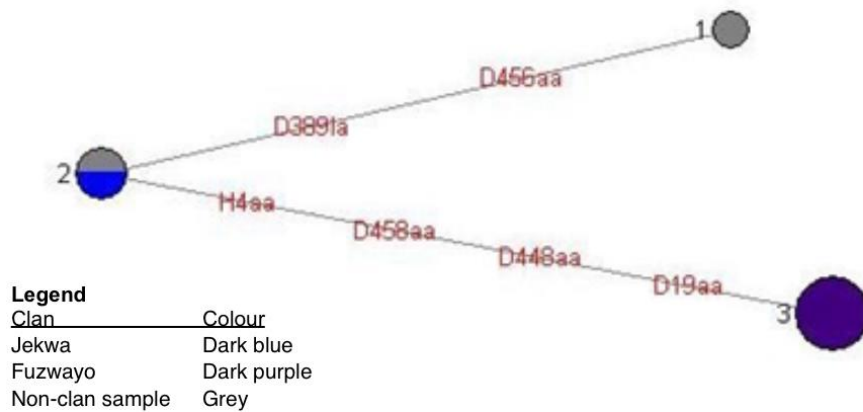


Figure 3.8. Haplogroup B2a1a1a1 (B-M152) RMJ network

3.2 Mitochondrial DNA findings

3.2.1 MtDNA haplogroups

All maternal lineages of clan members and non-clan affiliated individuals are exclusively of African origin, with the majority appearing within haplogroups L0d (32.34%) and L3e (23.75%) (Table 3.4). The frequencies of the major macro-haplogroups resolved are L0 (44.44%), L1 (2.02%), L2 (19.7%), L3 (33.35%) and L4 (0.5%). The frequencies observed for mtDNA sub-haplogroups are presented in Table 3.4. The distribution of haplogroups by clan are also described in the charts of Figure 3.9 below.

The frequency of the L0 clades are up to 40% in different south-eastern Bantu-speaking tribes (Schlebusch et al., 2009; Schlebusch et al., 2011). Haplogroup L0d is thought to be the oldest of the L0 clades and its distribution in southern Africa strongly points to an origin among Khoe-San ancestors, which occurred prior to the arrival of Bantu-speaking populations in southern Africa, and is found in the !Xun and Khwe peoples at frequencies of 51% and 16% respectively (Salas et al., 2002; Schlebusch et al., 2009). Haplogroup L3e comprises approximately one-third of all L3 types in sub-Saharan Africa and is the most widespread, frequent and ancient of the African L3 clades, which arose in Central Africa near Sudan around 35,000 years ago (Bandelt et al., 2001; Soares et al., 2011).

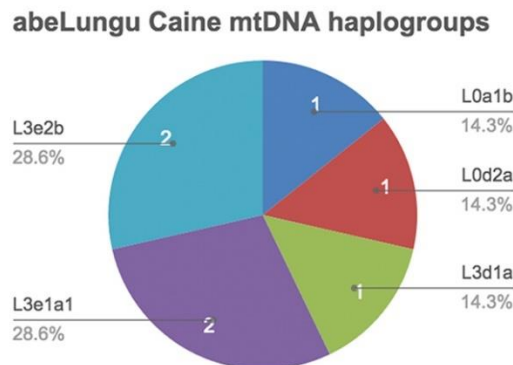
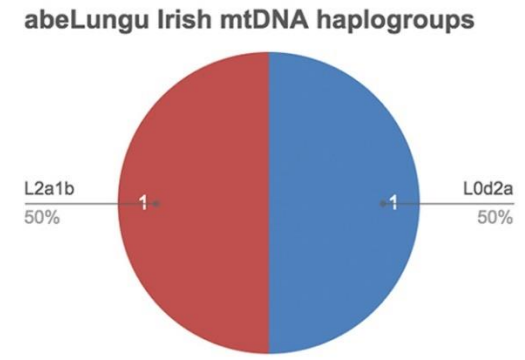
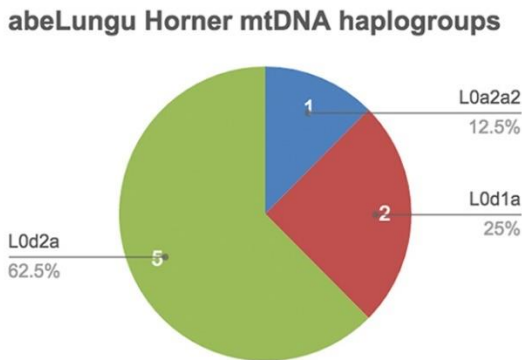
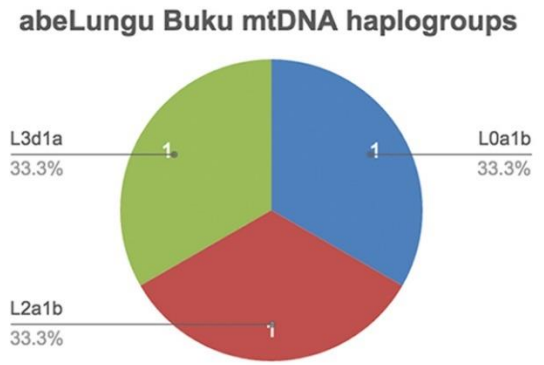
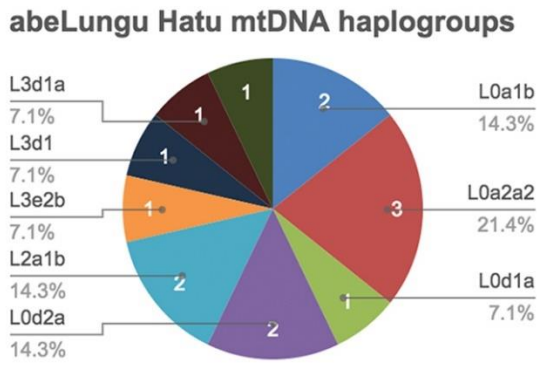
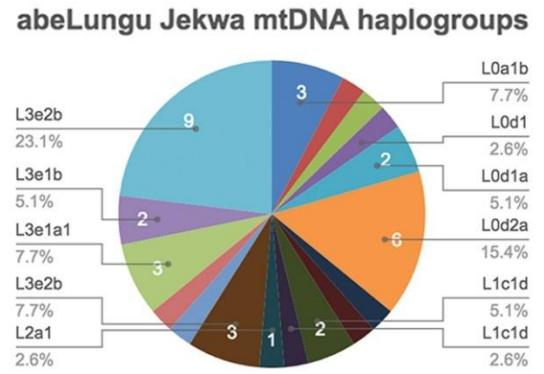
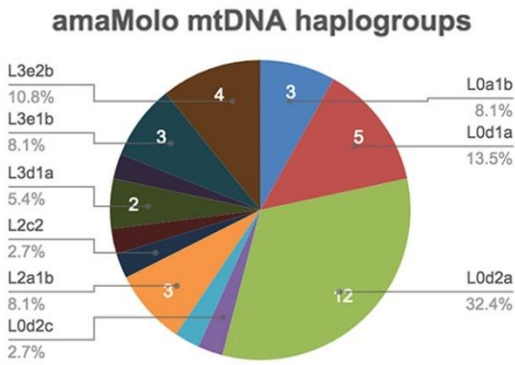
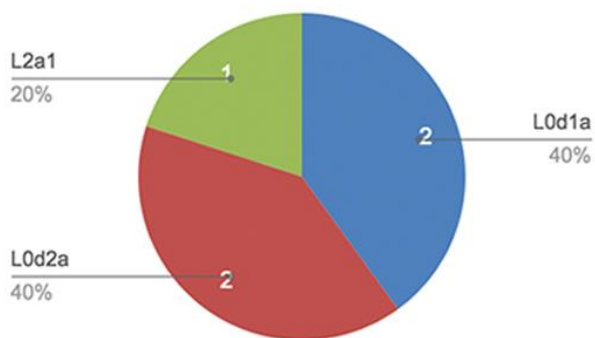


Figure 3.9. Distribution of mtDNA haplogroups by clan

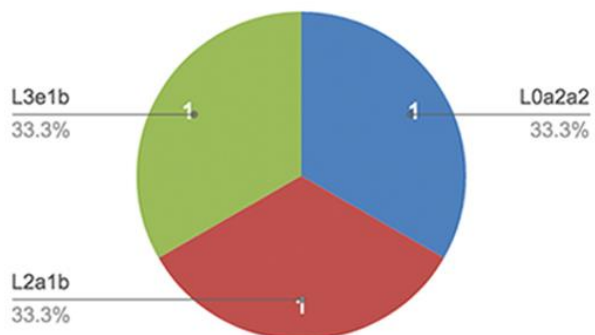
abeLungu France mtDNA haplogroups



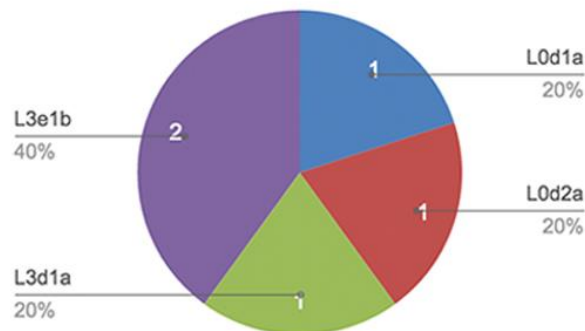
abeLungu Fuzwayo mtDNA haplogroups



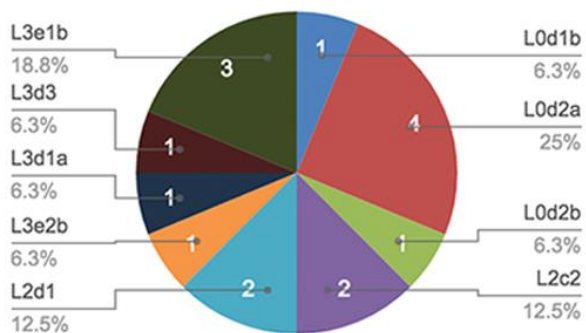
abeLungu Ogle mtDNA haplogroups



abeLungu Hastoni mtDNA haplogroups



abeLungu Sukwini mtDNA haplogroups



non-clan affiliated samples mtDNA haplogroups

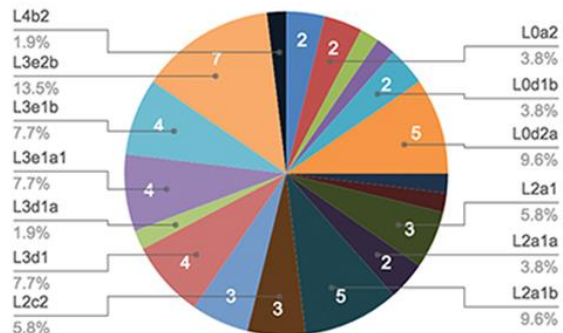


Figure 3.9 continued. Distribution of mtDNA haplogroups by clan

Table 3.4. MtDNA haplogroup frequencies

Macro-haplogroup	Subclade	MTDNA Haplogroup	N	Frequency	%
L0	L0a	L0a1b	13	0,07	6,57
		L0a2a2	10	0,05	5,05
	L0d	L0d1a	15	0,08	7,58
		L0d1b	3	0,02	1,52
		L0d2a	41	0,21	20,71
		L0d2b	2	0,01	1,01
		L0d2c	1	0,01	0,51
		L0d3	2	0,01	1,01
	L0f	L0f1	1	0,01	0,51
L1	L1c	L1c1d	2	0,01	1,01
		L1c3	2	0,01	1,01
L2	L2a	L2a1a	7	0,04	3,54
		L2a1b	13	0,07	6,57
	L2c	L2c2	6	0,03	3,03
	L2d	L2d1	7	0,04	3,54
	L2e	L2e2b	6	0,03	3,03
L3	L3d	L3d1a	16	0,08	8,08
		L3d3	3	0,02	1,52
	L3e	L3e1a1	10	0,05	5,05
		L3e1b	15	0,08	7,58
		L3e2b	21	0,11	10,61
		L3e3	1	0,01	0,51
L4	L4	L4b2	1	0,01	0,51

3.2.2 MtDNA haplotype diversity

The evolutionary relationships of the African mtDNA haplotypes are depicted in the neighbour-joining (NJ) tree, drawn using the mtDNA HVR I and HVR II sequences (Figure 3.10). MtDNA haplotype sharing between clans is observed to a much larger degree than with Y chromosome haplotypes, where none of the non-African Y-chromosome haplotypes were seen shared between clans and only several African Y-chromosome haplotypes were found shared among clans.

From the 198 individuals studied, there are 117 unique mtDNA haplotypes observed, 23 of which were found to be shared between multiple individuals - both within- and among- clans (Appendix D, Supplementary Table S2). Sixteen of these haplotypes were found to be shared between individuals from different clans and in 14 instances haplotypes were shared with non-clan affiliated samples as well. The two most frequent haplotypes are those which appear in a haplogroup L3e2b clade with HVR mutations 16172C, 16183C, 16189C, 16223T, 16320T, 16519C, 73G, 150T, 152C, 263G (appearing at a frequency of 0.068); and a clade within haplogroup L3e2b featuring the variants 16129A, 16187T, 16189C, 16212G, 16223T, 16230G, 16243C, 16311C, 16390A, 16519C, 73G, 146C, 152C, 195C, 198T, 247A, occurring at a frequency of 0.0625 (Appendix D, Supplementary Table S2). Out of the 21 sibling pairs, two pairs did not share the same parental haplotypes - a clan France sibling-pair segregated with haplogroups L0d2a and L0d1a, while a amaMolo sibling-pair segregated under haplogroups L0d1a and L3e2b suggestive of non-maternity, alternative modes of kinship or adoption (Appendix C, Figures S1 and S5).

