EPIDEMIOLOGY OF AFRICAN SWINE FEVER AT THE WILDLIFE-LIVESTOCK INTERFACE OF THE GORONGOSA NATIONAL PARK, MOZAMBIQUE

By

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SUMMARY

African swine fever (ASF) is a fatal and devastating viral disease that hampers pig production in most sub-Saharan African countries. The infection causes high mortality and morbidity rates in susceptible pigs. The disease is caused by a DNA Arbovirus classified as *Asfivirus*, the exclusive member of the family *Asfarviridae*.

The natural reservoirs of ASF in Africa are argasid ticks of the genus *Ornithodoros*, mainly if not entirely those of the *Ornithodoros moubata* complex, and wild suids, primarily warthogs (*Phacochoerus africanus*) and bush pigs (*Potamochoerus spp*) in which infection is unapparent. Three different scenarios for virus circulation were found to exist in southern Africa: a sylvatic cycle among wildlife involving argasid ticks and wild suids, a domestic cycle between soft ticks and domestic pigs and a domestic pig cycle without soft tick involvement.

The overall aim of this work was to elucidate the epidemiology of the disease at the wildlife/domestic interface of Gorongosa National Park (GNP), central Mozambique, by determining the prevalence of soft ticks (*Ornithodoros spp*) found in domestic and sylvatic habitats, contacts between wild and domestic pigs and soft ticks, the presence of ASF virus (ASFV) in different hosts and to demonstrate the genetic differences of ASFV isolates using PCR, nucleotide sequencing and phylogenetic analysis. Addictionaly, the performance of currently available serological diagnostic tests in their ability to detect ASFV antibodies in an endemic area for ASF in Mozambique was determined.

PCR-based virus detection and virus isolation methods were used to detect the presence of ASFV in 1662 *Ornithodoros porcinus porcinus* and 203 of *O. p. domesticus* specimens recovered in 29 warthog burrows in the GNP and 2 pig pens from GNP Buffer Zone respectively. A total of 72.4 %, 95% CI [52.8; 92.0] of ticks from the warthog burrows and all ticks from pig pens tested positive for ASFV DNA using nested PCR. However, using primary cell culture, haemadsorbing ASFV could only be isolated from 47.4 %, 95% CI [3.4; 60.4] of these burrows and half of the domestic habitats.

Twenty nine heamadsorbing viruses isolated from soft ticks were genotyped using a combination of partial *p72*, *p30* and *p54* gene sequencing and analysis of tetrameric amino-acid tandem repeats within the *B602L* gene. The isolates in this study clustered in three different genotypes: II (together with isolates from outbreaks that had occurred in Tete province-Mozambique, Mauritius, Malawi, Madagascar and the

recent outbreaks in the Caucasus and Russia), V (consisting of isolates circulating in Mozambique and Malawi) and one newly identified genotype XXIII.

The control of ASF in endemic settings relies on reliable diagnostic tools and the application of surveillance and control measures to prevent the spread of disease. Therefore, the sensitivity and specificity of available diagnostic tests under field conditions should be estimated during the design of an efficient surveillance program. Serum samples of 273 local breed domestic pigs from small scale pig farmers of Gorongosa District were tested simultaneously with an immunoblotting test (considered the gold standard), the OIE recommended Indirect ELISA (I-ELISA), a *p30* recombinant protein detection system (*rp30* ELISA) and a commercially available ELISA based on the *p72* protein detection (Blocking *p72* ELISA). The I-ELISA had sensitivity and specificity values of 88.2%, 95% CI [77.4; 99.1] and 97.5%, 95% CI [95.5; 99.5] respectively. The sensitivity and specificity for the *rp30* ELISA was 26.5%, 95% CI [11.6; 41.3] and 96.7%, 95% CI [94.4; 98.9] and for the Blocking *p72* ELISA these values were estimated at 50%, 95% CI [33.2; 66.8] and 98.3%, 95% CI [96.7; 100] respectively. An almost perfect agreement was observed between the I-ELISA and immuno-blotting assays (kappa value of 0.84, 95% CI [0.72; 0.93]), while the comparison of these tests with the Blocking *p72* ELISA showed good agreement with kappa values of 0.51, 95% CI [0.40; 0.67] and 0.58, 95% CI [0.46; 0.73] respectively. Moderate agreement was observed when the *rp30* ELISA was compared to the I-ELISA, immuno-blotting and Blocking *p72* ELISA (kappa values of 0.28, 95% CI [0.17; 0.42] 0.29, 95% CI [0.18; 0.44] and 0.38, 95% CI [0.26; 0.55]) respectively. The highest sensitivities were obtained with the parallel combination of the I-ELISA with the Blocking *p72* ELISA (sensitivity of 94.1%, 95% CI [91.3; 96.9]) and the I-ELISA combined with the *rp30* ELISA (sensitivity of 91.3%, 95% CI [87.9; 94.6]). The combination of the Blocking *p72* ELISA and the *rp30* ELISA gave a modest sensitivity estimate of 60.8%, 95% CI [55.0; 66.6].

To assess the sero-prevalence of ASF in pig populations within the study area, 634 domestic pigs from 314 small scale farms scattered in the different villages of Gorongosa District and 12 warthogs from GNP were bled and tested by means of I-ELISA for the presence of antibodies against ASFV. The sero-prevalence to ASFV in domestic pigs was approximately 9%, 95% CI [7.0; 11.7], in contrast with 75%, 95% CI [42.8; 94.5] in warthogs inside the GNP. Approximately 33%, 95% CI [29.2; 36.9] of pigs

tested sero-positive to salivary antigens of *Ornithodoros* spp. ticks, while in warthogs the sero-prevalence was just above 77%, 95% CI [40.0; 97.2].

This work confirms the existence of an ASFV sylvatic cycle at the wildlife/livestock interfaces in the central parts of Mozambique and provides evidence that this cycle acts as a permanent source of ASFV to domestic pigs in Mozambique. The presence of a new genotype in the ticks represents a potential risk for the occurrence of outbreaks caused by previously unknown genotypes. This could potentially contribute to failure of vaccines once they are developed based on current knowledge and warrants more research on the disease in Mozambique. The results of this study will also aid in the design and implementation of appropriate control strategies by the veterinary authorities of Mozambique.

SCIENTIFIC CONTRIBUTIONS EMANATING DIRECTLY FROM THIS WORK

Published articles in the international scientific journals derived from this work

1. **Quembo, C.J., Jori, F., Heath, L., Perez-Sánchez, R., Vosloo, W., 2014.** Investigation into the epidemiology of African swine fever virus at the wildlife-domestic interface of the Gorongosa National Park, Central Mozambique. Transboundary and Emerging Diseases. doi:10.1111/tbed.12289.

2. **Cubillos, C., Gómez-Sebastian, S., Moreno, N., Mulumba-Mfumu, L.K., Quembo, C. J., Heath, L., Etter, E., Jori, F., Escribano, J. M. and Blanco, E. 2013.** African swine fever virus serodiagnosis: a general review with a focus on analyses of African serum samples. Virus Research 173 : 159-167

Manuscripts presently in preparation and under review

1. **Quembo, C.J., Jori, F., Vosloo, W., Heath, L.,** Genetic characterization of African swine fever virus isolates from soft ticks found at the wildlife/livestock interface of

Gorongosa National Park, Central Mozambique. To be submitted to Emerging Infectious Diseases

2. **Quembo, C.J., Jori, F., Fostgate, G., Heath, L., Vosloo, W.** Bayesian evaluation of three different ELISAs to detect antibodies to African swine fever virus in an endemic setting in Central Mozambique

Results published in the abstracts of the proceedings presented in conferences

1. **Quembo, C. J., Vosloo, W., Jori, F., Heath, L., Pereira, C. L. and Souto, R. G. 2008.** Prevalência do virus da Peste Suína Africana em carraças do género *Ornithodoros* no Parque Nacional da Gorongosa, em Moçambique. *Proceeding of the XI International Veterinary Congress of Portuguese speaking countries in Maputo, Mozambique, pp?? (Paper in Portuguese)*

2. **Quembo, C. J., Vosloo, W., Costard, S. 2008.** Epidemiologia da Peste Suína Africana em Moçambique. *Proceeding of the XI International Veterinary Congress of Portuguese speaking countries in Maputo, Mozambique*. (Poster in Portuguese)

3. **Quembo, C.J., Vosloo, W., Heath, L., Jori, F., Pereira, C.L. 2009.** Epidemiology of African swine fever virus in soft ticks (*Ornithodoros spp.*) inhabiting warthog burrows and pig pens in Gorongosa District, Central Mozambique. *Proceeding of the I Veterinary post graduate student Symposium in Pretoria*, Republic of South Africa

4. **Quembo, C.J., Jori, F., Heath, L., Perez, R., Vosloo, W., 2012.** Epidemiology of African swine fever virus at the wildlife-domestic interface of Gorongosa National Park, Central Mozambique. *Proceeding of the II Veterinary post graduate student Symposium on One Health, Pretoria*, Republic of South Africa

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LIST OF ABREVIATION, SYMBOLS AND ACRONYMS

aa	Amino Acid
ARC	Agricultural Research Council
ASF	African Swine Fever
ASFV	African Swine Fever Virus
ºC	Degrees Celsius
CCR	Central Conserved Region
CD	Clustal of Differentiation – a subgroup of T cells
CI	Confidence Interval
CIRAD	Agricultural Research for Development
CSF	Classical Swine Fever
CSFV	Classical Swine Fever Virus
CTL	Cytotoxic T Lymphocytes
CVR	Central Variable Region
DAFF	Department of Agriculture Forestry and Fisheries
dsDNA	Double stranded Deoxyribonuase Nucleic Acid
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme Linked Immunosobent Assay
ESACWA	Europe, South America, the Caribbean and West Africa
EU	European Union
FAO	Food and Agriculture Organization of the United Nation
FCS	Foetal Calf serum
FTA	Flinders Technology Associates
GD	Gorongosa District
GNP	Goronogosa National Park
GNPBZ	Goronogosa National Park Buffer Zone
GPS	Global Positioning System
HAD	Haemadsorption Doses

HAD_{50}	50% Haemadsorbing Doses
ICDV	Icosahedral Cytoplasmic Deoxyribovirus
IEOP	Immuno-Electro-Osmophoresis
INFα	Interferon alfa
IL	Interleukins
IPT	Immuno-peroxidase Test
Kbp	Kilo Base Pairs
LAMP	Loop-Mediated Isothermal Amplification
LATER-PCR	Linear-After-The-Exponental Polymerase Chain Reaction
LVR	Left Variable Region
MGF	Multiple Gene Families
ml	Mililitre
mM	Milimolar
MS cells	Monkey Stable cells
n	Sample Size
NC	Negative Control
NDVS	National Directorate of Veterinary Service
NGOs	Non-Governmental Organizations
NK cells	Natural Killer cells
nm	Nanometre
NPV	Negative Predictive Value
OD	Optical Density
OIE	Office International de Epizooties/World Organization for Animal Health
OIE WAHID	OIE-World Animal Health Information Database
ORF	Open Reading Frame
OVI	Onderstepoort Veterinary Institute
p30, p54, p72	Proteins 30, 54, 72
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline

PBST	Phosphate Buffered Saline-Tween
PC	Positive Control
PCR	Polymerase Chain Reaction
pH	Unit measurement of acidity
PPV	Positive Predictive Value
RFLP	Restriction Fragment Length Polymorphism
RIA	Radio Immuno Assay
rp30	Recombinant Protein 30
rPCR	Real-time Polymerase Chain Reaction
ROD	Rest of District
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
RVL	Regional Veterinary Laboratory
RVR	Right Variable Region
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
TADP	Transboundary Animal Disease Programme
TFCA	Transfrontier Conservation Area
TIR	Terminal Inverted Repeats
TSGP	Tick Salivary Gland Proteins
UPL	Universal Probe Library
USA	United States of America
USD	United States Dollar
VP	Virus Protein
γSe	Sensitivity Covariance
%	Percentage
µl	Microlitre
µg	Microgram

CHAPTER 1

LITERATURE REVIEW

1.1. INTRODUCTION

African swine fever (ASF) is one of the most devastating viral diseases and a major limiting factor for pig production in most sub-Saharan African countries owing to its high morbidity and mortality rates (up to 100%) in susceptible pigs. This is compounded by the absence of an efficient vaccine (Penrith *et al*., 2004a). The infection is also associated with various clinical signs including haemorrhages in the skin, cyanosis, high body temperature, incoordination, high respiratory and heart rates and some pregnant sows may abort (Schlafer & Mebus, 1984; Penrith *et al.*, 2004a). Chronically infected pigs may show respiratory disorders, swelling around the leg joints as well as skin lesions (Boinas *et al*., 2004). However, the disease might change its characteristics when it becomes endemic in domestic swine, inducing considerably lower mortality (Thomson, 1985; Allaway *et al.*, 1995; Fasina *et al.*, 2010; Owolodun *et al.*, 2010a, 2010b).

ASF resembles classical swine fever (CSF) in its clinical signs and pathology. However, the latter is caused by a virus belonging to the family *Flaviviridae* resulting in different immunological and epidemiological patterns. As might be expected from diseases caused by unrelated viruses, there is no serological cross protection between CSF and ASF infections (Plowright *et al.*, 1994a).

The natural reservoirs of ASF in Africa are argasid ticks of the genus *Ornithodoros*, mainly if not entirely those of the *Ornithodoros moubata* complex and wild suids, primarily warthogs *(Phacochoerus africanus*) and bushpigs (*Potamochoerus larvatus)* in which infection is inapparent (Penrith *et al.*, 2004a).

African swine fever first appeared in Kenya in early 1921 (Montgomery, 1921) after which the disease was reported in southern African countries such as Angola in 1932 and South Africa in 1928 (reviewed in Penrith *et al.,* (2004a) and Penrith & Vosloo, (2009)).

The disease is highly infectious and considered a transboundary threat for the pig industry worldwide. In Europe, ASF was first reported in Portugal in 1957 (Wilkinson, 1989). Due the lack of efficient control, the disease spread from Portugal to Spain, France, Belgium, the Netherlands, Italy and Malta (Penrith *et al.,* 2004a). Subsequently, ASF was controlled in all those countries except in the Iberian peninsula where it became established causing outbreaks, some of these involving soft ticks, and was only eradicated 35 years later, in 1995 (Plowright *et al.,* 1994; Penrith *et al.,* 2004a). However, the disease is currently still circulating on the Italian Island of Sardinia where regular outbreaks have occurred since its first description in 1982 (Plowright *et al.,* 1994 and OIE WAHID, 2009). In 2007, ASF was identified in the Caucasus Republic of Georgia and since then, successive outbreaks have been reported in Armenia, the Russian Federation, Azerbaijan, Chechnya and Ukraine (Beltran Alcrudo *et al.*, 2008; Rowlands *et al.*, 2008; Malogolovkin *et al*., 2012; Gogin *et al.*, 2013). Additionally, the three wild boars were found positive to ASFV in Iran (Rahimi *et al.*, 2010).

Central America was threatened when the first ASF outbreak occurred in 1971 on the Caribbean Island of Cuba (Penrith *et al.*, 2004a). The pig industries in Brazil, Haiti and the Dominican Republic were also severely affected (Penrith *et al.,* 2004a) but these countries have been free from the disease since 1984 as a result of expensive control programs (Lyra, 2006).

In Mozambique, the disease was first reported in the mid 1950s based on clinical findings (Mendes, 1971). However, laboratory confirmation was obtained only during the outbreak that occurred in 1960 in Mutarara District in Tete Province (Abreu *et al.*, 1962). The disease has become endemic since then, with regular outbreaks occurring throughout the country, probably due to the movement of people and their animals as well as free ranging pigs which characterize most of the production systems in rural areas in the country; particularly in the northern and central provinces (Penrith *et al.,* 2004b, 2007; Matos *et al.,* 2011). During 1994 the disease crossed the Save River and reached the three southern provinces (Figures 1.1 and 1.2) causing massive outbreaks in commercial piggeries around Maputo City (Penrith *et al.,* 2004a*,* 2007).

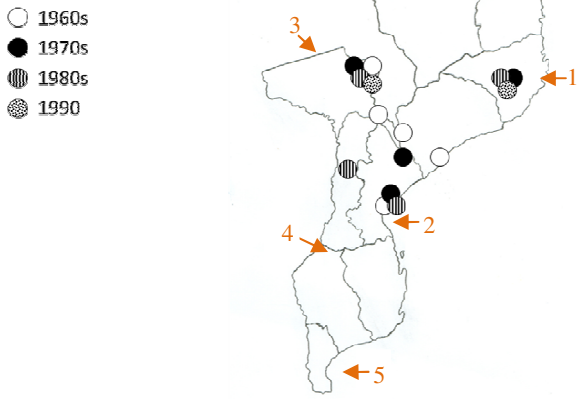

Figure 1.1.: Spatial distribution of ASF outbreaks in Mozambique between 1960 and 1993 (Penrith *et al*., 2007)
1-Nampula, 2-Beira, 3-Tete, 4-Save River
5- Maputo

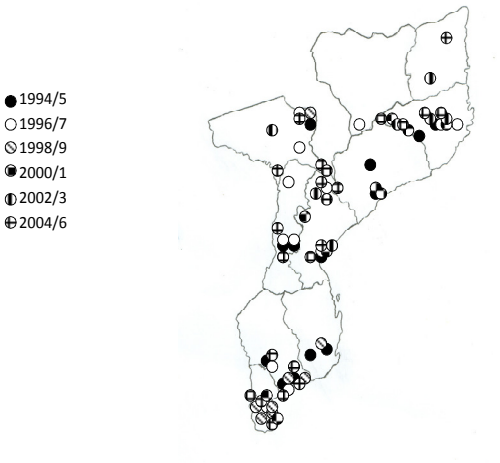

Figure 1.2.: Spatial distribution of ASF outbreaks in Mozambique from1994 to 2006 (Penrith *et al*., 2007)

1.2. AETIOLOGY AND THE BIOLOGY OF ASF VIRUS

1.2.1. Taxonomy and viral structure

African swine fever virus (ASFV) is the only double stranded DNA arbovirus (dsDNA) which now is classified as *Asfivirus* and it is the only member of the family *Asfarviridae* (Dixon *et al*., 2013). ASFV is also a unique icosahedral cytoplasmic deoxyribovirus (ICDV) (Dixon *et al.*, 2000).

The 170 to 193 kilobase pairs (kbp) long ASFV genome, with sizes varying depending on the isolates, is linear and encodes 113 proteins (Blasco *et al.*, 1989b; Yanez *et al.*, 1995; Dixon *et al.*, 2013). The genome is characterised by terminal inverted repeats (TIR) and hairpin loops at both ends. It consists of a central conserved region (CCR) of approximately 125 kbp flanked by a left variable region (LVR) and right variable region (RVR) that range from 38 to 47 kbp and 13 to 16 kbp respectively (Blasco *et al.,* 1989a).

The Central Variable Region (CVR) is a section of the 9RL open reading frame (ORF) which is characterized by variable numbers of tetrameric repeats within the ASFV genome (Irusta *et al.*, 1996; Nix *et al.*, 2006). The 9RL ORF encodes the *B602L* protein within the CVR that acts as chaperone necessary for the assembly the VP72 gene protein into ASFV virions (Cobbold *et al.*, 2001; Dixon *et al.*, 2013).

Fully developed virions are 200 nm in diameter with icosahedral symmetry. Five layers are present: a nucleoprotein core of less than 100nm in diameter, surrounded successively by a lipid inner membrane, double layers of capsomeres, an external icosahedral capsid layer of about 190 nm in diameter and a lipid envelope (Breese & Pan, 1978; Dixon *et al.*, 2000).

1.2.2. Important genes and viral proteins relevant to this study

The complete genome sequence of a Spanish ASFV isolate *BA71* revealed that between 150 to 167 ORFs are present within the ASFV genome (Yanez *et al.*, 1995; Dixon *et al.*, 2013). The 113 transcribed proteins are responsible for numerous functions such as viral assembly, replication and growth, virus host cell interaction, immune response and immune evasion mechanisms, pathogenesis and apoptosis including the mechanisms of survival and transmission to its hosts (Wardley & Wilkinson, 1980; Sanchez-Vizcaino *et al.*, 1981; Salas, 1999; Dixon *et al.*, 2013). Additionally, a large proportion (approximately 30%) of the ASFV genome encodes sets of multiple gene families (MGFs) representing multiple copies of paralogous genes varying according to each virus; probably having different functions (Yanez *et al.*, 1995; Dixon *et al.*, 2013).

The MGF 360 and MGF 530 genes are unique to ASFV; being the pig macrophage as well as soft tick range determinants (Neilan *et al*., 2002; Burrage *et al*., 2004). The wild types of these genes have been implicated in the survival of infected macrophages (Neilan *et al*., 2002). They are also required for the efficient replication in different soft ticks tissues, particularly those of the midgut, coxal gland hemolymph, salivary gland and or reproductive organs (Burrage *et al*., 2004). Therefore, they are an important mechanism of ASFV transmission from soft ticks to pigs. Delections of some copies of the MGF 360 and MGF 530 genes from the ASFV genome

lead to reduced virulence as well as reduced replication in pig macrophages and soft ticks (Afonso *et al*., 2004; Burrage *et al*., 2004). Further research revealed that gain or loss of copies of MGF genes may result in length variations in genomic DNA observed between different ASFV (Chapman *et al*., 2008). Consequently, these genes could be good candidates for describing ASFV strains isolated from ticks as well as clinical specimens.

A number of these proteins have been used for diagnostics and trial vaccine development. The capsid protein, VP72, encoded by *p72* gene, makes up approximately 32% of the total protein mass of the virion. The gene was initially mapped and sequenced by Lopez-Otin *et al.,* (1990) using a Spanish isolate, BA71V, adapted to grow in Vero cells. VP72 is antigenetically stable, contains neutralizing sites and has been used for diagnosis and molecular studies (Lopez-Otin *et al.*, 1990; Bastos *et al.*, 2003; Penrith *et al.*, 2004a; Lubisi *et al.*, 2005). The VP72 is highly immunogenic and plays an important role during natural infection with ASFV (Lopez-Otin *et al.*, 1990; Penrith *et al.*, 2004a).

An open reading frame (ORF), named *CP204L* encoding the VP30 protein, was found in the *EcoR*I C fragment on the sequenced genome of BA71V ASFV (Prados *et al.*, 1993). The *p30* fragment encodes for a protein of 204 amino acids rich in phosphorus with serine residues located in the 115 N-terminal end of the protein (Prados *et al.,* 1993). The protein is the most abundant and immunogenic viral protein, synthesized at the early stage of ASFV infection and it is considered an important tool for eliciting an effective immune response to the disease (Gomez-Puertas *et al.*, 1996; Gomez-Puertas *et al.*, 1998).

The VP54 protein is encoded by the *E183L* gene and contains a trans-membrane domain. The protein can be found in the early phase of infection (Rodriguez *et al.,* 2004), is highly antigenic and induces apoptosis in infected cells (Ovideo *et al.*, 1997; Bruno *et al.*, 2004).

The VP12 protein is synthesized during the early course of the disease, in conjunction with VP24 and VP54 and has been implicated in the mechanism of ASF virus attachment to cell membrane of the infected host cell (Gomez-Puertas *et al.*, 1996; Salas, 1999).

1.2.3. Virus replication

African swine fever virus replicates *in vivo* and *in vitro* primarily in the cytoplasm of infected antigen-presenting cells such as monocytes and macrophages of domestic and wild suids (Penrith *et al.*, 2004a; Dixon *et al.*, 2013). The virus has been shown to also replicate in other cell types namely glomerular cells, endothelial cells, renal collecting duct epithelial cells, hepatocytes, netrophils and megacaryocytes (Gómez-Villamandos *et al.*, 1995a, 1995b; Gomez-Puertas *et al.*, 1996). However, infection of these cell types is limited and it probably contributes to haemorrhages if the virus is released into the blood stream (Gomez-Villamandos *et al.*, 1997).

The replication cycle begins when the virus enters the cells by receptor mediated endocytosis. The loose fitting outer membrane of the host cell is lost and the virus is internalized in endosomes (Alcami *et al.*, 1989; Bernardes *et al.*, 1995). The viral particle entry and binding to the host cell was demonstrated in experimentally infected Vero cells (Valdeira *et al.*, 1998) and these events are carried out by early synthesis of the ASFV proteins VP12, VP30, VP54 and VP72 that are responsible for attachment and internalization (Borca *et al.*, 1994; Gomez-Puertas *et al.*, 1996, 1998; Rodriguez *et al.*, 2004).

A low pH is probably the optimal condition leading to the fusion between the viral enveloped membrane and the previously formed endosomal vesicle allowing the release of virus cores into the cytoplasm of the infected cell (Valdeira *et al.*, 1998). Early mRNA synthesis begins in the nucleus using enzymes and factors packaged in the virus core and early virus encoded enzymes required for viral DNA replication. This takes place in the nucleus as early as six hours post infection, then continues in the cytoplasm (Salas, 1999; Dixon *et al.*, 2004, 2013). During this period, head to head concatameric forms of the virus are found in the cells (Dixon *et al.*, 2013). The role of the early nuclear phase of DNA replication remains unclear. However, the available data indicate that virus replication does not occur in enucleated cells (Gomez-Villamandos *et al.*, 1997; Dixon *et al.*, 2013).

The microtubule network is required for formation of peri-nuclear factory areas (Dixon *et al.*, 2013). Following the onset of DNA replication in the factories, a shift occurs in the pattern of

gene transcription to late virus genes and virus assembly (Dixon *et al.*, 2004, 2013). These encode structural proteins and enzymes and factors which are packaged into virus particles for use during the next round of infection in other cells (Dixon *et al.*, 2013).

Replication of the full length ASFV genome occurs in the cytoplamic factory areas via head to head concatemers (Gomez-Villamandos *et al.*, 1997; Salas, 1999; Dixon *et al.*, 2013). Mature virions move on microtubules to the plasma membrane using the conventional kinesin motor. Virus particles bud through the plasma membrane gaining a loose fitting external envelope (Dixon *et al.*, 2013).

1.3. PATHOGENESIS AND PATHOLOGY OF ASF VIRUS INFECTION

The pathological effects of the disease are not the same in domestic and wild suids. For instance, warthogs and bushpigs show no clinical signs when challenged with highly virulent ASFVs compared to domestic pigs (Oura *et al.*, 1998). Adult warthogs develop lower levels of viraemia than young ones probably because they are less exposed to infected soft ticks compared to neonate warthogs (Jori & Bastos, 2009; Jori *et al.,* 2013), although both may harbour persistent virus in their lymphatic tissues (Plowright *et al.,* 1994). On the other hand, the pathology of the disease is more severe in domestic pigs with pronounced clinical signs followed by high mortality rates in all susceptible animals (Penrith *et al.*, 2004a).

1.3.1. Clinical signs, pathogenesis and pathology of the infection

1.3.1.1. General clinical signs

The skin, snout and oral cavity are the main routes of infection in domestic pigs. Pigs can become infected when exposed to infected animals, contaminated food or fomites and when bitten by infected soft ticks (Plowright *et al.*, 1969). The incubation period of the disease varies

between 5 to 15 days depending on the strain of the virus and the intensity of the exposure of the susceptible pig (Penrith *et al.*, 2004a). During this period, pigs may shed large amounts of virus into the environment; it is an important hazard for disease transmission because shedding could occur 48 hours before the onset of clinical signs (Penrith & Vosloo, 2009).

In a pig newly infected by a virulent strain via the oro-nasal route, the virus is initially detected in the pharyngeal tonsils and mandibular lymph nodes, as it first replicates in the reticulum-endothelial cells, monocytes and macrophages located in these tissues, mainly in the mandibular lymph nodes. ASFV shows a predilection for the antigen presenting cells of the macrophage-monocyte system (Boulanger *et al.*, 1967a; Greig, 1972a). After that, the virus begins its haematogenous dissemination, reaching different tissues and organs such as the visceral lymph nodes, spleen and kidney where substantial damage occurs. Some of the damage results in severe haemorrhages due to impairment of the endothelial cell function (Wilkinson & Wardley, 1978; Wilkinson, 1989). Oura and co-workers, (1998) and Carrasco *et al.,* (1996) demonstrated the occurrence of apoptosis of lymphocytes in bushpigs and domestic pigs experimentally infected with a highly virulent Malawian isolate. These changes could be responsible for leucopenia and lymph node lesions normally observed a few days after exposure to the virus (Carrasco *et al.*, 1996).

1.3.1.2. Acute and hyperacute forms

The acute form of the disease in susceptible domestic pigs is characterised by high mortality rate (usually 90 to 100%) and high body temperature which may reach 42° C within two days. Changes in the colour of the skin of the tail, ears, abdomen and limbs due to cyanosis, petechial haemorrhages and congestion occur (Penrith *et al.*, 2004a). Animals also show high cardiac and respiratory rates, blood-stained diarrhoea and nasal discharges, lung oedema and death (Penrith *et al.*, 2004a). Abortions and foetal lesions have been observed in experimentally infected and pregnant sows (Schlafer & Mebus, 1984, 1987), probably due to high fever and can be confused with other diseases, particularly in regions where ASF is endemic, if other reproductive infections agents or even if less virulent ASFV virus infections are present.