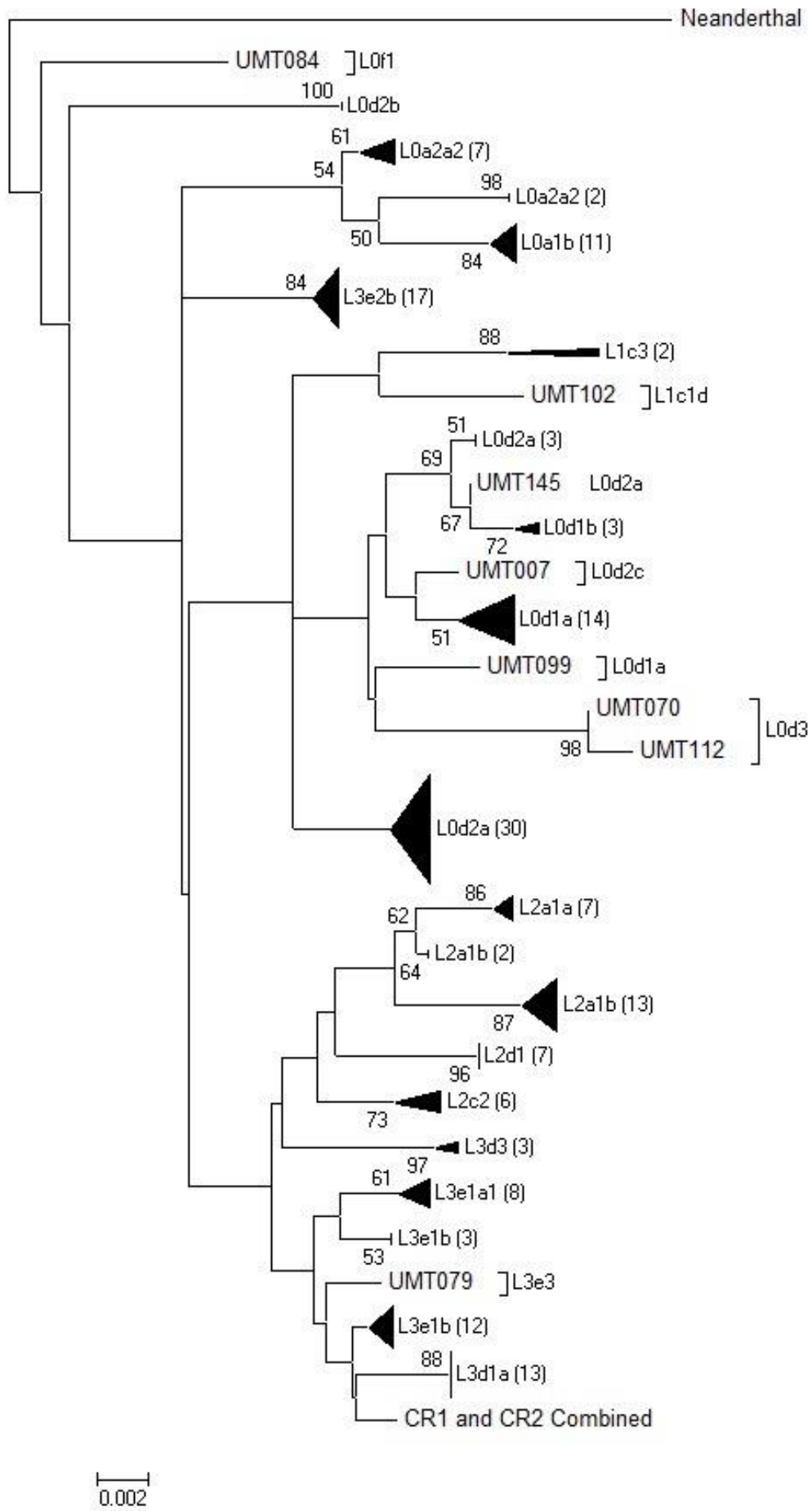


Figure 3.10. Neighbour-Joining (NJ) phylogenetic tree of 176 mtDNA haplotypes

Depicted in the mtDNA-haplotype NJ tree are distinct haplogroups seen clustering in the correct evolutionary topology; all abeLungu mtDNA haplotypes cluster alongside the *Neanderthal* mtDNA out-group (Figure 3.10). Haplogroup L0 sequences are seen branching off into their sub-clades, while L2 sequences are coupled near L3 sequences which cluster alongside the revised Cambridge Reference Sequence (rCRS). The rCRS is defined by its variant sites as a haplogroup H sequence, which clusters alongside haplogroup L3e sequences - its closest related sequence. This is also consistent with the correct evolutionary history of mtDNA ancestry markers as haplogroup L3 is the clade which migrated north during the Out of Africa expansion, and became every other haplogroup (Behar et al., 2008). Clades where multiple individuals share exactly the same haplotypes have been collapsed, with the frequency of individuals sharing the specific haplotype indicated in brackets (Figure 3.10).

CHAPTER 4

Discussion

4.1 Y chromosomes and genetic heritage

Y chromosome markers were used to trace the paternal ancestry of the abeLungu from the Wild Coast region of the Eastern Cape. This study was conceived following on discussions with Ms Janet Kalis, an anthropologist who has been conducting genealogical and historical research among the abeLungu, to test the claims based on their oral history about their “White” ancestry. Our laboratory subsequently engaged in collaborative research with Ms Kalis to use genetic tools to test and/or refine the oral and historical narrative. In this study Y chromosomes in a subset of males representing various clans among the abeLungu were tested to derive their Y chromosome profiles, which were then used to trace the most likely geographic region of origins of their Y chromosomes. Given that the abeLungu are a patriarchal society, Y chromosomes that are transmitted from fathers to sons, ought to segregate within clans. Therefore, in the first part of the study, Y chromosome data was used to test the oral history of the abeLungu, and also used to judiciously examine the transmission of Y chromosomes within the clans to assess genealogical relationships. Apart from determining the geographic regions of origins for the abeLungu, through the further analysis of microsatellite haplotypes of both within- and between- clans, as well as between clan members and Xhosa non-clan-affiliates, we were able to measure a degree of population sub-structure which displays typical diversity patterns of founder populations.

4.1.1 Y chromosomes and the founding fathers of the abeLungu

Throughout, the transmission of haplotypes generally remains consistent with the transmission clan name in the genealogies. However, the oral history which has been transferred across generations, depending solely on the memory of the present day individuals representing it, has exhibited ambiguity and distortion over time in the names, chronology and relations of clan members to their ancestors. This study, which made use of Y chromosome DNA and mtDNA data in conjunction with clan affiliated

genealogical data, was used to refine several anthropological questions pertaining to the history of the abeLungu.

Following SNP analysis of 146 abeLungu clan members, we were able to resolve their Y chromosomes using their global distribution patterns into African (30.12%) and non-African (69.86%) derived Y chromosomes. The commonest haplogroup was R1b (R-M343) which was found at a frequency of 41.10% (Figure 3.1). The amaMolo are associated with two haplogroups; the first an Eastern European haplogroup, R1a1a (R-M198), and the second with haplogroup Q (Q-M242) of West Asian origin. While the abeLungu (Jekwa, Hatu, Buku) and the amaMolo are considered to be the earlier mixed-race clans of the mPondoland, there are other mixed-race clans whose origins stem from more recent non-African contributions into the mPondo gene pool (Soga, 1930; Crampton, 2004). They are represented by four France clan members as well as six Horner individuals in our study who were found to have haplogroup I (I-M170) chromosomes, and two-thirds of Buku clan individuals segregated with haplogroup G (G-M201) chromosomes, which provides further evidence for non-African origins. A single Thaka clan member presented a haplogroup J-M172 profile which matches to two Eastern European individuals of Semitic origin in the YHRD, but does not contain the extended-CMH nor the CMH (the Cohen Modal Haplotype defined by 12 specific YSTRs originating from the *Kohanim*, who were the Jewish high priests) as described in Soodyall, (2013).

Two lines of evidence demonstrate a remarkable relationship between Y-chromosomal haplotypes and patrilineally-inherited cultural markers (clan names) - the low within-clan diversity and high non-African haplotype-sharing within abeLungu clans (Tables 3.2(a) and 3.2(b), as well as Figures 3.2 – 3.8), as well as a high degree of haplotypic variance observed between clans, and in particular between Xhosa non-clan affiliates and clan members (Tables 3.2(a) and 3.2(b), as well as Figures 3.2 – 3.8). It also demonstrates the powerful male-specific founder effects of the European and Eurasian castaways.

Regarding the rarity of haplotype matches to databases and published literature, the primary database queried was the Y Haplotype Reference Database, the YHRD, When abeLungu Y-haplotypes were queried against all populations of the YHRD using the maximum number of STRs, it proved difficult to find matches and abeLungu modal

haplotypes were found to cluster independently from nearest-match haplotypes, thus indicating their rarity and uniqueness.

Upon examining the abeLungu clan genealogies in conjunction with haplotype data several irregular aspects were discovered, while others were clarified. Clans which featured Y chromosome haplogroups that could conclusively be traced back to non-African founder individuals include the amaMolo, as well as the older abeLungu clans Jekwa, Hatu and Buku. Non-African ancestry also featured in the more recently established abeLungu clans Ogle, Caine, Irish, Horner, France and Thaka, however the forebears of these clans were introduced from Europe and Eurasia at a later time than that of the three primary abeLungu and amaMolo clans viz. variable time-depths of clan genealogies [Tables 3.2(a) and 3.2(b); (Appendix D; Figures S1-S6)]. European haplogroup R1b (R-M343) features in the primary abeLungu clans Jekwa and Hatu, as well as in the more recently established clans Caine, Irish, France, Horner, Ogle and Sukwini. Similarly, Eurasian haplogroups R1a1a (R-M198) and Q (Q-M242) feature in the amaMolo clan. European (and probably British) haplogroup I (I-M170) is found in France and Horner clan members while haplogroup G (G-M201) Y chromosomes feature in present-day clan Buku members, which indicates a more Middle-Eastern or South-West Asian ancestry. The fact that the vast majority of abeLungu and amaMolo clan members present with European and Eurasian ancestry ultimately validates the cherished, but damaged narrative of their origins passed down for ten-some generations, which states that their forebears were originally from very distant shores.

Conflicting versions of the oral history exist about the origins of the three men who had arrived with Bessie at Lambasi Bay (Soga, 1930; Crampton, 2004). A certain degree of clarity on the relations of castaways has been achieved, where previously there was suspicion from contradictory oral-historical details. Older beliefs were that they were black or Indian men, where in more recent recollections they are considered white (Soga, 1930; Crampton, 2004). The primary abeLungu clan founders Jekwa, Hatu and Buku, were thought to share a common, white ancestry, and they were believed to have survived the same shipwreck (Soga, 1930; Crampton, 2004). Crampton, (2004; p12), noted that “Theories of the clan’s origins are linked to the story of the arrival of

a young girl named Bessie on the Wild Coast along with three white men...the abeLungu proclaim that they are descendants of white European castaways...”.

Soga (1930) described abeLungu clan forefathers Buku and Jekwa, as both being descendants of Mbomboshe, from which we would expect their Y chromosome profiles to be the same. On the contrary, our data reveals that Jekwa clan individuals segregated with R1b (R-M343) Western-European ancestry, while the haplogroup G (G-M201) chromosomes of Eurasian origin genotyped in 66% of abeLungu Buku clan members, illustrates differing geographical origins and so, Buku was not a blood-relative to Jekwa through Mbomboshe as perceived in the oral history.

Soga (1930) had also described Hatu and Jekwa as clans having two independent origins from shipwreck survivors. Support for this claim is provided in the relationship of Y-haplotypes in the network for haplogroup R1b (R-M343), in which a clear separation with at least 10 mutational step differences occurring between haplotypes of individuals from the Jekwa and Hatu clans was observed (Figure 13, Table 11a and Appendix C, Figure S2 & S4).

Further support to Soga’s theory of the original founders being white castaways can be associated with the abeLungu clan-founder names. Kirby (1953) and Soga (1930) had noted that corruption of language through translation seems evident in the interaction between isiXhosa speaking individuals and Dutch speaking as well as English speaking individuals, primarily brought about through the phonetic linguistic differences of these languages. What this means is that it is possible that mPondo clans-people could have distorted the pronunciation of names of castaways and terms from other languages like Dutch and English, since isiXhosa has borrowed words from Khoisan click-sounding languages (Soga, 1930; Knight et al., 2003; Schlebusch et al., 2009; Schlebusch et al., 2011). If abeLungu ancestor names were derived from corrupted Anglicised roots, then the inherited names of the three English men may have been “*Xhosa-ised*” versions of their English names. Crampton (2004) suggests that *Badi* may have originally been Willem Billyert (or Bill Elliot), *Hatu* may have been Hendrik Clarke (or Henry Clark) and *Jekwa* most probably was Thomas Miller - the three men who had first assimilated with mPondo clans, and the same men who were believed to have arrived on the Wild Coast with Bessie.

The fact that the descendants of Hatu and Jekwa share differing haplogroup R1b (R-M343) haplotypes, illustrates that these two patriline stem from independent founder individuals originally from Western Europe, ultimately validating a portion of Soga's research. We thus infer that Jekwa, Hatu and Buku are in fact three independent founders who do not share a common ancestry, but who most probably arrived on the Wild Coast having sailed from the British Isles and the Middle-East, quite possibly aboard the same ship.

4.1.2 The amaMolo and their affiliation with the abeLungu

While socially and culturally the amaMolo are synonymous with the abeLungu, and while the forebears of these clan families both retain non-African ancestry, they originate from different geographical populations. The abeLungu clan-families segregate with a Western-European background, chiefly under haplogroup R1b (R-M343), while the amaMolo clearly segregate with a more Eurasian ancestral background, bearing haplogroups R1a1a (R-M198) and Q (Q-M242). Only several indigenous, deeply-rooted African haplotypes are seen shared between individuals of the abeLungu and amaMolo clan families - most of which had been introduced via gene-flow through extra-marital relations of abeLungu women with local Xhosa men of the surrounding region; however there has been no evidence of shared European or Eurasian haplotypes between the clan families. And so, we may conclude that the amaMolo have emerged from independent lineages of founders different to those of the abeLungu clan.

Soga's genealogical studies (1930) state that the ancestors of the amaMolo are believed to have originally come from either India or Malaysia. More recent accounts however, state that the original amaMolo forebears were white Europeans (Crampton, 2004). The assumption of the amaMolo's Indian origins however, is supported through the presence of R1a1a (R-M198) haplotypes in the Bhayi lineage of the amaMolo - although the exact geographical origin cannot be determined conclusively, as the distribution of haplogroup R1a1a is relatively broad in Western Asia and Eastern Europe (Klyosov, 2009). Previous studies by Sengupta et al., (2006) and Underhill, (2010) on the Indo-European language family also show that haplogroup R1a1a (R-M198) features in a large proportion of Indian males, which adds support to the notion of Indian ancestry and Soga's version of the amaMolo historical narrative. This may

also validate the beliefs held by Soga and Kirby that '*Molo*' may be derived from the word '*Moor*', pertaining to Indian Lascar slaves (Soga, 1930; Kirby, 1953).

The haplogroup Q (Q-M242) modal haplotype observed in eight amaMolo clan members delineates a second ancestral lineage of Asian descent, originating from another progenitor, Pita (Appendix C, Figure S1). There is a 100% effective father-son transmission rate for descendants of the haplogroup Q modal haplotype. The Asian haplogroup Q (Q-M242) is believed to have arisen in South Central Siberia, around the Altai Mountains area, between 17,000 and 31,000 years ago (Zegura, 2004).

Since there are two lineages which segregate with different backgrounds within the amaMolo clan, this clarifies that Bhayi and Pita were not biological siblings, but rather two distinctive founders, from Eastern-Europe/Eurasia and Asia respectively (Appendix C, Figure S1). These results also lend further support to Soga's claims that the amaMolo have black or Indian, rather than white ancestors.

4.1.3 Multiple founding events

The detection of Y-SNP markers like M170 and M343 in more recently established abeLungu clans, with a high degree of inter-clan haplotypic variance, proposes that clans originated from multiple founders of independent founding events and shipwrecks. The frequency of mutations in haplotypes reflects the time-scale from the point of divergence of castaways from their population of origin to their establishment in the Eastern Cape. Anthropological evidence agrees with molecular data in supporting the theory that secondary waves of non-African shipwreck survivors also reached the Wild Coast, assimilated with the mPondo community and became founders of other abeLungu clans at later points in time (Appendix C, Figure S3, S5 and S6). This is because the genealogies of more recently established clans are proportionally shorter - having a decreased time-depth with fewer generations dating back to respective clan founders than the genealogies of the three primary abeLungu and amaMolo clans (Soga, 1930; Kirby 1953; Crampton, 2004). The more recently established clans Caine, Irish, Ogle, Horner, France, Hastoni, Fuzwayo, Sukwini, and Thaka feature genealogies which average three to five generations to the point where their clan founders had arrived in mPondoland, while the older abeLungu clan

genealogies (Hatu, Jekwa, Buku and amaMolo) trace back on average ten generations to their respective founders.

4.1.4 Clan-affiliated Africans

The presence of ~30% of sub-Saharan African haplogroups A-M51, B-M52, E-M2, E-M85, E-M191, and E-M35 in abeLungu clans may have been as a result of differential gene flow and/or admixture from Y chromosomes of neighbouring, non-abeLungu Xhosa clans - which acts to diminish non-African ancestry diversity, as well as the degree of clan-name/haplotype coancestry. Input into the mPondo gene-pool by non-African founders is restricted to abeLungu clan affiliates. This is shown by the general absence of non-African haplogroups, and a high frequency of African haplotypes in the non-clan affiliated individuals ($n=42$), which were sampled from the surrounding regions of abeLungu clan *lalis* (homesteads). Only few African haplotypes were seen shared amongst Xhosa non-clan-affiliated individuals (Table 3.2(b)). These clan-affiliated individuals which possess African haplotypes, hence “African Africans”. These are also examples of possible non-patrilineal transmissions.

Given that kinship relations and genealogical information were not sourced for non-clan affiliated samples, it is possible that cousins or other male relatives were included, resulting in higher type-sharing of African haplotypes which are common to the region and its cohabiting populations. Fuzwayo, Hastoni and Sukwini are three abeLungu clans which exhibit almost entirely African ancestry. Only three Sukwini individuals carried Western-European haplogroups (Table 3.2(a); Appendix C, Figure S6). This brings into question the true ancestral identity of these clans, and whether they actually had non-African ancestry in their patriline to begin with, which may have been diluted out through various processes such as non-patrilineal transmissions - or whether the notion of their foreign ancestry was an artefact of their cultural associations and geographical proximity with other abeLungu clans.

Alternatively, this could be an example of other Y chromosome patterns of transmission representing the social practices and customs of the mPondo. It is not to be taken for granted that we have an exact measurement of father/son pairs, because culturally, what may be interpreted as a father/son relation, may not be the case

biologically. Regarding terms of kinship in the amaXhosa, the term *brother* (“*bhuti*” in isiXhosa) has a broader use and a greater social impact in Xhosa culture than in Western society, which generally uses the term to describe a biologically related male sibling. In Xhosa culture, a brother is the son of a man related to an individual’s father through paternal kinship, which includes an individual’s brother, uncle, cousin, or a man with a shared father's clan name. *Bhuti* is also a term used for showing respect for hierarchy by age and to address an older male individual. *Bhuti* also refers to a young man who has returned from initiation school, and may also be used when referring to another man who had undergone initiation prior to him (Young and Jackson, 2011). This may create conflicting inheritance patterns for inferring and understanding relations when Y data are checked against genealogical data. These types of scenarios, in which social relatedness and biological relatedness do not correlate, need to be addressed delicately. In the event of such a situation, the Chief of the clan was informed prior to the individual, as they are in each instance aware of all relations and have knowledge of any “illegitimacies”.

4.1.5 Factors which shape clan diversity

Factors affecting clan diversity, which are inherent to small groups of individuals, may lead certain haplotype lineages to extinction and thus reduce the Y chromosome genetic diversity, while others may act to increase the Y chromosome diversity within- and between- clans (Chaix, 2007).

One limiting factor was the sampling geography - where sampling of families in remote locations was more often than not the circumstance, and there was great difficulty in assembling subjects at one location for sampling. Translation was necessary to convey the premise of the study, but the process was time-consuming, which limited the number of families which could be sampled in a day.

Other factors which shape diversity include non-patrilineal transmissions (NPTs) and multiple founders for names (where individuals with the same clan-name have different haplotypes). Progeny having a certain ancestral background, with their fathers exhibiting a different one, are most likely a result of illegitimacy, non-paternity, maternal surname inheritance, name changes or adoption. Together we refer to these as non-patrilineal transmissions (NPTs), which will act to introduce exogenous

haplotypes into a surname-lineage or clan (King and Jobling, 2009). The incidence of non-paternity was found to have been low; only six out of the 146 clan-affiliated individuals sampled were NPTs (4.1%). Two instances where NPTs were observed were in the Jekwa and Hatu clan genealogies (Appendix C, Figures S2 and S4). In the Jekwa clan, two individuals carrying European R1b_5 haplotypes were found bearing progeny with African haplotypes, namely E2b1a_9 and E2b1a_5. Similarly, in clan Hatu, an E2b1a_4 individual and an E1b1a1a_14 individual, were recorded as progeny of individuals carrying the modal haplotype R1b_18, which is also European in origin (Appendix C, Figure S4). These individuals are not related biologically to their “fathers”, and were most likely the result of NPTs such as infidelity or adoption. There are six clear-cut examples of NPTs observed in clan genealogies (Appendix C; Figures S1-S6), however there is possible indication for a higher frequency of non-patrilineal events due to the presence of African haplogroups in clans, however father or sib-information is not available to definitively state in case whether these events are what they seem. The informed consent pages distributed prior to sampling did not state that NPTs could be discovered or disclosed to the subjects, however the matter was discussed with clan elders. In report-backs to clan members all NPT cases and possible NPTs were relayed back to clan elders first, who then took it upon themselves to disseminate the information back to specific clan-members and families as they were the ones who were informed of all kinship relations and cases of adoption and non-paternity.

“Daughtering-out” is a process which also results in stochastic variation in the number of sons fathered by different men, which over many generations can lead to the extinction of Y chromosome lineages and the increase in frequency of others within clans (King and Jobling, 2009). Absence of migration of men among clan descent groups further exacerbates the strength of genetic drift. Thus, although some Y chromosome haplotypes might go to extinction, others might reach rapidly high frequencies within a clan and thus give rise to the so-called identity core-haplotypes (Chaix, 2007). Similarly, genetic drift which occurs due to random changes in haplotype frequencies over the generations acts to either reduce/increase diversity.

Mutation rates also affect haplotype diversity and can be used to infer whether a random or a causative change in inheritance has occurred, which distinguishes truly

coancestral haplotypes from stochastic variants (Jorde et al., 1998). Jorde et al., (1998), Gray et al., (2000) and Kayser et al., (2000) show that the average STR mutation rate (of $\pm 2.5 \times 10^{-3}$ per locus per generation) is much higher than that of single nucleotide polymorphisms (which mutate at a rate of 10^{-7} to 10^{-8} per generation). While SNPs mutate more slowly and provide an indication for deeper ancestry, microsatellites have average mutation rates about five times higher and therefore one may expect to see mutations on the timescale of this type of surname (clan-name) study (Klyosov et al., 2009). Rates of loci also vary depending on their structural make-up (i.e. whether they are tri-, tetra- or penta- nucleotide repeats) (Butler et al., 2002). An example of a comparatively slowly mutating marker is trinucleotide repeat-marker DYS388 which is more impactful than other hypervariable markers (Klyosov 2009). The STR markers selected in the STR YFiler™ and Multiplex II PCR panels reflect: i) the consideration of the rates of change of STR markers within a broad enough haplotype and ii) the estimated time-span from which the founders purportedly established clans.

A striking difference in average STR mutation rates is observed, depending on whether the evolutionary estimate is used, or whether the rates were calculated by direct count in deep-rooted pedigrees (while assuming one generation consists of 25 years) (Jorde et al., 1998; Forster et al., 2000; Zhivotovsky et al., 2004). Therefore, a direct-count of the father-son transmission rate of Y chromosome haplotypes in genealogies reflects the time-depth of the constructed clan genealogies, and is a more suitable indicator than an evolutionary mutation rate for this study. The occurrence of mutations in within haplogroups that give rise to the diversity of haplotypes within haplogroups probably reflects the time-scale from the point of introduction of a lineage from castaways to the present-day population established in the Eastern Cape. If we use the date which the oral and documented history places the primary abeLungu clans' founding to be in 1723 (Soga,1930), which was 287 years before present-day clan members were sampled in 2010, the time span equates to ~11 generations ago, with one generation consisting of approximately 25 years (Kayser et al., 2000). This is consistent with primary abeLungu clan genealogies, which exhibit on average 11 generations between the clan progenitor and present-day clan members.

An example that testifies to this is an amaMolo individual who carried haplotype R1a1a_5 which was markedly different from other R1a1a (R-M198) haplotypes (Figure 3.2), particularly with that of his alleged “brother” (according to the genealogy and consent form information) who segregated with the R1a1a_2 modal haplotype (Appendix C, Figure S1). The 16 mutational step differences between these two haplotypes shows a divergence too great for these individuals to be biological siblings, which implies illegitimacy or non-paternity and reduces the father/son transmission efficacy of the modal haplotype in Bhayi’s lineage. Assuming the average STR mutation rate estimated by Jorde et al., (1998), Gray et al., (2000) and Kayser et al., (2000) is $\sim 2.5 \times 10^{-3}$ per locus per generation, it is not possible to have observed related haplotypes bearing 16 mutational differences within the given historical time-frame, which reaffirms that the R1a1a amaMolo brothers are in fact not related. An alternative explanation for the presence of this haplotype may be from another haplogroup R1a1a (R-M198) founder, possibly from an entirely different shipwrecking, which was introduced into the clan at a later stage.

4.2 The maternal legacy of the abeLungu

While the main focus of the study was to assess the concordance between the oral history (including genealogical records) of the abeLungu and patterns of Y chromosome variation within and among clans, we also made use of mtDNA to examine the maternal ancestries of the abeLungu. Given that that written records claim, for example the story of Bessie (Crampton, 2004), that some women who survived ship wrecks were also integrated into the local community, an assessment of mtDNA haplogroups (using their known geographic patterns of distribution), would help us trace the origins of woman who contributed to the mtDNA pool of the abeLungu.

These results showed that all individuals had mtDNA haplogroups that were traced exclusively to African origin, with the majority being found in haplogroups L0d and L3e (Table 3.4). The presence of L0d lineages would suggest that the maternal history of the abeLungu is associated with recent admixture from the Khoi and San groups (Schlebusch et al., 2009). MtDNA variation patterns indicate high within-clan genetic diversity, low levels of among-clan differentiation, suggesting virtually random female

mediated gene flow among clans of deeply-rooted ancestral mtDNA haplotypes. Their mtDNA is bound to be from different mothers married into the clan, hence the increased diversity. Two factors have an effect on mtDNA diversity patterns: Polygyny, which is traditionally practiced in abeLungu clans (with the number of wives often depending on the wealth of the husband) may result in higher mtDNA diversity (Soga, 1930; Crampton, 2004; Jackson, 2005). The increased levels of mitochondrial diversity observed in clans might also be a consequence of the complex rules of exogamy in practice in abeLungu clans. Traditionally, a man must choose a bride so that he will not share a common ancestor on the paternal lineage with her for a given number of past generations (Crampton, 2004; Chaix, 2007). This number is usually close to the genealogical depth of a lineage (five to ten generations depending on the population), so that in practice the bride usually belongs to a different lineage (lineage exogamy). These rules of exogamy imply that, at each generation, a significant number of women migrate from one descent group to another, amounting to increased diversity (Chaix, 2007). The molecular evidence is congruent with oral history in that a non-African input is clearly detected in the patriline, and that only Southern-African haplogroups are witnessed in the mtDNA lines of the abeLungu clans. This indicates that, apart from a small non-maternity rate, abeLungu women maintained fidelity and their African maternal ancestral legacy, which has stood through the tests of changing male population demographics.

As a corollary to examining the Y chromosome lineages which reflect the transmission of clan name, it would have been interesting to detect non-African ancestry in the mtDNA of clans-people, which may or may not have been derived from Bessie and her marriage into the amaTshomane clan; however, this has not been observed. Bessie's story tells of how she became part of the mPondo people, had to learn their language and practice their customs, her marriage into the amaTshomane clan, the death of her husband and her subsequent remarriage to his brother Sango. Her story contains several mysterious elements like the unconfirmed identity of the "three Englishmen" who supposedly accompanied her upon her arrival on the Wild Coast, as well as putative links to other shipwrecks' surviving castaways, with particular interest placed upon one of the more famous ships, the Grosvenor wrecked in 1782 and its legendary bounty, which to date remains a mystery (Soga, 1930; Crampton, 2004). Soga recalls Bessie's affiliation (the only link, which has no relevance unfortunately):

“They were given isiXhosa names; the men were called Jekwa, Badi and Hatu, and the girl, Gquma. Having all come from the same ship (interpreted locally as a house), they were considered to be family, with Badi and Jekwa seen to be brothers and Gquma, the daughter of Badi”.

Bessie married and bore children, including her daughter Bessy, but her offspring would have belonged to the clan of their father - amaTshomane – and not abeLungu, making her inclusion as a major founder not only unusual, but also fundamentally contradictory to the principles of patrilineal descent. The little that is known about Bessy’s family tree has survived the generations and has been passed down by oral history. Soga (1930) accounts: “Gquma died at Mgazi about 1770. Her daughter Bessy married Mjikwa, son of Wose, Chief of the amaNkumba clan, and Principal Son of the Great House of Zwetsha - the premier clan of the amaBomvana. Xwebisa and Gquma’s family, claiming foreign heritage, still retains by virtue of descent through the male line the original clan name of the amaTshomane, derived from Tshomane, Xwebisa’s father” (Soga, 1930, p.380) (Figure 20). No trace of Bessie’s European history was found in the mtDNA haplogroups of maternal lines. This too was expected, considering no direct efforts to trace Bessie’s descendants could be made due to the patriarchal mechanisms of inheritance of Xhosa clan name, and the lack of a social marker to parallel mtDNA inheritance.

Further collaborative steps can be made, beginning with deeper and more widespread anthropological research, so as to attempt to trace the mitochondrial lines stemming from Bessie and other potential female survivors. This would be a difficult task, considering there are no female cultural markers to parallel the patrilineal inheritance of the abeLungu clan name. Tracing maternal ancestry has been successful in several studies, however the proposed reconstruction of social relations, especially that of matrilineal societies, is based on very thin evidence for traditional - that is, pre-1900 times (Pollock, 2009). Some of the seminal studies on populations that exhibit matrilineal modes of inheritance include that of Godard (1867) on the Sudanese Nubians, the study on Iroquois and Hopi Native Americans by Freire-Marrecco (1914), the Vanatinai of Papua New Guinea by Lepowsky (1981), and the Rapanui in Polynesia by Hage & Marck (2003). More recently, Starck, (2013) studied Minangkabau of West Sumatra who form the largest matrilineal society in the world. The life in the core areas was

defined by a matrilineal way of life. This means there are certain kinship groups which follow the female descent of a mother. The woman's brother is responsible for her children rather than her husband. These studies have investigated these cultural groups which exhibit matrilineal and matrilineal inheritance of clan name, so as to trace their matrilineal origins. These studies are the biological and socio-cultural counterparts to the ancestry studies of those like this study on the abeLungu, and they need to be used as guides for future studies on the abeLungu for the discovery of Bessie's maternal legacy.