The hyperacute form of the infection in domestic pigs is characterized by superficial respiratory rate, profuse, frothy or watery and bloody nasal discharge, lung oedema leading to death of an animal. Addictionally, the mucopurulent ocular exudates may also be present (Penrith *et al.*, 2004a).

1.3.1.3. Subacute and chronic forms

These forms often happen when the disease lasts three to four weeks; it may appear in domestic pigs that survive outbreaks or in endemic situations (reviewed by Penrith *et al.*, 2004a). Infected animals may exhibit enlargement (due to hyperplasia) of lymph nodes, interstitial and fibrinous pneumonia, skin ulcers, arthritis, severe emaciation, growth retardation of piglets, intermittent fever and finally death (Wilkinson, 1989; Plowright *et al.*, 1994; Penrith *et al.*, 2004a). During the course of the infection the animals develop antibodies to ASFV, and due to the persistence of virus for up to several weeks, may represent a potential source of infection for susceptible pigs (Wilkinson, 1989; Penrith *et al.*, 2004a).

1.3.1.4. ASFV in soft ticks

Soft ticks become infected with ASFV after taking a blood meal from infected domestic or wild suids. The virus replicates initially in the gut (particularly in phagocytic cells in the midgut epithelium) and then spreads to the salivary and coxal glands and reproductive organs of the soft ticks. Tick-to-pig transmission is through blood feeding of infected soft ticks on pigs (Greig, 1972b; Kleiboeker *et al.*, 1999). Kleiboeker and colleagues (1998) believed that ASFV is successfully transmitted from the arthropod host to pigs if there is a high titre of virus in salivary glands and coxal fluids. These data also indicated that the ASFV is well adapted to soft ticks as it can replicate in different tissues and cells of the arthropod without substantial harm and mortality to this vector. It has been demonstrated experimentally that it is important for the virus to replicate in the midgut epithelium for successful transmission to pigs (Kleiboeker *et al.*, 1998).

1.4. EPIDEMIOLOGY OF THE DISEASE

1.4.1. Transmission

Three different scenarios for virus circulation can exist independently or concomitantly: i) A sylvatic cycle among wildlife involving argasid or so-called soft ticks (*Ornithodoros spp*) and wild suids, particularly warthogs (*P. africanus*) and bushpigs (*P. larvatus*), ii) a domestic cycle between soft ticks and domestic pigs (Haresnape & Wilkinson, 1989; Kleiboeker *et al.*, 1998), and iii) a domestic pig cycle without tick involvement (Thomson, 1985; Penrith *et al.*, 2004a). The potential pathways of ASF transmission in Mozambique are indicated in the Figure 1.3 (adapted from Abreu *et al.,* 1962; Penrith *et al.,* 2007).

1.4.1.1. Sylvatic cycle, the role of *Ornithodoros* ticks and wild suids

All African wild suids, i.e. warthogs (*P. africanus*), bushpigs (*P. larvatus*) and the giant forest hog (*Hylochoerus meinertzhageni*) are able to harbour ASFV (Anderson *et al.*, 1998; Jori & Bastos, 2009). However, while the role of warthogs in East and southern Africa has been well described, the role of this species in other parts of Africa and the role of other wild African swine remains obscure (Jori & Bastos, 2009; Jori *et al*., 2013).

Vertical and horizontal transmission between warthogs is unlikely to occur (Thomson, 1985; Plowright *et al.*, 1994). Warthog piglets are born apparently free from the virus (Plowright *et al.*, 1975; Thomson, 1985) and are likely to be infected by soft ticks feeding on young warthogs which spend the first 4 - 6 weeks of their life inside the burrow (Jori & Bastos, 2009). This is followed by a complex cycle of ASF infection between warthog and soft ticks.

The ancient relation between warthogs and soft ticks (*O. moubata* complex) as well as its significance in the epidemiology of the disease has been documented in specific countries of eastern and southern Africa (Plowright, 1984; Jori & Bastos, 2009; Jori *et al.*, 2013). However, no consistent data are available regarding the occurrence of a sylvatic cycle for many other areas of Africa. This is the case for Mozambique and several countries in West or Central Africa

(Penrith *et al.*, 2004a; Jori *et al.*, 2007, 2013; Jori & Bastos, 2009) except Senegal were *Ornithodoros sonrai* has been identified in pig pens and rodent burrows (Vial *et al.*, 2006, 2007; Jori *et al.*, 2013). The active search in warthog burrows in Senegal and other West African countries failed to demonstrate the presence of *Ornithodoros* ticks (Vial *et al.*, 2007). Additionally, a few serum samples from warthogs were tested for the presence of antibodies to the salivary gland antigens of soft ticks and no antibodies were detected to ASFV (Jori *et al.*, 2013). Despite the fact that these results were not conducted from a complete and systematic sampling strategy, it seems that contact between soft ticks and warthogs is limited and the ASFV is not circulating among these wild suids.

The mechanism of ASFV transmission within the sylvatic cycle (i.e. ticks to wild suids) is complex. It has been demonstrated that viraemic neonatal warthogs may provide a source of ASFV for soft ticks and the infection rate increases with the volume of blood meal and the frequency of feeds which normally takes not more than 45 minutes (Plowright, 1984; Thomson, 1985; Vial, 2009). Furthermore, the soft ticks could maintain infectious virus and transmit the same to wild or domestic pigs (Oleaga-Perez *et al.*, 1990).

Warthog burrows are often colonized by significant quantities of soft ticks (Butler *et al.*, 1984; Butler & Gibbs, 1984; Vial *et al.*, 2006, 2007; Vial, 2009; Jori *et al.*, 2013) that are infected with ASFV (Vial *et al.*, 2007; Bastos *et al.*, 2009). The virus is maintained in successive generations of ticks by trans-ovarial, venereal (male to female ticks) and trans-stadial transmission (Plowright *et al.*, 1974; Wilkinson, 1989; Kleiboeker *et al.*, 1998; Rennie *et al.*, 2001).

Transmission from the sylvatic cycle to domestic pigs is via infected soft ticks that drop off from warthogs in the vicinity of domestic pigs where they have access to these pigs. The soft ticks may transmit the virus during the process of blood meal feeding, consequently starting a domestic pig/soft tick cycle (Plowright, 1984, 1994; Kleiboeker *et al.*, 1998; Penrith *et al.*, 2004a, 2004b; Jori & Bastos, 2009).

In contrast to experimentally infected bushpigs, adult warthogs show at most a low level of viraemia (not exceeding 10^2 HAD_{50}/ml) and thus shed little if any virus through secretions (Thomson, 1985; Oura *et al.*, 1998). Therefore, the only good source of virus for oral transmission would be the tissues, particularly superficial or visceral lymph nodes, of very young

warthogs, which can develop high levels of viraemia (Jori *et al.,* 2013). However, the young viraemic animals are usually confined to burrows and are generally not hunted (Thomson, 1985; Plowright *et al.*, 1994; Penrith *et al.*, 2004a). Therefore, in the absence of ticks, the likelihood of domestic pigs becoming infected by direct contacts with infected warthogs is limited.

Data on the transmission of ASFV from the *O. moubata* complex to the desert hog (*Phacochoerus aethiopticus*), which is only confined in a small area in the Horn of Africa (d'Huart & Grubb, 2001), is not well documented (Jori & Bastos 2009).

Studies have shown that bushpigs remain viraemic for a maximum of 90 days post infection, long enough to infect *Ornithodoros* ticks, and that these ticks are able to transmit infection to a susceptible animal during feeding (Plowright, 1984). It has also been shown experimentally that bushpigs may transmit ASF viruses to domestic pigs of local breed by close contact (Jori and Bastos 2009). For instance, bushpigs experimentally infected with LIL 20/1 virus may transmit it to pigs by close contact, but did not transmit the infection if they were infected with the VIC T90/1 virus (Anderson *et al.*, 1998).

Transmission from bushpigs to domestic pigs seems unlikely to occur in the field, as the bushpig population is relatively lower in number (Wilkinson *et al.*, 1989; Roger *et al.*, 2001). They are nocturnal, usually sedentary hence difficult to be in contact with domestic pigs and rarely encounter argasid ticks because they do not inhabit burrows, but build nests from grass (Jori *et al.*, 2007, 2013; Jori & Bastos, 2009). The presence of ticks in such habitat has never been demonstrated (Penrith *et al.*, 2004a). Furthermore, a survey conducted in bushpigs in Madagascar found no antibodies to ASFV and *O. moubata* salivary antigens and spleen tissues were negative for the presence of ASFV DNA (Ravaomanana *et al.,* 2011).

As far as viraemia and pathogenesis are concerned, ASFV infection in bushpigs results in low levels of replication, less virus spread, less tissue damage as well as apoptosis in lymphoid tissues (Anderson *et al*., 1998; Oura *et al*., 1998). Despite all these facts, bushpig carcasses also represent a potential risk for ASFV transmission to domestic pigs, as viraemia is longer than that recorded in warthogs and the virus may be present in spleen with the maximum ASFV load reaching $10^{4.9}$ HAD_{50}/ml (Jori and Bastos 2009; Jori *et al.,* 2013), sufficient to induce infection in susceptible domestic pigs (Anderson *et al*., 1998). This may possibly happen in areas where

parts of wild pig carcasses are fed to domestic pigs (Plowright *et al.*, 1969; Thomson, 1985; Jori & Bastos 2009).

It is suspected that in some parts of Africa, bushpigs may cross-breed with free-ranging domestic pigs resulting in hybrids that could become resistant to and serve as an additional reservoir host for ASFV; this situation requires further investigation (Jori & Bastos, 2009; Jori *et al.*, 2013).

Other blood sucking arthropods such as Ixodid ticks, mosquitoes, lice and mites have not been shown to transmit ASFV to domestic or wild pigs. The possible role of the blood-sucking mite (*Triagom gerstaekeri*) in transmitting the virus was investigated and it was incapable of transmitting the virus to pigs during feeding, although it was able to retain ASFV for four days (Plowright *et al.,* 1994). Only the stable fly (*Stomoxys calcitrans*) has been shown to maintain and transmit ASFV to domestic pigs for 24 – 48 hours after ingestion of infected blood (Mellor *et al.,* 1987). Full clarification on the exact role of these insects (particularly stable fly commonly found in the pig pens) in the transmission of the disease is needed.

A sero-epidemiological survey conducted in ASF endemic areas in South Africa did not find any positive sera among selected wild carnivores and herbivores (Thomson, 1985). Nevertheless, some animals such as porcupines (*Hystrix* spp) and spotted hyenas (*Crocuta crocuta*) may occupy warthog burrows and can be the source of blood meal for *Ornithodoros* ticks (Penrith *et al.*, 2004a).

1.4.1.2. Domestic pig/soft tick cycle

African swine fever virus is thought to be brought to pigs via infected ticks, since direct contact between warthogs and domestic pigs failed to result in transmission (Plowright *et al.*, 1969; Thomson, 1985; Haresnape *et al.,* 1988). However, adult warthogs may transport infected soft ticks from the burrow to the domestic pig premises or grazing areas, exposing them to the virus (Thomson *et al.*, 1983; Thomson, 1985; Jori & Bastos 2009). This represents important risk factors for disease spread in areas where the sylvatic cycle is present (Penrith *et al.*, 2004a, 2013; Penrith & Vosloo, 2009; Fasina *et al.*, 2012).

A survey carried out in Malawi, adjacent to the endemic area in Mozambique, revealed that between 0.3 and 12% of *Ornithodoros* ticks collected in the pig pens were infected with ASFV (Haresnape *et al.*, 1988). Another investigation also carried out in domestic pig pens within three villages in Malawi indicated that 24% of soft ticks were positive to ASFV shortly after an outbreak of the disease (Haresnape & Wilkinson, 1989). In Madagascar, ASFV was isolated from soft ticks found on a previously abandoned pig farm after four years (Ravaomanana *et al.*, 2010). In these circumstances, the risk of ASF infection may be reduced when tick populations become extinct following the absence of hosts over an extended period of time (Oleaga-Perez *et al.*, 1990).

1.4.1.3. Domestic cycle

A model developed by Wilkinson (1989) and later modified by Penrith *et al.,* (2004a), indicated that ASF can become established and spread in a pig population in three possible ways: The first event may occur when direct contact between infected and naïve domestic pigs occurs. The second possibility is through indirect transmission by fomites which can occur by various methods, namely transportation of pigs using vehicles that have not been well cleaned and disinfected, farm workers whose hands, footwear and clothing are contaminated by pig secretions and excretions or waste. A third possibility is through iatrogenic transmission by veterinarians or other animal health workers. A review carried out by Penrith *et al*., (2007) revealed that commercially farmed domestic pigs in Mozambique likely get ASFV infection from the infected pigs kept under free-range production system.

Once ASFV reaches naïve domestic pigs, different scenarios may take place that vary between the disease killing almost 100% of the population to some pigs surviving during outbreaks and becoming chronically ill, representing a potential source of virus to other susceptible pigs (Thomson, 1985; Gogin *et al*., 2013). Additionally, the virus may be adapted in a domestic host by becoming less virulent and enabling some pigs to survive infection and persistently spreading the virus (Oura *et al*., 2013). This was reported in outbreaks that occurred outside Africa induced by viruses that had become less virulent (Gogin *et al*., 2013; Oura *et al*., 2013).

Highly or less virulent ASFV may be excreted from the respiratory tract of live infected pigs (Wilkinson, 1989), as well as in all secretions and excretions. Dead pigs are also a potent source of contamination of pig pens and the environment (Penrith *et al.*, 2004a). It has been shown in Europe and elsewhere that the virus can also spread through slurry, commonly used as a fertilizer, and uncooked pork products (McKercher *et al.*, 1978; Penrith *et al.*, 2004a). Airborne transmission is negligible in conditions other than the intensive and close contact pig production systems (Wilkinson, 1989).

In West African countries particularly in Senegal, Cameroon and Nigeria, there is evidence of the maintenance of the disease in domestic pig populations, with low mortality rates and chronically ill pigs (Costard *et al.*, 2009a, 2013; Etter *et al.*, 2011). Nevertheless, when ASF is (re-) introduced into non-endemic areas of the region, it causes outbreaks with high morbidity and mortality rates (Costard *et al.*, 2013).

The pattern of ASF in endemic regions, where wild suids and the vectors are absent, is sometimes charactrized by less severe outbreaks (with low mortality) contrasting with high mortalities, particularly in naïve domestic pigs, when they have contact with wild reservoirs (Penrith *et al.,* 2004a, 2007). This scenario corroborate with recent data indicating that in endemic regions, mortality rates are decreasing and subclinical or chronic ASFV infections have become more frequent (Thomson, 1985; Allaway *et al.*, 1995; Fasina *et al.*, 2010; Owolodun *et al.*, 2010b).

In most of regions of central Mozambique the source of ASFV for the warthog- and tick-free areas where domestic pigs are kept still needs to be clarified. Molecular studies tracking the origin of outbreaks using virus genotyping tools revealed that viruses share similarities with those identified in neighboring countries namely Malawi, Zambia and Zimbabwe as well as those found in the Indian Ocean Islands, notably, Mauritius and Madagascar (Bastos *et al.*, 2003, 2004; Lubisi *et al.*, 2005, 2009).

Lately, some authors have also found links between Mozambican viruses and those introduced in Eastern Europe (Rowlands *et al.*, 2008).

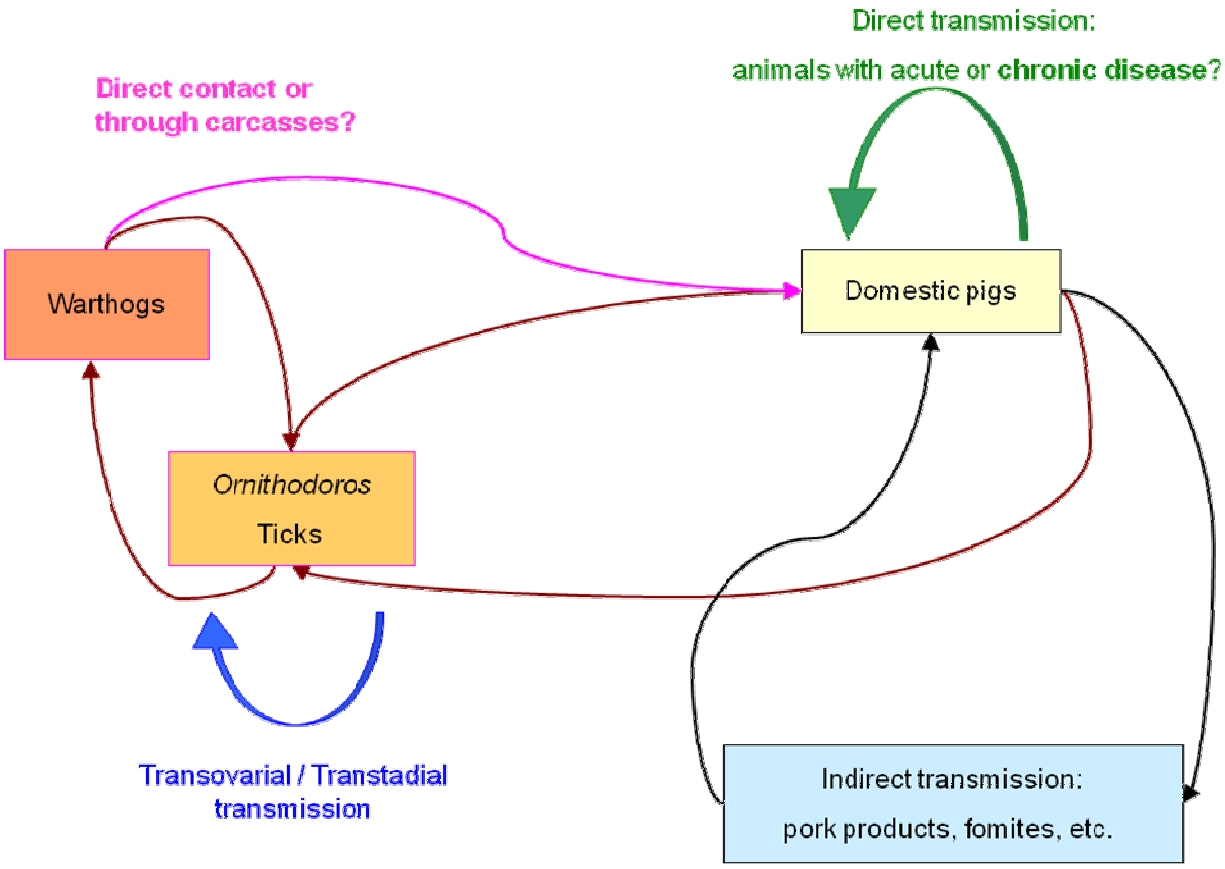

Figure 1.3.: Potential transmission pathways of ASFV in Mozambique (adapted by Costard S. and Quembo C.J. from Abreu *et al.,* (1962); Penrith *et al.,* (2007))

Many risk factors for the occurrence of ASF in countries of sub-Saharan Africa (including Mozambique) are related to the type of farming system where free-ranging pigs scavenge for food, feeding swill that could contain infected pork, compulsory slaughter of pigs to control ASF with no compensation or compensation at less than the market value and lack of control of movement of pigs and their products (McKercher *et al.*, 1978; Penrith *et al.*, 2004a, 2007). Penrith *et al.,* (2013) believed that there are significant relationships between the size of pig populations and the probability of introduction of ASFV in a certain country as there is more chance of contacts between infected pigs and non-infected pig population in larger pig populations.

1.4.1.4. The epidemiology of ASF in Mozambique

Little is known about the epidemiology of ASF in Mozambique. The involvement of a warthog–tick cycle, as it occurs in other countries in the region, was suspected in the first outbreaks (Abreu *et al.*, 1962; Thomson, 1985; Plowright *et al.*, 1994). However, up to date, its role in the occurrence and maintenance of the disease has not been confirmed. In Mozambique wildlife areas with large populations of warthogs are localised in a few national parks. Those areas offer numerous opportunities of interactions between warthogs, soft ticks and domestic pigs. Therefore, incursions of virus from a wild host origin into the domestic pig population are likely to occur. Nevertheless, factors permitting maintenance of the virus among domestic pigs are much more common and more likely to play an important role as a cause of outbreaks of ASF in Mozambique (Penrith *et al.,* 2007).

The endemic areas of Angónia, Caia and Mutatara Districts in Tete and Sofala Provinces (in Central Mozambique, Figure 1.1) as well as the Mchinje district of Malawi, adjacent to Angónia, are characterised by regular outbreaks that cause lower mortality in domestic pigs of local breed, of which a high proportion (sometimes more than 50%) may be serologically positive for antibodies to ASFV (Haresnape *et al.*, 1987; Penrith *et al.*, 2004a; Matos *et al.*, 2011). These pigs are apparently healthy, and although the presence of antibodies indicates past exposure to infection, there is some evidence that such pigs have some degree of natural resistance to the pathogenic effects of the virus, are possibly infected by less virulent strains or do not develop clinical signs of disease and may only be short term carriers (Penrith *et al.,* 2004a, 2004b).

The way in which ASF is maintained in domestic pigs in these areas has not been clarified, since, unlike in Malawi, to date association with ticks of the *O. moubata* species complex has not been documented (Penrith *et al.,* 2004a, 2004b). It appears unlikely that healthy recovered pigs act as long-term carriers of the virus, since these pigs rapidly become virologically negative and are unable to infect other, susceptible pigs (Penrith *et al.,* 2004a, 2004b). The evidence of endemicity in domestic pigs in Angónia and Gorongosa Districts, as well as the simultaneous circulation of two highly virulent viruses in 1998, suggest that these districts and other neighbouring districts may be focal points for ASF in Mozambique (Bastos *et al.*, 2004; Matos *et al.*, 2011). This is supported by the involvement of both of those viruses in subsequent outbreaks,

one of them in the provinces of Zambézia and Sofala, and the other in Nampula, Cabo Delgado, and Maputo (Penrith *et al.*, 2007).

1.4.1.5. Molecular epidemiology of ASF

Molecular epidemiological tools make it possible to study and track the origin of outbreaks and the relationships between outbreaks. Early studies on the epidemiology of ASF at molecular level were based on restriction fragment length polymorphism (RFLP) assays, where the virus genome is fragmented into various sizes by restriction enzymes and electrophoresed (Dixon & Wilkinson, 1988; Blasco *et al.*, 1989a; Weaver, 1999). Restriction fragment length polymorphism was widely used to track the origin of the ASFV occurred in the Iberian Peninsula, South America and Caribean (Blasco *et al.*, 1989a). Based on this method, different isolates of ASF virus were phylogenetically assessed and the results indicated similarities between isolates (Blasco *et al.*, 1989a). Therefore, the viruses introduced into these regions appeared to have a common origin (Wesley & Tuthil, 1984; Blasco *et al.*, 1989a, 1989b). Furthermore, it has also been demonstrated that persistent outbreaks of ASF that occurred in domestic pig populations in Cameroon were due to the re-introduction of closely related viruses isolated from infected areas outside the country (Ekue & Wilkinson, 2000). However, this method is time consuming, was found to have low level of resolution (Penrith *et al*., 2004a) and therefore not the most suitable for rapid viral epidemiological characterisation during outbreaks.

The PCR-sequencing based methods are alternatives convenient for molecular epidemiological studies of ASFV as it gives the necessary information about the source of virus within 48 hours, allow identification of the major viral genotypes linked to outbreaks of ASF and do not rely on virus isolation (Bastos *et al.*, 2003, 2004).

Four different gene regions namely: *p72*, *p30*, *p54* and B602L were targeted to detect ASFV phylogenetic relationships with different levels of precision (Irusta *et al.*, 1996; Bastos *et al.*, 2004; Nix *et al*., 2006; Gallardo *et al.*, 2009a, 2011).

The nucleotide sequences of the *p72* gene have extensively been used for genotyping ASF viruses in different regions of Africa (Bastos *et al.*, 2003; Lubisi *et al.*, 2005, 2007; Boshoff *et al.*, 2007; Owolodun *et al.*, 2010a, 2010b). Twenty two ASFV *p72* genotypes have been previously described and these viruses clustered into three distinct evolutionary lineages (Boshoff *et al.,* 2007).

The *p72* data showed that a group of genetically homogeneous viruses have been circulating in Europe, South America, the Caribbean and West Africa, also known as Europe, South America, the Caribbean and West Africa (ESACWA genotype) (Bastos *et al.,* 2003), indicating that in the absence of the sylvatic cycle less variation is observed in the recovered ASF viruses than in other parts of Africa (Gallardo *et al.*, 2009a, 2011). Furthermore, Boshoff *et al.,* (2007) demonstrated the presence of fourteen *p72* genotypes, six of them considered new and confined to the southern African region and shared between countries. The rest of the genotypes were country-specific based on viruses obtained from outbreaks in pigs between 1973 and 1999. Lubisi *et al.,* (2005) showed that in East Africa, at least 13 *p72* genotypes exist, with 10 being country specific. The previously described pig associated ESACWA genotype (Bastos *et al.,* 2003) was found in sylvatic hosts in East Africa, indicating that this genotype may have originated from there (Lubisi *et al.*, 2005).

Four different *p72* genotypes (II, V, VI, VIII) have previously been identified in ASFV isolated from domestic pigs (Bastos *et al.*, 2003, 2004; Lubisi *et al.*, 2005) in different regions of Mozambique as well as in the neighboring countries. Among these genotypes, only genotype VI seemed to be confined to Mozambique (Bastos *et al.*, 2004), whereas genotype II shared homology with isolates from outbreaks that occurred elsewhere in Mozambique (Nampula Province), Mauritius, Malawi, Madagascar (Bastos *et al.*, 2003, 2004; Lubisi *et al.*, 2005, 2009) and a recent outbreak that occurred in the Caucasus region of Georgia in 2007 (Rowlands *et al.*, 2008). The virus has spread rapidly into vast areas of Russia (Malogolovkin *et al*., 2012; Gogin *et al.* 2013) and more recently, Ukraine and Belarus (Oura, 2013). This progressive spread from east to west represents a considerable threat to European Union (EU) and Asian countries (Costard *et al.,* 2009a; Oura, 2013). Despite of broad spread of this strain, no reports of it circulating in the sylvatic cycle in the ASF endemic countries including Mozambique exist.

Genotype V formed a cluster of viruses of domestic origin in Mozambique (Beira, Maputo, Manica and Tete) as well as viruses recovered from warthogs (Tengani) and domestic pigs (Mpemba) in Malawi (Bastos *et al.*, 2004; Lubisi *et al.*, 2005). The *p72* genotype VIII was also present in the outbreaks of ASFV that occurred in domestic pigs in Tete and Zambezia Provinces in Mozambique, as well as from soft tick samples and domestic pig tissues collected in different locations in Malawi and Zambia (Lubisi *et al.*, 2005).

Although the *p72* gene is suitable to identify major ASFV genotypes, it does not allow differentiation between viruses within the same genotype with reasonable precision. Both *p30* and *p54* genes have been employed to enhance discrimination, with intermediate resolution, of ASFV isolated from soft ticks as well as clinical specimens in East Africa (Gallardo *et al.*, 2009a, 2011). Nix *et al.,* (2006) studied the molecular epidemiology of ASFV by analyzing the variable B602L gene.

A two-step approach for epidemiological studies at molecular level has been carried out by targeting the *p72* gene and a section of the 9RL open reading frame (ORF) aimed at resolving the intra-genotype relationships of the virus causing outbreaks in East and southern Africa (Lubisi *et al.,* 2007; Boshoff *et al.,* 2007). Irusta *et al.,* (1996) and Phologane *et al.,* (2005) found that most African viruses contained a high degree of CVR length polymorphism with product sizes varying between 360 to 686 bp in all genotypes. Therefore, it makes the CVR region suitable for distinguishing between different viruses that belong to the same *p72* genotype within countries or proposed study areas (Bastos *et al.,* 2004; Lubisi *et al.,* 2005, 2007; Boshoff *et al.,* 2007).

Moreover, *p54* and *p30* gene sequencing in conjunction with *p72* and CVR gene regions have proved to be a valuable method to enhance discrimination of ASFV isolates from domestic pigs in West Africa and from domestic pigs, warthogs and soft ticks in East Africa (Rowlands *et al.*, 2008; Gallardo *et al.*, 2009a, 2011). The intermediate resolution for genotyping ASFV using *p54* and *p30* in West Africa where *p72* genotype I is present is thought to be homogeneous (Gallardo *et al.*, 2009a). However, a comparative sequence analysis of full-length *p54* and *p30* genes from warthog serum samples, domestic pig tissue samples and soft ticks done Kenya (East Africa), found that ASFV was highly heterogeneous (Gallardo *et al.,* 2011). Similar results were also obtained using the *p72* gene region. The intermediate resolution of ASFV genotyping using the *p54* and *p30* genes should also be expanded to the sylvatic cycle to understand the role of this

cycle in the areas where it is present in the southern part of Africa, particularly in endemic areas of Mozambique.

The current genotyping and nomenclature of ASFV was recently reassessed by targeting the *p72*, *p30* and *p54* gene regions using both maximum likelihood and Bayesian coalescence methods that allows the construction of bifurcative phylogenetic trees and haplotype network analysis of different isolates (Michaud *et al*., 2013). This study revealed the presence of a novel lineage and precisely demonstrated the relationships of isolates within genotypes or between genotypes (Michaud *et al*., 2013). However, further studies should be conducted by combining this approach with the CVR gene region sequencing analysis.