The only detail that historical data provide is that it is suspected that Bessie was a crew member aboard one of the Dutch East India (VOC) vessels which became wrecked sometime around 1737, which remains unconfirmed to date. To investigate the maternal line deriving from her daughter Bessy who married Mjikwa would be the one means at discovering European maternal ancestry. However, the family line leads up to an individual Sizungazane, who died in 1921 (Figure 4.1). There are no records as to whether this individual had any offspring who may have continued the maternal ancestral legacy of Gquma (Bessie), her daughter Bessy and their European origins (Soga, 1930). Even if there are historical examples of female European castaways, their non-African maternal ancestry would most likely be diluted out within a few generations, due to admixture from the mtDNA haplotypes of local Xhosa women which clan males had married.

Examples of matrilocality exist within the amaXhosa, albeit sparsely. Kalis, in her interviews recorded in 2009: "...For example, a friend whose patrilineal family resides in the Tsomo village in which his amaThembu great-great-great-grandmother was born. She married an Englishman by the name of Jonas (possibly a trader) in the mid-nineteenth century, and their descendants took their mother's clan-name, amamTolo". Kalis also has a colleague whose mother's family hails from Holy Cross and traces its descent from a shipwreck survivor. Through simply discussing her further research plans with people in and around Mthatha, Kalis had also received suggestions of additional clan names which require following up.

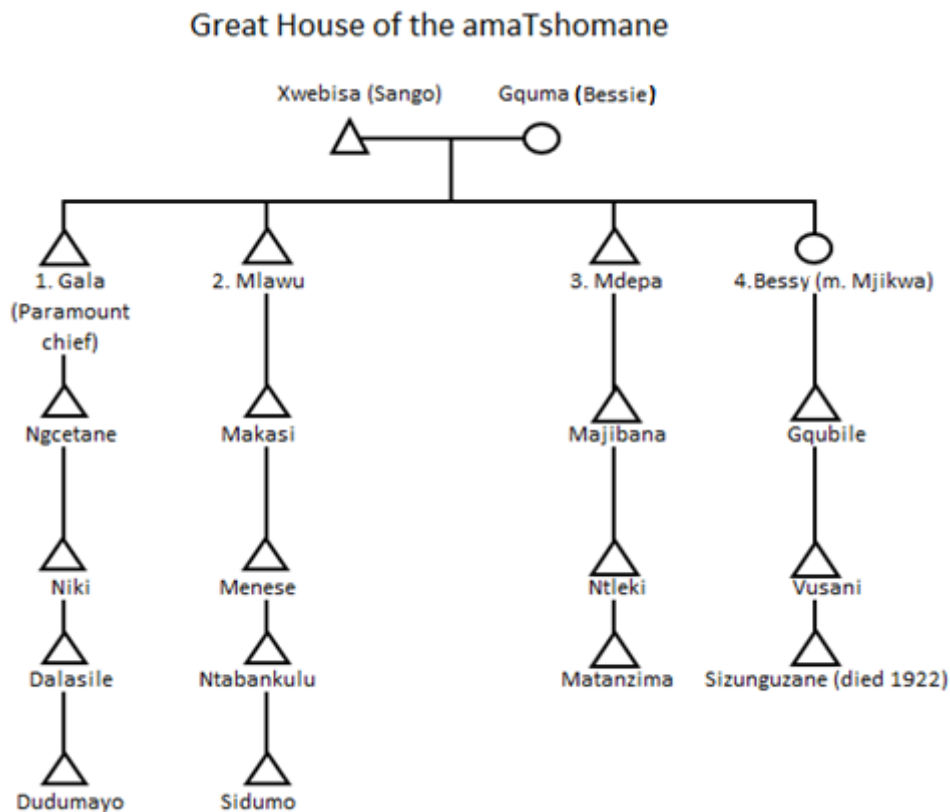


Figure 4.1. The amaTshomane clan genealogy featuring the lineages and the progeny born from Xwebisa (Sango) and Gquma (Bessy) (adapted from Soga, 1930, p.380)

4.3 In summary of the findings

These demographic and genetic processes may explain not only the existence of the core identity haplotypes at the Y chromosome and mtDNA levels in clans, but also their overall lower Y chromosome diversity compared to non-clan affiliates in neighbouring, cohabiting regions, as well as the higher diversity of deeply-rooted mtDNA haplotypes. The prevalence of non-African haplogroups in the vast majority of abeLungu and amaMolo clan members with coancestral haplotypes ultimately validates the hypothesis put forward in the documented and oral history (Soga, 1930; Kirby, 1953; Crampton, 2004). Analysis of the results provides evidence for the relevance of the dual inheritance model (culture and genetics) in understanding patterns of human genetic variation, as inferred by gene-culture coevolution theory (Jobling, Rasteiro & Wetton, 2015). Analyses indicate that the dynamics of patrilineal descent groups imply different male and female socio-demographic histories, as well as the fact that patrilocality, NPTs and polygyny are primarily responsible for these sexually-asymmetric genetic patterns.

4.4 Future Studies

Sampling of a wider variety of clan names and genealogical histories will contribute to alleviating any current social or geographical bias, leading to interesting new insights of cultural and demographic history (King and Jobling, 2009[b]).

Admixture analysis would be needed to further refine origins and relations within clans of the Pondoland region as well. Autosomal Ancestry Informative Markers (AIMs) are distributed abundantly throughout the genome, and have shown to retain geographically restricted allele frequency distributions which serve as indications for the likely parental populations of the samples being investigated (Phillips, 2007; Hinds, 2005). Autosomal SNPs with population specificity to those of likely parental populations of the samples can be screened for using an array of autosomal ancestry informative markers (AIMs), selected from published data, found in European, African, Asian, Eurasian, Middle Eastern and Oceanic populations, so as to uncover any possible genetic substructure and/or admixture, in order to complement findings from Y chromosome and mtDNA data. In these tests, AIMs are examined and compared with frequency data of these markers in the surveyed groups. The results of this comparison are then statistically analysed to produce a break-down in the form of percentages of different ancestries (Nash, 2006). These percentages are therefore not a measure of the number of ancestors of different backgrounds within an individual's genealogy, but rather an estimate that depends on the initial selection of subjects, the examination of particular markers, complex statistical calculations and the system of ordering patterns of human diversity into the main continental groups (Nash, 2006).

Fejerman (2005) suggests that if an admixture event had occurred many generations ago, then African alleles would be expected to be widespread among individuals. However, if it took place only two or three generations ago (as in the case of some of the secondary abeLungu clans, with more recent ages of establishment), we would expect to observe a small proportion of individuals in the sample with a relatively high probability of having African ancestors. He attributes this to the fact that the 'immigrant' alleles do not have time to become established among individuals of the endemic population, but rather tend to remain concentrated within families. Autosomal tests do provide a degree of statistical certainty about the proportions of different groups in

one's ancestry; however, the results are more of an artefact of a series of approximations that underestimate the complex ways in which ethnical and racial identities are socially defined and experienced. Another misappropriation of admixture analyses is that they can easily be interpreted as supporting to the idea that racial or ethnic categories have a genetic basis (Fejerman, 2005; Nash, 2006).

Comparative diversity analysis between populations will tell us about differences in their histories, and knowledge from multi-allelic marker mutation rate analyses will allow for estimates of the age of the most recent common ancestor of the group of chromosomes examined. These kinds of studies have implications beyond the field of Y chromosome research, as they can reveal signals of population structure and history which are important in choosing populations for mapping genes underlying complex traits (Jobling and Tyler-Smith, 2000). Most new advances will emerge from the exploitation of recent technological developments. Improvements to the methods of analysis of ancient DNA should enable the testing of genealogical links between living individuals and putative patrilineal ancestors and also among archaeological human remains (Fejerman, 2005; Nash, 2006). High-resolution Y chromosome typing and mitochondrial DNA sequencing, together with whole-genome SNP analyses, should enable reliable reconstructions of genealogies; these will include the establishment of links across the sexes, which cannot be achieved by the analysis of uni-parentally inherited markers alone. In terms of relatedness, surname/clan-name—ascertained cohorts of men who share Y-chromosomal coancestry lie between the traditional pedigree and the population substructure, and application of whole-genome typing to such groups could be useful in understanding the history of recombination, and for genetic epidemiological purposes. With the decrease in cost of sequencing, private individuals will fund their own genome projects, and it seems inevitable that SNPs specific to surnames, clan names or their lineages will be identified, providing powerful resources for genealogical research.

4.5 The impact of human population diversity and genetic genealogy studies

No single record of the past is more important than another, but each one records different features of the past. The utility of DNA over time is similar to that, in archeological terms, of bones which have been exhumed, as it also provides us with a clearer image of our past. Heritable clan-names, like surnames, are unique cultural markers of coancestry, that represent a rich resource for the analysis of human diversity (Kayser et al., 2003; Jobling, 2015), archaeology (Paabo et al., 2004; Green et al., 2010), history (Moore et al., 2006), genealogical descent (Foster et al., 1998; King and Jobling, 2009 [a and b]) and disease (Pritchard et al., 2001). The effort to understand human origins and history of migration is intrinsic to our basic human nature and curiosity.

As we track the ancestors from geographical stopping points we uncover the history of the migration routes of anatomically modern humans that left Africa between 35,000 and 89,000 years ago (Underhill, 2000). A minority of contemporary East Africans and Khoisan represent the descendants of humankind's most ancestral patrilineages. Examining the utility of heritable DNA on a more recent time scale allows for the detection of location and migration of clan-groups and surname lineages. Clan-names are patrilineal, and so men sharing surnames might be expected to share related Y chromosome haplotypes, because these are also passed down from father to son (Jobling and Tyler-Smith, 2003). However, the strength and structure of the relationship between the two could be influenced by a number of additional factors (Jobling, 2001). Mutation will alter haplotypes through time, although, on the timescale of clan-names, this will only affect rapidly mutating markers such as short tandem repeats (STRs) (Jobling, 2015). Knowledge of mutation rates and processes allows this to be taken into account. Similarly, differences in the number of founders at the time of surname/clan-name establishment within a given population could affect the number of descendant lineages within a clan-name (King and Jobling, 2009[b]).

There are several advantages to using molecular data when investigating population structure, namely: molecular entities are strictly heritable and the description of molecular characters (mutations) is unambiguous, unlike oral history which is subject to large degrees of distortion and incoherence. Molecular data are abundant and

amenable to quantitative treatment - we can statistically measure support for hypotheses. Homology assessment is easier and more reliable with molecular data than with morphological traits, which have been typically relied upon in the past. There is some regularity to the evolution of molecular traits as well, which allows for accurate measurements of demographic expansion timings. The population-specificity of binary markers used in combination with microsatellites in Europe and elsewhere has proved useful in analysing clan-names that are thought to reflect origins outside a particular region, such as for those formerly British clan-names like Ogle, Caine (or McCaine), Irish and Horner might suggest.

4.6 Genealogy testing and its limitations

In more recent times there has been an upsurge of commercial ancestry testing companies, offering services aimed to situate individuals within global patterns of human genetic diversity, locate genetic origins and sort out true biological relatedness from practiced kinship (Shriver and Kittles, 2004; King and Jobling, 2009). Most of these companies assert that DNA evidence can provide a link between possible branches of a family tree when there is difficulty in establishing a connection by other means (Shriver and Kittles, 2004; Nash, 2004).

Ancestry test results are depicted through the familiar graphics of the human family tree and explained via recognised but newly geneticised notions of human reproduction, ancestry and inheritance. Ancestry testing defines the most recent association of popular and scientific models of ancestry and descent in geneticised genealogy, and signifies the cultural work of authorising genetic answers to questions of relatedness and identity.

However, as these tests are novel and still under-developed, much of the public is often apprehensive to undergo testing due to the controversies associated with personalised genetic histories - but for the most part, these controversies have more to do with the complex history of race, discrimination and prejudice than the science behind genetic ancestry itself. The outcomes are variable: for some individuals, the results may be disconcerting and the experience at attempting to interpret the meaning of the test results for personal or familial interpretations of origin and identity, is

sometimes complicated and often unsatisfactory. For others still, they can be only marginally interesting, or end up even having very little significance, while for certain people the tests provide, as testing companies assure, a meaningful and significant sense of ancestral origin (Nash, 2004; Shriver and Kittles, 2004). Regarding the clan-affiliated subjects who received their ancestry reports the outcome was welcomed and one of gratitude from the abeLungu for contributing to validating their unique ancestry.

Traditional anthropological genealogy should in theory be able to provide a family tree that includes all the sets of great-grandparents, great great-grandparents and so on, whose genetic material has been mixed together and passed on to a present day individual. Genetic genealogy however cannot inform the public about the complex blend of genetic material people have inherited from all the preceding ancestors of that individual. The narrowness of the tests and their dependence on the form of direct transmission, from fathers to sons in the case of the Y chromosome, and from mothers to children in the case of mtDNA, makes them useful in exploring patterns of descent. However, this specificity also means that the tests only focus on a small portion of DNA which is inherited directly (Nash, 2006).

Technically more advanced tests that employ genome-wide association to infer genetic 'ancestry painting', admixture analyses, or one's percentage 'global similarity' to other people in the world, oversimplify the genetic data (and as a result the individual's identity), reducing it to something as scientifically lacking, but historically marked, as 'European' (Bhattacharya, 2010). Also, considering the time-scale of genetic ancestry tests and the results of a genetic genealogy test alone, without comparison to other socio-cultural genealogical narratives, these tests do not provide information on more recent ancestors who could have lived in many different places (Nash, 2004).

It is noteworthy for clients of genetic ancestry tests to consider that while haplogroups are presented as personal results which suggest their special link to the customer, (through claims that their result is their 'unique genetic signature'), these haplogroup designations are in fact shared with millions of other people. The estimated genomic variation of any one person differs from that of another by only 0.1% to 1%, which in turn means that the vast majority of the genes that are said to make us who we are,

are invariably shared with every other human. This means that the axiom that most testing companies exploit, namely 'Genes make you who you are as a unique individual' actually have very little discrimination capacity, other than in ascertaining 'ethnic or geographical origins', or in some instances, the compositional breakdown thereof (Shriver and Kittles, 2004; Nash, 2006; Jobling, 2015).

Genetic genealogy tests provide individual results which may have implications for other relatives who, as discussed above, in the case of Y-chromosome tests share paternal descent, and in the case of mtDNA tests share maternal descent. The information that the results suggest about ethnic or geographical origins that a person receives is therefore pertinent to other family members who may not have chosen to acquire this knowledge, and for whom it may complicate or disturb their particular sense of cultural or ethnic identity. Examples exist, such as is frequently the case, where Y-chromosome tests results can point to a white male ancestor for African-American or British black men - as is the case with the *abeLungu*, who rather praise their foreign ancestry than feign it. In other cases, when the oral history seems irrefutable, the DNA evidence may sometimes be conflicting, and may provide unwelcome news of an illegitimacy - examples of which have been discovered in this particular study- and so these instances need to be treated with cultural and ethical sensitivity.

Despite the limitations which are found in genetic ancestry testing currently available, the power and resolution capacity of DNA analyses used in conjunction with traditional historical research in genealogy will continue to grow. Genetic tests will become more affordable and more sophisticated, and genetic databases will expand, incorporating more markers representing a better geographical coverage of global diversity (Jobling, 2015). It must be noted - the finding of matching haplotypes needs careful interpretation and, in particular, consideration must be taken of the frequency and resolution of the haplotype in a haplogroup. Ideally, the question of whether a mismatch is due to mutation, or whether it represents an exclusion of a recent common ancestor, should be considered in terms of locus-specific mutation rates (Jobling, 2015). It is also crucial to keep in mind how genetic ancestry inference is a biotechnologically assisted process that is based on a socially constructed process, and

the two disciplines should aim to corroborate each other so as to define clearer ancestral migration and demographic histories.

4.7 Biomedical and forensics impact of population diversity studies

An understanding of how genetic diversity is structured in the human species is not only of anthropological and political importance, but also of medical relevance (Jobling, 2000; Lu et al., 2016). Much of the population diversity literature recently points out that individuals of various cultural and ethnic origins may often respond differently to medical treatments where major differences in allele frequencies exist between populations (Wilson et al., 2001; Shriver and Kittles, 2004; Jobling, 2015).

While the majority of polymorphisms investigated in population diversity studies are probably neutral, they can be used to query for associations with particular phenotypes and - to examine the reverse - to ask if there are specific phenotypes which influence the distributions of polymorphisms within populations (Jobling, 2000). The first approaches to quantifying biological differences were based on crude physical measurements that were heavily biased in their execution, using characteristics that define racial classification to which human perception is most directly amenable, namely phenotypic traits such as skin colour, eye shape and colour, hair colour and texture, etc. Until much more recently, direct and objective methods of quantifying genetic variation (as opposed to “physical” characteristics) were non-existent (Lu et al., 2016).

The first in a series of large public efforts that began to shift the field of medical genetics away from purely descriptive documentation of patients’ phenotypes, coupled with ineffective and time-consuming examination of a small subset of patients’ potentially disease-causing genes, was marked by the successful completion of the Human Genome Project, in 2003 (Lu et al., 2016). Genome-wide association studies (GWAS) provided the opportunity to efficiently and comprehensively assay genetic variants common to a population, and to identify those variants more frequent in patients with a particular disease, than in controls without the disease (Manolio et al. 2009; Lu et al., 2016). Numerous population-specific studies of disease have obtained conclusive results for population affinity of alleles, which include the investigation of myocardial infarction in Icelanders, or to prostate cancer in African-Americans, which

have capitalised on the disease-susceptibility of specific alleles in more genetically homogeneous populations (Jobling and Tyler-Smith, 2000; Lu et al., 2016). Similarly, the autosomal recessive disease sickle cell anemia, for example, was shown to be largely restricted to African, Mediterranean, and South Asian populations (Lu et al., 2016). As early as in 1966 it was already known about another example of population-affinity for a disease genotype which is that of Ashkenazi Jews, who are statistically more susceptible to carry the mutant alleles causing autosomal recessive Tay–Sachs disease (Lu et al., 2016; Myriantopoulous and Aronson, 1966). Another renowned case study for treatment response to disease for which there is population specificity is hepatitis C virus (HCV) infection, for which it was established that African individuals respond more poorly to HCV drug treatment than Caucasian and Asian individuals (Lu et al., 2016).

In studies such as these, the appropriate choice of the control population is of intrinsic importance. While the Y chromosome is highly stratified by geography, it might also be stratified by other factors such as social-class, which could amount to ascertainment biases in the attendance of subjects at clinics, as an example. An approach to this would be to use male, non-blood relatives from within the subjects' families, as control subjects (Jobling and Tyler-Smith, 2000).

An understanding of population structure is critical for the identification of disease genes by association with marker loci. Advances in DNA sequencing technologies and analyses have driven the recent rise of genomics in medicine which is aimed at finding genetic causes of common complex diseases, so as to develop marketable cures for them (Lander and Schork, 1994; Cardon and Bell, 2001; Serre and Paabo, 2004; Bhattacharya 2010; Jobling, 2015; Lu et al., 2016).

In another application of surname/clan-name—genotype association studies, a list of surnames (clan-names) with associated Y-STR haplotypes could enable a Y profile to be matched with one or more surnames (clan-names). This might allow the surname of the depositor of DNA evidence to be deduced, in conjunction with the identification of genes involved in certain phenotypic traits such as pigmentation, so as to provide a means to prioritise a suspect list for crime investigation (King and Jobling, 2009).

Y-STR haplotypes will increase the success rate of identifying the male component in male/female cell mixtures in body fluids where other discrimination methods were unsuccessful or too risky; for example, highly degraded samples or samples with very low sperm counts (Kayser et al., 1997).

King and Jobling (2009[b]) proposed that a database of surnames and associated Y profiles would have forensic utility. However, for more frequent and common names (those with greater than 6,000 bearers), predictive power is poor, due to high haplotype diversity. However, for rarer names (those with less than 50 bearers) databases would be ineffective, as crime-scene samples are relatively unlikely to be deposited by bearers of these more unique names, so for the most practical reasons and for optimum efficacy of such a database, a solution would be to incorporate surnames of intermediate frequency. Regarding a database for forensic utility based on haplotype-surname relationships, Bhatti et al., (2016) feel that current databases are not very effective in forensic analysis, due to the currently limited sample size and unknown geographical ethnic origins of the populations. Further studies need to be conducted so as to expand and update the current population-specific databases at ethnic and geographical level by generating a DNA data-set with higher resolutions of discrimination capacity.

4.8 Social cohesion and making a new South African demographic history

As humans we are all descendants from the same human-species tree. However, still today we in South Africa observe racial tensions based on the systemic violence, displacement, racial formation and institutions of social control entrenched by apartheid, because this enforced separation had resulted in radical racialised notions of cultural identity. Race has the implied meaning only in the sense that its members share common ancestry distinct from other groups. Races also share many things besides genes, to the extent that the concept is inextricably cultural in nature (Kittles and Weiss, 2003).

People often understand themselves in a capacity of not only what is genetically inherited, but also in terms of a much more comprehensive set of social relationships, namely narratives of what is culturally inherited, traditions, attitudes, as well as in terms of the impact of their childhood and lifelong experiences (Nash, 2006; Bhattacharya,

2010). Genetic genealogy has the power to clarify and reinforce existing understandings of the significance of ancestry with the public's sense of identity as individuals or as members of ethnic groups.

There is a need for social and molecular scientists to step up and dispel myths, by investigating modest objectives such as confirming relationships between people with similar surnames to answering specific questions like "what was life like for humans 10,000 years ago?". This, as well as evaluating oral histories of traditional clans, which would challenge our perceptions of race, ethnicity and culture. Molecular biology, when used as a tool coupled with detailed anthropological history, can act to corroborate and refine demographic history, which for South Africa (and indeed the world today) can be used to illustrate the arbitrary nature of racial differences which are insignificant in comparison to much more fundamental commonalities of the human race.

This study has been in line with such intentions. Through the analyses of the DNA of current clan descendants in conjunction with genealogical data, certain aspects of the oral story of the origins of the *abeLungu* and *amaMolo* which have survived ten generations have been affirmed, with other aspects invalidated. With this in mind, we confirm that molecular (genetic) methods can be used to validate and refine genealogical history of an example in human history where non-African shipwreck survivors and immigrants underwent a harmonious integration into a deeply rooted Xhosa culture, which contrasts with much of South Africa's recent political history.

CHAPTER 5

Concluding remarks

5.1 Testing the oral history of the abeLungu

Oral history was, and is, a form of entertainment as well as a tool for passing on a cultural identity and a system of values, the details of which have been added and subtracted throughout the course of its telling and re-telling. The premise of this study was to evaluate the congruency of the history passed down from abeLungu progenitors primarily through the cultural medium of historical and genealogical narration, by investigating the molecular evidence found in the DNA of its contemporary clan members.

The genetic data supports the anthropological information regarding introgression of non-African genes into the gene pool of the abeLungu (Appendix C, Figure S1-, S6). It has been shown that the DNA narrative is in convincing agreement with the oral history narrative, which contributes great value to affirming and refining the identity and culture of the abeLungu people.

Present-day descendants of the abeLungu and the amaMolo exhibit high efficacy in father/son chromosome transmission, in that they show continuity of transmission of patrilineal haplotypes in parallel with the transmission of clan name, without dilution across approximately ten generations (Appendix C, Figures S1-S6). The distinct feature of tight clustering of the abeLungu ancestral modal-haplotypes with few variants was consistent for most RMJ networks, which were resistant to repetition of the analysis with randomised inputs (namely non-clan affiliates from the greater mPondo region), as suggested in Bandelt et al., (1999).

In addition to affirming the non-african male ancestry of the abeLungu clans, this study has also demonstrated how the cultural subdivision in patrilineal descent groups has left its footprints on Y chromosome diversity of patrilocal populations, without affecting mitochondrial diversity. The male abeLungu population is experiencing a demographic history of lineal fissions of descent groups without subsequent migrations between descent groups, and this results in so-called “identity cores” and to a reduction of Y chromosome diversity (Chaix, 2007). At each generation the female population undergoes migration flows between lineages of clans, as a result of the social rules of exogamy in practice in the abeLungu, and therefore obstructs the social structure from imprinting mitochondrial structure. This presents as an obstacle to investigating the maternal origins of clan members.

In addition to forming a better understanding of the history of these unique groups of Xhosa clans who feature foreign origins, a better picture of South Africa’s demographic history is painted which we can only hope will demonstrate the superficiality of the antiquated concept of race, provide a greater sense of unity for the future of our country and provide a deeper understanding of our human origins for our unity as a species.

As Haraway observes: *‘Epistemophilia, the lusty search for knowledge of origins, is everywhere’* (Nash, 2004).

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APPENDICES

Appendix A : Ethics



UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Mr David de Veredicis

CLEARANCE CERTIFICATE

M120364

PROJECT

Tracing the Ancestors of mPondo Clans along
the Wild Coast of the Eastern Cape

INVESTIGATORS

Mr David de Veredicis.

DEPARTMENT

Division of Human Genetics

DATE CONSIDERED

30/03/2012

DECISION OF THE COMMITTEE*

Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 30/03/2012

CHAIRPERSON


(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Prof H Soodyall

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.

I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

Appendix B: SNP-marker panels for SBE multiplex assays

Table S1





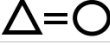



Assay	SNP	Haplogroup	Alternate Name	Ancestral allele	Derived allele
Y SNP1	SRY1083.1	BT	-	C	G
	M168	CT	-	C	T
	M89	F	F-M89	G	A
	M201	G	G-M201	G	T
	M69	H	H-M69	T	C
	M170	I	I-M170	A	C
	M172	J2	J-M172	T	G
	M9	K	K-M9	C	G
	M207	R	R-M207	A	G
	M198	R1a1a	R-M198	C	T
M343	R1b	R-M343	C	A	
HG-A	M91	A	A-M91	T	A
	M31	A1a	A-M31	C	G
	M14	A1b1a1	A-M14	A	G
	M114	A1b1a1a2a	A-M114	A	G
	P28	A1b1a1a1	A-P28	C	T
	M28	A1b1b1	A-M28	A	C
	M51	A1b1b2a	A-M51	C	T
	M13	A1b1b2b	A-M13	G	C
	M171	A3a2	A-M171	C	G
	M118	A1b1b2b1	A-M118	A	T
HG-B	M60	B	B-M60	A	T
	M146	B1a	B-M146	T	G
	M182	B2	B-M182	C	T
	M150	B2a	B-M150	C	T
	M152	B2a1a1a1	B-M152	C	T
	M108	B2a2a1	B-M108.1 (or B-M108.2)	A	G
	M43	B2a2a1a	B-M43	A	G
	M112	B2b	B-M112	G	A
HG-B2b	P6	B2b1b	B-P6	G	C
	M115	B2b1a1a	B-M115	C	T
	M30	B2b1a1b	B-M30	G	A
	P7	B2b1a	B-P7	T	C
	P8	B2b1a2	B-P8	G	A
	M211	B2b1a1c	B-M211	C	T

Table S1 continued

HG-E	M40	E	E-M40	C	T
	M33	E1a	E-M33	A	C
	M44	E1a1	E-M44	C	G
	M75	E2	E-M75	G	A
	M41	E2a	E-M41	G	T
	M85	E2b1a	E-M85	G	T
	P2	E1b1	E-P2	G	A
	M2	E1b1a1	E-M2	T	C
	M35	E1b1b1	E-M35	C	G
HG-E1b1a (prev E3a)	M58	E1b1a1a1a1a	E-M58	G	A
	M116.2	E1b1a1a1b	E-M116.2	A	C
	M149	E1b1a1a1c	E-M149	G	A
	M154	E1b1a1a1a2a1a3b	E-M154	A	G
	M155	E1b1a1a1d	E-M155	C	T
	M10	E1b1a1a1a1b	E-M10	T	C
	M191	E1b1a1a1a1c1a1	E-M191	T	G
HG-E1b1b1 (prev E3b)	M78	E1b1b1a1	E-M78	C	T
	M148	E1b1b1a1b2a1	E-M148	T	C
	M81	E1b1b1b1a1a	E-M81	C	T
	M107	E1b1b1b1a	E-M107	A	G
	M165	E1b1b1b1a1a~	E-M165	T	C
	M123	E1b1b1b2a	E-M123	G	A
	M34	E1b1b1b2a1	E-M34	G	T
	M136	E1b1b1b2a1a1	E-M136	G	A
	M281	E1b1b2	E-M281	C	T

Appendix C: Clan genealogies

Figures S1 – S6 are the partial genealogies of the 13 clan families. The legend below describes the symbols found in the clan genealogy diagrams:

Legend	
Symbol	Description
	Male, not sampled
	Male, sampled
	Female (spouse), not sampled
	Spouse, sampled
	Marriage
	Deceased
	Relation unknown
	Relation unknown

Clan-affiliated individuals that are part of the sample set are demarcated with a sample code (UMT prefix) and their unique haplotype, while the remainder of the individuals in the genealogies are demarcated by a numbering system where the roman numeral indicates a particular generation, and the subsequent numbers designate the particular individual within that generation.

Note: The amaMolo and abeLungu Jekwa genealogies (Figures S1 & S2) have been split and resized as opposed to having featured them in landscape orientation on the page, so as to illustrate them with an optimal aspect ratio.