1.5. IMMUNOLOGY

In theory the immune responses to a particular disease can occur as a response to any antigen. In general, both cellular and humoral immune mechanisms contribute to protection against viral infection and clear the virus from infected hosts (Escribano *et al.*, 2013; Takamatsu *et al.*, 2013). Studies showed, however, that not all immune responses contribute to protective immunity, and some immune responses may be responsible for pathology (Takamatsu *et al.*, 2013).

African swine fever virus generates complex immune response mechanisms in the infected host that are characterised by the antagonism between immunity and defects in the immune system (Childerstone *et al.*, 1998). The monocyte/macrophage lineages, which are preferred by ASFVs, are functionally defective as professional antigen presentation cells (Childerstone *et al.*, 1998). In addition, apoptosis of both B and T lymphocytes also impairs the immune response to ASFV infection (Oura *et al.*, 1998).

1.5.1. Humoral immunity to ASFV

Three to seven days post infection, IgM and IgG type antibodies are detected in infected domestic pigs (Wardley & Wilkinson, 1980). Antibodies and virus can be detected simultaneously throughout the course of acute infection since virus replication and dissemination occur rapidly. Early investigations have revealed that these antibodies are unable to neutralise ASFV (De Boer, 1967; De Boer *et al.*, 1969) but in contrast, research carried out in the early 1990s demonstrated neutralising activity of antibodies in the serum of pigs infected with a low virulence Spanish isolate (E75) (Zsak *et al.*, 1993) that is also considered an early event that delays disease onset (Onisk *et al.*, 1994).

Protection via passive humoral immunity was demonstrated when suckling piglets received antibodies through colostrum intake (Schlafer *et al.*, 1984). In addition, ASFV infection was successfully prevented by transferring immunoglobulins derived from pigs recovered from the infection to a susceptible domestic pig followed by infection with the homologous ASFV (Onisk *et al.*, 1994). However, the mechanism behind this event is not fully understood (Penrith *et al.*, 2004a). Available data from *in vitro* experiments indicate that there are a number of factors influencing the susceptibility of ASFV to neutralisation; the most important being the composition of phospholipids of virion membranes which varies according to different levels of passages (low and high passage) in cell culture which in turn affects the protein recognition by the host's antibodies (Escribano *et al.*, 2013).

Early observations of the humoral immune responses of infected animals revealed that the VP12, VP30, VP54, and VP72 proteins were involved in inhibition of viral attachment and internalization, which delayed the appearance of clinical signs (Gomez-Puertas *et al.*, 1996, 1998; Gomez-Puertas & Escribano, 1997). However, more recent work demonstrated that neutralising antibodies to ASFV proteins VP30, VP54 and VP72 are not sufficient to provide protection when pigs are challenged with virulent ASFV if cell-mediated immune response is not triggered (Neilan *et al.*, 2004; Escribano *et al.*, 2013). The immune response to these proteins should be further investigated in order to possibly develop a suitable vaccine.

1.5.2. Cell-mediated immunity to ASFV

Pathogenesis studies on the acute course of disease have demonstrated that ASFV targets monocyte and macrophage cells resulting in necrosis and apoptosis which leads to major defects in the immune system (Wardley & Wilkinson, 1980; Sanchez-Vizcaino *et al.*, 1981; Oura *et al.*, 1998).

Martins *et al.,* (1993) experimentally demonstrated that ASFV infection triggers cytotoxic T lymphocytes (CTL). Experiments carried out *in vitro* indicated that the measurements of T cells responses are usually more efficient when challenged with homologous virus compared to heterologous ASFV (Gonzalez *et al.*, 1990; Martins *et al.*, 1993). These cells were able to efficiently destroy infected macrophages with the virulent Lisbon 60 ASFV (L60) but unable to cause lysis of macrophages infected with the DR-II and Tengani isolates, suggesting a virus specific immune response. In another experiment, Oura *et al*., (2005) also found that pigs exposed to attenuated/avirulent OURT/88/3 isolate and depleted of $CD8^+$ T lymphocytes become unprotected from virulent OURT/88/1 challenge. This is an indication of the key role of these types of cells in the immunity to ASFV infection since antibody mediated immune response proved to be insufficient to protect pigs against ASFV infection (Escribano *et al.*, 2013; Takamatsu *et al.*, 2013).

The host is also armed with potential tools to counteract the infection and Dixon *et al.*, (2004) found that some ASFV proteins, namely CD2v and A238L, are involved in host defense system evasion, thus favoring virus replication and dissemination throughout the body. Further investigation is necessary in order to fully understand this and other possible mechanisms and pathways of immune evasion.

In general, infection with ASFV in domestic pigs leads to acute or per-acute haemorrhagic disease and death and as a consequence, there is not sufficient time to trigger an immune response (Escribano *et al.*, 2013; Takamatsu *et al.*, 2013). Yet, several reports showed that pigs can survive infection with less-virulent ASFV and become chronically infected indicating a certain degree of protective immunity which is linked to the secretion and activity of natural

killer (NK) cells and cytokines such as interferon alfa (INFα), interleukins (IL) 6 and 12 (Afonso *et al.*, 2004; Zhang *et al.*, 2006; Gil *et al.*, 2008).

1.6. DIAGNOSIS OF ASF VIRUS

African swine fever virus infection causes a wide range of unspecific syndromes and produces non-pathognomonic signs. For these reasons, it is difficult to recognise the disease in the field, particularly if other haemorrhagic syndromes such as CSF are present in the same geographic region (Wilkinson, 1989; Plowright *et al.*, 1994; Penrith *et al.*, 2004a). Therefore, field diagnosis should be confirmed in the laboratory in order to set up proper control measures.

The ASF epidemiology in a particular country or area should be taken into consideration when selecting laboratory procedures. For example, if ASF is an emerging and sporadic disease in an area or country, virus isolation and identification by genotyping are essential for tracking the origin of the virus and its genetic relationship with other ASFVs (Bastos *et al.*, 2003, 2004; Lubisi *et al.*, 2005, 2007, 2009; Boshoff *et al.*, 2007). Furthermore, in countries where the disease is endemic in domestic pig populations, sero-diagnosis can be used to estimate prevalence but it gives limited information on the risk factors involved in disease transmission. Therefore, virus isolation plus genotyping and sero-diagnosis can be combined if there are cases of ASF based on less obvious clinical signs (Wilkinson, 1989). In addition, diagnostic tools provide information on the contact between different hosts within a transmission cycle (Canals *et al.*, 1990; Penrith *et al.*, 2004a; Jori *et al.*, 2013).

Diagnostic samples for ASFV detection often require a cold chain for sample preservation up to the laboratory (Wilkinson, 1989). This represents a challenge for most countries particularly in the tropics. Since the maintainance of cold chain is not always possible, samples can be submitted in glycerol-saline, however this may decrease the likelihood of virus isolation (Oura *et al.*, 2013). Another investigation has showed that filter papers can be used for sample collection and preservation (Michaud *et al.*, 2007). Recently, the potential use of oral fluid samples from pigs has been experimentally tested (Mur *et al.*, 2013). Oral fluid diagnostic sample collection relies on a pig's instinct to explore new objects by chewing (Kittawornrat and Zimmerman,

2011). This diagnostic approach is well-suited to small scale farmers and para-veterinarians (particularly in Mozambique) because it does not require restraining and bleeding of pigs.

The laboratory diagnostic systems for ASFV are divided in two groups: virological and serological. The first procedure includes virus isolation as well as the detection of viral antigens and viral genome (OIE, 2012; Oura *et al.*, 2013). The serological methods are used to detect antibodies against ASFV (Filgueira-Perez *et al.*, 2006; OIE, 2012; Oura *et al.*, 2013) as well as salivary proteins of soft ticks (Canals *et al.*, 1990; Díaz-Martín *et al.*, 2011).

1.6.1. Virus isolation

Malmquist and Hay (1960) observed that ASFV is able to grow in swine bone marrow and buffy coat cells. These infected cells produce two distinct phenomena, namely haemadsorption followed by cytolysis, or cytopathic effects if non-haemadsorbing viruses are involved, therefore, mimicking the natural ASFV infection. Since not all ASFV isolates are haemadsorbing viruses, specimens that test negative for virus isolation should be confirmed by means of polymerase chain reaction (PCR) or direct immunofluorescence to avoid false negative results (Sánchez-Vizcaíno & Mur, 2013).

Swine alveolar macrophages were considered an alternative source of primary porcine cells (Carrascosa *et al.*, 1982). These cells are obtained through a lavaging process of pig lungs and are used to culture and titrate ASFV (Carrascosa *et al.*, 1982). Continuous porcine cell lines from pig alveolar macrophages have been developed as tools for isolating and characterizing different porcine viruses including ASFV and CSFV (Weingartl *et al.*, 2002). However, these cells are not used in practice as they are only sensitive to certain ASFV isolates, particularly attenuated forms of the virus namely: Hinde and Uganda as well as wildtype of E70 and CC83 strains (Weingartl *et al.*, 2002).

Virus may be detected in infected macrophage cultures by direct immunofluorescence, antigen ELISA or PCR (Plowright *et al.*, 1994a; Penrith *et al.*, 2004a). The first two assays are considered of lower sensitivity compared to PCR (Oura *et al.*, 2013) and are widely used to

differentiate ASF from CSF in countries where both diseases occur (Penrith *et al.*, 2004a; Oura *et al.*, 2013).

Despite the convenience of peripheral blood mononuclear cells (PBMC) to culture ASFV for diagnostics, not all isolates are well adapted to these cells (de León *et al.*, 2013). However, the reproducibility of the results obtained in these cultures and the amount of virus to be harvested are limited. Therefore, different monkey-derived cell lines, particularly Monkey stable (MS) and Vero cells are extensively used in diagnostics or biochemical studies as alternative to overcome these issues (Hess *et al.*, 1965; de León *et al.*, 2013). Nevertheless, those cells are limited to be used in cell-adapted virus strains (de León et al., 2013).

Further biological studies of the ASFV were carried out in COS-1 cells (Hurtado *et al.*, 2010). These cells have been shown to be sensitive when used for diagnostics and virus titration even at low concentration of virus or poorly preserved samples (de León *et al.*, 2013). In addition, the cells can be used for the preparation of ASFV suspensions as antigen for indirect ELISA and immuno-peroxidase (IPT) tests used to detect specific antibodies to ASFV in domestic pig serum samples (Gallardo *et al.*, 2013) as well as during optimization of plaque assays (Hurtado *et al.*, 2010).

1.6.2. Antigen detection

Complement fixation (Boulanger *et al.*, 1967b), direct immunofluorescence from infected cell cultures or impression of clinical specimens onto microscope slides (Boulanger *et al.*, 1967c), immunodiffusion (Malmquist, 1963; Boulanger *et al.*, 1967a), enzyme linked immunosorbent assay (ELISA) and radio immuno-assay (RIA) (Wardley *et al.*, 1979) are techniques that have been developed for antigen detection, but none of these has the sensitivity of the haemadsorption assays used to grow virus (Wardley *et al.*, 1979; Penrith *et al.*, 2004a). However, the sandwich ELISA based on a monoclonal antibody against VP72 protein was sensitive for the detection of homologous antigen during the laboratory trials (Vidal *et al.*, 1997). Conversely, the polyclonal antibodies used in the indirect sandwich ELISA to detect antigens from field isolates was more sensitive compared to the assay using the monoclonal antibody (Hutchings & Ferris, 2006).

Nevertheless, most of the laboratories in Africa employ the direct immunofluorescence test as a rapid technique for virus detection in case of an ASFV outbreak (Penrith *et al.*, 2004a).

1.6.3. Antibody detection

In the absence of a vaccine for the disease, the presence of ASFV antibodies provides an indication of preceding infection (Penrith *et al*., 2004a; OIE, 2012). These antibodies are produced as early as 10 days post infection (OIE, 2012) and may persist for several months making antibody detection techniques extremely useful in diagnosing the sub-acute and chronic form of disease (Penrith *et al*., 2004a, 2007; Cubillos *et al.*, 2013).

The immuno-electro-osmophoresis (IEOP) assay was the earliest serological test developed in routine diagnostic procedures for the detection of antibodies to ASFV. The assay is based on antigen extracted from Vero cells infected with ASFV (Pan *et al.*, 1972). The sensitivity of this assay was considered higher compared to the agar gel double-diffusion precipitation test and complement fixation technique (Ferris *et al.*, 1980). However, the sensitivity is lower compared to more recent ELISAs, and therefore suitable as screening test for larger pig populations (OIE , 2008).

ELISAs are most commonly used for examining serum from domestic and wild suids that have been infected by viruses (Wardley *et al.,* 1979; OIE, 2012; Oura *et al*., 2013). A number of ELISA have been developed and validated, these include a highly sensitive and specific commercial ELISA kit based on a competition format where ELISA plate wells are coated with a purified *p72* protein, the OIE recommended indirect ELISA and new ELISAs based on the ASFV recombinant proteins (Filgueira-Perez *et al.*, 2006; Gallardo *et al.*, 2009b; OIE , 2012).

The OIE recommended ELISA for testing sera is based on crude antigen extracted from purified ASFV and is the prescribed test for international trade of pigs (OIE, 2012). However, this test relies on virus antigen obtained from live virus and could be seen as a risk in countries that do not have access to suitable high containment facilities when propagating virus. In addition, the OIE ELISA lacks specificity when testing poorly preserved sera often encountered in developing countries (Arias *et al.*, 1993; Alcaraz *et al.*, 1995; Gallardo *et al.*, 2009b). As a result, alternative

and more robust systems for the detection of antibodies for ASFV have been developed (Gallardo *et al.*, 2009b, 2013), although only a few of them are routinely used in diagnostic laboratories (Pastor *et al.,* 1989; Escribano *et al.,* 2013).

A recombinant ASFV protein *p30* expressed in insect larvae of *Trichosplusia ni* has been used as coating antigen in ELISA and Western Blotting assays (Barderas *et al.*, 2000; Filgueira-Perez *et al.*, 2006). It is considered an alternative for whole virus particles that need to be cultured in specialized facilities where tissue culture facilities are needed (Barderas *et al.*, 2000; Filgueira-Perez *et al.*, 2006). However, validation of this method was done using only serum samples from domestic and wild pigs collected in Nigeria, Uganda and Spain (Filgueira-Perez *et al.*, 2006). Therefore, full validation with the inclusion of sera from the southern region of Africa is warranted.

An anti-tick ELISA has been developed to detect humoral responses in pigs to the European soft tick, *O. erraticus.* The test can be used to evaluate the importance of the soft tick cycle for the transmission of disease in pig populations by determining if pigs have been in contact with soft ticks (Canals *et al.*, 1990). In addition, the salivary lipocalin protein TSGP1 from *O. moubata* has been characterized and assessed for its diagnostic performance under experimental conditions (Diaz-Martin *et al.,* (2011). To date, the test has been used to assess the epidemiological role of the *O. moubata* complex in several areas such as Senegal, Mauritius (Jori *et al.,* 2013) or North-western Madagascar (Ravaomanana *et al.,* 2011). However, this technique has never been implemented using samples from domestic pigs or wild pigs in Mozambique nor to evaluate the potential role of *O. porcinus porcinus* and *O. p. domesticus* in the epidemiology of ASFV.

1.6.4. Molecular assays

Many laboratories employ viral DNA detection systems e.g. PCR. These methods are highly sensitive and suitable to detect viral DNA even in the absence of viable viral particles and presence of circulating antibodies (Pastor & Escribano, 1990; Atuhaire *et al.*, 2013). A wide

variety of molecular techniques have been used to detect viral DNA in tissues, serum as well blood of infected pigs (Steiger *et al.*, 1992; Agüero *et al.,* 2003; Oura *et al.*, 2013).

1.6.4.1 Conventional PCR

Polymerase chain reaction has become the method employed for rapid detection of ASFV in several laboratories and may be suitable for diagnosis in the absence of biosafety containment facilities needed for handling live virus during isolation process (Steiger *et al.*, 1992; Penrith *et al.*, 2004a; Oura *et al.*, 2013). These assays enable the diagnosis of haemadsorbing as well as non-haemadsorbing viruses, detect low virulence isolates and are considered biologically safe and rapid methods (Steiger *et al.*, 1992; Oura *et al.*, 2013).

The first PCR assay for the detection of ASFV infection was reported in early 90's based on a set of primers designed to amplify a 740 bp of DNA fragment from the conserved region of ASFV genome using organs and plasma obtained from ASFV-infected pigs, ASFV-infected cell cultures, as well as cloned DNA fragments samples. The assay proved to be highly sensitive and specific compared to virus isolation (Steiger *et al.*, 1992). Other uniplex diagnostic PCRs, targeting conserved regions of the *p72* gene, were developed and validated (Agüero *et al.,* 2003; Bastos *et al*., 2003; OIE, 2012). The protocol was further used for molecular epidemiology studies (Bastos *et al.,* 2003, Boshoff *et al.,* 2007; Lubisi *et al.,* 2005).

Basto and colleagues (2006) developed a nested PCR and its internal control for the detection of ASFV in *O. erraticus*. The assay revealed improved sensitivity compared to virus isolation from soft ticks and the PCR protocol from the OIE (Agüero *et al.*, 2003). The tool can be adapted for use in tissues, blood, serum and *O. p. porcinus* or *O. p. domesticus* samples as the internal controls assess the presence of PCR-inhibitory substances (Zsak *et al.*, 2005).

Additionally, a highly sensitive and specific one-step hot start gel-based multiplex Reverse Transcription-PCR (RT-PCR) have been developed to simultaneously detect ASFV and CSFV in clinical samples (Agüero *et al.*, 2004). The method uses two primer sets, each one specific for the corresponding virus and generating different fragment lengths of PCR products (Agüero *et*

al., 2004; Oura *et al.*, 2013). However, it still needs to be validated with field samples (Agüero *et al.*, 2004; Zsak *et al.*, 2005; Oura *et al.*, 2013).

Lastly, a direct PCR assay was developed by Agricultural Research for Development (CIRAD) in France to assess the long term storage of genomic material under tropical conditions (Michaud *et al.*, 2007). Blood specimens were obtained from domestic pigs and goats and spotted onto Flinders Technology Associates (FTA®) and Whatman 3 MM filter papers. The sensitivity of FTA® cards protocol were relatively higher compared to Whatman 3 MM filter papers (Michaud *et al.*, 2007). However, the pre-treatment using elution buffer needed before PCR in FTA® cards increases the cost comparing the Whatman 3 MM filter papers (Michaud *et al.*, 2007), therefore making FTA® cards unsuitable for routine large-scale epidemiological surveys (Michaud *et al.*, 2007). These protocols can be extensively used for virus genotyping and are useful to send diagnostic material to laboratories for further evaluation.

Despite the wide range of available assays, the extensive validation of all newly developed PCR assays is warranted in order to ensure that they do not crossreact with other pig viruses (for example CSFV, porcine circoviruses and porcine reproductive and respiratory syndrome virus), and to guarantee that they detect all the known genotypes of ASFV that are currently circulating (Oura *et al.*, 2013).

1.6.4.2 Real-time PCR

A single tube Real-time PCR (rPCR) assay has supplanted the conventional OIE recommended PCR and has the added advantage of avoiding the use of agarose gels, increased sensitivity as well as rapid and low risk of cross contamination (King *et al.*, 2003; Oura *et al.*, 2013). The assay, targeting the *p72* gene, was first developed and validated by King *et al.,* (2003). The technique provides fast and accurate diagnosis of ASFV and is suitable for differential diagnosis of ASF and CSF in the regions where both diseases are simultaneously present (Zsak *et al.*, 2005). It is useful for active surveillance in domestic and sylvatic cycles of ASFV or in case of disease outbreak management and emergency situations (Zsak *et al.*, 2005). It also enables assessment of the presence of ASFV DNA derived from clinically healthy pigs blood samples

collected onto FTA® cards (Braae *et al.*, 2013). In order to increase analytical and diagnostic sensitivity of rPCR assay, Tignon *et al.,* (2011) developed another diagnostic rPCR that also targets the *p72* gene and uses the ß-actin as internal control. This assay proved to be more sensitive compared to the OIE recommended assays (Agüero *et al.*, 2003, 2004; King *et al.*, 2003; Tignon *et al.*, 2011). Recently a rPCR was developed using a universal probe library (UPL) that allows differentiation of ASFV from other clinically similar viruses (Fernández-Pinero *et al.*, 2013). The assay proved to be more sensitive for the early detection of viral DNA in experimentally infected animals and proved to be 10% more sensitive than the TaqMan rPCR (Fernández-Pinero *et al.*, 2013).

Ronish *et al*. (2011) developed a Linear-After-The-Exponential PCR (LATE-PCR) assay to amplify the *p72* gene of ASFV. This technique is a simple and rapid antigen detection system based on the direct amplification of large amount of single stranded DNA; it is considered an advanced form of asymmetric PCR and the PCR products are verified by melting curve analysis that emit fluorescent signals. The assay was validated based on tissue samples (spleen, tonsil, and liver) from experimentally infected pigs and was found to have a high analytical sensitivity (1 and 10 copies) that can be used either in the laboratory or in the field by using a portable PCR thermocycler (Ronish *et al*., 2011; Oura *et al.*, 2013).

1.6.4.3. Isothermal amplification assays

Isothermal amplification technologies may be considered an alternative to PCR-based detection techniques for the diagnosis of ASFV and do not demand highly sophisticated equipment. Therefore, reactions can be performed in an ordinary water bath at a single temperature (Oura *et al.*, 2013). Isothermal assays have been described for the diagnosis of ASF. A closed tube isothermal Invader® method able to accurately detect and quantify DNA targets with high sensitivity and specificity was developed by Hjertner *et al.*, (2005). The invader assay is associated with a probe that emits a signal if the specific DNA target has been detected and read by a fluorescent plate reader (Hjertner *et al.*, 2005; Oura *et al.*, 2013). A loop-mediated isothermal amplification (LAMP) assay was developed for ASFV detection (James *et al.*, 2012).

The latter appears to be more sensitive and rapid compared to the Invader® method. Furthermore, the LAMP was found highly sensitive in a comparative assessment relative to convental PCR for the detection of ASFV in clinical samples from domestic pigs in Uganda (Atuhaire *et al*., 2014). However, further validations in field samples for both assays are needed in order to assess their effectiveness particularly in endemic areas.

1.7. CONTROL OF THE DISEASE

In the absence of an effective vaccine, owing to the inability of the virus to produce protective neutralizing antibodies (De Boer, 1967; De Boer *et al.,* 1969; Gomez-Puertas *et al.,* 1996), control in high-risk areas depends on rigorous prevention of contact between domestic pigs and free-living reservoirs (by double fencing and confining animals in pig pens for example) and massive "stamping out" of sick, surviving and in contact animals together with the establishment of quarantine zones and disinfection procedures when disease does occur (Penrith *et al.*, 2004a; Lubisi *et al.*, 2009). Strict movement control as well as appropriate legislation and a compensation scheme for slaughtered animals, combined with permanent surveillance, are key factors for control ASF (Plowright *et al.*, 1994; Penrith *et al.*, 2004a; Oura *et al*., 2013).

In the endemic areas where a sylvatic cycle occurs, control and eradication appear to be difficult in extensive production systems (free-ranging pigs and farmers that provide low input for animal production). Small scale pig farmers often lack information and awareness of ASFV transmission mechanisms and in most developing countries, the farmers allow potential buyers onto the premises and speculators often move from one farm to the next without disinfection (Penrith *et al.*, 2007, 2013). This is one example of lack of biosecurity measures where pigs are more likely to become infected through contact within marketing chains (Penrith *et al.*, 2007; Costard *et al.*, 2009b; Penrith & Vosloo, 2009; Penrith *et al.*, 2013). During surveys conducted in Madagascar, farmers mentioned that as soon as pigs started dying from a suspected disease outbreak, most villagers sold their stock alive or slaughtered, sold the carcasses in the local market and did not report outbreaks to the authorities hence increasing the risk of spreading the disease (Radriamparany *et al.*, 2005). In such scenarios, the control of the disease should be focused initially on the most important risk factors of disease spread, namely live animal

markets, open access abattoirs particularly in rural areas, farm visits (potential buyers or traders) and free-ranging domestic pigs particularly if the pig population is large enough (Costard *et al.*, 2009b; Penrith & Vosloo, 2009; Penrith *et al.*, 2013).

In many countries in the southern region of Africa, changing pig production practices among small scale producers into more intensive husbandry methods, or the re-organization of the pig industry in addition to well-developed marketing networks that link small scale pig producers to city markets, are measures that could reduce uncontrolled pig movements hence reducing the potential risk for ASF transmission (Plowright *et al.*, 1994; Penrith *et al.*, 2004a, 2013; Costard *et al.*, 2009b; Penrith & Vosloo, 2009). These approaches can be applied in conjunction with the implementation of surveillance and monitoring programmes based on more reliable antibody detection assays (ELISA) to demonstrate either past infection with ASFV or contact with the vectors (Allaway *et al.*, 1995; Barderas *et al.*, 2000) in order to identify potential sources of virus. It must be emphasized, however, that the presence of antibodies to ASFV in a healthy pig does not imply that the animal is infectious, as it has been shown that the virus disappears within two months after infection in pigs and those mostly recover completely (Wilkinson, 1989; Penrith *et al.,* 2004a).

In the presence of the sylvatic and domestic pig cycles, the contact between natural hosts and domestic pigs may be prevented either by confinement or building infrastructure with double-fenced barriers (Costard *et al.*, 2009b; Penrith *et al.*, 2013). Additionally, the control of soft ticks, which includes application of acaricides in domestic pig premises, may be another option (Plowright *et al.*, 1994; Penrith *et al.*, 2013).

African swine fever outbreaks are conventionally controlled by applying strict movement control, quarantine, culling all infected as well as in contact pigs and compensating farmers at market values (Costard *et al.*, 2009b; Penrith *et al.*, 2013). These are highly effective in the case of a single focus of event but do not always lead to effective control when the disease is widespread (Penrith *et al.*, 2013). Nevertheless, the prerequisites are resources in terms of veterinary infrastructure such as diagnostic laboratories, well trained personnel, legislation and cooperation from pig farmers (Plowright *et al.*, 1994; Penrith *et al.*, 2004a, 2013; Penrith & Vosloo, 2009; Fasina *et al.*, 2012).

1.8. SOCIO-ECONOMIC SIGNIFICANCE OF THE DISEASE

In European and Latin American countries where eradication programmes were undertaken as a result of the introduction of ASF into Portugal from Africa in 1957, the cost of control measures such as culling, compensation to the farmers, hygienic measures, restriction of movement of pigs and their products was estimated to be approximately 100 million USD a year (Arias & Sanchez-Vizcaino, 2002; Costard *et al*., 2009b). Cuba, for example, lost about 400 000 pigs in 1971 as result of the eradication campaign (Plowright *et al.*, 1994).

African swine fever causes high mortality and morbidity and can threaten food security, as pigs represent a good source of cheaper protein and a source of income in large parts of Africa (Costard *et al.,* 2009b). In Africa and other non-developed nations, ASF can have severe consequences for both commercial and small scale farmers since they often lack resources to implement prevention and control measures and are frequently unable to restart production following outbreaks (Edelsten & Chinombo, 1995). Furthermore, in countries where the pig industry is well developed, the impact of the disease is more visible in terms of elevated mortality rates, high costs for control or eradication campaigns and loss of status for international trade (Costard *et al.,* 2009b; Penrith *et al.,* 2013).

In countries that are contiguous to Mozambique the impact of ASF outbreaks has also been severe. Edelsten & Chinombo, (1995) estimated that in two years (from 1989 to 1990), 45% of the 70 000 pig population were killed in Malawi as a result of the direct effect of ASF outbreaks and stamping out. In addition, in the pig-dense, highly populated areas of the western Province of Cameroon, where 63 % of the country's 1.1 million pigs are kept, approximately 3 762 pigs farmers reported losses and 54 432 pigs were reported to have died during the ASF epidemic of 1982, and these losses are estimated to the amount of US$ 2.5 million (Nana-Nukechap & Gibbs, 1985). However, it is believed that these figures are underestimated because many farmers did not reveal their losses (Nana-Nukechap & Gibbs, 1985).

The pig population in Mozambique is estimated to be about 1.3 million (F. Rodrigues, personal communication). The national pig industry is composed of commercial piggeries representing 10% of the pig production in the country and relatively poor, small-scale pig producers which keep 90% of the pig population (G. Dias, unpublished data). Commercial pig farming is mainly

concentrated in Maputo City (in the South), Beira and Sussundenga District (in the central Provinces of Sofala and Manica respectively), and the total annual pork production is estimated to be approximately 879 tonnes of meat (National Directorate of Veterinary Services report, 2011).

The first ASF outbreak in Mozambique occurred in the mid 1950s in the Angónia District, northeast of Tete Province (Mendes, 1971). It spread to areas adjacent to the epicentre and resulted in the loss of more than 3 000 pigs (Abreu *et al.*, 1962). Until 1993, repeated outbreaks have been reported to the World Organization for Animal Health (OIE) mainly in Tete, Sofala, Manica, Nampula and Zambezia provinces. The disease was reported for the first time in 1994 in the provinces south of the Save River. Most pigs in the commercial and smallholder sector in the vicinity of Maputo City and the neighbouring Gaza Province were decimated, representing losses of more than 4 600 pigs in a total pig population not exceeding 5 000 (National Directorate of Veterinary Services, unpublished data). However, these figures are thought to be an underestimation, particularly in the remote areas where veterinary infrastructure, communication and personnel are scarce. Added to this, lack of compensation to the farmers in case of stamping out, discourages disease reporting (Matos *et al.,* 2011; Penrith *et al.,* 2013).