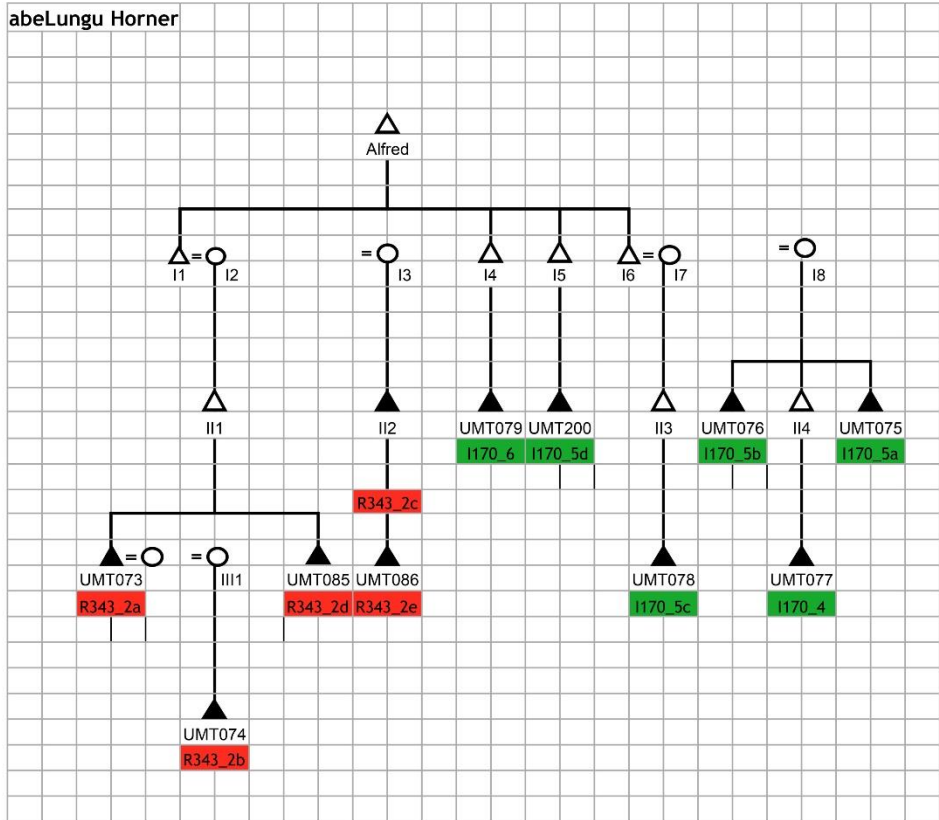
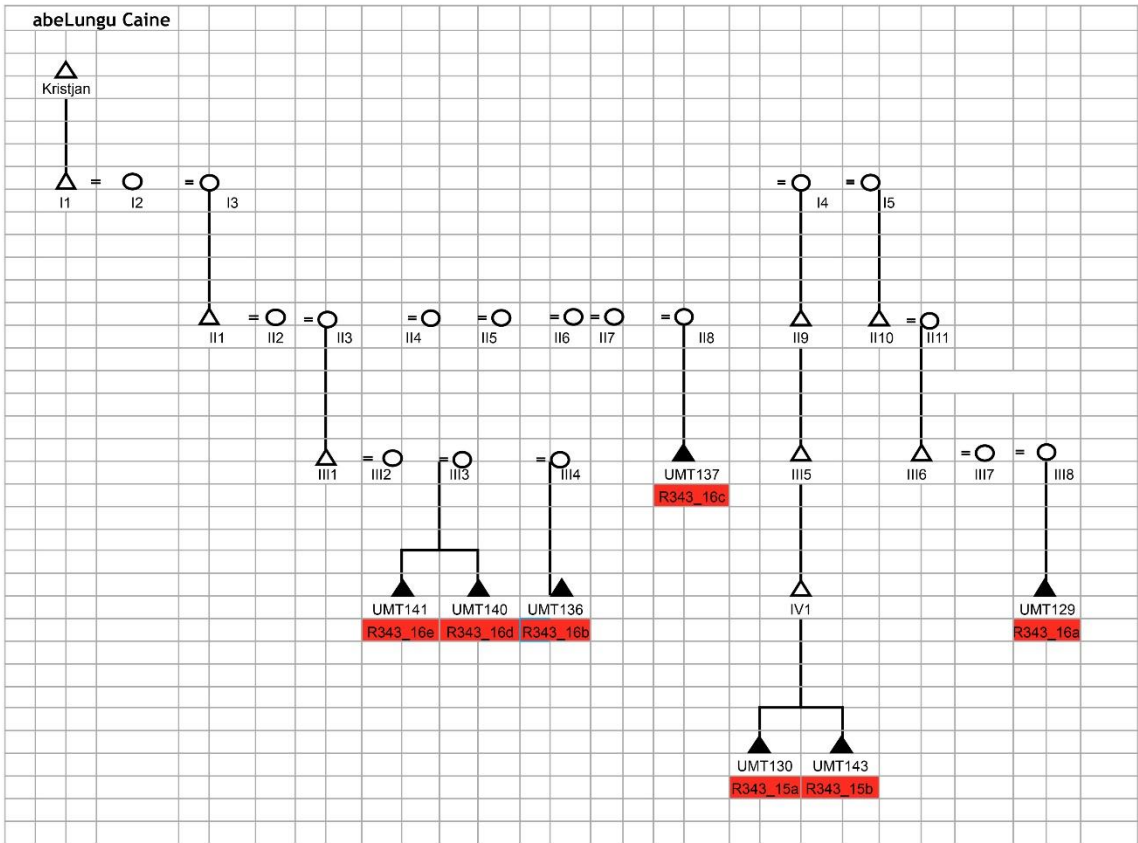


Figure S3 - The abeLungu Caine and Horner clan genealogies

abeLungu Hatu

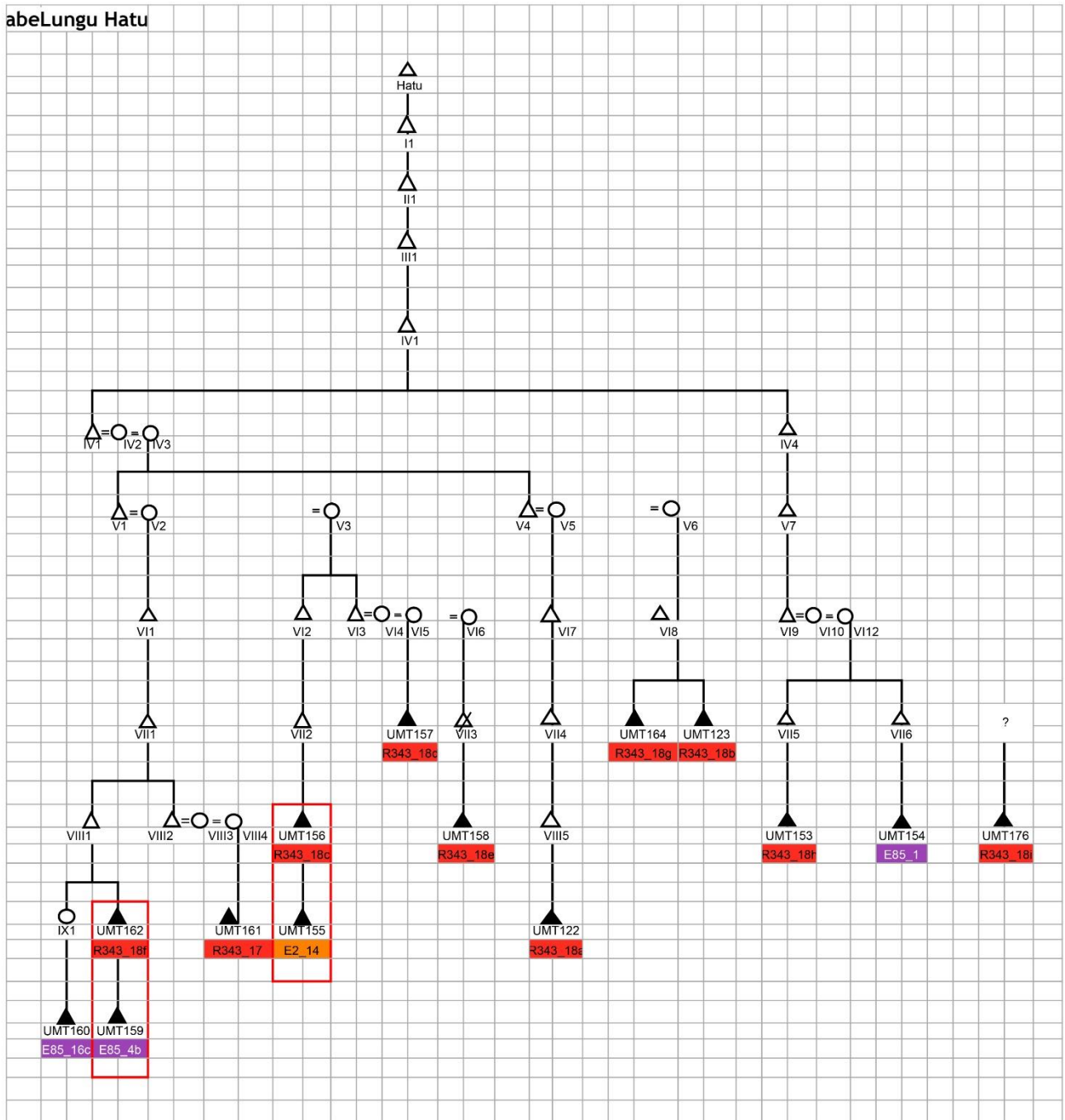


Figure S4 - The abeLungu Hatu clan genealogy

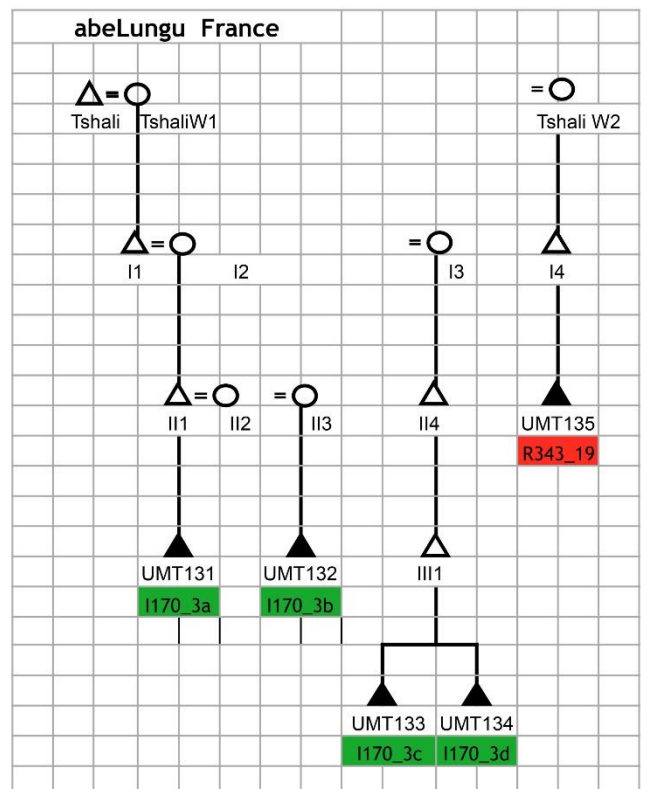
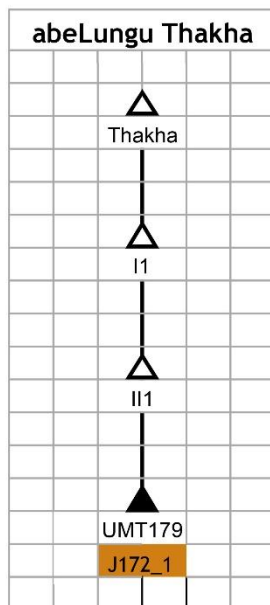
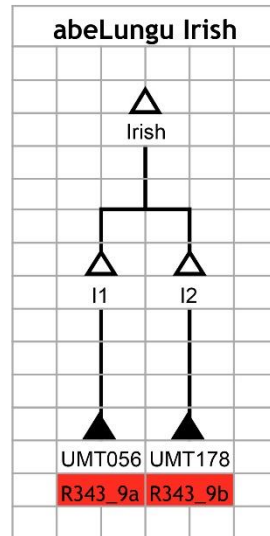
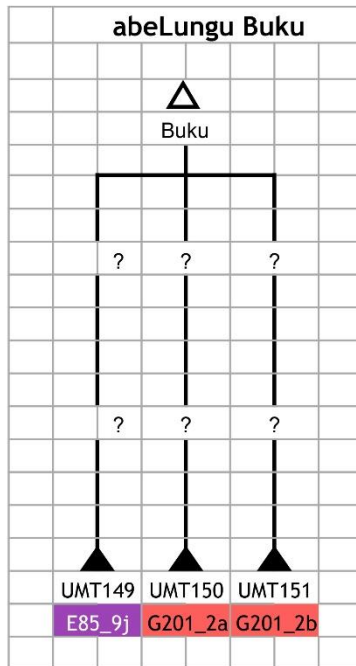
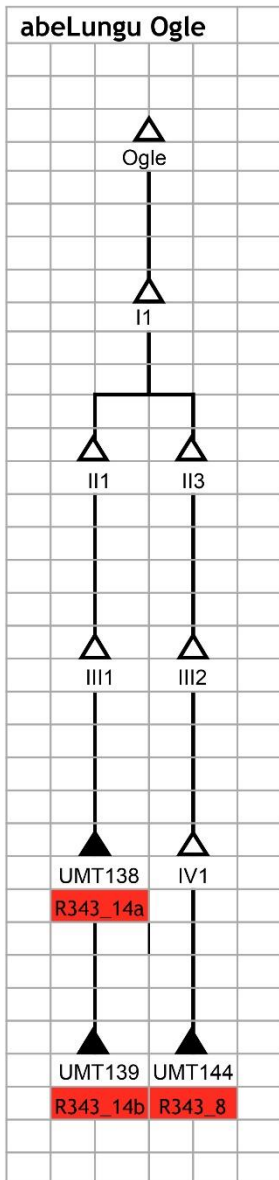
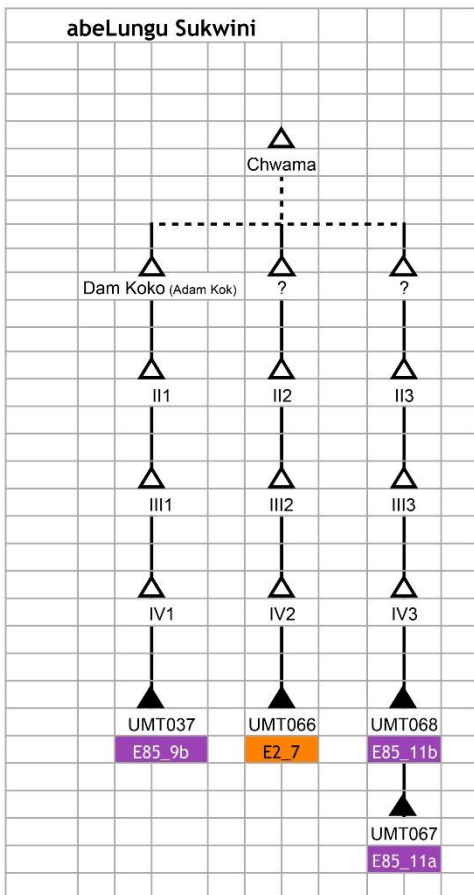
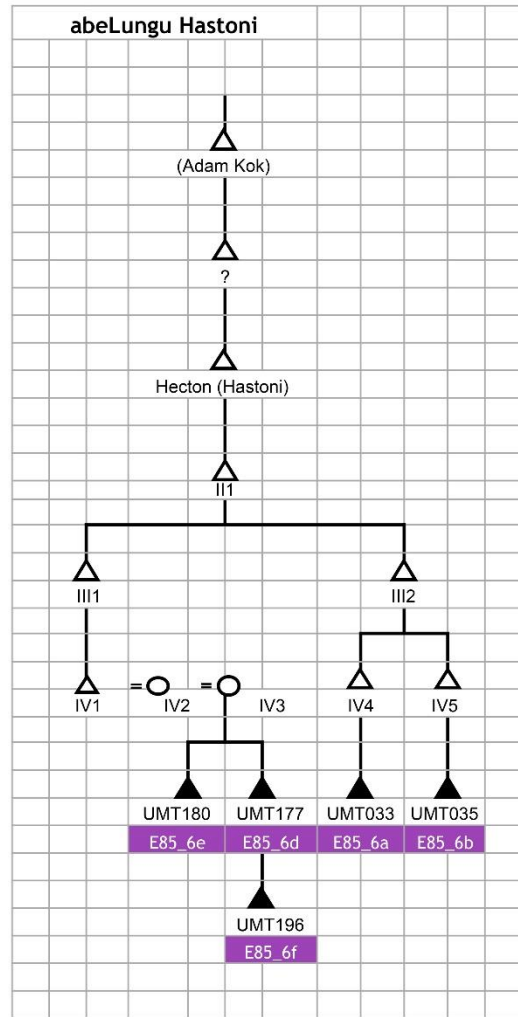
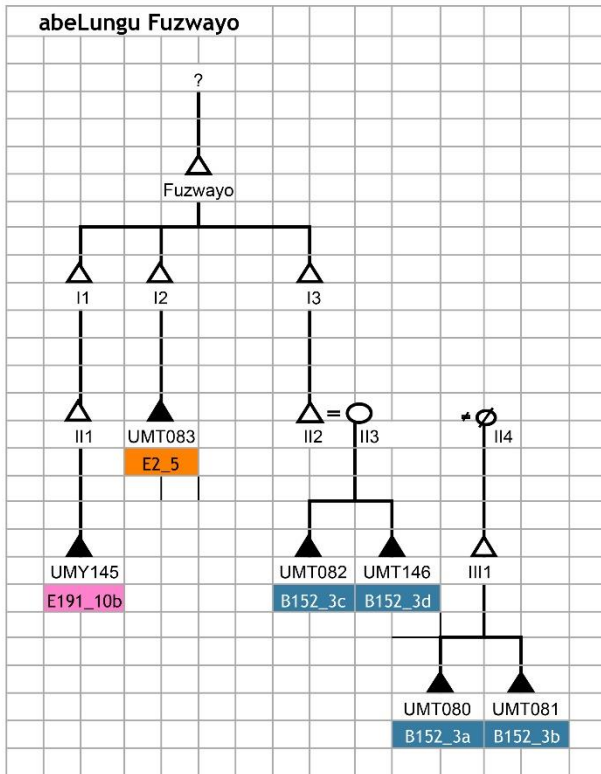


Figure S5 - The abeLungu Ogle, Irish, France, Buku and Thakha clan genealogies



Sukwini, where relationships are unknown:

UMT189	A51_1a
UMT190	A51_1b
UMT181	E2_8a
UMT186	E2_8b
UMT187	E2_8c
UMT182	E191_7
UMT193	E191_5a
UMT194	E191_5b
UMT188	E85_10
UMT183	R343_11
UMT184	R343_12
UMT185	R343_10

Figure S6 - The abeLungu Fuzwayo, Hastoni and Sukwini clan genealogies featuring African haplotypes

Appendix D: Variant sites of unique mtDNA haplotypes

Table S2

HG	HT	HT freq.	HVR1 Variant Sites	HVR2 Variant Sites
L0a1b	1a	2	16129A, 16148T, 16168T, 16172C, 16187T, 16188G, 16189C, 16223T, 16230G, 16278T, 16293G, 16311C, 16320T	73G, 146C, 152C, 195C, 247A
	1b	1	16129A, 16148T, 16168T, 16172C, 16187T, 16188G, 16189C, 16223T, 16230G, 16278T, 16293G, 16311C, 16320T	73G, 146C, 152C, 195C, 263G
	1c	1	16129A, 16148T, 16168T, 16172C, 16187T, 16188G, 16189C, 16223T, 16230G, 16278T, 16293G, 16311C, 16320T	73G, 150T, 185A, 189G, 263G
	1d	1	16129A, 16148T, 16168T, 16172C, 16187T, 16188G, 16189C, 16223T, 16230G, 16278T, 16293G, 16311C, 16320T	73G, 185A, 236C, 247A, 263G
	1e	1	16129A, 16148T, 16168T, 16172C, 16187T, 16188G, 16189C, 16223T, 16230G, 16278T, 16293G, 16311C, 16320T	73G, 95C, 189G, 198T, 247A, 263G
	1f	1	16129A, 16148T, 16168T, 16172C, 16187T, 16188G, 16189C, 16223T, 16230G, 16278T, 16293G, 16311C, 16320T	89C, 93G, 95C, 185A, 189G, 236C, 247A, 263G
	1g	2	16129A, 16148T, 16168T, 16172C, 16187T, 16188G, 16189C, 16223T, 16230G, 16278T, 16293G, 16311C, 16320T	93G, 95C, 185A, 189G, 236C, 247A, 263G
	1h	1	16129A, 16148T, 16168T, 16172C, 16187T, 16188G, 16189C, 16223T, 16230G, 16278T, 16293G, 16311C, 16320T, 16519C	93G, 95C, 185A, 189G, 236C, 247A, 263G
	1i	1	16129A, 16148T, 16168T, 16172C, 16188G, 16189C, 16223T, 16230G, 16278T, 16293G, 16311C, 16320T	93G, 95C, 185A, 189G, 236C, 247A, 263G
	L0a2a2	2a	2	16129A, 16148T, 16169T, 16172C, 16187T, 16188A, 16189C, 16223T, 16230G, 16261T, 16278T, 16311C, 16320T, 16519C
2b		1	16148T, 16172C, 16187T, 16188G, 16189C, 16223T, 16230G, 16311C, 16320T	93G, 152C, 189G, 204C, 207A, 236C, 247A, 263G

	2c	2	16148T, 16172C, 16187T, 16188G, 16189C, 16223T, 16230G, 16311C, 16320T, 16519C	64T, 93G, 152C, 189G, 204C, 207A, 236C, 247A, 263G
	2d	1	16148T, 16172C, 16187T, 16188G, 16189C, 16223T, 16230G, 16311C, 16320T, 16519C	73G, 150T, 185A, 189G, 195C, 263G
	2e	1	16148T, 16172C, 16187T, 16188G, 16189C, 16223T, 16230G, 16311C, 16320T, 16519C	73G, 152C, 195C, 263G
	2f	1	16148T, 16172C, 16187T, 16188G, 16189C, 16223T, 16230G, 16311C, 16320T, 16519C	73G, 93G, 146C, 150T, 152C, 182T, 183G, 195C, 198T, 263G, 325T
	2g	1	16148T, 16172C, 16187T, 16188G, 16189C, 16223T, 16230G, 16311C, 16320T, 16519C	73G, 93G, 152C, 189G, 204C, 207A, 236C, 247A, 263G
L0d1a	3a	1	16129A, 16187T, 16189C, 16209C, 16230G, 16234T, 16243C, 16266A, 16311C, 16519C	73G, 146C, 153G, 199C, 247A
	3b	1	16129A, 16187T, 16189C, 16230G, 16234T, 16243C, 16266A, 16284G, 16311C, 16519C	73G, 146C, 152C, 199C, 247A, 310C, 311T
	3c	1	16129A, 16187T, 16189C, 16230G, 16234T, 16243C, 16266A, 16284G, 16311C, 16519C	73G, 150T, 185A, 189G, 263G
	3d	1	16129A, 16187T, 16189C, 16230G, 16234T, 16243C, 16266A, 16311C, 16464C, 16519C	73G, 146C, 195C, 199C, 247A
	3e	2	16129A, 16187T, 16189C, 16230G, 16234T, 16243C, 16266A, 16311C, 16519C	73G, 146C, 152C, 199C, 247A
	3f	3	16129A, 16187T, 16189C, 16230G, 16234T, 16243C, 16266A, 16311C, 16519C	73G, 146C, 195C, 199C, 247A
	3g	1	16129A, 16187T, 16189C, 16230G, 16234T, 16243C, 16266A, 16311C, 16519C	73G, 146C, 195C, 199C, 247A, 318C
	3h	1	16129A, 16187T, 16189C, 16230G, 16234T, 16243C, 16266A, 16311C, 16519C	73G, 150T, 195C, 263G
	3i	1	16129A, 16187T, 16189C, 16230G, 16234T, 16243C, 16266A, 16311C, 16519C	73G, 185A, 236C, 247A, 263G
	3j	1	16129A, 16187T, 16189C, 16230G, 16234T, 16243C, 16266A, 16311C, 16519C	93G, 146C, 150T, 185A, 189G, 195C, 204C, 207A, 236C, 247A, 263G
	3k	1	16129A, 16187T, 16189C, 16230G, 16234T, 16243C, 16266T, 16311C, 16318T, 16519C	73G, 146C, 195C, 199C, 247A
	3l	1	16187T, 16189C, 16223T, 16230G, 16234T, 16243C, 16249C, 16311C, 16519C	73G, 146C, 152C, 195C, 247A

L0d1b	4a	1	16129A, 16187T, 16189C, 16218T, 16223T, 16227G, 16239T, 16243C, 16294T, 16311C, 16478G, 16519C	73G, 146C, 152C, 195C, 247A
	4b	2	16129A, 16187T, 16189C, 16218T, 16223T, 16239T, 16243C, 16294T, 16311C, 16519C	73G, 146C, 152C, 195C, 247A
L0d2a	5a	1	16093C, 16129A, 16187T, 16189C, 16212G, 16223T, 16230G, 16243C, 16311C, 16390A, 16519C	73G, 146C, 152C, 195C, 198T, 247A
	5b	1	16129A, 16145A, 16187T, 16189C, 16212G, 16223T, 16230G, 16243C, 16311C, 16390A, 16519C, 16524G	73G, 146C, 152C, 195C, 198T, 247A
	5c	1	16129A, 16154G, 16187T, 16189C, 16212G, 16223T, 16230G, 16243C, 16311C, 16390A, 16519C	73G, 146C, 152C, 195C, 198T, 247A
	5d	1	16129A, 16179T, 16187T, 16189C, 16212G, 16223T, 16230G, 16243C, 16311C, 16390A, 16519C	73G, 146C, 152C, 195C, 198T, 247A
	5e	4	16129A, 16187T, 16188G, 16189A, 16212G, 16223T, 16230G, 16243C, 16311C, 16390A, 16519C	73G, 146C, 152C, 195C, 198T, 247A
	5f	1	16129A, 16187T, 16189C, 16212G, 16221T, 16223T, 16230G, 16243C, 16311C, 16390A, 16519C	73G, 146C, 195C, 199C, 247A
	5g	2	16129A, 16187T, 16189C, 16212G, 16223T, 16230G, 16243C, 16311C, 16320T, 16390A, 16519C	73G, 146C, 152C, 195C, 198T, 247A
	5h	1	16129A, 16187T, 16189C, 16212G, 16223T, 16230G, 16243C, 16311C, 16390A, 16519C	185A, 189G, 236C, 247A, 263G, 324G, 348G
	5i	1	16129A, 16187T, 16189C, 16212G, 16223T, 16230G, 16243C, 16311C, 16390A, 16519C	73G, 146C, 152C, 195C, 198T, 227G, 247A
	5j	11	16129A, 16187T, 16189C, 16212G, 16223T, 16230G, 16243C, 16311C, 16390A, 16519C	73G, 146C, 152C, 195C, 198T, 247A
	5k	1	16129A, 16187T, 16189C, 16212G, 16223T, 16230G, 16243C, 16311C, 16390A, 16519C	73G, 146C, 152C, 199C, 247A, 310C, 311T
5l	1	16129A, 16187T, 16189C, 16212G, 16223T, 16230G, 16243C, 16311C, 16390A, 16519C	73G, 146C, 153G, 199C, 247A	

5m	1	16129A, 16187T, 16189C, 16212G, 16223T, 16230G, 16243C, 16311C, 16390A, 16519C	73G, 150T, 185A, 189G, 263G	
5n	1	16129A, 16187T, 16189C, 16212G, 16223T, 16230G, 16243C, 16311C, 16390A, 16519C	73G, 150T, 195C, 263G	
5o	1	16129A, 16187T, 16189C, 16212G, 16223T, 16230G, 16243C, 16311C, 16390A, 16519C	73G, 93G, 146C, 152C, 195C, 236C, 247A, 263G	
5p	1	16129A, 16187T, 16189C, 16212G, 16223T, 16230G, 16243C, 16311C, 16390A, 16519C, 16549G	73G, 146C, 152C, 195C, 198T, 247A	
5q	1	16129A, 16187T, 16189C, 16223T, 16230G, 16239T, 16243C, 16294T, 16311C, 16519C	73G, 146C, 152C, 195C, 198T, 247A	
5r	1	16129A, 16187T, 16189C, 16223T, 16230G, 16239T, 16243C, 16294T, 16311C, 16519C	73G, 146C, 152C, 195C, 247A	
5s	1	16129A, 16187T, 16189C, 16223T, 16230G, 16239T, 16243C, 16294T, 16311C, 16519C	73G, 150T, 195C, 263G	
5t	1	16129A, 16187T, 16189C, 16223T, 16239T, 16243C, 16294T, 16311C, 16519C	73G, 146C, 152C, 195C, 247A	
L0d2b	6a	2	16069T, 16126C, 16129A, 16169T, 16182C, 16183C, 16189C, 16212G, 16223T, 16230G, 16243C, 16258C, 16291T, 16311C, 16519C	73G, 146C, 195C, 247A, 265C
L0d2c	7a	1	16129A, 16187T, 16189C, 16223T, 16230G, 16243C, 16311C, 16519C	73G, 146C, 195C, 247A, 294A
L0d3	8a	1	16187T, 16189C, 16223T, 16230G, 16243C, 16256T, 16274A, 16278T, 16290T, 16300G, 16311C, 16362C, 16519C	73G, 146C, 150T, 195C, 247A
	8b	1	16187T, 16189C, 16223T, 16230G, 16243C, 16256T, 16274A, 16278T, 16290T, 16300G, 16311C, 16519C	73G, 146C, 150T, 195C, 247A, 316A
L0f1	9a	1	16129A, 16169T, 16172C, 16174T, 16182C, 16183C, 16189C, 16223T, 16230G, 16278T, 16311C, 16327T, 16368C, 16519C	93G, 151T, 152C, 189G, 207A, 247A, 263G
L1c1d	10a	1	16038G, 16187T, 16189C, 16223T, 16278T, 16293G, 16294T, 16311C, 16360T, 16519C	73G, 151T, 152C, 182T, 186A, 189C, 195C, 198T, 247A, 263G, 297G, 316A
L1c3	11a	1	16129A, 16182C, 16183C, 16189C, 16215G, 16223T, 16278T, 16294T, 16311C, 16360T, 16519C	73G, 152C, 182T, 186A, 189C, 247A, 263G, 316A