As in the other countries, the disease caused a significant impact in small scale pig producer's economy, as well as the food security. Unfortunately, the occurrence of ASF in the country discouraged some development agencies from financing subsistence pig farmers and it hampers a traditional and culturally rooted source of income in rural communities (Penrith *et al.,* 2007).

1.9. JUSTIFICATION OF THE STUDY

This study was needed for the following reasons:

ASFV is an endemic disease in Mozambique which seriously hampers food security and impacts on the well-being of small scale farmers and the development of pig farming. The Sofala Province in Central Mozambique is particularly prone to frequent outbreaks. Several reviews of ASF in Mozambique indicated the frequent occurrence of ASF outbreaks in areas adjacent to the Gorongosa National Park (GNP) in the Gorongosa District (GD), in the central Sofala province

(Penrith *et al.*, 2007, 2013; Penrith & Vosloo, 2009). However, the possible source of those outbreaks has never been investigated.

In addition to the risk factors for ASF occurrence listed below, the GD was selected for the study on account of the high number of domestic pigs farmed in the area (17 348, shown in DADAR, 2005). This, together with its proximity to a wildlife reserve, made the GD an ideal setting to evaluate the role of a potential sylvatic cycle in the maintenance and dissemination of ASF virus strains into the domestic pig chain.

In addition, there is a paucity of data on the genetic characterization of circulating viral strains of ASFV in Mozambique since outbreaks of ASF often remain underreported with few samples submitted to the laboratory (Penrith *et al.*, 2007). Information on the genetic diversity of circulating strains of ASFV in the study area as well as the seroprevalence of ASFV in domestic pig can be used to assist the authorities in designing proper control measures (Haresnape *et al.*, 1987; Allaway *et al.*, 1995; Bastos *et al.*, 2002, 2003, 2004; Etter *et al.*, 2011). Furthermore, the identification of endemic areas and regions where the ASFV is actively circulating and presence of sub-acute and chronic forms of the disease (Haresnape *et al.*, 1987; Etter *et al.*, 2011), can enable the veterinary authorities to implement active surveillance and monitoring strategies to prevent disease spread through the control of movements of pigs and their products to other important areas for pig production in the country. Indeed, the renovation of the tar road (N1) connecting Sofala and Zambézia provinces through GD is under way and means that this district is located on the route that facilitates the distribution of live pigs and pig-meat to the City of Beira (Sofala Province Capital city). This represents a potential risk factor for occurrence but also and perhaps more importantly, for spread of the virus.

In the absence of an effective vaccine, those surveillance and monitoring methods should be based on the most reliable diagnostic methods available to detect past circulation of the virus. However, the performance of available diagnostic techniques has never been assessed in a specific endemic setting in Mozambique. This information is fundamental to provide veterinary authorities with the most convenient and best test combination to detect antibodies against the local circulating virus strains.

1.10. OBJECTIVES

Overall objective for this investigation is:

To investigate and characterize the epidemiology of ASF at the wildlife-domestic animal interface and determine whether a sylvatic cycle exists and furthermore to evaluate the different diagnostic tests, therefore improving the detection of antibodies to ASFV in a representative area of central Mozambique.

Specific objectives are as follows:

1. To determine whether a sylvatic cycle exists by:
    a. verifying the presence of soft ticks *(Ornithodoros spp)* in both wildlife and domestic areas and determine their morphological taxonomy in the GNP interface
    b. assessing potential contacts between wild and domestic pigs and soft ticks by detection of anti-tick antibodies in domestic and wild pig sera
    c. establishing whether wild and domestic pigs and soft ticks are infected with ASFV;
2. To determine the molecular epidemiology of the isolated ASFV in the area, by sequencing different gene regions and comparing phylogenetic outputs;
3. To evaluate the performance of currently available diagnostic tests in their ability to detect ASFV antibodies in an endemic setting of Central Mozambique.

CHAPTER 2*

INVESTIGATION INTO THE EPIDEMIOLOGY OF AFRICAN SWINE FEVER VIRUS AT THE WILDLIFE – DOMESTIC INTERFACE OF THE GORONGOSA NATIONAL PARK, CENTRAL MOZAMBIQUE

2.1. SUMMARY

An epidemiological study of African swine fever (ASF) was conducted between March 2006 and September 2007 in a rural area adjacent to the Gorongosa National park (GNP) located in the Central Mozambique. Domestic pigs and warthogs were sampled to determine the prevalence of antibodies against ASF virus and the salivary antigens of *Ornithodoros spp*. ticks while ticks collected from pig pens were tested for the presence of ASFV. In addition, 310 farmers were interviewed to gain a better understanding of the pig value chain and potential practices that could impact on the spread of the virus. The sero-prevalence to ASFV was 12.6% on farms and 9.1% in pigs while it reached 75% in warthogs. Approximately 33% of pigs and 78% of warthogs showed antibodies against salivary antigens of ticks. The differences in sero-prevalence between farms close to the GNP, where there is greater chance for the sylvatic cycle to cause outbreaks, and farms located in the rest of the district, where pig to pig transmission is more likely to occur, were marginally significant. *Ornithodoros spp.* ticks were found in only 2 of 20 pig pens outside the GNP and both pens had ticks testing positive for ASFV DNA. Interviews carried out among farmers indicated that biosecurity measures were mostly absent. Herd sizes were small with pigs kept in a free-ranging husbandry system (65%). Only 1.6% of farmers slaughtered on their premises but 51% acknowledged allowing visitors into their farms to purchase pigs. ASF outbreaks seemed to have a severe economic impact with nearly 36% of farmers ceasing pig farming for at least one year after a suspected ASF outbreak. This study provides the first evidence of the existence of a sylvatic cycle in Mozambique and confirms the presence of a permanent source of virus for the domestic pig value chain.

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2.2. INTRODUCTION

African swine fever (ASF) is a highly contagious viral disease affecting domestic and wild pigs (*Sus scrofa*). It is characterised by fever and extensive haemorrhages throughout different organs and often leads to high mortalities in domestic pigs. The disease is caused by the ASF virus (ASFV) which is the only member of the *Asfarviridae* family, genus *Asfivirus*. African swine fever is endemic in most sub-Saharan African countries where it causes major economic losses, threatens food security and limits pig production (Penrith *et al*., 2004a, 2007; Costard *et al.*, 2009b). In 2007, ASF spread to the Caucasus region and Russia, increasing the risk of introduction to Europe and Asia (Rowlands *et al*., 2009; Rahimi *et al*., 2010).

The high mortality rate associated with the disease, coupled with the highly infectious nature of the virus, makes it one of the most serious threats to the swine industry worldwide. In large parts of sub-Saharan Africa, subsistence livestock farming involves raising small herds of pigs. However, in areas where ASF is endemic, the disease impacts negatively on the sustainability of these practices. Since the mid-1990s, ASF has caused severe losses in southern Mozambique (Penrith *et al*., 2013), Madagascar (Roger *et al*., 2001; Ravaomanana *et al.,* 2010) and in several countries in West Africa (Etter *et al*., 2011), dramatically reducing pig numbers in these countries.

African swine fever virus is maintained in three epidemiological cycles (Penrith *et al*., 2004a). The sylvatic cycle, involving warthogs *(Phacochoerus africanus)* and argasid ticks belonging to the *Ornithodoros moubata* complex, is restricted to regions where the two species coexist, predominantly central, east and southern Africa (Jori & Bastos, 2009). Warthog piglets are born free from the virus (Thomson, 1985, Kleiboeker *et al*., 1998) and are infected by ticks during the first 6–7 weeks spent inside the burrow (Thomson, 1985; Jori & Bastos, 2009). As the natural arthropod host of ASFV, infected soft ticks also represent the link between wild suids and domestic pigs. In areas where domestic pigs are kept within the home range of wild suids, the spread of ASFV to pigs is often facilitated by soft ticks (Plowright *et al.*, 1994; Kleiboeker *et al*., 1998; Penrith *et al*., 2004b). When pig shelters are infested with ASFV-infected *Ornithodoros moubata* ticks, the disease can be maintained between domestic pigs and the arthropod host in

the absence of warthogs. Once established in the domestic pig populations the virus can be maintained independently of the wild suids and ticks.

The first description of a disease resembling ASF in Mozambique appeared in the mid-1950s. However, it wasn't until 1960 that the disease was confirmed by laboratory diagnosis following an outbreak in Tete Province, located in the Central West region of the country. Since this time, Mozambique has experienced regular outbreaks of the disease resulting in ASF being considered endemic in that country (Abreu *et al*., 1962; Penrith *et al.*, 2007). A study conducted in the Angonia district, close to the border with Malawi, found that the disease is exclusively maintained in domestic pigs (Penrith *et al.*, 2004b). In contrast, the frequent occurrence of ASF in the areas surrounding the Gorongosa National Park (GNP) suggests that a sylvatic cycle may also contribute to the maintenance of the disease in Mozambique (Penrith *et al.*, 2007). Here we report on the prevalence of antibodies against ASFV in warthogs and domestic pigs found at the domestic/wildlife interface of the GNP and highlight the potential risk factors contributing to the dissemination of ASF in the region.

2.3. MATERIALS AND METHODS

The study was approved by the joint ARC-Onderstepoort Veterinary Institute (OVI) and Faculty of Veterinary Science, University of Pretoria Animal Ethics Committees (Ref. 21/2006) prior to execution.

2.3.1. Study area

The field study was conducted in the Gorongosa District, located in the central province of Sofala at 18^0 45'/19^0 15' latitude South and 33^0 30'/34^0 45' longitude East (Figure 1). The district was selected as a study area based on the high number of small-scale domestic pig farms and its proximity to the GNP. The GNP is an unfenced wildlife conservation area located within the

district. It covers a total area of 3770 km^2 and in 2006 the warthog population was estimated to be approximately 4,000 (Pereira, C.L. personal communication).

According to the Ministry of Agriculture, there were no commercial pig farms in the area and the subsistence farmer held, on average, a herd composed of a maximum of 8 pigs (often a sow and offspring) per farm (District Agricultural Directorate, 2005). Pigs are predominantly reared under an extensive production system in which the animals are left roaming free, except during the rainy season (between October to April) when crops are still in the field and the pigs are kept indoors to protect the season's production. The typical pig pens, also called *Tanga* in the local language, are constructed from mud and wooden poles and are covered by grass or corrugated iron. Each farm generally maintains only one pen without internal divisions, located in the backyard.

2.3.2. Sampling protocol

Pigs

The protocol used to determine the sampling frame was based on the information provided by the District Agricultural Directorate, Mozambique. The pig population in 2005 in the area surrounding the GNP was estimated to be 17,348 animals owned by approximately 10,990 small-scale farmers distributed in a total of 19 villages. Since the pig population size in each of the different villages was not available, it was decided to distribute the number of farmers equally across the villages, and farmers were selected for the survey following a multistage sampling approach. Of the 19 villages, one was excluded from the study as the pig farms had been depopulated. The remaining 18 villages were grouped according to their proximity to the GNP boundary. Six villages located at the edge of the GNP (less than 10 km) are referred to as the Gorongosa National Park Buffer Zone (GNPBZ). The additional 12 villages, at an average of 15 km from the GNP boundaries, are referred to as Rest of District (ROD) (Figure 2.1). The GNPBZ is characteristically rural with a lack of basic infrastructure such as electricity and roads, low human population density, and crops interspersed with bushy vegetation. In contrast, the ROD is mostly a peri-urban area with a high human population density, basic facilities

(electricity) close to a tarmac road and less cropland. We hypothesized that an environment more influenced by human activities, such as the one found in ROD, would influence the abundance of warthogs in the area and potential contacts between warthogs and domestic pigs would be more frequent in the GNPBZ compared to the ROD.

To estimate the required sample size for detecting at least one diseased pig, a prevalence of 50% for ASFV antibodies was assumed with an accepted error of 5% and a 95% level of confidence. Villages were considered as clusters of pig farms. In each village, pig farms were chosen randomly following a multistage sampling approach, and up to 5 pigs per farm were sampled. The minimum sample size required was 385 pigs as calculated using the free software Win Episcope 2.0 (www.clive.ed.ac.uk/winepiscope).

2.3.3. Sample and data collection

Warthogs

Twelve warthogs from 4 different locations aged less than 1 year (n=3) and older than 1 year (n=9) were sampled opportunistically inside the GNP during 2006. Capture locations were chosen based on the accessibility of the site and abundance of animals. Warthogs were captured by darting them from the vehicle with a compressed air dart gun and plastic darts (Daninject®) at a distance of 5 to 8 m. A dose of 250 mg of Zoletil® was used to induce anaesthesia, topped up with a combination of 100 mg of Ketamine and 20 mg of Azaperone to allow a light level of anaesthesia, good immobilisation and reduction of recovery time (Kock & Burroughs, 2012). Approximately 5 ml of blood was obtained from the anterior *vena cava* in each animal using 10 ml plain Vacutainer® tubes.

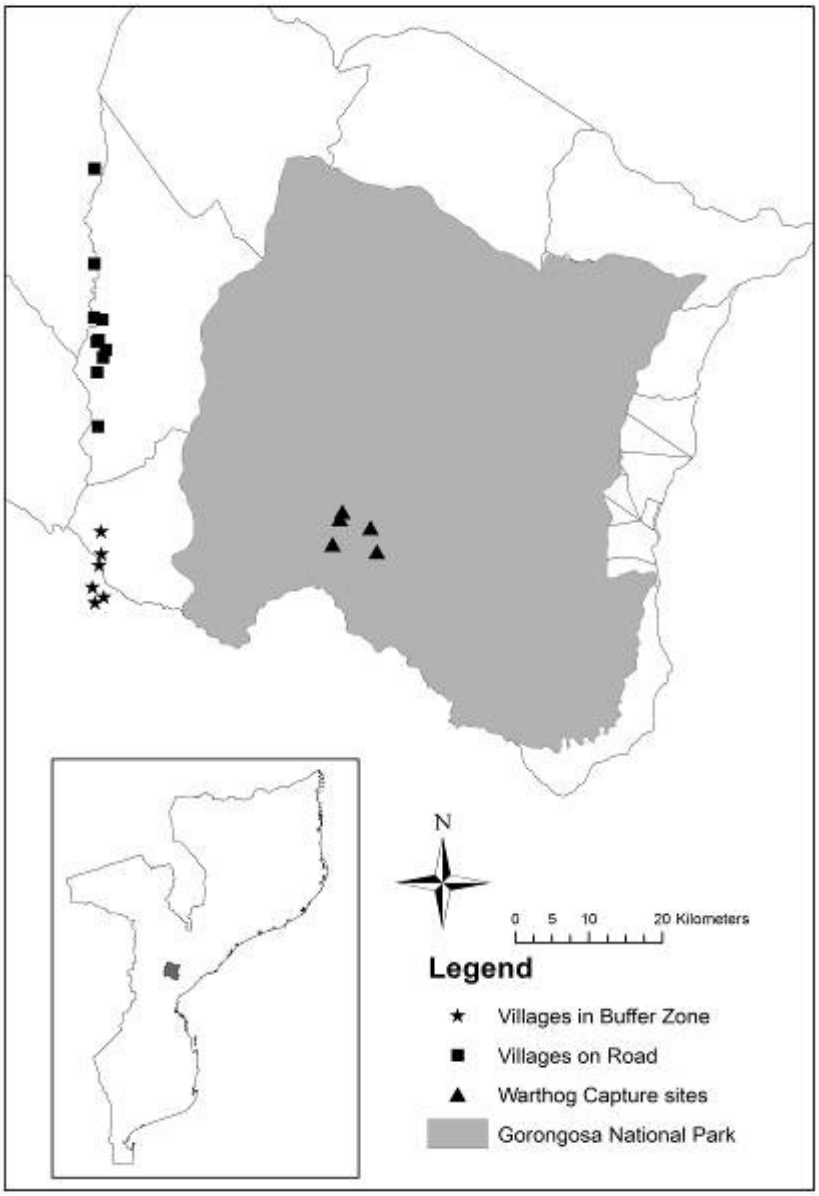

Figure 2.1.: Map of the Gorongosa District, including the area of the Gorongosa National Park, and the locations where warthogs and pig farms were sampled

Soft ticks in pig pens

Twenty farms from the study area (GNPBZ and ROD) were assessed for the presence of soft ticks using the vacuum aspiration method (Butler & Gibbs, 1984; Vial *et al.*, 2007, Ravaomanana *et al.*, 2010; Jori *et al.*, 2013). A petrol-powered mulching blower/vacuum was

used to collect material from different parts of pig pens and small mammal burrows in close proximity to the pen. Spades were used to collect bigger volumes of material where appropriate. Soft ticks were collected by spreading litter on black plastic sheets exposed to the sun. Specimens were placed in a plastic sample bottle with sand at the bottom and kept in cool, dark conditions until they were dispatched to the Transboundary Animal Disease Programme (TADP), OVI in South Africa for further analysis. The specimens were transported under a permit issued by the South African Department of Agriculture, Forestry and Fisheries.

Pigs

A total of 629 pigs of local breed ranging from 6 months to 3 years old were sampled from 310 small-scale farmers clustered in 18 villages. Sera were obtained by standard methods, transferred to labelled cryotubes and stored at -20ºC until sent to the TADP to be tested for antibodies against ASFV. Aliquots of these sera were also sent to the *Instituto de Recursos Naturales y Agrobiologia de Salamanca* (CSIC) in Spain to be tested for antibodies to the salivary proteins of *Ornithodoros* spp. ticks.

Questionnaire

At the time of blood collection, 314 pig owners were interviewed using an extensive questionnaire. The GPS coordinates of the farms were recorded and the presence of soft ticks or other ecto-parasites was also noted. Information about the sampled animals, including age, sex, health status, clinical signs of haemorrhage, herd size, breed and the suspicion of ASF or recent fatalities was gathered. In addition, information on the farm type, husbandry and feeding practices was obtained to identify possible risk factors for the transmission of ASFV. A presumptive diagnosis for ASF was assessed according to clinical details given by the pig owner. Soft ticks were shown to farmers to assess whether similar parasites have been observed in their premises or village.

2.3.4. Sample and data processing

2.3.3.1. ASFV antibody detection using Enzyme-linked Immunosorbent Assay (ELISA)

Serologic analysis was performed using an indirect ELISA following the protocol described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (2012). The antigen used in the assay consisted of purified ASFV isolate Zaire collected in 1977 and subsequently adapted on Vero cells. The results were considered positive when sera had an absorbance value of more than twice the mean absorbance value of the control negative sera on the same plate.

2.3.3.2. ASFV and DNA detection in soft ticks

For pig pens where ticks were found, a sample of soft ticks of adults (male or female) and pools of immature (N1 to N5) soft ticks was crushed in a 1.5 ml Eppendorf tube containing 1 ml of phosphate buffered saline (PBS) supplemented with 1% foetal calf serum (FCS) and 1% of a combination of antibiotics and an antimycotic. The soft tick homogenates were centrifuged at 10 000 x g for 1 min and the supernatant frozen at -70° C until further use.

ASFV was isolated from soft ticks using a haemadsorption assay previously described (Malmquist and Hay, 1960). One hundred µl of supernatant from tick homogenates were inoculated into peripheral blood mononuclear cells (PBMC) cultured in flat bottom 96 well plates, according to standard procedures. The plates were observed for up to seven days, virus harvested when haemadsorption was observed and stored at -80^{o}C. All samples were subjected to three serial passages on PBMCs, with each passage performed on a weekly basis. Samples were considered negative for ASF virus if no haemadsorption was observed.

DNA was extracted from 200 µl of each tick homogenate and recovered in a final volume of 50 µl DNA solution using the Qiamp kit (Qiagen GmbH, Hilden), according to the manufacturer's instructions. A nested PCR that targets the C terminal end of the *p72* gene was used to screen soft tick samples for the presence of ASFV DNA (Basto *et al*., 2006). All DNA samples were tested for tick mitochondrial 16S rDNA according to previously published methodology (Black

& Piesman, 1994, Vial *et al*., 2007) to exclude the occurrence of false-negative results due to inhibitors in the tick supernatant.

2.3.3.3. Detection of antibodies against tick salivary protein

The presence of antibodies against the salivary proteins of *Ornithodoros* spp. ticks was assessed in domestic pig and warthog sera using the ELISA developed by Díaz-Martín *et al* (2011), which is based on a recombinant salivary lipocalin protein (rOmTSGP1) of *O. moubata* complex. This assay demonstrated 99.4% specificity and 100% sensitivity in detecting antibodies 3 months after exposure to tick bites (Díaz-Martín *et al.,* 2011).

2.3.3.4. Statistical analysis

In order to determine the influence of age on the detection of ASFV antibodies in the study area (GNPBZ and ROD), animals were classified into two groups: younger than 1 year (n=522) and older than 1 year (n=112). The number of months between time of sampling and the last reported outbreak of ASF on farms was calculated to determine any effect of the time elapsed after the outbreak on the prevalence results. Farms were subsequently divided into those with pigs sampled less than 8 months after a reported outbreak (n=132) and those sampled more than 8 months after a reported outbreak (n=84). The remaining sampled animals (n=418) were from areas where suspicions or reports of ASF outbreaks had not been recorded. A farm was considered to have been at risk for the disease or exposure to the arthropod host when at least one pig was found positive to one of the two serological tests used. Associations between sero-positivity and the different variables such as pig age, location and the time between sampling and reported outbreaks were tested with the chi square test and odds ratio calculations for homogeneity of two populations (Fischer exact test). Values of $p < 0.05$ were considered significant.

Prevalence of tick infestation in warthog burrows and pig pens and tick infection with ASFV with 95% confidence intervals (CI) were calculated with Epi-Info v.3.5.3, 2011 (CDC, Atlanta, US).

2.4. RESULTS

2.4.1. Questionnaire

A herd was considered as the total number of pigs belonging to the same owner. The average herd size per farm was calculated to be 8 pigs (median: 6, IQR [2;10]). Some variations in herd size and composition (sexes and age groups) were identified between farms ($p<0.05$). Approximately 20.2% (128/629) of the sampled populations of pigs were adults with 85.9% female pigs (110/128) and 14.1% boars (18/128). Juveniles (less than one year) represented 79.8% (506/629) of the total sampled population with 55.7% (282/506) fatteners (i.e. after weaning and before being used for reproduction) and 44.3% (224/506) suckling piglets. All respondents mentioned that the pig reproduction was by natural mating using own or borrowed boars. Pigs were not fed commercial feed supplements, but 100% of questioned pig-owners mentioned giving maize bran, approximately 48% gave their stock post-harvest by-products and between 29 to 35% of respondents fed kitchen leftovers and wild legumes, respectively. Most of the 314 interviewed pig farmers kept other animals on their premises with poultry accounting for 49% of the species, small ruminants (goats and sheep) for 19.7%, beef cattle 2.3% and 29.6% kept dogs and cats.

In many cases (76.4%), pigs were reported to share the same space with other animals. In addition, farmers acknowledged that their pigs were left free ranging (65%) and free mating (47%) and 72.3% declared the common practise of lending/borrowing boars. A few farmers (7.0%) shared materials and equipment and 1.6% slaughtered pigs on their premises. Concerning biosecurity measures and risk factors in favour of ASFV transmission, 51.3% of the farmers reported that visitors entered the premises to purchase pigs.

Pigs were not treated for any diseases and 100% of respondents indicated that they do not administer any prophylactic treatment such as vaccination, de-worming or iron supplement.

None of the pig owners in the villages acknowledged having seen soft ticks. However, 100% of the respondents confirmed having seen hard ticks (*Ixodid* group). Pigs were also often affected by lice, fleas and mange.

Over 39% (123/314) of respondents mentioned having experienced what they suspected to be ASF outbreaks in the past and reported pig deaths. An outbreak reportedly occurred prior to 2005 and was subsequently followed by two outbreaks in November 2006 and February 2007. Amongst farmers suspected to have experienced ASF outbreaks, 40.7% (50/123) mentioned that not all animals died during the outbreaks. Almost 35.8% (44/123) of affected farmers stopped rearing pigs for at least one year after an outbreak due to high mortality and losses. The remaining 60.8% (191/314) of pig farmers answered that they had never experienced mortalities which they suspected to have been due to ASF. These 191 farmers were mostly from the ROD (80.6%) while 19.4% were from the GNPBZ.

2.4.2. Detection of antibodies against ASFV

Warthogs

The prevalence of antibodies directed against ASFV in all warthogs tested (n=12) was 75%, 95% CI [42.8-94.5]. The sero-positivity in adult animals (n=9) was 66.7%, 95% CI [29.9-92.5] and in individuals less than 1 year old (n=3) it was 100%.

Domestic pigs

The mean number of pigs sampled per village was 35 (median: 33, IQR [16;55]) and the mean number of pigs bled per farm was 2 (median:1, IQR [1;3]). During 2006, 50 pigs were sampled in the GNPBZ and 74 in the ROD compared to 120 sampled in the GNPBZ and 390 in the ROD in 2007.

In total, 629 pig serum samples were collected from 310 farms in 18 villages (Table 2.1): 170 pigs from 79 farms were sampled in 6 villages from the GNPBZ and 459 pigs from 235 farms were sampled in 12 villages in the ROD. The bias in sampling towards the ROD was due to the

relatively low number of farms and pigs in the GNPBZ. The overall ASFV antibody prevalence in domestic pigs was 9.1% 95% CI [7-11.7]. The ASFV sero-prevalence in pigs from GNPBZ was 12.5%, 95% CI [7.4-17.6] and in pigs from the ROD was 7.8%, 95% CI [5.4-10.3] (Table 2). However, these differences were only marginally significant (OR=0.59, p=0.05).

Of the 314 farms tested, 12.6%, 95% CI [8.9-16.3] had pigs that were sero-positive to ASFV. There was no significant difference in sero-prevalence based on region (Table 2.2; OR=0.97, p=0.53) with 12.8%, 95% CI [5.4-20.2] sero-prevalence in farms from GNPBZ (n=79) and 12.5%, 95% CI [8.2-16.8] in farms from the ROD (n=231).

When the sero-prevalence in different age groups was compared, 91.2%, 95% CI [80.7-97.1] of the sero-positive animals were younger than one year with the remaining 8.8%, 95%, CI [2.9-19.3] older than one year (OR=0.42, p=0.043).

There was a strong association between the reported occurrence of recent outbreaks resulting in 40.4%, 95% CI [27.6-54.2] of the positive animals being pigs that had been exposed to an outbreak between 1 and 8 months prior to sampling (OR=5.7, p=0.004). There was also a significant association (OR=1.93, p=0.0276) between those farms which experienced recent outbreaks and the presence of piglets (younger than 6 months).

2.4.3. Soft tick collection and ASFV and DNA detection

Two of the 20 inspected pig pens in the GNPBZ and ROD were found to be infested with 203 *Ornithodoros* spp. ticks, corresponding to an infestation prevalence of 10%, 95% CI [3.1 -23.1]. In both cases the pens were in the GNPBZ. African swine fever virus DNA was detected in homogenates of ticks from both of the infested pig pens and live virus was isolated from one pool (results not shown). All samples, with the exception of the negative controls, were positive in the 16S soft tick mitochondrial gene PCR confirming the integrity of template DNA and precluding the possibility of false negative results (results not shown).

Table 2.1. Summary of the villages, number of farms and percentage sero-positivity for ASFV and tick salivary proteins in the Gorongosa District

Village	No. of pigs	No. of farms	% pigs with abs to ASFV	% farms with pigs sero-positive to ASFV	% pigs with abs to tick salivary proteins	% farms with pigs sero-positive to tick salivary proteins
Aerodromo	64	13	1.6	7.7	9.4	38.5
Canda	60	15	0	0	51.7	80.0
Gorongosa sede	74	34	2.7	5.9	33.8	38.2
Madibe	39	34	0	0	25.6	29.4
Missão Cristo Rei	11	1	18.2	100	N/D	N/D
Mukodza	42	18	0	0	54.8	72.2
Mutukuduri	27	17	33.3	41.2	14.8	23.5
Nhanguo	2	2	0	0	100	100
Nhataca	4	4	0	0	N/D	N/D
Tambarara	72	49	19.4	24.5	27.3	26.4
Twassicana	22	14	22.7	14.3	13.6	21.4
Vunduzi	42	30	7.3	10.0	9.5	10.0
Matchisso	14	10	0	0	28.6	40.0
Mbulawa	23	3	0	0	N/D	N/D
Mutiwambamba	45	16	13.3	37.5	40.0	56.3
Nhambita	6	1	0	0	N/D	N/D
Pungue	59	45	3.4	2.2	64.4	73.3
Tsiquiri	23	4	59.1	75.0	17.4	75.0
Total	**629**	**310**	**9.1** (7.0-11.7)*	**12.6** (8.9-16.3)*	**32.7** (29.2-36.9)*	**42.0** (36.4-47.7)*

In dark grey, villages located in the Gorongosa National Park Buffer Zone, N/D=not done, CI=confidence interval, abs=antibodies. *Presented as point estimate with the corresponding 95% CI

2.4.4. Detection of antibodies against tick salivary protein

Warthogs

The prevalence of antibodies against tick salivary proteins in the sampled warthog population was 77.8%, 95% CI [40.0-97.2]. When different age groups of warthogs were compared, all warthogs less than 1 year (n=3) and 66.7%, 95% CI [22.3-95.7] of older animals (n=9) showed sero-positivity against the rOmTSGP1 antigen.