	11b	1	16129A, 16183C, 16189C, 16209C, 16215G, 16223T, 16278T, 16294T, 16311C, 16360T, 16519C	73G, 150T, 189G, 263G
L2a1a	12a	1	16092C, 16223T, 16278T, 16286T, 16294T, 16309G, 16390A, 16519C	73G, 146C, 152C, 195C, 198T, 247A
	12b	3	16092C, 16223T, 16278T, 16286T, 16294T, 16309G, 16390A, 16519C	73G, 146C, 152C, 195C, 263G
	12c	1	16092C, 16223T, 16278T, 16286T, 16294T, 16309G, 16390A, 16519C	73G, 150T, 189G, 200G, 263G
	12d	1	16223T, 16278T, 16286T, 16294T, 16309G, 16390A, 16519C	73G, 146C, 152C, 195C, 198T, 247A
	12e	1	16223T, 16278T, 16286T, 16294T, 16309G, 16390A, 16519C	73G, 146C, 152C, 195C, 263G
L2a1b	13a	1	16051G, 16182C, 16183C, 16189C, 16192T, 16223T, 16278T, 16290T, 16294T, 16309G, 16390A	73G, 146C, 152C, 195C, 263G
	13b	1	16051G, 16182C, 16183C, 16189C, 16223T, 16278T, 16290T, 16294T, 16309G, 16390A	73G, 146C, 152C, 195C, 263G
	13c	1	16182C, 16183C, 16189C, 16194C, 16195A, 16223T, 16278T, 16290T, 16294T, 16309G, 16390A	73G, 146C, 152C, 195C, 263G
	13d	1	16182C, 16183C, 16189C, 16223T, 16278T, 16290T, 16294T, 16309G, 16380T, 16390A	73G, 146C, 150T, 152C, 182T, 183G, 195C, 198T, 263G, 325T
	13e	1	16182C, 16183C, 16189C, 16223T, 16278T, 16290T, 16294T, 16309G, 16380T, 16390A	73G, 146C, 152C, 195C, 263G
	13f	4	16182C, 16183C, 16189C, 16223T, 16278T, 16290T, 16294T, 16309G, 16390A	73G, 146C, 152C, 195C, 263G
	13g	1	16182C, 16183C, 16189C, 16223T, 16278T, 16290T, 16294T, 16309G, 16390A	73G, 150T, 152C, 185A, 189G, 263G
	13h	1	16182C, 16183C, 16189C, 16223T, 16278T, 16290T, 16294T, 16309G, 16390A	73G, 150T, 185A, 189G, 195C, 263G
	13i	1	16182C, 16183C, 16189C, 16223T, 16278T, 16290T, 16294T, 16309G, 16390A	73G, 150T, 185A, 189G, 263G
	13j	1	16182C, 16183C, 16189C, 16223T, 16278T, 16290T, 16294T, 16309G, 16390A	73G, 93G, 146C, 152C, 195C, 236C, 247A, 263G

	13j	1	16182C, 16183C, 16189C, 16223T, 16278T, 16290T, 16294T, 16309G, 16390A	73G, 93G, 146C, 152C, 195C, 263G
	13k	1	16182C, 16183C, 16189C, 16223T, 16278T, 16290T, 16294T, 16390A	73G, 146C, 152C, 195C, 263G
	13l	1	16189C, 16223T, 16278T, 16294T, 16309G, 16390A, 16519C	73G, 146C, 152C, 195C, 263G
	13m	1	16189C, 16223T, 16278T, 16294T, 16309G, 16390A, 16519C	93G, 152C, 189G, 204C, 207A, 236C, 247A, 263G
L2c2	14a	1	16223T, 16264T, 16265G, 16278T, 16311C, 16390A	73G, 146C, 150T, 152C, 182T, 183G, 195C, 198T, 263G, 325T
	14b	1	16223T, 16264T, 16265G, 16278T, 16311C, 16390A	73G, 150T, 195C, 263G
	14c	1	16223T, 16264T, 16265G, 16278T, 16311C, 16390A	73G, 93G, 146C, 150T, 152C, 182T, 183G, 195C, 198T, 263G, 325T
	14d	1	16223T, 16264T, 16265G, 16278T, 16311C, 16390A	73G, 93G, 146C, 150T, 152C, 182T, 195C, 198T, 263G, 325T
	14e	1	16223T, 16264T, 16278T, 16311C, 16390A	73G, 93G, 146C, 150T, 152C, 182T, 183G, 195C, 198T, 263G, 325T
	14f	1	16223T, 16264T, 16278T, 16390A	73G, 93G, 146C, 150T, 152C, 182T, 195C, 198T, 263G, 325T
L2d1	15a	4	16093C, 16223T, 16278T, 16294T, 16311C, 16390A, 16399G, 16519C	73G, 143A, 146C, 152C, 182T, 195C, 263G
	15b	1	16093C, 16223T, 16278T, 16294T, 16311C, 16390A, 16399G, 16519C	73G, 146C, 152C, 195C, 198T, 247A
	15c	1	16093C, 16223T, 16278T, 16294T, 16311C, 16390A, 16399G, 16519C	73G, 146C, 152C, 195C, 247A
	15d	1	16093C, 16223T, 16278T, 16294T, 16311C, 16390A, 16399G, 16519C	73G, 146C, 152C, 195C, 263G
L3d1a	16a	1	16124C, 16223T, 16319A	73G, 146C, 152C, 195C, 198T, 247A
	16b	2	16124C, 16223T, 16319A	73G, 146C, 152C, 195C, 263G
	16c	1	16124C, 16223T, 16319A	73G, 146C, 195C, 247A, 294A

	16d	7	16124C, 16223T, 16319A	73G, 150T, 152C, 263G
	16e	1	16124C, 16223T, 16319A	73G, 150T, 185A, 189G, 263G
	16f	1	16124C, 16223T, 16319A	93G, 95C, 185A, 189G, 236C, 247A, 263G
L3d3	17a	1	16124C, 16183C, 16189C, 16223T, 16278T, 16304C, 16311C	73G, 152C, 195C, 263G
	17b	1	16124C, 16183C, 16189C, 16223T, 16278T, 16304C, 16311C, 16430C	73G, 152C, 195C, 263G
	17c	1	16124C, 16183C, 16189C, 16223T, 16278T, 16304C, 16311C, 16430C	93G, 146C, 150T, 185A, 189G, 195C, 204C, 207A, 236C, 247A, 263G
L3e1a1	18a	2	16185T, 16223T, 16265G, 16311C, 16327T, 16519C	73G, 150T, 189G, 200G, 263G
	18b	1	16185T, 16223T, 16311C, 16327T	73G, 150T, 185A, 189G, 200G, 263G
	18c	1	16185T, 16223T, 16311C, 16327T, 16519C	73G, 146C, 152C, 195C, 263G
	18d	2	16185T, 16223T, 16311C, 16327T, 16519C	73G, 150T, 185A, 189G, 263G
	18e	1	16185T, 16223T, 16311C, 16327T, 16519C	73G, 150T, 189G, 200G, 263G
	18f	1	16185T, 16223T, 16311C, 16327T, 16519C	73G, 150T, 189G, 263G
L3e1b	19a	1	16223T, 16239T, 16325delT	73G, 146C, 152C, 195C, 198T, 247A
	19b	1	16223T, 16239T, 16325delT	73G, 150T, 152C, 185A, 189G, 263G
	19c	2	16223T, 16239T, 16325delT	73G, 150T, 185A, 189G, 195C, 263G
	19d	5	16223T, 16239T, 16325delT	73G, 150T, 185A, 189G, 263G
	19e	1	16223T, 16239T, 16325delT	73G, 150T, 195C, 263G
	19f	2	16223T, 16239T, 16325delT, 16519C	73G, 150T, 185A, 189G, 263G
	19g	3	16223T, 16325delT, 16327T	73G, 150T, 185A, 189G, 263G
L3e2b	20a	1	16070C, 16172C, 16183C, 16189C, 16223T, 16320T, 16519C	73G, 150T, 195C, 263G

	20b	1	16172C, 16182C, 16183C, 16189C, 16223T, 16320T, 16519C	73G, 150T, 195C, 263G
	20c	1	16172C, 16183C, 16189C, 16223T, 16320T, 16519C	73G, 146C, 152C, 195C, 244G, 263G, 340T
	20d	12	16172C, 16183C, 16189C, 16223T, 16320T, 16519C	73G, 150T, 152C, 263G
	20e	1	16172C, 16183C, 16189C, 16223T, 16320T, 16519C	73G, 152C, 182T, 186A, 189C, 247A, 263G, 316A
	20f	1	16172C, 16183C, 16189C, 16223T, 16320T, 16519C	73G, 95C, 189G, 198T, 247A, 263G
L3e3	21a	1	16223T, 16265T, 16519C	73G, 150T, 195C, 263G
L4b2	22a	1	16051G, 16114T, 16189C, 16207G, 16223T, 16293T, 16311C, 16316G, 16355T, 16362C, 16399G, 16519C	73G, 146C, 152C, 195C, 244G, 263G

Appendix E: Comparative data sources

Table S3

Publication	Sample size	Geographical Populations	Extended haplotype frequencies	Number of Y-STRs markers in each haplotype
Roewer 2008	396	Archangelskaja	J (M172) - 16	17
		Brianskaja	I (M170) - 117	
		Iwanovskaja	G (M201) - 10	
		Lipezkaja	R1a1a - 253	
		Nowgorodskaja		
		Orlovskaja		
		Penzenskaja		
		Smolenskaja		
		Tambovskaja		
		Tverskaja		
		Vologodskaja		
Jarve 2009	86	Khakassia	Q (M242) - 4	17
		Tajikistan	R1a1a (M198) - 82	
		Tajikistan		
		Turkmenistan		
		Estonia		
		Slovakia		
		Ukraine		
		Caucasus		
		Iran		
		Turkmenistan		
		Tajikistan		
		Altay		
		Tuva		
		Punjab		
		Gujarat		
West Bengal				
Sri Lanka				
Varzari 2013	43	Moldovians	J2 (M172) - 6 Q (M242) - 1 R1a1a (M198) - 36	13
Cadenas 2008	16	UAE	R1a1a (M198) - 16	17
		Qatar		
Capelli 2005	14	Italy	R1a1a (M198) - 14	6
		Cyprus		
		Lebanon		
		Tunisia		
Msaidie 2010	12	Comores	R1a1a (M198) - 12	17
Nebel 2001	34	Middle East	R1a1a (M198) - 34	6
Qamar 2002	233	Pakistan	R1a1a (M198) - 233	16
Sengupta 2006	168	India	R1a1a (M198) - 168	10
		Pakistan		
Thanseem 2006	46	India	R1a1a (M198) - 46	12
Zalloua 2008	31	Lebanon	R1a1a (M198) - 31	11
		Cyprus		
		Syria		
		Palestine		
Di Gaetano 2009	13	Sicily	R1a1a (M198) - 13	10

Comparative data from In-house (HGDDRU) projects

Table S3 continued

Data from various In-house projects	Sample size	Geographical Populations	Extended haplotype frequencies	Number of Y-STRs markers in each haplotype
	405	Central African Republic 1994/1995	G (M201) - 36	17
		Madagascar	I (M170) - 59	
		Democratic Republic of Congo 1998	J2(M172) - 106	
		Maldives 2005	R1a1a (M198) - 76	
		Zanzibar 2006	R1b (M343) - 135	
		Simonstown 2007		
		Riversdale 2010		
		Colesburg, Northern Cape 2006		
		Western Cape 2008		
		University of Witwatersrand, Johannesburg		
		Uitenhage 2010		

Appendix F: Preparation of Solutions

70% Ethanol Solution

96% Ethanol Solution 729ml

*Make up to 1000ml with ddH₂O

0.5M Ethylenediamine Tetra-acetic Acid (EDTA)

Na₂EDTA.2H₂O 93.05g

dH₂O 300ml

Final volume (dH₂O) 500ml

* pH adjusted to 8.0 with 10M NaOH and then autoclaved

1 X TE buffer

10 ml 1 M Tris-HCl pH8

2 ml 0.5 M EDTA

Make up to 1000ml with dH₂O and autoclave

10 X TBE buffer

108 g Tris

55 g Boric acid

7.44 g EDTA

Make up to 1000ml with dH₂O and autoclave

1 X TBE (1:10 dilution)

40 ml 10 X TBE

Make up to 200ml with ddH₂O

10M NaOH

NaOH pellets 4g

dH₂O 10ml

2% Agarose Gel

Agarose 0.5g

1 x TBE	50ml
EtBr	0.5µl

Ficoll loading Dye

Sucrose	50%
EDTA	50mM
Bromophenol blue	0.10%
Ficoll	10%

1 M Tris-HCl

121.1 g Tris
1 L dH₂O
Autoclave

1 M MgCl₂

101.66 g MgCl₂
500 ml dH₂O
Autoclave

Proteinase K (10 mg/ml)

100 mg Proteinase K stock (100 mg/ml)*
10 ml ddH₂O

*Available from Roche Diagnostics

Proteinase-K mix

For 16 extractions:

400µl 10% SDS

16µl 0.5 M EDTA

2.8 ml autoclaved dH₂O

Add 800 µl Proteinase K (10 mg/ml stock) just before use

Saturated NaCl

100 ml autoclaved dH₂O

Slowly add 40 g NaCl until absolutely saturated (some NaCl will precipitate out)

Before use, agitate and let NaCl precipitate out

Bromophenol blue Ficoll dye

50 ml dH₂O

50 g sucrose

1.86 g EDTA

0.1 g bromophenol blue

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10 g Ficoll

Dissolve

Adjust volume to 100 ml with dH₂O, stir overnight

pH to 8.0

Filter through Whatmann filter paper

Store at room temperature

10 mg/ml Ethidium bromide (EtBr)

Add 1 g of ethidium bromide to

100 ml of ddH₂O

Stir until completely dissolved

Store at 4°C wrapped in aluminum foil

1kb size standard

285 µl 1kb ladder (GibcoBRL)

143 µl Ficoll dye

2400 µl 1 X TE