Domestic pigs

Of the 629 pig sera collected in the field, 44 from 9 farms (Table 2.1) were not tested as there was insufficient serum available to perform the analysis. The overall prevalence of antibodies to tick salivary proteins among the 585 domestic pigs tested was 32.7%, 95% CI [29.2-36.9], with 42.0%, 95% CI [36.4-47.7] of farms positive. Approximately 45% of pigs (64/140 sera) in the GNPBZ were sero-positive to tick salivary proteins (95% CI [37.3-54.3]), compared to 28.9%, 95% CI [24.8-33.4] of pigs (129/445) in the ROD. The on-farm prevalence in the GNPBZ and the ROD was 66.2%, 95% CI [54.3-76.8] and 34.2%, 95% CI [28.1-40.7], respectively (Table 2). Significant differences were observed in the proportion of sero-positive domestic pigs (OR=0.48, p=0002) and sero-positive farms (OR=0.27, p=0.0001) between the two areas (Table 2.2).

Table 2. 2. Comparison of sero-prevalence to ASFV and tick salivary proteins (rOmTSGP1) in the Gorongosa National Park Buffer Zone (GNPBZ) and the rest of the district (ROD)

Seroprevalence (%)	**GNPBZ**	**ROD**	**OR**	***p value***
ASFV (pigs)	12.5	7.8	0.59	0.05
ASFV (farms)	12.8	12.5	0.97	0.53
rOmTSGP1 (pigs)	45.7	28.9	0.48	0.0002
rOmTSGP1(farms)	66.2	34.2	0.27	0.0001

When the sero-prevalence of tick-salivary proteins in pigs of different ages was compared, antibodies were found in 32.0%, 95% CI [27.9-36.3] of the animals younger than one year (n=493) and 38.0%, 95%, CI [28.1-48.8] of the animals older than one year, respectively but these differences were not significant (p=0.16).

2.4.5. Correlation between prevalence of antibodies to ASFV and tick salivary proteins

Among the 585 sera tested for the presence of antibodies to ASFV and the rOmTSGP1antigen, only 20.0%, 95% CI [10.4-33.0] contained antibodies to both antigens. The association between the tests was statistically significant (OR=0.48, p=0.01). Of the 301 farms, 28.6%, 95% CI [14.6-46.31] had pigs that tested positive in both assays. However, this association was only marginally significant (OR=0.51, p=0.056). Sera collected from pigs in four villages in the ROD and one village in the GNPBZ only contained antibodies to tick antigens.

2.5. DISCUSSION

The socio-economic impact of ASF is due to high mortality and morbidity rates that can threaten food security, as pigs represent a source of cheap protein and income to many poor people (Costard *et al.*, 2009a, 2009b). In African countries and other developing nations ASF impacts negatively on the sustainability of pork production. Farmers lack the resources to implement prevention or control measures and are often unable to restart production following an outbreak (Edelsten & Chinombo, 1995). This is demonstrated by the fact that 36% of farmers interviewed in this study ceased to farm with pigs for at least 1 year following an ASF outbreak. The questionnaire used in this study also revealed that most villagers sold their stock alive or slaughtered without reporting the disease to local veterinary authorities. Similar behaviour by farmers following ASF outbreaks has previously been shown to contribute to the spread of ASF in other developing countries (Nana-Nukechap & Gibbs, 1985; Costard *et al.*, 2009b; Fasina *et al.*, 2010; Penrith *et al.*, 2013) and could account for the low number of adult pigs observed in our study.

The combination of serology and a questionnaire provided a good indication that ASFV was circulating in the area a few months before blood samples were collected, as >40% of ASFV sero-positive animals had been exposed to an ASFV outbreak less than three months prior to sampling. The overall sero-prevalence of ASFV (9.1%) observed in domestic pigs in the GD was slightly lower compared to the results obtained in the Angónia District (14.3%) of Mozambique (Penrith *et al*., 2007; Matos *et al.*, 2011) and significantly lower when compared with the serological data of a survey carried out in the Mchinje District (48%) of Malawi (Haresnape *et al.,* 1985). African swine fever is endemic in both these areas and regular outbreaks of the disease occur, often characterised by lower than usual mortality rates in adult domestic pigs (Haresnape & Wilkinson, 1989; Penrith *et al.*, 2004a).

The results of this study strongly suggest that the sylvatic cycle is present in the GNP. The serological survey in warthogs presented in this study, despite its limited size, is the first described in Mozambique to date. The high sero-prevalence to ASFV (75%) and tick salivary proteins (78%) is consistent with previous observations in warthog populations from other parts of East and southern Africa (Penrith *et al.*, 2004b; Jori & Bastos, 2009; Jori *et al.*, 2013). Although the GNP is not fenced, warthogs do not venture too far away as they are hunted for food by local rural communities once they reach the GNPBZ and ROD.

The prevalence of ASF per farm was highly similar in both areas, however, a marginally significant (p=0.05) higher sero-prevalence was detected in the GNPBZ compared to ROD (12.5% versus 7.8%). Furthermore, 80% of farmers who reported not having experienced any outbreaks on their farm were located in the ROD area. This suggests that ASFV infections could be more common in the buffer zone farms, which are located closer to the park and to a permanent source of virus from wild host than the farms in the ROD.

The results of the serological assessment of antibodies to tick salivary protein provided evidence that 42% of the farms had pigs that had been bitten by soft ticks recently and support the premise that contact between soft ticks and domestic pigs is widespread in the area. In addition, the significant differences in prevalence of antibodies to the tick antigen in the GNPBZ compared to the ROD suggest that the proximity of wildlife areas is a major contributing factor to the exposure of domestic pigs to soft ticks. The detection of ASFV DNA and infectious virus in ticks collected from two farms outside GNP provide evidence of the presence of the infected

ASFV vector in pigsties outside wildlife areas. Pigs from a number of farms did not have antibodies to ASFV, but were positive for antibodies to the rOmTSGP1 antigen, which could indicate that the ASF infection rate in ticks may be low and needs further study.

2.6. CONCLUSIONS

Results from this study showed that ASFV is maintained within the sylvatic cycle involving warthogs and *Ornithodoros spp.* ticks in the GNP as evidenced by the presence of ASFV infected ticks in warthog burrows and the high percentage of warthogs with antibodies to the virus. The demonstration of antibodies to salivary proteins of *Ornithodoros* spp. ticks and finding ASFV infected *Ornithodoros* spp. ticks in pig pens suggest that domestic pigs are periodically exposed to soft ticks which may lead to the dissemination of the virus beyond the sylvatic cycle. However, the contribution of the domestic pig/tick cycle in the epidemiology of the disease in our study area seems to be limited considering the low level of infestation of pig pens with infected ticks. Conversely, the survival rate among infected pigs is higher than would be expected suggesting that domestic pigs in GD may have developed a degree of resistance to the pathogenic effect of ASFV, similar to what has previously been described for Angonia and the Mchinje district of Malawi.

It is likely that the presence of this sylvatic cycle at the GNP interface acts as a permanent source of ASFV for domestic pigs. Further studies are necessary in Mozambique to identify the role of other wildlife areas as sources of ASF in order to design management plans to control and prevent the transmission of the virus to the domestic pig value chain and its dissemination at regional, national or international level.

CHAPTER 3

GENETIC CHARACTERIZATION OF AFRICAN SWINE FEVER VIRUS ISOLATES FROM SOFT TICKS AT THE WILDLIFE/DOMESTIC INTERFACE OF GORONGOSA NATIONAL PARK, MOZAMBIQUE

3.1. SUMMARY

African swine fever (ASF) is a serious infectious diseases for the swine industry worldwide and is endemic in Mozambique. The role of the sylvatic cycle in the maintenance and dissemination of ASF virus (ASFV) strains to the domestic pig has not been sufficiently investigated. To address this gap, a study was conducted to detect ASFV from *Ornithodoros* spp. ticks present in wild and domestic pig habitats at the interface of the Gorongosa National Park (GNP), Central Mozambique. A total of 1662 *O. porcinus porcinus* and 203 *O. p. domesticus* specimens recovered from 29 warthog burrows in the GNP and 2 pig pens respectively, were tested for the presence of live virus and viral DNA. African swine fever viral DNA was confirmed by nested PCR in 18.8% of *O. p. porcinus* and 15.4% of *O. p. domesticus*. The 19 haemadsorbing viruses isolated were genotyped using a combination of partial gene sequencing (*p72, p30* and *p54)* and analysis of the central variable region (CVR) of the *B602L* gene. Eleven of the isolates were classified as genotype II and were homologous to contemporary isolates from southern Africa, the Indian Ocean and eastern Europe. Three isolates grouped within Genotype V and were similar to isolates from Mozambique and Malawi. The remaining 5 isolates constituted a new genotype previously unidentified. This work confirms for the first time the capacity of *Ornithodoros* ticks from natural and domestic environments in Mozambique to act as a permanent source of ASFV infection to domestic pigs in Mozambique and beyond. These findings justify the need to continue studying the sylvatic cycle in other protected areas of Mozambique in order to identify potential hot spots of ASF emergence and target surveillance and control efforts.

3.2. INTRODUCTION

African swine fever (ASF) is an infection caused by the ASF virus (ASFV), the only member of the Asfarviridae family, genus Asfivirus (Dixon *et al*., 2000), which often leads to high mortalities (Penrith *et al.*, 2004a, 2013) and cause devastating impacts in the pig industry of many countries in sub-Saharan Africa, the Indian Ocean and more recently, the Balkans and eastern Europe (Costard *et al.,* 2009b; Gogin *et al.,* 2013). The disease is highly contagious; the affected pigs showing symptoms characterized by fever and hemorrhagic lesions in affected organs (Penrith *et al.,* 2004a).

The disease is maintained through a sylvatic cycle involving warthogs (*Phacochoerus africanus),* which are resistant to ASFV and do not show clinical symptoms, and soft ticks of the *Ornithodoros moubata* complex (Thomson, 1985; Jori & Bastos, 2009). The virus is transmitted to ticks by feeding on infected warthogs and maintained in successive generations by trans-ovarial, venereal and trans-stadial transmission (Plowright *et al*., 1974; Wilkinson, 1989; Kleiboeker *et al.*, 1998; Rennie *et al*., 2001). It has been shown that the probability of infection in domestic pigs significantly increases in areas where a warthog–tick cycle occurs (Jori & Bastos, 2009). Transmission of the virus from the sylvatic cycle to domestic pigs occurs via tick bites from infected soft ticks (Plowright, 1984; Kleiboeker *et al.*, 1998; Penrith *et al*., 2004a). The boundaries of protected areas in East and southern Africa where large populations of warthogs and *O. moubata* ticks are present (referred in the literature as wildlife/domestic interface) represent key areas for ASFV dissemination between domestic and wild species (Jori *et al*., 2007, 2011, 2013; Jori & Bastos, 2009). The ASF sylvatic cycle has been reported and described in detail in many countries in East and southern Africa (Okoth *et al*., 2013), with the exception of Mozambique where interactions between wild pigs, soft ticks and the domestic pig have only been reported very recently (Penrith *et al*., 2007; Quembo *et al*., 2014).

A baseline survey conducted at the wildlife/domestic interface of Gorongosa National Park (GNP) showed that outbreaks in that area occurred repeatedly over time (Penrith *et al*., 2007; Quembo *et al*., 2014). In addition, our study area encompassed several risk factors known to be associated with the occurrence and dissemination of ASF infection such as a) an extensive

production system where pigs roam freely (Quembo *et al*., 2014); b) an absence of bio-security measures on the pig premises and c) the existence of a main road used as a trade route for live pigs and their products between the GD and a major pig production and consumption region such as the coastal City of Beira. Small-scale pig farmers on the outskirts of GNP mentioned that the persistence of ASF outbreaks in the region may be due to pigs moving inside GNP and getting in contact with wildlife and its ASFV associated vector (Penrith *et al*., 2007). However, this hypothesis has never been supported scientifically until recently (Quembo *et al*., 2014). Additionally, no information has been published to date on the presence and genetic characteristics of ASF viruses maintained among wild hosts in Mozambican wildlife areas.

African swine fever virus is the only double stranded DNA-containing arbovirus (dsDNA) with a genome of 170 to 193 kilobase pairs (kbp), the size depending on the isolate (Blasco *et al*., 1989a; Dixon *et al*., 2013). The genome is characterized by terminal inverted repeats (TIR) and hairpin loops at both ends (Blasco *et al*., 1989b). The central region of the genome is highly conserved and is flanked by a left variable region (LVR) and right variable region (RVR) where most genes are located that have been used for genotyping ASF viruses (Yanez *et al*., 1995). Four different gene regions namely *p72, p30, p54* as well as the *B602L* gene, located in the central variable region (CVR) of the 9RL open reading frame (ORF), have been targeted to detect ASFV phylogenetic relationships with different levels of precision (Irusta *et al*., 1996; Bastos *et al*., 2004; Nix *et al*., 2006; Gallardo *et al*., 2009a, 2011). Molecular analysis of ASFV strains worldwide has suggested that genetic homogeneity within genotypes appears more frequently in areas where only the domestic pig cycle is present, without the involvement of wild hosts (Bastos *et al*., 2003, 2004; Lubisi *et al*., 2007). Conversely, in certain endemic areas of southern and eastern Africa where the warthog–tick sylvatic cycle exists, the genetic diversity of ASFV circulating strains has been reported to be higher (Bastos *et al*., 2009; Penrith & Vosloo, 2009, Gallardo *et al.,* 2011; Penrith *et al*., 2013).

Therefore, the aims of this investigation were a) to identify the presence of soft ticks at the wildlife/domestic interface of GNP, b) to determine the infectious status of these vectors with regards to ASFV, c) to characterize the genetic diversity of viruses found in soft ticks, d) to compare the genetic diversity of these viruses with those found in other locations.

3.3. MATERIALS AND METHODS

3.3.1. Study area

The field work was carried out in GD, Province of Sofala, located in the central area of Mozambique, between latitude 18^0 45'/19^0 15' South and longitude 33^0 30'/34^0 45' East. The GD covers a total area of 7 659 km² with approximately 92 550 habitants, widespread in 19 villages (District Agricultural Directorate, unpublished report). At least 30% of its population are small-scale farmers (with an average 1.5 ha of land per farmer), who mainly live from subsistence agricultural activities such as raising livestock (mainly local cattle breeds, free-range poultry and pigs) and growing crops (District Agricultural Directorate, unpublished report).

Half of the GD surface is occupied by the Gorongosa National Park (GNP), which covers a total area of 3 770 km^2 represented by a diversity of habitats such as humid rainforest on the slopes of Mount Gorongosa, dry woodland savanna on the Cheringoma Plateau and extensive, flat grasslands on the floodplains of Lake Urema (www.Gorongosa.org - accessed on the 21st November 2013). A predominant dry tropical climate is influenced by altitude on the western side of the district and by the presence of tropical rainforest in the eastern boundary. Average rainfall varies from 900 to 2 000 mm per year particularly during November and April, and the average daily temperature varies from 25 to 33^0C (www.inam.gov.mz - accessed on the 21st November 2013). The pig production systems in the district, including the GNPBZ, are characterized by basic small-scale pigsties where approximate 17 348 pigs are left to roam freely throughout the year, except during the cropping season from October to April (Quembo *et al.*, 2014).

The wildlife/domestic interface of the GNP encompasses 6 villages which are located less than 10 km from the GNP. This area, where interactions between domestic pigs and wild hosts are considered to be common, is hereafter named the GNP Buffer Zone (GNPBZ). This area has experienced several suspected outbreaks of ASF since the year 2005, although those were not confirmed by laboratory testing (Penrith *et al.*, 2007; Quembo *et al.*, 2014).

3.3.2. Sampling protocol

Tick sampling in the GNP was carried out in warthog burrows. To determine the sample size, we considered an estimated population of 4 000 warthogs in the GNP (Pereira, C.L. personal communication), an average size of 4 individuals per warthog sounder and an average of 3 burrows used by a sounder (Estes, 1999). Therefore, the estimated numbers of burrows in the park was expected to be at least 3 000. The expected prevalence of warthog burrows infested with *O. moubata* soft ticks was estimated to be between 30% and 88% on the basis of previous reports from areas of East and southern Africa (Plowright *et al*., 1994). Using Win Episcope 2.0 (Thrusfield *et al*., 2001), the minimum sample size estimated to detect at least one infested burrow, considering a minimum tick infestation rate of 30% with a 95% level of confidence, was calculated to be at least 9 burrows.

The sampling frame and the characteristics of domestic pig farms were described before (Quembo *et al*., 2014). A prevalence of 30.7% of soft tick infested farms, reported in domestic pig pens in other locations (Haresnape & Wilkinson, 1989; Oleaga-Perez *et al*., 1990), was used as the expected prevalence in our area. A minimum sample of 9 pig pens per village to detect at least one infested domestic pig pen was estimated with the level of 95% of confidence, based on Win Episcope 2.0 (Thrusfield *et al*., 2001). Therefore, 162 pig pens were expected to be inspected for soft tick sampling.

3.3.3. Tick sampling

In September 2006, a survey was undertaken in 32 warthog burrows in the GNP to collect soft ticks. The sampling was performed by driving along the available roads in the GNP and locating areas with sparse vegetation that allowed walking and searching for warthog burrows. The geographic coordinates for each burrow were recorded and plotted using a GPS (Figure 3.1). Two combined sampling methods were employed to collect ticks from the burrows: first, loose sand and litter were removed manually using a spade, followed by vacuum aspiration to collect

material from cracks in the walls and lining of the warthog burrows (Butler & Gibbs, 1984; Ravaomanana *et al*., 2010; Jori *et al*., 2013).

Random sampling for soft ticks in domestic pig pens was conducted between March 2006 and September 2007 in the ROD and GNPBZ as recently described (Quembo *et al*., 2014).

The ticks collected by either method were placed in 10 ml labeled containers filled with sand and covered with a perforated lid. These specimens were dispatched live from Mozambique to the Transboundary Animal Disease Programme (TADP), ARC-Onderstepoort Veterinary Institute (OVI) in South Africa for further analysis, under a permit issued by the South African Department of Agriculture Forestry and Fisheries (DAFF).

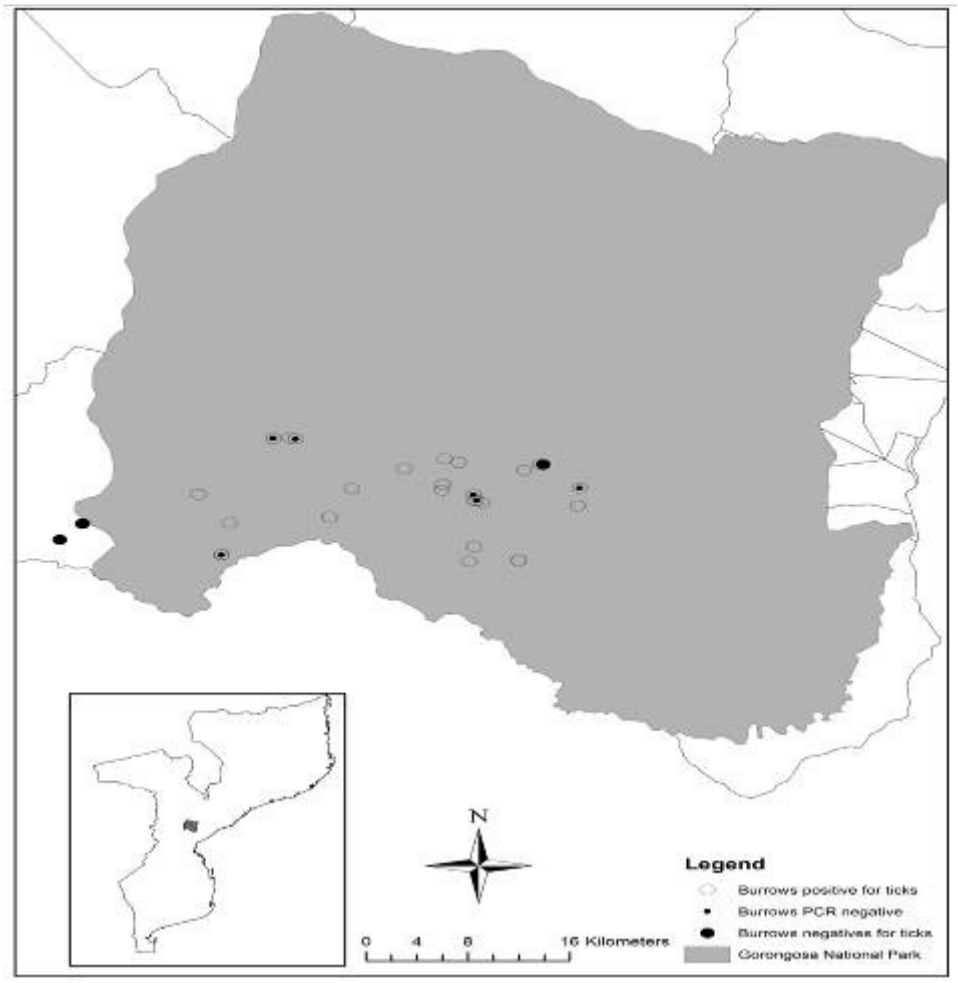

Figure 3.1. Warthog burrows locations in the GNP.

3.3.4. Tick sample classification

The soft ticks were sorted into different categories depending on their stage of development using standard methods and previously published guidelines (Walton, 1962, 1979). Adult ticks (males and females) were examined in pools of three specimens per habitat (warthog burrow or pig pen) whilst the immature stages (N1 to N5), were pooled in groups of maximum 6 individuals per developmental stage and habitat. Pools of all eggs collected from each sampling point were tested. Each pool was crushed in a 1.5 ml Eppendorf tube containing 1 ml of phosphate buffered saline (PBS) supplemented with 1% foetal calf serum (FCS) and 1% of a combination of antibiotics and anti-mycotics. The homogenates were clarified by centrifugation at 10 000 x g for 1 min and the supernatant stored at -70°C.

3.3.5. Virus isolation

ASF viruses were isolated from soft ticks using an haemadsorption assay previously described (Malmquist & Hay, 1960; Quembo *et al*., 2014). A portion of the supernatant (100 µl) from the tick homogenates prepared as described in the previous chapter section 2.3.3.2. was inoculated onto peripheral blood mononuclear cells (PBMC) cultured following standard procedures (Malmquist & Hay, 1960; OIE, 2012). All samples were subjected to three blind serial passages on PBMC (Quembo *et al*., 2014).

3.3.6. DNA extraction and PCR

DNA was extracted using two different methods, either directly from 200 µl of each infected cell culture by using a commercial kit (Qiagen GmbH, Hilden) or from 200 µl aliquots of tick homogenates following a silica/guanidium-based nucleic acid extraction protocol (Boom *et al.,* 1990) then stored at -80° C.

A nested PCR was used to screen soft tick samples for the presence of ASFV DNA (Basto *et al*., 2006; Quembo *et al*., 2014). In order to exclude the occurrence of false-negative results, all DNA samples were tested for tick 16S rDNA as previously published (Black & Piesman, 1994; Vial *et al*., 2007; Ravaomanana *et al*., 2010). A pig farm or a warthog burrow was considered positive to ASFV when at least one soft tick from such farm or burrow was found positive.

Samples that tested positive were subsequently used to amplify various gene regions for nucleotide sequencing. Genomic amplification using DNA recovered from infected cells and those that were positive on nested PCR was carried out in a 50 µl volume in the presence of 2x Kappa ready mix (Biosystems), 10 pmol/µl of each primer *p72*-D and *p72*-U which generate an amplicon of 478 bp within *p72* gene (Bastos *et al*., 2003), for intermediate genotyping resolution the sets of primers BamHI/EcoRI and E183F/R were used to generate 561 bp and 681 bp amplicons of *p30* and *p54* genes respectively (Rodriguez *et al*., 1994; Ovideo *et al*., 1997) and for higher resolution, the CVR1/CVR2 primer set was used to amplify *B206L* gene (Nix *et al*., 2006) expecting amplicons ranging from 300 to 500 bp. PCR products were loaded on a 1% agarose gel and electrophoresed against a 100 bp DNA ladder (stained by 0.5% µg/ml of ethidium bromide).

3.3.7. DNA sequence and phylogenetic analysis

Polymerase chain reaction products of each amplified gene region were excised from the gel and purified using the Nucleospin Extract Kit (Macherey-Nagel, GmbH & Co. KG (Düren, Germany) as per the manufacturer's instructions. The nucleotide sequence of the purified products was obtained by automated sequencing at Inqaba Biotech™ (Pretoria, South Africa) using the Big Dye terminator kit (Applied Biosystems, USA) and the same primers used for amplification.

Fifty two ASFV isolates representative of 22 *p72* genotypes isolated in this study and others from several countries from sub-Saharan Africa (Bastos *et al*., 2003, 2004), the Indian Ocean (Lubisi *et al*., 2009) and Europe (Lubisi *et al*., 2005, 2009; Nix *et al*., 2006; Boshoff *et al*., 2007;

Chapman *et al*., 2008) were included in the analysis (Table 3.1). From this dataset, 44 isolates were chosen to perform the phylogenetic analysis for the *p30* and *p54* genes and 22 for the CVR-PCR analysis (Table 3.1).

Nucleotide sequences were edited and aligned using Sequencher™ Gene Codes Version 4.8 (Gene codes Corporation, Michigan, USA) and Mega 5.0 (Tamura *et al*., 2007) software packages respectively. The latter software was also used to construct neighbour-joining (NJ) phylogenetic trees of *p72*, *p30* and *p54* gene regions, algorithm with nodal support being assessed by bootstrap 1000 replicates, minimal evolution (ME) and Bayesian inference (Tamura *et al*., 2007).

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Table 3.1: List of the 52 ASFV isolates from different origin and those obtained in our study collected in soft ticks at the GNP and the interface

Virus Name	Place/Country of origin	Host species (Soft tick stage reference)	*P72* Genbank accession Number	*P72* Genotype	Reference	*CVR* Genbank accession Number	Length of aa for *CVR*	Reference	*P30* Genbank accession Number	Reference	*P54* Genbank accession Number	Reference
Mozambique/1960	Tete/Mozambique	Domestic pig	AF270708	V	Bastos *et al*., (2004)	AY274465	88	Bastos *et al*., (2004)	EU874309	Unpublished	EU874378	Unpublished
Tengani/60	Tengani/Malawi	Warthog	AF301541	V	Bastos *et al*., (2003)	AY538727	28	Bastos *et al*., (2004)	EU874296	Unpublished	EU874318	Unpublished
MAL/1/02	Mpemba/Malawi	Domestic pig	AY494553	V	Lubisi *et al.,* (2005)	N/A	N/A	N/A	EU874311	Unpublished	EU874373	Unpublished
Mozambique/1979	Beira/Mozambique	Domestic pig	AF270709	V	Bastos *et al*., (2004)	AY274470	48	Bastos *et al*., (2004)	EU874372	Unpublished	EU874310	Unpublished
SPEC265	Maputo/Mozambique	Domestic pig	AF270710	VI	Bastos *et al*., (2003)	AY274467	64	Bastos *et al*., (2004)	EU874264	Unpublished	EU874344	Unpublished
MOZ/8/94	Manica/Mozambique	Domestic pig	AF270712	VI	Bastos *et al*., (2004)	AY274469	64	Bastos *et al*., (2004)	EU874276	Unpublished	EU874343	Unpublished
MOZ/1/94	Maputo/Mozambique	Domestic pig	AF270711	VI	Bastos *et al*., (2003)	AY274468	64	Bastos *et al*., (2004)	EU874263	Unpublished	EU874342	Unpublished
Lillie	RSA	Domestic pig	DQ250109	XX	Boshoff *et al.*, (2007)	DQ250109	52	Boshoff *et al.*, (2007)	EU874306	Unpublished	EU874341	Unpublished
RSA/1/99/Thab	Thabazimbi/RSA	Warthog	AF302818	IV	Bastos *et al*., (2003)	DQ250108	52	Boshoff *et al.*, (2007)	EU874307	Unpublished	EU874369	Unpublished
RSA/1/98	Potgietersrus/RSA	Domestic pig	AF302818	VII	Bastos *et al*., (2003)	DQ250106	56	Boshoff *et al.*, (2007)	EU874312	Unpublished	EU874374	Unpublished
MAD/1/98	Madacascar	Domestic pig	AF270706	II	Bastos *et al*., (2003)	AY274471	40	Bastos *et al*., (2004)	N/A	N/A	N/A	N/A
MAU/2007/1	Mauritius	Domestic pig	FJ528594	II	Lubisi *et al*., (2009)	N/A	N/A	Unpublished	EU874316	Unpublished	EU874384	Unpublished
MOZ/2/02	Nampula/Mozambique	Domestic pig	AY351518	II	Lubisi *et al*., (2005)	N/A	N/A	Unpublished	EU874274	Unpublished	EU874376	Unpublished
ZIM/1/92	Gweru/Zimbabwe	Domestic pig	DQ250119	XVII	Boshoff *et al.*, (2007)	DQ250097	48	Boshoff *et al.*, (2007)	N/A	N/A	EU874345	Unpublished
Lisbon/60	Lisbon/Portugal	Domestic pig	AF301539	I	Bastos *et al*., (2003)	AM259405	N/A	Nix *et al*., (2006)	EU874273	Unpublished	EU874321	Unpublished
BEN/1/97	Benin	Domestic pig	AF302816	I	Bastos *et al*., (2003)	AM712239	144	Chapman *et al*., (2008)	N/A	N/A	EU874382	Unpublished
ANG/70	Angola	Domestic pig	AF301542	I	Bastos *et al*., (2003)	AM259411	56	Nix *et al*., (2006)	EU874271	Unpublished	EU874327	Unpublished
SPEC/205	Windhoek/Namibia	Domestic pig	DQ250114	I	Boshoff *et al.*, (2007)	DQ250092	44	Boshoff *et al.*, (2007)	EU874291	Unpublished	EU874359	Unpublished
NYA/12	Kalumo/Zambia	Ticks	AY351555	XIV	Lubisi *et al*., (2005)	N/A	N/A	N/A	EU874302	Unpublished	EU874330	Unpublished
TAN/2/03	Arusha/Tanzania	Domestic pig	AY494551	XVI	Lubisi *et al*., (2005)	N/A	N/A	N/A	EU874255	Unpublished	EU874355	Unpublished
TAN/1/01	Tanzania	Domestic pig	AY494552	XV	Lubisi *et al*., (2005)	N/A	N/A	N/A	EU874303	Unpublished	EU874356	Unpublished
SUM/1411	Sumbu Park/Zambia	Ticks	AY351542	XIII	Lubisi *et al*., (2005)	N/A	N/A	N/A	EU874287	Unpublished	EU874357	Unpublished
MZI/1/92	Mzinda/Malawi	Domestic pig	AY351543	XII	Lubisi *et al*., (2005)	N/A	N/A	N/A	EU874288	Unpublished	EU874360	Unpublished
KAB/6/2	Livingstone/Zambia	Ticks	AY351522	XI	Lubisi *et al*., (2005)	N/A	N/A	N/A	EU874289	Unpublished	EU874331	Unpublished
SPEC/257	Ellisras/ South Africa	N/K	DQ250120	III	Boshoff *et al.*, (2007)	DQ250098	52	Boshoff *et al.*, (2007)	EU874265	Unpublished	EU874338	Unpublished
RSA/96/3	Pienaarsrivier/RSA	N/K	DQ250127	XIX	Boshoff *et al.*, (2007)	DQ250105	52	Boshoff *et al.*, (2007)	EU874283	Unpublished	EU874375	Unpublished
RSA/96/1	Gravelotte/RSA	N/K	DQ250125	XXI	Boshoff *et al.*, (2007)	DQ250103	40	Boshoff *et al.*, (2007)	N/A	N/A	EU874339	Unpublished
SPEC/245	Louis Trichardt/RSA	N/K	DQ250117	XXII	Boshoff *et al.*, (2007)	DQ250095	52	Boshoff *et al.*, (2007)	N/A	N/A	EU874381	Unpublished
NAM/1/95	Windhoek, Namibia	N/K	DQ250122	XVIII	Boshoff *et al.*, (2007)	DQ250100	84	Boshoff *et al.*, (2007)	N/A	N/A	N/A	N/A
MOZ/1/98	Tete/Mozambique	Domestic pig	AF270705	VIII	Bastos *et al*., (2003)	N/A	92	OVI unpublished	EU874317	Unpublished	EU874385	Unpublished
BUR/1/84	Burundi	Domestic pig	AF449463	X	Bastos *et al*., (2003)	AM259422	90	Nix *et al*., (2006)	EU874298	Unpublished	EU874364	Unpublished
UGA/1B/03	Uganda	Domestic pig	AY351564	IX	Lubisi *et al*., (2005)	AM259420	N/A	Nix *et al*., (2006)	N/A	N/A	N/A	N/A
UGA/1/95	Mulindue/Uganda	Domestic pig	AF449475	IX	Bastos *et al*., (2003)	N/A	N/A	N/A	EU874300	Unpublished	EU874362	Unpublished
Moz 1/2006	Gorongosa National Park	Soft tick (N2)		II	This study		84	This study		This study		This study
Moz 2/2006	Gorongosa National Park	Soft tick (N4)		II	This study		40	This study		This study		This study
Moz 3/2006	Gorongosa National Park	Soft tick (N5)		II	This study		40	This study		This study		This study
Moz 4/2006	Gorongosa National Park	Soft tick (N5)		II	This study		40	This study		This study		This study
Moz 5/2006	Gorongosa National Park	Soft tick (N1)		II	This study		40	This study		This study		This study
Moz 6/2006	Gorongosa National Park	Soft tick (N4)		II	This study		N/A	This study		This study		This study
Moz 7/2006	Gorongosa National Park	Soft tick (N5)		II	This study		40	This study		This study		This study
Moz 8/2006	Gorongosa National Park	Soft tick (N5)		II	This study		40	This study		This study		This study

Moz 9/2006	Gorongosa National Park	Soft tick (eggs)	II	This study	40	This study	This study	This study
Moz 10/2006	Gorongosa National Park	Soft tick (Male)	XXIII	This study	36	This study	This study	This study
Moz 11/2006	Gorongosa National Park	Soft tick (N4)	XXIII	This study	N/A	This study	This study	This study
Moz 12/2006	Gorongosa National Park	Soft tick (N4)	II	This study	40	This study	This study	This study
Moz 13/2006	Gorongosa National Park	Soft tick (Female)	II	This study	40	This study	This study	This study
Moz 14/2006	Gorongosa National Park	Soft tick (Female)	V	This study	48	This study	This study	This study
Moz 15/2006	Gorongosa National Park	Soft tick (N4)	V	This study	84	This study	This study	This study
Moz 16/2006	Gorongosa National Park	Soft tick (N4)	XXIII	This study	44	This study	This study	This study
Moz 17/2006	Gorongosa National Park	Soft tick (N3)	V	This study	48	This study	This study	This study
Moz 18/2006	Buffer zone	Soft tick (N3)	XXIII	This study	144	This study	This study	This study
Moz 19/2006	Buffer zone	Soft tick (N4)	XXIII	This study	N/A	This study	This study	This study

N/A=Not available
N/K=Not known

3.4. RESULTS

3.4.1. Soft tick collection

A total of 1662 argasid ticks were collected from the GNP found in 29 out of 32 inspected warthog burrows. Therefore, the apparent prevalence of soft tick infestation was 90.6%, 95% CI [82.2 - 99.0] in warthog burrows. Due to suspicions of recently occurring ASF outbreaks and the presence of abundant mud in domestic pigsties during the rainy season, only 20 could be inspected for soft ticks. The apparent prevalence in that sample was 10%, 95% CI [3.1 -23.1].

The proportions of tick specimens collected by zone (GNP and GNPBZ), sex and stage of development and location are shown in Table 3.2. The majority (89.1%, 95% CI [87.7 -90.5]) was identified as *O. porcinus porcinus*, while only 10.9%, 95% CI [9.5 -12.3] was classified as *O. p. domesticus.* Speciation was confirmed by means of 16S rDNA gene sequencing (data not shown). Numbers for soft tick eggs were not recorded as there were too many.

The most predominant tick stages collected in warthog burrows and pig pens were immature or nymph specimens (N1 to N5), whereas adults were less common. Female soft ticks were the most abundant among the adults while N5 nymphs were the most represented of the immature stages (Table 3.2).

Additionally, 15 hard ticks of the genera *Amblyomma*, *Haemophysalis* and *Rhipicephalus* were found in the burrows only, but were not included for ASF viral analysis.

Table 3.2: Observed prevalence of ASFV in different development stages of *Ornithodoros* spp. found at the GNP interface.

	GNP				GNPBZ			
Development stage	**N° of soft ticks collected**	**N° of pools of tested soft ticks**	**N° and (%) positives for nested PCR ***	**N° and (%) of ASFV isolation [1]**	**N° of soft ticks collected**	**N° of pools of tested soft ticks**	**N° and (%) positives for nested PCR ***	**N° and (%) of ASFV isolation [1]**
Male	115	61	4 (6.6)	2 (50.0)	6	3	0	0
Female	130	52	10 (19.2)	5 (50.0)	9	3	0	0
N1	26	9	4 (44.4)	2 (50.0)	0	0	0	0
N2	78	16	2 (12.5)	1 (50.0)	31	3	1 (33.3)	0
N3	250	41	14 (34.1)	4 (28.6)	14	2	1 (50.0)	1 (100)
N4	353	43	13 (30.2)	7 (53.8)	62	7	2 (28.6)	1 (50.0)
N5	710	77	8 (10.4)	5 (62.5)	81	8	0	0
Eggs	Not counted	5	2 (40.0)	1 (50.0)	0	0	0	0
Total	1662	304	57 (18.8)	27 (47.4)	203	26	4 (15.4)	2 (50.0)

**** Percentage of positive samples using nested PCR as described by Basto et al., (2006) in relation to the number of individuals (male or female) and pools (immature stages) of tested soft ticks. [1] Percentage of positive isolations was calculated in relation to the number of individuals or pools of ticks positive to Nested PCR. The soft tick eggs were so many and difficult to record***

3.4.2. Analysis of ASFV infection in collected soft ticks

A total of 1865 soft ticks were collected from two clusters (GNP and GNPBZ) and a total of 330 pools were made. Out of these, ASFV genetic material was recovered from 61 pools and a total of 29 ASFV isolates were obtained. Only 2 of the 29 viruses originated from soft ticks collected from pig pens in the GNPBZ with the rest recovered from warthog burrows, representing a virus recovery rate of 47.4% (Table 3.2).

Fifty seven of the 304 pooled samples collected from warthog burrows tested positive of ASFV DNA, representing an overall prevalence of infection of 18.8%, 95% CI [14.4-23.1]. Soft ticks collected from two pig pens in the buffer zone tested positive of ASFV DNA. The prevalence of ASFV detected by PCR in the 26 pooled samples collected in two pigsties was calculated to be 15.4%, 95% CI [1.53-29.3].

3.4.3. *p72* Gene relationships between ASFV from soft ticks in different habitats

In order to classify the 29 virus isolates obtained from soft ticks at the GNP into the previously described major ASFV genotypes, the C-terminal of the *p72* gene was amplified and sequenced. Multiple isolates from individual habitats (warthog burrow and pig pens) were found to be identical across the sequenced portion of the gene. Finally, 19 unique isolates of ASFV from soft ticks representing 19 out of 29 virus isolates within different warthog burrows or pig pens (17 specimens from the GNP and 2 from the GNPBZ; Table 3.1) were chosen and compared to 33 viruses representing all 22 (I-XXII) previously described *p72* genotypes (Boshoff *et al.,* 2007). A summary of the 52 viruses used in the phylogenetic analysis is provided in Table 3.1. The final dataset consisting of 52 taxa was used to construct a phylogenetic tree using NJ, ME with Bayesian inference. All trees had similar topology and the optimal tree with the sum of branch length of 0.29 is shown in Figure 3.2.

The viruses clustered into three distinct evolutionary lineages (Figure 3.2), each one confined to a broad geographical area (Boshoff *et al.,* 2007) and supported by bootstrap values ranging from 69% to 100%. Lineage I comprised 13 genotypes associated with viruses from West and southern Africa, while lineage II was associated with viruses from East Africa and lineage III, with viruses from the Great Lakes Region of East and Central Africa (Bastos *et al*., 2003, 2004; Lubisi *et al*., 2005, 2007, 2009; Boshoff *et al.,* 2007). Most of the viruses historically isolated in Mozambique fell into lineage I, except for Moz/1/98 (genotype VIII) that was included in lineage II (Bastos *et al*., 2003). All ASFV strains from our study were classified within Lineage I.

Previously, ASFV genotypes were defined as groups of isolates with a minimum between-genotype pairwise difference of 0.96% and greater than 80% bootstrap support (Bastos *et al*., 2003). Based on this definition, 22 of the *p72* genotypes represented the Roman numeral I to XXII were previously described (Boshoff *et al*., 2007). The ASFV viruses characterized in this study clustered into 3 genotypes (Figure 3.2). Eleven of the 19 viruses clustered within genotype II with 4 being 100% homologous over the region sequenced and indistinguishable from other viruses previously classified as belonging to genotype II (Mad/1/98, Moz/2/02 and Mau2007/1) (Bastos *et al*., 2003; Lubisi *et al.,* 2005, 2009). The remaining 7 viruses were all genetically distinct and differed between 1.25 to 2.75% nucleotides (Appendix I). Three viruses from this study clustered within genotype V and were genetically related (nucleotide identitity >97%) to viruses previously isolated from warthogs in Malawi (Tengani/60) and ASF outbreaks in Mozambique (Mozambique/1960/1979) and Malawi (Mal/1/02) (Bastos *et al*., 2003, 2004; Lubisi *et al*., 2005). The remaining 5 viruses grouped as a separate cluster and new genotype defined as genotype XXIII (bootstrap suport of 75% and <3% nucleotide divergence).

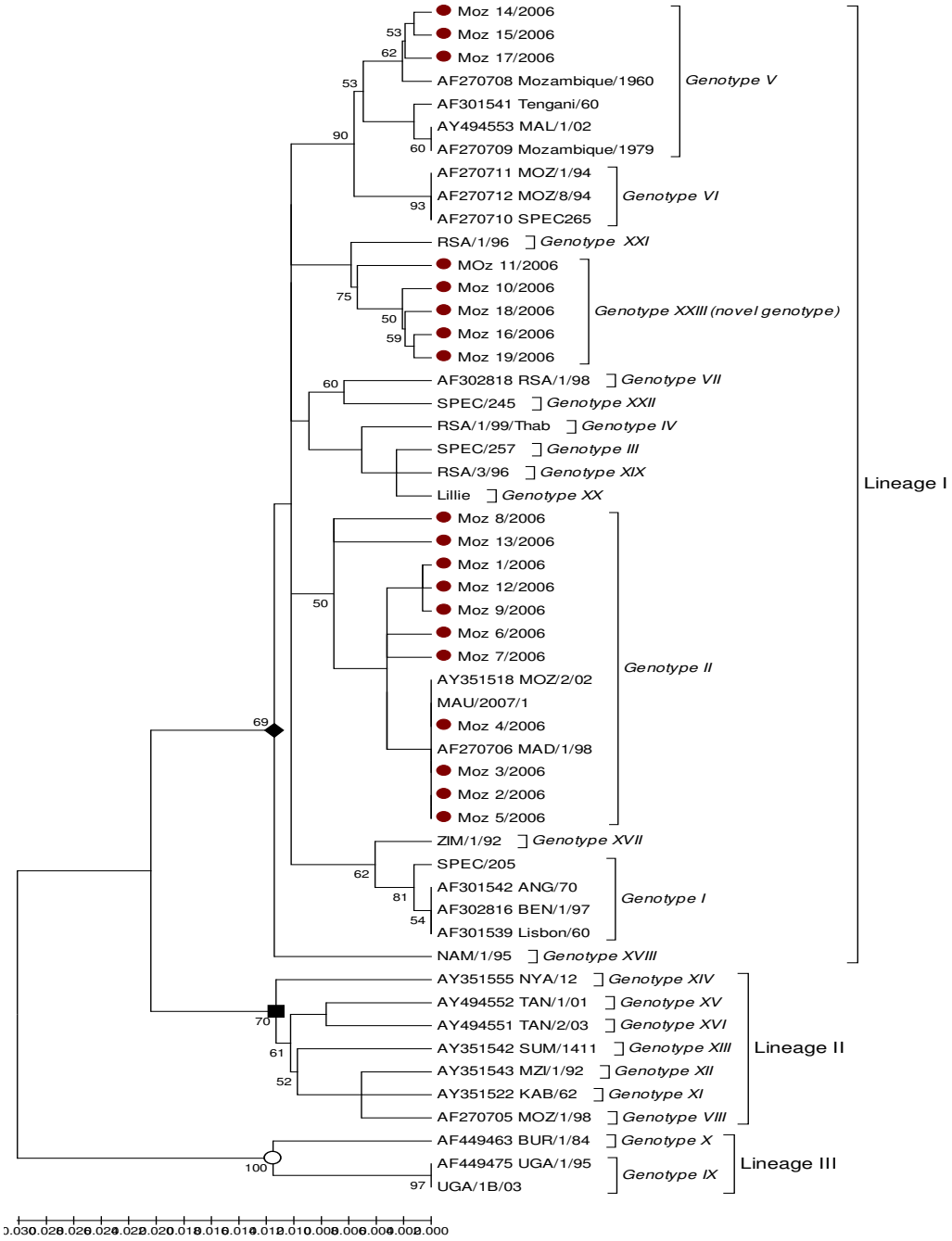

Figure 3.2.: Phylogenetic tree based on the ***p72*** gene. The evolutionary history was inferred using the Neighbor-Joining method, Minimal Evolution and Bayesian inference. The optimal tree with the sum of branch length = 0.29246972 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. The analysis involved 52 nucleotide sequences. GNP and GNPBZ isolates from soft ticks characterized in this study are marked (●). The 22 known genotypes are according to (Bastos *et al*., 2003, 2004; Lubisi *et al*., 2005, 2009; Boshoff *et al.*, 2007).

3.4.4. Molecular epidemiology of the viruses isolated from Mozambique based on the *p30* and *p54* genes

The *p30* and *p54* genes have previously been used as an additional method for determining phylogenict relationships between ASF isolates (Rowlands *et al*., 2008; Misinzo *et a*l., 2010; Gallardo *et al*., 2011). In this study a smaller data set (44 taxa) was used to infer the phylogenies based on the *p30* and *p54* gene sequences due to failure to sequence a number of isolates and the smaller data set of historical isolates.

Similarly to *p72* genotyping, all ASFV *p30* gene sequences clustered into three evolutionary lineages with high bootstrap support of 100% (Lineage Ia, IIa, IIIa; Figure 3.3). All the Mozambican isolates from this study clustered into Lineage 1a and further into four previously identified genotypes, and in addition, three novel genotypes were identified (XXIIIA, XXIIIb and XXIIIc).

Based on *p54* gene characterization, three different evolutionary lineages were identified (bootstrap support ranging from >70% to 98%; Figure 3.4) with all Mozambican isolates in Lineage 1b. A number of the ASFV isolates from this study differentiated into 3 previously described genotypes (IIA, IIB, IIC) (Bastos *et al*., 2003; Lubisi *et al*., 2005, 2009; Rowlands *et al*., 2008) while three novel genotypes were identified (XXIIIA, XXIIIB and XXIIIC) with <8.3% nucleotide divergence compared to the former genotypes (Appendix I). Similar to *p72* and *p30* genotyping, the data on the *p54* gene sequence indicated that the ASFV isolated from soft ticks in our study showed >92% nucleotide identitity with MAUR/07/1 from Mauritius (Lubisi *et al*., 2009) and Moz/02/2 from northern Mozambique (Bastos *et al*., 2003).

Although the clustering of the isolates based on these genes were similar to that generated using *p72*, individual *p30* or *p54* genotypes were generally different using the criteria of pairwise difference of 0.96% and greater than 80% bootstrap support to define a genotype. This suggests that the genetic diversity within these gene regions are not sufficient to infer the phylogenetic or evolutionary relatedness of ASF viruses.

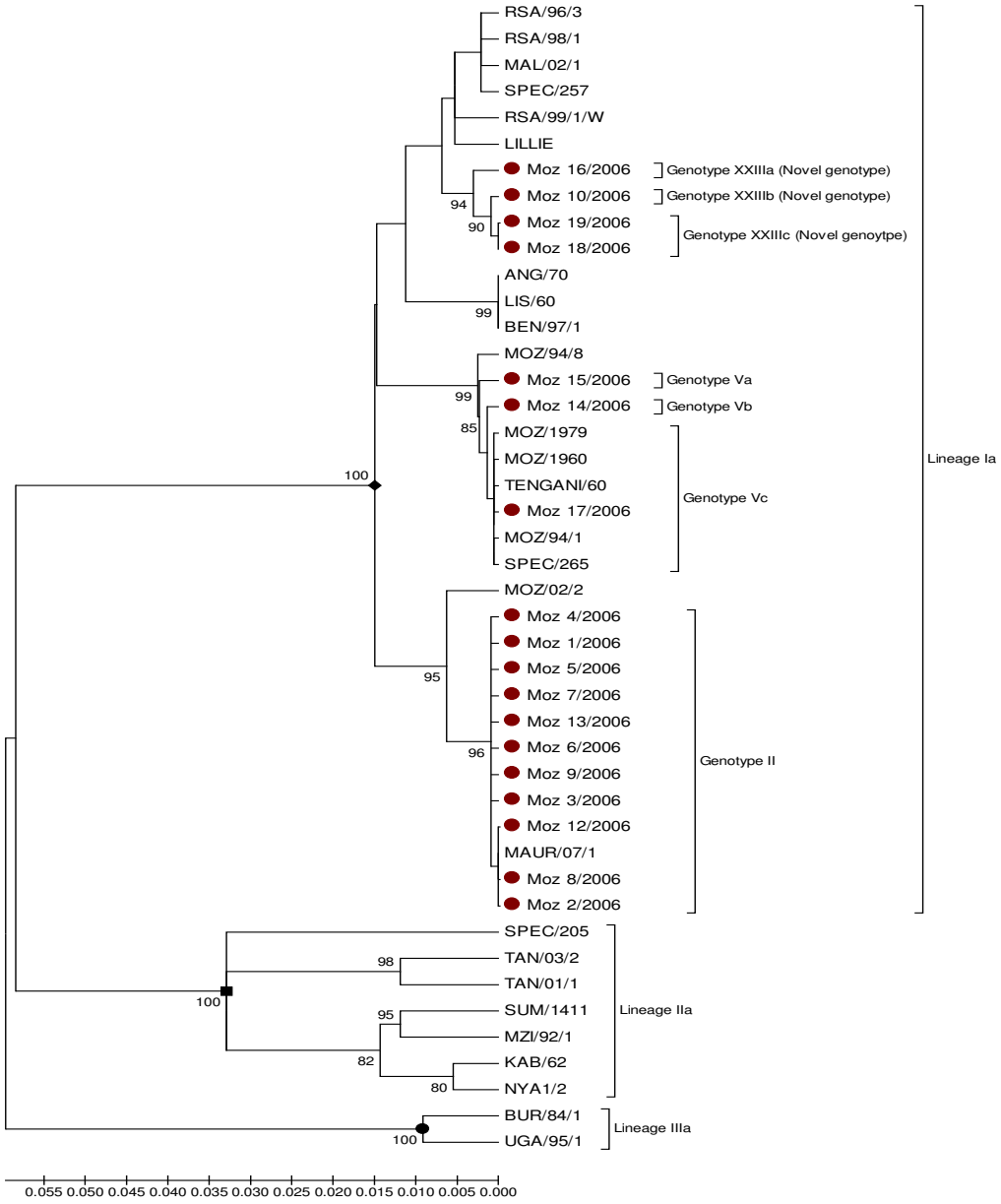

Figure 3.3. Phylogenetic tree based on the ***p30*** gene. The evolutionary history was inferred using the Neighbor-Joining method, Minimal Evolution and Bayesian inference. The optimal tree with the sum of branch length = 0.39012296 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. The analysis involved 44 nucleotide sequences. GNP and GNPBZ isolates from soft ticks characterized in this study are marked (●). The 22 known genotypes are according to (Bastos *et al*., 2003, 2004; Lubisi *et al*., 2005, 2009; Boshoff *et al.*, 2007) and the novel genotype was defined from the *p72* genotyping.

Figure 3.4. Phylogenetic tree based on the ***p54*** gene. The evolutionary history was inferred using the Neighbor-Joining method, Minimal Evolution and Bayesian inference. The optimal tree with the sum of branch length = 0.51508011 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. The analysis involved 44 nucleotide sequences. GNP and GNPBZ isolates from soft ticks characterized in this study are marked (●).The 22 known genotypes are according to (Bastos *et al*., 2003, 2004; Lubisi *et al*., 2005, 2009; Boshoff *et al.*, 2007) and the novel genotype was defined from the *p72* genotyping.

3.4.5. Using the *B602L* gene encoded amino acid sequence to compare isolates from the study area and southern Africa

The number of amino acid (aa) tandem repeats in the *B602L* gene region is a highly variable genetic marker and was used to differentiate closely related *p72* genotypes. The *B602L* gene of 16 ASFV isolates collected in this study was sequenced. These were compared to six additional closely related viruses previously described (Bastos *et al*., 2004; Nix *et al*., 2008) and unpublished data at OVI belonging to the same *p72* genotype (II, V and new genotype XXIII). Together, these isolates were classified into eight subgroups, labeled XXI, XXIa, XXIb, XXIc, XXXII, XXXIII, XXXIIIa, XXXIIIb (Table 3.3), based on their length and sequence of aa tetramer repeats. Four of the isolates from our study were closely related to subgroup XXI previously described by Nix *et al*., (2006).

The differences were based on the number of tetrameric aa repeats that varied between 9 and 36. The isolates from this study were classified into 6 of the 8 subgroups namely XXIa, XXIb, XXXII, XXXIII, XXXIIIa and XXXIIIb (marked in bold in the Table 3.3). All isolates that clustered in *p72* genotype II belonged to CVR subgroup XXXII, except Moz 1/2006 that fell into CVR subgroup XXIa together with Moz15/2006 (*p72* genotype V). Three ASFV that had been described from outbreaks in Madagascar (1998), Mauritius (2007) and Mozambique (2002) also fell into CVR subgroup XXXII, with 10 aa repeats, indicating the close relationship between these viruses. Three of the isolates from the novel genotype XXIII had their own CVR subgroups that have not been described before.The *p72* genotype V also formed 3 CVR subgroups; two of these were found in the ASFV isolated in soft ticks in our study.

Seventeen different aa tetramers were found: **B**=CADT; **N**=NVDT/NVGT; **D**=CASM; **A**=CAST; **L**=CTST; **H**=NEDT; **P**=NADT; **S**=SAST; **O**=NASI; **F**=NAST; **Q**=NADI; **V**=NANT; **M**=NANI; **T**=NVNT; **C**=GAST; **K**=CANT. The most common tetramers encoded by isolates of subgroup XXXII were CADT and NVDT (coded as B and N in Table 3.3). The duplet of tetramers coded BN was repeated several times in isolates within this subgroup, confirming the homogeneity among the *p72* genotype II isolates from soft ticks in this study and other viruses that caused outbreaks in islands of the Indian Ocean (MAD/1/98), MAU/2007/1) (Bastos *et al*., 2004).

Table 3.3. Central Variable Regions of the 9RL ORF tetrameric amino acid repeat alignment from the isolates collected at the GNP interface. The single letters refers to the code of each tetrameric repeat: **B** = CADT; **N** = NVDT/NVGT; **D** = CASM; **A** = CAST; **L** = CTST; **H** = NEDT; **P** = NADT; **S** = SAST; **O** = NASI; **F** = NAST; **Q** = NADI; **V** = NANT; **M** = NANI; **T** = NVNT; **C** = GAST; **K** = CANT (Bastos *et al*., 2004; Boshoff *et al*., 2006; Chapman *et al.,* 2008; Nix *et al*., 2006).

Isolate	Place/country of origin	Species of origin	P72 Genotype	CVR tetrameric aminoacids sequences	N° of repeats	CVR sugroups	Reference
Moz 2/2006	Gorongosa Park	Ticks	II	BNDBNDBNAL	10	XXXII	This study
Moz 3/2006	Gorongosa Park	Ticks	II				This study
Moz 4/2006	Gorongosa Park	Ticks	II				This study
Moz 5/2006	Gorongosa Park	Ticks	II				This study
Moz 7/2006	Gorongosa Park	Ticks	II				This study
Moz 8/2006	Gorongosa Park	Ticks	II				This study
Moz 9/2006	Gorongosa Park	Ticks	II				This study
Moz 12/2006	Gorongosa Park	Ticks	II				This study
Moz 13/2006	Gorongosa Park	Ticks	II				This study
MAD/1/98	Madacascar	Domestic pig	II				Bastos *et al*., (2004)
MAU/2007/1	Mauritius	Domestic pig	II				OVI-unpublished
MOZ/2/02	Mozambique	Domestic pig	II				OVI-unpublished
Tengani/60	Tengani/Malawi	Warthog	V	ABABNBABHAL	11	XXI	Nix *et al*., (2006)
Moz 1/2006	Gorongosa Park	Ticks	II	APSPSOPNAFNOFFNFOPNAFNOFFNQVQMV	31	XXIa	This study
Moz 15/2006	Gorongosa Park	Ticks	V				This study
Moz 14/2006	Gorongosa Park	Ticks	V	ABHABNBABHAL	12	XXIb	This study
Moz 17/2006	Gorongosa Park	Ticks	V				This study
Moz/1979	Beira/Mozambique	Domestic pig	V				Bastos *et al*., (2004)
Moz/1960	Tete/Mozambique	Domestic pig	V	ABNAAAALBNBNBABNBABHAL	22	XXIc	Bastos *et al*., (2004)
Moz 10/2006	Gorongosa Park	Ticks	XXIII	BNAABNBNA	9	XXXIII	This study
Moz 16/2006	Gorongosa Park	Ticks	XXIII	ABHAABNBBHAL	12	XXXIIIa	This study
Moz 18/2006	Gorongosa District	Ticks	XXIII	ABTAAAACBNAAAAACBNAAAAACKTAAAACBNAKA	36	XXXIIIb	This study

The isolates from this study are **marked in bold**

The ASFV isolates Moz14/2006, Moz15/2006 and Moz17/2006 within *p72* genotype V were separated into subgroups XXIa and XXIb. These two subgroups were distinct in the number of aa tetramer repeats: thirty one for the subgroup XXIa and twelve for the subgroup XXIb. The other distinct characteristic pattern found in Moz15/2006 was the presence of SAST, NADT, NASI, NAST, NADI, NANT and NANI (coded S, P, O, Q, V and M respectively) repeats which were not present in other isolates assembled in the subgroup XXIb. These isolates were divergent (in number and sequences of aa repeats) to those isolates previously obtained from outbreaks that had occurred in Central Mozambique and from warthogs in Malawi (Bastos *et al*., 2004).

The *B602L* gene subgroups XXXIII, XXXIIIa and XXXIIIb, representing the newly identified *p72* genotype XXIII (isolates Moz10/2006, Moz16/2006 and Moz18/2006 respectively), were unique in terms of number of aa tandem repeats and sequences. For instance, within subgroup XXXIII, the Moz10/2006 isolate contained nine aa tandem repeats in its CVR sequence that were made of two copies of CAST flanked by duplets tetramer CADT/NVDT (coded B,N) and followed by a triplet of CADT/NVDT/CAST (coded B, N, A). On the other hand, the subgroup XXXIIIb comprised 36 repeats that contained NVNT and CANT (coded T and K) aa tetramers that were not present in the rest of the 22 ASFV isolates, and therefore, this would be considered a new subgroup (Table 3.3).

3.5. DISCUSSION

In this study, the occurrence of ASFV infected soft ticks within warthog burrows and domestic pig pens at the wildlife/domestic interface of GNP in central Mozambique was clearly demonstrated. The genetic heterogeneity among ASFV isolated from soft ticks was discriminated by means of four different genes that conferred different levels of resolution.

Malawi is the closest country to the previously described ASF endemic areas of Mozambique located in the north and west of the country. An investigation conducted in an endemic area of Malawi revealed that ASFV is maintained in certain areas where warthogs are present. However, in areas where they are absent, the virus is maintained in the domestic cycle with the involvement of soft ticks (Haresnape, 1984; Haresnape *et al*., 1985, 1988). ASF in Mozambique

was first diagnosed in 1960 in Mutarara District (Abreu *et al*., 1962) adjacent to the endemic area in Malawi (Haresnape *et al*., 1985). Since then, several outbreaks have occurred in the northern and central provinces of the country and these outbreaks were always suspected to be linked with a virus spill-over from a potential sylvatic cycle (Abreu *et al*., 1962; Valadão, 1969). The first appearance of disease in the southern provinces was in 1994. Currently sporadic outbreaks are occurring and more frequently in endemic areas (Penrith *et al*., 2007).

Despite the suspected importance of wild hosts as a source of ASFV (Bastos *et al*., 2004; Penrith *et al*., 2007), information about the ecology of ASF in Mozambique is scarce. The present study provides the first and most complete data on the genetic diversity of ASFV isolated from soft ticks in Mozambique.

Although tick sampling in the GNP was performed only close to accessible routes, the prevalence of soft tick infestation among warthog burrows was higher (90%) than what has been reported in many other wildlife conservation areas in South Africa, Kenya, Uganda and Zambia (Wilkinson *et al*., 1988; Plowright *et al.,* 1994). Therefore, we can assume that the probability of soft ticks moving from wild to domestic habitats could be relatively high in our study area. However, soft ticks could not be found in most of the domestic pig pens searched (Quembo *et al*., 2014).

Soft ticks identification was conducted using conventional methods (Walton, 1962, 1979) based on their morphological characteristic. For instance, the soft ticks (*O. p. porcinus*) collected in the wild were found morphologically different compared to those *O. p. domesticus* from the domestic pig pens. Furthermore, this classification was supplemented by 16S rDNA sequences and the results indicated that both subspecies of soft ticks are phylogenetically very close to *O. moubata* (data not shown). Similar findings were observed in Madagascar where *O. p. domesticus* ticks were collected in domestic pig pens and they were closely related to Tanzanian *O. moubata* (Ravaomanana *et al.,* 2010). However, it is necessary to conduct further research of soft tick taxonomy at molecular level.

The contacts between soft ticks and domestic and wild pigs in the same study area was recently assessed by the detection circulation of antibodies against salivary gland of soft ticks in pigs and warthogs living at the GNP interface. In that study, a significantly higher proportion of domestic

pigs was positive to anti-tick antibodies in areas closer to the GNP, suggesting that pigs living close to the GNP are likely to be more exposed to *Ornithodoros spp.* bites than those distant from the GNP borders (Quembo *et al*., 2014).

ASFV were found in nearly 50% of the soft ticks collected in warthog burrows and domestic pig pens (Table 3.1). The prevalence of ASFV in those ticks confirms previous reports that suggested the presence of *Ornithodoros*–warthog cycle in that region (Penrith *et al*., 2007; Quembo *et al*., 2014). Additionally, this result suggests that the soft ticks can maintain and transmit ASFV to domestic pigs in the study area.

The genetic analysis of the viruses isolated in this study targeting four independent genomic regions of ASFV, *p72, p30*, *p54* and *B602L*, is the first assessment to intra-and inter-genotypic variability of ASFV field isolates from an endemic setting. Our results suggested potential epidemiological links between viruses present in the sylvatic cycle of GNP and ASFV isolates from outbreaks in different parts of Africa, the Indian Ocean and eastern Europe (Bastos *et al*., 2004; Boshoff *et al*., 2007; Lubisi *et al*., 2007; Rowlands *et al*., 2008).

Genetic characterization based on the C-terminal end of the *p72* gene indicated that within genotype II, there was a high level of homology between a number of our recovered viruses and those that have caused outbreaks in Mozambique (Moz 2/02), Madagascar (MAD/1/98), Mauritius (MAU/2007/1) (Bastos *et al*., 2003, 2004; Lubisi *et al*., 2005, 2009) the Caucasus and the Russian Federation (Rowlands *et al.*, 2008; Malogolovkin *et al*., 2012; Gogin *et al.,* 2013). A similar finding was obtained in the phylogenetic data using the *p30* and *p54* genes (Figures 3.3 and 3.4 respectively) as well as the sequence data analysis of the *B602L* gene, indicating that these viruses were genetically similar and probably from a common wild host origin.

Interestingly, viruses within genotype V had been reported in warthogs and several ASF outbreaks in domestic pigs in Mozambique and Malawi more than 50 years ago (Abreu *et al*., 1962; Bastos *et al*., 2004), which indicates that this genotype can be maintained for at least half a century in the sylvatic cycle with relative sequence conservation. The higher level of resolution using the *p30, p54* and *B602L* gene sequences (Figure 3.3 and 3.4 and Table 3.3) indicated more variation across all isolates within this genotype compared to *p72* genotyping. This variation was confirmed by the presence of four subgroups (XXI, XXIa, XXIb and XXIc) within *B602L* gene

sequences from GNP isolates. In common with previous studies, the viruses in genotype V appeared to be geographically confined within central provinces of Mozambique and Malawi (Bastos *et al*., 2003, 2004).

The presence of a novel genotype in different isolates from soft ticks is an indication of the possible presence of more genotypes that may be circulating in the sylvatic cycle and represents a potential risk of spill-over of previously unknown genotypes to domestic pigs. This might pose a problem if a vaccine should become available, as cross protection to whole spectrum of ASF viruses is less likely to occur (King *et al.*, 2011). Therefore, extensive surveillance among wild hosts in Mozambique and other countries to identify the most commonly circulating ASFV genotypes is crucial.

Genetic variation within the *p72, p30* and *p54* gene sequences of ASFV isolates from our study was found to be as high as 6.2, 10.0 and 9.1% nucleotide differences respectively (Appendix I). The viruses were also divided into six subgroups within the *B602L* gene sequence. This high level of diversity is consistent with the soft tick acting as permanent source of viruses and it is in line with previous studies (Bastos *et al*., 2003; Misinzo *et al*., 2010; Gallardo *et al*., 2011). However, further studies should be conducted in other hosts (warthogs and domestic pig) to precisely identify all circulating ASFV isolates within that endemic area.

Additionally, the central location of the GNP and the existence of a national road (N1) that links this area with other large pig production areas close to major cities such as Beira and Caia (South East and North of GD, respectively), is suspected to facilitate the potential dissemination of ASFV strains from wild hosts into other pig production areas of the country (Penrith *et al*., 2007). Furthermore, at the end of this chain, the City of Beira could play a role in the spread of ASFV far beyond its boundaries since international trade from its airports and harbours is intense. Although there is no evidence to confirm the source and origin of ASV dissemination in Mozambique, it is nevertheless clear that ASFV strains of eastern African origin have reached different countries in the Indian Ocean and the Caucasus most likely through infected products transported by ships (Bastos *et al*., 2003, 2004; Lubisi *et al*., 2005, 2009; Rowlands *et al.*, 2008). Therefore, movement control of pigs should be reinforced and the incentive for better pig production (good husbandry practices, training farmers and animal feed resources) in

conjunction with well organized slaughter houses within GD could be alternatives to prevent the spread of ASF and other diseases.

In conclusion, the results obtained in this study suggest that in addition to the existence of a domestic pig cycle that can maintain the virus by itself (Haresnape *et al*., 1985; Penrith *et al*., 2004a, 2004b), the occurrence of isolates with genetic heterogeneity in soft ticks from natural and domestic habitats, can be a permanent source of ASF virus for pigs reared in free ranging conditions. These results suggest that the quantity and diversity of circulating viruses are high and that a significant proportion of soft ticks had the opportunity to feed on viraemic mammal hosts (Gallardo *et al*., 2011), most likely warthogs, but also domestic pigs if they are available. It also provides evidence of the importance of the sylvatic cycle in harboring and disseminating virus strains in the Mozambican pig value chain and supports previous publications that demonstrated that the genetic diversity of ASFV is originally from sylvatic hosts (Bastos *et al*., 2009; Penrith & Vosloo, 2009; Penrith *et al*., 2013). Furthermore, live pig transportation for marketing in the City of Beira may have contributed to the spread of ASF within Mozambique and this could contribute to transcontinental spread of ASF.

This is the first study of the ecology of ASF in Mozambique and the role played by wild hosts living in protected area in the center of the country. However, Mozambique encompasses 8 big national parks in its territory. Therefore, further studies to characterize the possible role played by wild hosts in other protected areas of its territory are necessary in order to complete the identification of high risk areas of ASFV maintenance and emergence. This information should allow targetted surveillance and control efforts to mitigate potential dissemination of viral strains towards important pig production areas in Mozambique and beyond its boundaries. Future research analysing the pathogenicity of the new ASFV genotype described for the first time in this thesis would be interesting. Experimental infection of pigs would determine the rate and time of seroconversion, persistence of antibodies and possible serological immunodeterminants.

CHAPTER 4

EVALUATION OF THREE DIFFERENT ELISAS FOR ANTIBODY DETECTION AGAINST AFRICAN SWINE FEVER VIRUS IN NATURALLY INFECTED PIGS FROM THE GORONGOSA DISTRICT, CENTRAL MOZAMBIQUE

4.1. SUMMARY

In the absence of a vaccine, the control of African swine fever (ASF) in endemic settings relies on efficient diagnostic programs and the application of surveillance and control measures to prevent the spread of disease. In that context, knowledge of the sensitivity and specificity of available diagnostic tests under field conditions is instrumental to design an efficient surveillance program. In this study, the sensitivity and specificity of three different tests to detect antibodies against ASF were estimated relative to an immunoblotting test as the gold standard. In addition, conditional dependence estimates of different test combinations were reported in a sample of naturally exposed and infected pigs from a region of Central Mozambique where regular outbreaks have been confirmed. Samples of 273 local breed domestic pigs from small scale pig farmers of Gorongosa District were tested simultaneously with an immunoblotting test (considered the gold standard), the OIE recommended Indirect ELISA (I-ELISA), a laboratory made ELISA based on a *p30* recombinant protein detection (*rp30* ELISA) and a commercially available ELISA based on the *p72* protein detection (Blocking *p72* ELISA). The immuno-blotting was used as reference assay to assess the performance of above mentioned ELISAs. Kappa statistics revealed that the I-ELISA and immuno-blotting assay showed an almost perfect agreement (kappa value of 0.84, 95% CI [0.72; 0.93]), while the comparison of these tests with the Blocking *p72* ELISA showed good agreement with kappa values of 0.51, 95% CI [0.40; 0.67] and 0.58, 95% CI [0.46; 0.73] respectively. Furthermore, moderate agreement was observed when the *rp30* ELISA was compared to these two tests (kappa values of 0.28, 95% CI [0.17; 0.42] and 0.29, 95% CI [0.18; 0.44] respectively). The I-ELISA had sensitivity and specificity values of 88.2%, 95% CI [77.4; 99.1] and 97.5%, 95% CI [95.5; 99.5] respectively compared to the immune-blotting assay. The sensitivity and specificity for the *rp30* ELISA was 26.5%, 95% CI [11.6; 41.3] and 96.7%, 95% CI[94.4; 98.9] and for the Blocking *p72* ELISA these values were 50%, 95% CI [33.2; 66.8] and 98.3%, 95% CI [96.7; 100] respectively. The highest sensitivities were obtained with the parallel combination of the I-ELISA with the Blocking *p72* ELISA (sensitivity of 94.1%, 95% CI [91.3; 96.9]) and the I-ELISA combined with the *rp30* ELISA (sensitivity of 91.3%, 95% CI [87.9; 94.6]). The combination of the Blocking *p72* ELISA and the *rp30* ELISA gave a modest sensitivity of 60.8%, 95% CI [55.0; 66.6]. Therefore, in endemic settings of Central Mozambique, the best test choice among all the available ELISAs is the I-ELISA test and the best test combination to increase sensitivity is the

parallel combination of this test with the *p72* ELISA-based assays. The current use of the *rp30* ELISA for the detection and control of ASF in Mozambique is questionable and demands further studies to evaluate its performance in other endemic settings.

4.2. INTRODUCTION

African swine fever (ASF) is endemic in most provinces in the central and northern parts of Mozambique where outbreaks occur regularly. These outbreaks are often characterized by low mortality rates, particularly among indigenous pigs (Penrith *et al*., 2004a, 2007).

Previous investigations in Mozambique document the frequent occurrence of ASF (in 2001, 2006 and 2007) on the outskirts of the Gorongosa National Park (GNP) and the peri-urban area of the Gorongosa District (GD) (Penrith *et al.*, 2007, 2013; Penrith and Vosloo, 2009). This might be due to a sylvatic cycle that represents a permanent source of virus for pig populations and the high number of domestic pigs farmed under a free-range system (Penrith *et al.*, 2007). Therefore, different indicators suggest that our study area is likely to be an endemic area for ASF.

It is believed that the acquisition of immunity to ASFV requires a combination of both humoral and cellular mechanisms (Escribano *et al.*, 2013; Takamatsu *et al.*, 2013). The complexity of the immune response to the virus in pigs has impaired the development of an effective vaccine. The detection of antibodies to ASFV can be used as an indicator of the disease status of an animal due to the lack of an available vaccine (Penrith *et al*., 2007). Serology is an instrumental tool to detect and monitor the disease and control spread among new susceptible populations. Therefore, the success of disease control in Africa, particularly in endemic areas, requires reliable antibody detection methods to identify potential sources of infection (Allaway *et al*., 1995; Barderas *et al*., 2000) and infected areas. In endemic countries of sub-Saharan Africa, the epidemiology is further complicated by the presence of virus strains with reduced virulence and subsequent

presence of asymptomatic infected animals (Etter *et al*., 2011). Pig breeds that are resistant to ASFV might also be present in the region (Penrith *et al*., 2004b; Uttenthala *et al*., 2013). These factors make serological diagnosis an instrumental tool for disease control.

Many laboratories in developing countries use the OIE-approved ELISA to detect ASFV-specific antibodies with or without western blotting (or immuno-blotting assay) as a confirmatory test (OIE, 2012). Major limitations of the OIE ELISA include the requirement of level 3 biosecurity facilities for the production and handling of the pathogen for antigen preparation (Barderas *et al*., 2000; Filgueira-Perez *et al*., 2006), the requirement of confirmation by a second serological test and the occurrence of false-positive results in poorly preserved sera (Cubillos *et al*., 2013).

Other serological methods for the detection of ASFV antibodies have been developed and include an ELISA based on a recombinant ASF viral protein *p30* from an attenuated E70 Spanish isolate or Morara/Georgia *CP204L* gene, expressed in insect larvae of *Trichosplusia ni* (Filgueira-Perez *et al.*, 2006; Cubillos *et al*., 2013). A commercially available ELISA based on the purified capsid protein *p72* (Ingenasa, Madrid, Spain) has been used in many endemic countries including Madagascar (Ravaomanana *et al*., 2011), Senegal (Etter *et al*., 2011), Tanzania (Uttenthala *et al*., 2013) and Uganda (Atuhaire *et al.,* 2013).

The performance of several ASF ELISA tests has been assessed using pig sera of European and African origin (Gallardo *et al*., 2009, 2013; Cubillos *et al*., 2013). However, given the variability and diversity of the virus on the African continent (Abreu *et al*., 1962; Bastos *et al.*, 2003, 2004; Lubisi *et al*., 2005; Boshoff *et al*., 2007), it is important to validate serological diagnostic tests in endemic settings of Africa where genetically distinct viruses are circulating in order to accurately estimate sensitivity and specificity.

Prior to the utilization of a new test, either in diagnostic or surveillance programs, it is necessary to determine its specificity and sensitivity. The specificity of a test is defined as the probability of obtaining negative test results when applied to non-affected animals. The sensitivity of a test is the probability of the test returning a positive result when applied to an affected animal (Toma *et al.* 1999). In the case of a highly infectious disease such as ASF, it is often preferable to maximise sensitivity so that disease can be detected more efficiently to reduce the spread (Thrusfield, 2005; Smith, 2005). When multiple diagnostic tests are performed on an individual

or a population, it is possible to combine the information of two or more diagnostic tests assuming that the tests are not perfectly dependant (Gardner *et al.,* 2000), to improve the sensitivity.

This chapter aims to i) to evaluate the performance of three different ELISAs for antibody detection against ASFV using domestic pig sera obtained from an endemic area at the interface of the GNP (Central Mozambique), ii) to determine the best test choice or combination in order to maximize the detection of truly sero-positive pigs.

4.3. MATERIALS AND METHODS

4.3.1. Data and sample collection

Serum samples were collected under the approval of the Onderstepoort Veterinary Institute and Faculty of Veterinary Science, University of Pretoria Animal Ethics Committees (21/2006). During March 2006 and September 2007 a total of 634 pigs of local breed ranging from 6 months to 3 years of age were sampled from 314 small scale farms located in 18 different villages in the GD, Sofala Province (18^0 45'/19^0 15' latitude South and 33^0 30'/34^0 45' longitude East). Six were typical rural villages located 10 km from the GNP boundary termed the Gorongosa National Park Buffer Zone (GNPBZ). The remaining twelve villages were located in a peri-urban area with high human population density at more than 10 km from the GNP limits, and they are referred to as Rest of District (ROD). All sampling sites were recorded using a Global Positioning System (GPS) and plotted on a map (Figure 4.1).

Approximately 5 ml of blood was collected in plain Vacutainer® tubes from the anterior *Vena cava.* Sera were extracted by spinning at 3000 rpm and stored at -20ºC until sent to the Transboundary Animal Diseases Programme (TADP), ARC-Onderstepoort Veterinary Institute (OVI) in South Africa to be tested simultaneously for the presence of antibodies against ASFV. The specimens were transported under a permit issued by the South African Department of Agriculture, Forestry and Fisheries (DAFF).

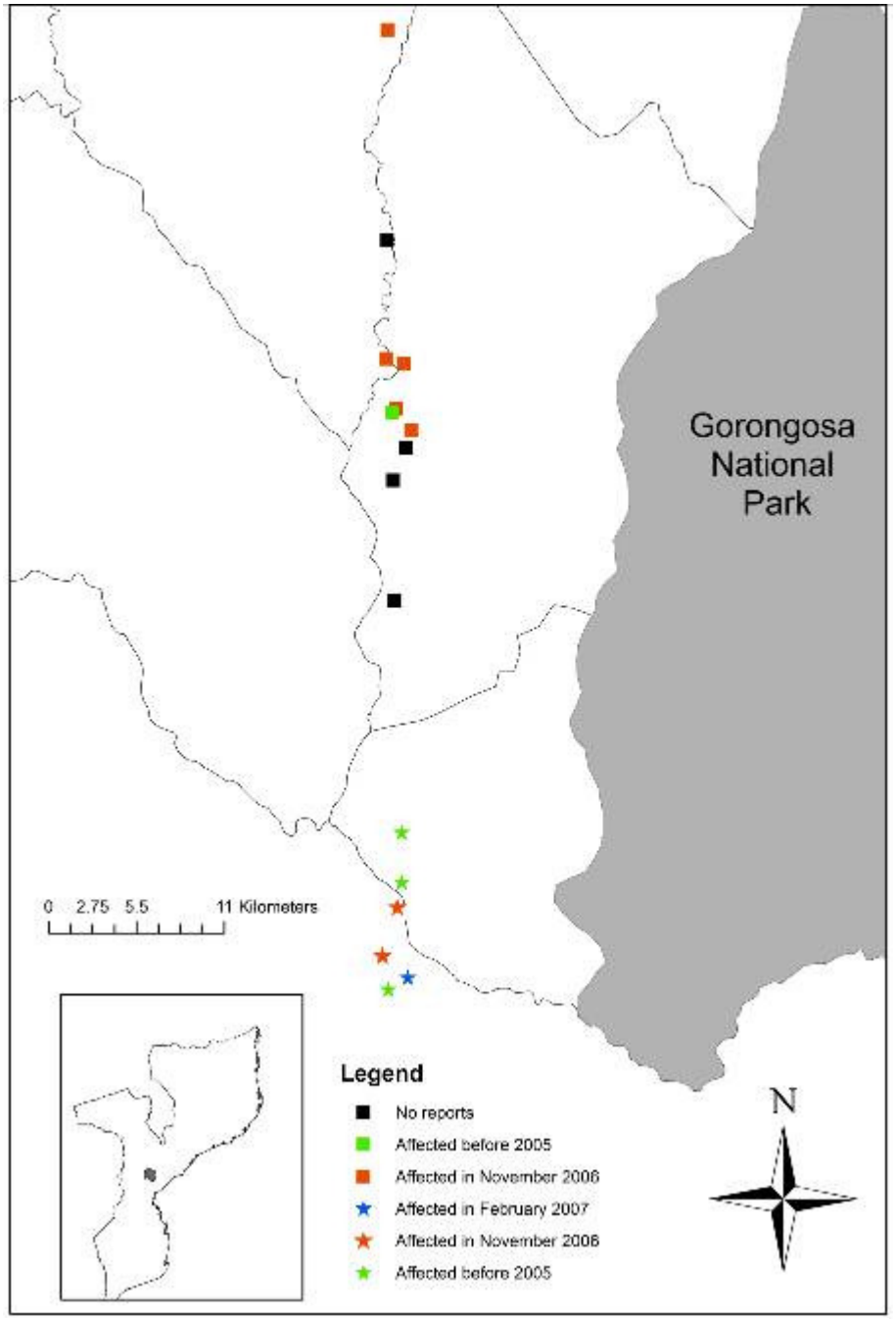

Figure 4.1 Map of the Gorongosa District including the locations where pig farms were sampled

4.3.2. Diagnostic tests

4.3.2.1. Algenex *rp30* indirect ELISA (*rp30* ELISA)

The Algenex *rp30* indirect ELISA (*rp30* ELISA) is an indirect ELISA where the antibody present in the sample is sandwiched between the recombinant protein antigen coated on the plate and an enzyme labelled conjugate (Filgueira-Perez *et al.,* 2006; Cubillos *et al*., 2013). Recombinant baculovirus expressed *p30* protein (*rp30*) of the attenuated E70 Spanish isolate is used as antigen (Filgueira-Perez *et al.,* 2006). The ELISA micro-plates (Polysorp, Nunc, Denmark) were coated either with 100 µl 1:20 dilution (in 50 mM Carbonate/Bicarbonate buffer - Appendix II) containing antigen (Ag+) or the same volume of buffer without antigen and incubated overnight at 4ºC. The plates were washed three times with approximately 300 µl of Phosphate Buffered Saline-Tween (PBST; Appendix II), then used immediately or stored at -20ºC until needed.

Serum samples as well as control sera were diluted 1:100 in blocking buffer (Appendix II) and 100 µl of the dilution was dispensed in duplicate wells. The positive control serum was obtained from a domestic pig infected with 10^4 of 50% haemadsorbing doses (HAD_{50}) of an avirulent or low-virulent ASFV to induce a chronic infection, while the commercially available negative control was purchased from Difco® (Voigt Global Distribution, Inc. USA). Each serum sample was tested against both antigen-positive and antigen-negative coated wells and incubated for 1 hour at 37ºC followed by 3 washing steps using PBST. Protein G conjugate (1:25 stock, Sigma-Aldrich, USA) was then diluted 1:10 in blocking buffer and 100 µl dispensed to each well, incubated for 1 hour at 37ºC under constant agitation and the plates were washed 5 times using PBST.

For the substrate reaction, 100 µl/well of substrate solution (Appendix II) was added (including the clean blanking ELISA micro-plate) and the peroxidase reaction was allowed to incubate for 5 min at room temperature or until solid colour developed in the positive control well (the negative control must remain clear). After the incubation period, the reaction was stopped by adding 50 µl/well of stopping solution (Appendix II). Finally, reactions were read at an optical density of 405 nm in an ELISA micro-plate reader, (Multiskan EX, Thermo Electron Corporation, USA). The ELISA result (R) was evaluated in relation to the negative antigen. Therefore, for each

serum it was calculated as the ratio of the optical density (OD) of sample loaded in the positive antigen well and sample loaded in the negative antigen well. Samples were considered positive for antibodies to the *rp30* antigen of ASFV if $R \geq 2$.

4.3.2.2. OIE-recommended Indirect ELISA (I-ELISA)

The conventional indirect-ELISA for antibody detection of ASFV was performed following the protocol described by the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (2012) and optimized at TADP. The antigen used in the assay was purified ASFV, isolate Zaire, which was collected in 1977 and subsequently adapted on Vero cells (Escribano *et al.,* 1989; OIE, 2012). One hundred microlitres of positive and negative antigens diluted at 1:25 in 50 mM Carbonate/Bicarbonate buffer was distributed to alternative rows of the ELISA micro-plates and incubated overnight at 4ºC. Plates were washed in three cycles with PBST and stored at -20ºC until use.

Sample sera and appropriate positive and negative controls (described in 4.3.2.1) were diluted at 1:32 with 0.5% of PBS-casein (Appendix II) and 100 µl of diluted serum was pipetted into each well. Pig sera were placed in columns of four wells on the ELISA micro-plates, thus giving a duplicate test against both antigen-positive and antigen-negative coated wells, then incubated for 1 hour at 37ºC followed by three washing steps with PBST.

The lyophilized commercial Protein G conjugate (Sigma-Aldrich, USA) was dissolved in 100 ml of casein. This was subsequently diluted to 1:25 of a stock solution in PBS-casein. One hundred microlitres of the Protein G conjugate suspension was dispensed into each well and incubated for 1 hour at 37ºC followed by 5 washing steps using PBST.

Immediately before the end of the previous incubation step, the substrate/chromogen solution was prepared. For the substrate reaction, 100 µl/well of substrate/chromogen solution was added to all the wells including the clean blanking ELISA micro-plate. After incubation at room temperature for 15 minutes, the reaction was stopped by the addition of 50 µl of stopping solution. Finally, the ELISA micro-plates were read with the ELISA micro-plate reader (mentioned in 4.3.2.1) at a wave length of 450 nm.

The cut-off point for each plate was calculated as follows: The optical density (OD) of negative serum x 1 + OD of positive serum x 0.20. Sera with an optical density below the cut off were considered negative (OIE, 2012).

4.3.2.3. Ingezim PPA Compac K3 ELISA Kit (Blocking *p72* ELISA)

Antibodies against ASF virus were detected using the commercial competitive ASFV antibody Blocking *p72* ELISA (manufactured by Ingenasa, Madrid, Spain), which is based on purified *p72* protein. All reagents including diluents, positive and negative sera controls and ELISA micro-plates were supplied by the manufacturer. The washing solution concentrate provided in the kit was diluted 1:10 in sterile distilled water. The domestic pig serum samples and control sera were diluted at 1:2 in diluent prior to use. The dilution was done directly on the ELISA micro-plates by adding 50 µl of diluents and sera into each well (in duplicate) followed by an incubation step for 1 hour at 37ºC.

The conjugate was prepared at a dilution of 1:100 using the supplied diluent. One hundred microliter of conjugate was dispensed into each well followed by incubation of 30 minutes at 37ºC. This was followed by 5 washing cycles.

Lastly, 100 µl of substrate was added to each well (described in 4.3.2.2) and kept at room temperature for 15 minutes until the appearance of colour in the negative controls (positive controls must remain uncoloured). The reaction was stopped by adding 100 µl of stop solution followed by reading the plates at a wave length of 450 nm.

The test could be considered valid when the OD of the negative control (NC) is higher than 0.7 and at least 4 times higher than the OD of the positive control (PC) according to the manufacturer.

The cut-off point for each plate was calculated based on the following formulas:

Positive cut off = CN - [(CN - CP) x 0.5] Negative cut off = CN - [(CN - CP) x 0.4]

Where: CN = OD of negative control serum; CP = OD of positive control serum

Serum samples with an OD lower than positive cut off were considered as positive.

4.3.2.4. OIE recommended Immuno-blotting assay (Immuno-blotting)

The procedure was performed as described in Chapter 2.8.1 of the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (2012). Antigen strips were prepared using 10 µg of cytoplasmic soluble ASFV protein fraction obtained from MS cells (monkey stable cell line) infected with the Zaire isolate. Protein fractions were electrophoresed through 12% denaturing Sodium Dodecyl Sulphate-Polyacrylamide gels (SDS-PAGE) with a standard protein molecular weight marker and then transferred onto nitrocellulose membranes (NitroBind, Osmonics Inc., USA) using a Semi-Phor transfer cell (Hoefer Scientific Instruments Inc., USA). The membrane was soaked (labeled side up during the immune-reaction) in a 2% of PBS casein solution and incubated at 37°C for 30 minutes, rinsed with PBS, cut into 5 mm strips and air dried.

Pig sera and controls (as per 4.3.2.1) were diluted 1:40 in blocking buffer, added to each antigen strip and incubated at 37°C for 45 minutes with continuous agitation. Incubation was followed by four washing cycles in blocking buffer with the final wash lasting 5 minutes. Protein-A/horseradish-peroxidase conjugate (Sigma-Aldrich, USA) diluted at 1:1000 in blocking buffer was added to all antigen strips, incubated at 37°C for 45 minutes with continuous agitation and washed as previously described.

Substrate solution was added and antigen strips were incubated at room temperature for 5– 15 minutes with continuous agitation. The reaction was stopped using distilled water when the protein bands became visible.

Results were considered valid when serum samples and positive control showed a similar protein pattern and intensity of colour (OIE, 2012).

4.3.3. Statistics and data analysis

4.3.3.1. Estimation of sensitivity, specificity and agreement of ELISAs

The 3 ELISA tests were evaluated against the immuno-blotting test as the "*Gold Standard*" (Filgueira-Perez *et al.,* 2006; OIE, 2012). The calculations described in Smith (2005) and Thrusfield (2005) were applied to obtain the performance values of specificity and sensitivity of the ELISA tests. Furthermore, apparent prevalence as well as the negative and positive predictive values were calculated (Win Episcope version 2.0) and reported with 95% confidence intervals.

Kappa values were used to assess the agreement between all assays. Kappa values of 0 represent chance-only agreement, values of 0.01–0.20 represent low agreement, 0.21–0.40 moderate agreement, 0.41–0.60 good agreement, 0.61–0.80 substantial agreement and values of 0.81–1.00 represent almost perfect agreement (Smith, 2005; Thrusfield, 2005).

4.3.3.2. Parallel testing

The parallel interpretation of results from two different tests was employed in effort to increase overall sensitivity. The overall sensitivity when using parallel testing was obtained through the following formula (Gardner *et al*., 2000):

$$Combined\ sensitivity\ test\ A\ and\ test\ B = 1 - (1 - Se\ A) * (1 - Se\ B) - ySe$$

The sensitivity covariance (ySe) value was calculated according to the formula described in Table 4.1.

Table 4.1: Results of pairwise test comparison of two tests (A and B) to be used in parallel and calculation formulas of test sensitivities and conditional dependence of Se according to Gardner *et al*., (2000).

Infected animals			
	Test B		
	+	**-**	
Test A +	a	b	a+b
-	c	d	c+d
	a+c	b+d	n1=a+b+c+d

$$SeTest\ A = \frac{(a+b)}{n1} \qquad SeTest\ B = \frac{(a+c)}{n1}$$

$$ySe = \frac{a}{n1} - \frac{(a+b)*(a+c)}{n1*n1}$$

4.4. RESULTS

4.4.1. Test population characteristics

Although a total of 629 pig sera were tested for the presence of antibodies to ASFV using the three ELISAs in this study, only 273 sera were also tested with the immuno-blotting due to limited volumes (Table 4.2 and Appendix III).

Table 4.2: Results of domestic pig sera from the GD tested with different ELISAs and the immuno-blotting (n=273)

Diagnostic tests	Total positives	Total negatives
I-ELISA	36	237
Blocking *p72* ELISA	21	252
rp30 ELISA	17	256
Immuno-blotting	34	239

The immuno-blotting assay is considered the gold standard for ASF antibody detection and therefore was used as the reference for the evaluation of the three ELISAs. The accuracy of the I-ELISA, Blocking *p72* ELISA and *rp30* ELISA, relative to the OIE recommended immuno-blotting are presented in Table 4.3.

Table 4.3: Comparison of immuno-blotting (Test A) results with the I-ELISA (Test B), Blocking *p72* ELISA (Test C) and *rp30* ELISA (Test D) (n=273)

	Test A			Test A			Test A	
	+	-		+	-		+	-
Test B +	30	6	**Test C +**	17	4	**Test D +**	9	8
-	4	233	**-**	17	235	**-**	25	231

4.4.2. Estimates of sensitivity, specificity, prevalence, agreement between tests and predictive values

The estimates of sensitivity and specificity for each of the three ELISAs based on the 273 sera from domestic pigs are shown in Table 4.4. The I-ELISA had the best sensitivity and specificity (88.2%, 95% CI [77.4; 99.1] and 97.5%, 95% CI [95.5; 99.5] respectively). The sensitivity and specificity of the *rp30* ELISA were 26.5%, 95% CI [11.6; 41.3] and 96.7%, 95% CI [94.4; 98.9] and those of the Blocking *p72* ELISA were 50.0, 95% CI [33.2; 66.8] and 98.3%, 95% CI [96.7; 100] respectively. In addition, the negative predictive values (NPV) were higher in the I-ELISA and Blocking *p72* ELISA compared to those found in the *rp30* ELISA (98.3%, 95% CI [96.7; 100] and 93.3%, 95% CI [90.2; 96.4] vs. 90.2%, 95% CI [86.6; 93.9] respectively, Table 4.4).

Table 4.4: Estimates of sensitivity, specificity, apparent prevalence and predictive values of three ELISAs relative to the reference immuno-blotting assay for ASF based on domestic pig sera from Gorongosa.

Parameter estimates	**I-ELISA***	***rp30* ELISA***	**Blocking *p72* ELISA***
Sensitivity	88.2% [77.4; 99.1]	26.5% [11.6; 41.3]	50% [33.2; 66.8]
Specificity	97.5% [95.5; 99.5]	96.7% [94.4; 98.9]	98.3% [96.7; 100]
Apparent prevalence	13.2% [9.2; 17.2]	6.2% [3.4; 9.1]	7.7% [4.5; 10.9]
Positive Predictive Value	83.3% [71.2; 95.5]	52.9% [29.2; 76.7]	81.0% [64.2; 97.7]
Negative Predictive Value	98.3% [96.7; 100]	90.2% [86.6; 93.9]	93.3% [90.2; 96.4]

*Presented as point estimate with the corresponding 95% confidence interval.

The higher performance of the I-ELISA with respect to the other two ELISAs was confirmed with kappa statistics value. The almost perfect agreement, with the kappa value of 0.84, 95% CI [0.72; 0.93], was observed between the I-ELISA and OIE recommended immuno-blotting assay (Table 4.5). The lowest agreement was between I-ELISA and *rp30* ELISA (0.28, 95% CI [0.17; 0.42]) results. A similar level of agreement (kappa value of 0.29, 95% CI [0.18; 0.44]) was observed when the former assay was compared to the OIE recommended immuno-blotting. The two new assays (*rp30* ELISA and Blocking *p72* ELISA) had moderate agreement with a kappa value of 0.38, 95% CI [0.26; 0.55].

Table 4.5: Agreement among four serological tests for antibodies against ASF virus detection as measured by the kappa statistic and corresponding 95% confidence interval.

Diagnostic tests	n=273
Immuno-blotting *versus* I-ELISA	0.84 [0.72; 0.93]
Immuno-blotting *versus rp30* ELISA	0.29 [0.18; 0.44]
Immuno-blotting *versus* Blocking *p72* ELISA	0.58 [0.46; 0.73]
I-ELISA *versus rp30* ELISA	0.28 [0.17; 0.42]
I-ELISA *versus* Blocking *p72* ELISA	0.51 [0.40; 0.67]
Blocking *p72* ELISA *versus rp30* ELISA	0.38 [0.26; 0.55]

4.4.3. Conditional dependence and test combinations

To determine whether the tests are conditionally independent or dependent, the sensitivity covariance (ySe) was calculated. Two tests are considered independent when the sensitivity covariance value is zero or negative. When the sensitivity covariance is above zero, the two tests can be considered conditionally dependant (Gardner *et al*., 2000). A 95% confidence interval calculated for the covariance term that does include 0 indicates statistically significant conditional dependence.

The I-ELISA and *rp30* ELISA were conditionally independent (ySe values below zero) and so were with the I-ELISA and the Blocking *p72* ELISA. The Blocking *p72* ELISA and *rp30* ELISA were conditionally dependent. The combination that maximised overall sensitivity was the parallel use of the I-ELISA and Blocking *p72* ELISA, which gave a sensitivity of 94.1%, 95% CI [91.3; 96.9] (Table 4.6).

Table 4.6: The combined test sensitivity values in parallel testing scheme and the respective the conditional covariance

Combination of tests	ySe (95% CI)	Combined sensitivity tests*
I-ELISA or *rp30* ELISA	- 0.265 [- 0.601; 0.071]	91.3% [87.9;94.6]
Blocking *p72* ELISA or *rp30* ELISA	0.025 [0.007; 0.044]	60.8% [55.0;66.6]
I-ELISA or Blocking *p72* ELISA	- 0.183 [- 0.519; 0.153]	94.1% [91.3;96.9]

*Presented as point estimate with the corresponding 95% confidence interval.

4.5. DISCUSSION

In the GD, pigs are kept under free-range production systems in the presence of natural reservoirs for ASFV (warthogs and infected soft ticks) and the virus may circulate among different hosts (Penrith *et al.*, 2007; Penrith & Vosloo, 2009). Therefore, frequent outbreaks could be expected. Based on the information gathered during this study, nearly 40% of pig farmers that were interviewed during the pig sampling process had apparently experienced outbreaks of ASF (Chapter 2).

With no vaccine or treatment available, ASF can only be controlled and eradicated by strict biosecurity measures and depopulation (Penrith *et al.,* 2004a, 2007). However, this control method appears less practical in most rural areas of the country. Accurate knowledge of the epidemiology and a good risk assessment is necessary to perform and implement procedures that prevent the introduction and dissemination of ASF (Wieland *et al.,* 2011). Serological assays such as ELISA are the most frequently applied methods to track disease spread, due to relatively low cost, availability and implementation in many laboratories (Filgueira-Perez *et al*., 2006; Sanchez-Vizcaino and Mur, 2013). A number of ELISA are available for antibody detection to ASFV (Filgueira-Perez *et al*., 2006; Etter *et al*., 2011; OIE, 2012; Cubillos *et al*., 2013; Sanchez-Vizcaino and Mur, 2013). These assays are often employed as an aid to disease control strategies and are valuable for disease surveillance in both endemic and non-endemic areas.

Estimations of specificity and sensitivity are required prior to the introduction of new diagnostic tests for disease surveillance (Toma *et al.*, 1999). The OIE recommended immuno-blotting was used as the reference test to estimate the performance of the OIE recommended ELISA, the Blocking *p72* ELISA and the *rp30* ELISA. Furthermore, sensitivity and specificity values can vary based on local conditions including host-pathogen interactions and the performance of these tests had not been evaluated in an endemic area of Mozambique.

The specificity estimates for the Blocking *p72* ELISA and the *rp30* ELISA were high and similar to the I-ELISA (98.3%, 95% CI [96.7; 100] and 96.7%, 95% CI [94.4; 98.9] respectively vs. 97.5%, 95% CI [95.5; 99.5], Table 4.4). The enhanced specificity of these assays is important in those tropical countries challenged by deficient cold chain for the preservation and shipment of serum samples as these assays have proven performance in poorly preserved sera (Filgueira-Perez *et al.*, 2006).

The *p30* and *p72* proteins are highly immunogenic and known to be expressed early during infection (Lopez-Otin *et al.*, 1990; Prados, *et al.,* 1993; Gomez-Puertas, *et al.,* 1996; Filgueira-Perez *et al.,* 2006) leading to an expectation of high sensitivity. However, both assays had poor sensitivity, suggesting that the infected pigs either had no antibodies to these proteins or the level of antibodies against these proteins was below the positive threshold. It has also been suggested that this may be due to the fact that only a single protein is used in each assay compared to the whole viral particles in the I-ELISA and immuno-blotting (Pastor *et al.,* 1989). Furthermore, the poor performance of these tests could also be due to sequence and antigenic differences between the isolates (E70 Spanish isolate circulating in Europe and West Africa) used to develop the ELISAs compared to those circulating in our study area. Cubillos *et al.* (2013) found three amino acid changes in Morara/Georgia isolates when compared to E70 Spanish isolate. These amino acid substitutions determine the antigenic variability among isolates. It is therefore possible that improved sensitivity could be obtained using antigen derived from the Morara/Georgia isolates or an assay based on local isolates when testing domestic pig sera from endemic areas of Mozambique.

By testing 273 serum samples taken from domestic pigs that had prior exposure to ASFV, good agreement was observed between the purified *p72* protein ELISA (the Blocking *p72* ELISA) and

both the OIE prescribed ELISA and immuno-blotting (kappa values of 0.51, 95% CI [0.40; 0.67] and 0.58, 95% CI [0.46; 0.73] respectively). The use of the Blocking *p72* ELISA likely underestimates the local prevalence when comparing apparent prevalence values in the table 4.4. Further work should be done to improve its performance.

Only the Blocking *p72* ELISA and *rp30* ELISA were conditionally dependent. It is hypothesized that two tests that measure different biological events are expected to be conditionally independent or only weakly dependent (Gardner *et al*., 2000; Claes *et al*., 2005). However, all ELISA assays assessed here detect antibodies against ASF virus and are therefore expected to be conditional dependent since they are based on a similar biological principle (Claes *et al*., 2005).

In conclusion, this is the first time that the different available ELISA tests were compared in an endemic African setting. Despite the limited number of sera available and the methodology implemented, our assessment suggested that the I-ELISA test provides the best performance when used alone or in combination with other tests. The parallel testing combination of I-ELISA and the Blocking *p72* ELISA greatly increased sensitivity of the latter. For this reason, veterinary authorities in Mozambique are advised to use these tests alone or in combination to survey domestic pigs in the endemic areas in order to maximize the chances of detecting positive animals and therefore reduce disease spread. Parallel testing would be applicable during quarantine, when animals are to be transported from the endemic regions to non-endemic regions (Smith, 2005). However, the possible large scale utilization of the Blocking *p72* ELISA for disease surveillance may be hampered by the cost, particularly in developing countries with limited resources (Cubillos *et al*., 2013; Oura *et al.*, 2013). The *rp30* ELISA for the detection and control of ASF in Mozambique performed poorly compared to the other two tests and it should not be used alone. Therefore, its application was found inappropriate for testing pigs in the ASF endemic area either alone or in combination with other tests due to increased false-negative results. Limitations of the I-ELISA and immuno-blotting in developing countries include the necessity of appropriate bio-containment facilities for antigen production and manipulations. However, these assays can be used if the regional ASF reference laboratories provide the antigen-coated ELISA plates.

This evaluation allowed us to have a good overview of the performance of different ASF diagnostic tests in an endemic area of Mozambique. However, our sample size was relatively small as this kind of diagnostic assessment typically requires several hundreds of samples. To account for a potential bias due to the small sample size, further confirmation of those results is recommended using more sophisticated and robust statistical methods such as latent class analysis (Branscum *et al.*, 2005; Toft *et al.*, 2007; Gari *et al.*, 2008).

CHAPTER 5

GENERAL DISCUSSION

African swine fever, with its devastating impact on livelihoods in Africa and beyond, has been extensively reviewed in Chapter 1. Since no vaccine is available, effective disease control is reliant on rapid and reliable laboratory detection systems (Sanchez-Vizcaino and Mur, 2013) and the introduction of basic husbandry practices as well as the control of movement of pigs and their products (Penrith *et al.,* 2004a, 2007; Wieland *et al*., 2011). The management of ASF in domestic pig populations in a specific area will depend on the nature of the epidemiological cycles of the disease and also on the predominant production systems and marketing patterns, which in the case of central Mozambique, is influenced by the presence of a large number of small scale farmers. Therefore, it is important to understand the components and epidemiology of the disease in the study area, since this information gives some insights in the potential dynamics of the disease in order to design more efficient control strategies.

This study aimed to investigate the role of sylvatic hosts in the epidemiology of ASF at the wildlife-domestic animal interface in a representative protected area of central Mozambique (the GNP). Several approaches were used:

- Domestic pigs and warthogs were tested for antibodies to ASFV and the salivary antigens of soft ticks and their respective habitats were searched for the presence of soft ticks to confirm the existence of a sylvatic cycle in the study area.
- African swine fever virus was detected from soft ticks in warthog burrows from the GNP and pig pens from the GNPBZ. In addition, the genetic differences of ASFV isolates from soft ticks were determined based on sequences of four gene regions namely: the *p72*, *p30*, *p54* and the CVR that gives different level of resolution of genotyping the ASFV (Bastos *et al*., 2003, 2004; Nix *et al*., 2006; Rowlands *et al*., 2008; Gallardo *et al*., 2009a, 2011) to further elucidate the epidemiology of the disease in Mozambique and the genetic diversity of viruses circulating in our study area. Furthermore, a questionnaire to gain more information on the current status of ASF and its linkage with the production system in the study area was applied.

- The I-ELISA, the Blocking *p72* ELISA and the *rp30* ELISA were compared to determine which test or combination of tests is most suitable for the detection of antibody against ASFV to be use in southern Africa.

The study area in Central Mozambique (GNP and its buffer zone) was selected on account of the frequent occurrence of ASF outbreaks in domestic pigs. The GNP, with an estimated population of 4000 warthogs (Sanparks, 2008), suggested the potential existence of a sylvatic cycle as described in other sites in southern and East Africa (Vial *et al*., 2007; Jori & Bastos 2009; Jori *et al.,* 2013), which could explain the endemic patterns of the disease reported in that area. In both areas of the GD (GNPBZ and ROD), pigs were kept in extensive free-range production systems. The domestic/wildlife interface where domestic pigs, warthogs and soft ticks potentially interact can act as a permanent source of ASFV (Penrith *et al.*, 2004) resulting in frequent outbreaks. The abundance of warthogs close to the GNP could potentially influence the likelihood of contacts between domestic suids, warthogs and infected soft ticks (*O. moubata*), facilitating transmission of ASFV from the sylvatic cycle.

The demonstration of antibodies against salivary gland antigens of soft ticks in domestic pigs and warthogs is an indication of previous exposure to the vector (Canals *et al.,* 1990; Diaz-Martin *et al*., 2011) (Chapters 2 and 3). The results indicated that possibly 42%, 95% CI [36.4; 47.7] of the farms had pigs bitten by soft ticks and support the hypothesis that contact between soft ticks and domestic pigs occurs in the area and is probably widespread. In addition, 77.8%, 95% CI [40.0; 97.2] and 75.0%, 95% CI [42.8; 94.5] of sampled warthogs had antibodies to soft tick salivary proteins and ASFV respectively, providing more evidence for continual exposure to infected soft ticks in the GNP as all farms that tested positive for antibodies against salivary gland antigens of soft ticks in domestic pigs were the locations where soft ticks were also sampled. Therefore, physical barriers (by confining domestic pigs) to separate domestic pigs from wild suids and their associated soft ticks should be established to prevent the occurrence of disease transmission. The control method should be supplemented by acaricide treatment or burning of the domestic pig pens to reduce the burden of soft ticks and other pests. However, the former method have proven less effective since soft ticks often occupy crevices which are inaccessible to chemical treatment (Penrith *et al*., 2004a, 2013).

The data resulting from the assessment of the presence of *Ornithodoros spp.* in wild and domestic habitats (Chapter 3) confirmed that over 90%, 95% CI [36.4; 47.7] of inspected warthog burrows and 10%, 95% CI [3.1; 23.1] of domestic pig pens were infested by soft ticks. The infestation rate of warthog burrows in the GNP was higher compared to those found in game reserves in South Africa, Kenya, Uganda and Zambia (Wilkinson *et al*., 1988; Plowright *et al.*, 1994) but similar to levels found in the Serengeti National Park (Tanzania) and Kafue National Park in Zambia (Wilkinson *et al*., 1988). Therefore, the potential risk of infected vectors spreading from wild habitats to the domestic habitats could be relatively higher in our study area than those of game reserves of southern African countries.

ASFV could be isolated from soft ticks in 47.4 %, 95% CI [3.4; 60.4] from warthog burrows and half of the domestic pig habitats. The molecular epidemiological investigation of ASFV isolated from soft ticks involved an initial genotyping approach consisting of partial sequencing of the *p72* gene region to determine the phylogenetic relationships between the ASF viruses present in our study area and those obtained in eastern Europe and other African regions (Bastos *et al.*, 2003; Bastos *et al.*, 2004; Lubisi *et al.*, 2005). Two previously described genotypes (II and V) and one novel genotype XXIII were found in domestic and natural habitats of our study area. Genotype II has previously been associated with domestic pig outbreaks in Mozambique, some Indian Ocean countries (Bastos *et al*., 2003, 2004; Lubisi *et al*., 2005, 2009), the Caucasus, the Russian Federation, (Rowlands *et al*., 2008; Oganesyan *et al*., 2013) and more recently the Balkans (OIE WAHID, 2014). Equally, genotype V was described in warthogs in Tengani (Malawi) and domestic pigs in central Mozambique (Bastos *et al*., 2003, 2004; Lubisi *et al*., 2005). Genotypes II and V may not be host specific since they have been found in both domestic and wild hosts. The geographic spread of the novel genotype XXIII described for the first time in this study still needs to be determined.

In order to improve the phylogenetic resolution, additional gene regions of ASFV were sequenced. These included nucleotide sequences of the *p30, p54* and CVR genes that were compared to those previously obtained from ASFV isolates in Europe and Africa (Appendix IA, IB, IC). The three homogenous *p72* genotypes II, V and novel XXIII resolved into seven *p30* and nine *p54* genotypes and 6 subgroups by the CVR gene region sequence data (Chapter 3). Based on these data, it appears that the presence of a sylvatic cycle in the study area could have

contributed to the observed genetic diversity within a relatively small area confirming the observations reported in East Africa (Gallardo *et al.,* 2011). The presence of a novel genotype in domestic pig pens and warthog burrows and the possibility of the presence of more genotypes that may be circulating in the sylvatic cycle, represent a potential risk of spill-over of previously unknown genotypes to domestic pigs. This might pose a problem if a vaccine should become available, as it has already been established that vaccine candidates do not provide cross protection to the whole spectrum of ASF viruses (King *et al.*, 2011). Therefore, it is important to conduct extensive surveillance among wild hosts in Mozambique and other countries to identify the most commonly circulating ASFV genotypes.

In endemic areas, selecting appropriate serological diagnostic tools is crucial to ensure early disease detection during surveillance, which would help veterinary authorities to implement appropriate control measures to prevent the spread of the disease to other susceptible populations in the event of an outbreak (Smith, 2005; Jori *et al*., 2013). The I-ELISA showed a high degree of sensitivity and specificity values of 88.2%, 95% CI [77.4; 99.1] and 97.5%, 95% CI [95.5; 99.5] respectively when using a panel of known sera. The results of this test were in almost perfect agreement with those of the "*gold standard"* immuno-blotting test for ASFV (with the kappa value of 0.84, 95% CI [0.72; 0.93]) and was subsequently used as 'gold standard' to compare three different ELISAs (described in the Chapter 4) to detect antibodies against ASF in naturally infected pigs in the endemic area of Mozambique. The results indicated that the I-ELISA can be used as a standalone test for disease surveillance only in endemic situations or possibly where low virulent infection persists, but not during outbreaks. The Blocking *p72* ELISA and *rp30* ELISA lacked sensitivity (50%, 95% CI [33.2; 66.8] and 26.5%, 95% CI [11.6; 41.3] respectively) and can only be used in combination with the OIE assay unless their sensitivity is improved.

The best test combination to increase sensitivity for ASF antibody detection is the parallel combination of the I- ELISA and the Blocking *p72* ELISA. This test combination improved the sensitivity to 94.1%, 95% CI [91.3; 96.9] for the detection of antibody against ASFV (Chapter 4). Therefore, these assays can be recommended in extensive sero-surveillance programs in order to identify and map endemic regions of ASFV and facilitate the identification of regions where ASFV is circulating in apparently healthy pig populations. However, using both tests in parallel

may not be economically sustainable particularly in countries like Mozambique with limited resources.

The I-ELISA was used to determine the sero-prevalence of ASF in domestic pigs. The overall sero-prevalence to ASFV in apparently healthy pigs was 9.1%, 95% CI [7.0; 11.7] and most of sampled pigs (522 out of 629) were less than one year of age. As was expected, the prevalence of antibodies to ASFV in the GNPBZ (12.5%, 95% CI [7.4; 17.6]) was relatively higher compared with the ROD (7.8%, 95% CI [5.4; 10.3]) although only marginally significant (OR=0.59, p=0.05). The overall sero-prevalence of our study area was similar to those results obtained using either the I-ELISA or Blocking *p72* ELISA in the Angónia District (14.3%) of Mozambique (Penrith *et al*., 2007, Matos *et al.*, 2011) and Mbeya Region (5.4%) of Tanzania (Uttenthal *et al*., 2013) and considerably lower when compared with the serological data of a survey carried out in the Mchinje District (48.0%) of Malawi (Haresnape *et al.*, 1985) and surveillance conducted in Kampala City (Uganda) where the sero-prevalence was 53.6% (Atuhaire *et al*., 2013).

These results may suggest a lower survival rate of infected pigs (particularly in adult pigs) in our study area, especially if one considers that the last outbreak prior to this study occurred only 8 months before sampling. In addition, there was also a significant association (OR=1.93, p=0.0276) between those farms which experienced suspicions of recent outbreaks and the presence of piglets (younger than 6 months). It was demonstrated that some pigs may survive infection caused by less virulent ASFV and become chronically ill probably due to activities of NK cells and cytokines. Furthermore, the presence of in-contact, but apparently healthy animals that escaped slaughtering, could become another source of transmission (Afonso *et al*., 2004; Zhang *et al*., 2006; Gil *et al*., 2008). A low survival rate inferred in this study could be due to new virulent virus strains coming from a natural reservoir.

The outcome of the questionnaire indicated that most pig farmers had pig pens in their yards, although 65% are accustomed to keeping their pigs outdoors during the dry season when there are no crops in the field, increasing the risk of interactions among pigs with resultant disease transmission. Therefore, the confinement of pig herds and implementation of basic husbandry practices may be enhanced with sufficient training. Farmers should be trained on how to source innovative, alternative food resources during feed scarcity. The high mortality of pigs experienced among 40% of the small-scale farmers may be due to ASF possibly associated with

high rates of free-ranging activities. For small-scale farmers the existence of affordable feed for pigs is one of the pre-requisites that drive the implementation of confinement of domestic pigs. For instance, 29% of the interviewed pig farmers that permanently confined their animals, fed with kitchen leftovers. This practice is likely to reduce the probability of encounters between domestic pigs, wild hosts and infected ticks from warthogs. However, if enclosed pigs are fed with products contaminated with ASFV (Costard *et al*., 2009a, b; Penrith *et al*., 2013) may increase the risk of infection with ASFV. This is most likely to occur if those kitchen leftovers contain meat or offal from infected domestic pigs, unless the swill is suitably heat-treated to ensure that the virus is inactivated and farmers need to be trained to so implement this activity. The risk from warthog carcasses has never been investigated, but adult warthogs are unlikely to contain sufficient amounts of virus in their tissues and fluids to infect domestic pigs (Penrith *et al.,* 2004a).

Considering that all pig farmers interviewed mentioned maize bran as a food source and nearly half gave their stock post-harvest by-products, an additional possible measure to reduce the risk of introduction via swill could be to train rural farmers on animal feed formulations based on locally available ingredients and promotion of growth of crops such as velvet bean (*Mucuna pruriens*) or moringa (*Moringa oleifera*) and other legumes. Alternatively, the promotion of agricultural projects to financially empower small scale farmers (for example micro credits, better market access of agricultural products and cooperative societies) can facilitate the use of industrial feeds for animals (through the reactivation of the local maize milling and animal feed plant) and allowing the farmers to integrate better husbandry practices that reduce the risk of disease introduction and increase the income from pig production (FAO, 2012; Penrith *et al*., 2013). Overall, it is important to raise awareness among rural small scale farmers on practices that should be avoided in order to mitigate the risk of introduction of ASF in their pig herds.

Outcome from the questionnaire also suggested that the pig marketing activities in the GD are predominantly done by individuals who buy live animals from local producers to sell mainly in Beira City. Over 51% of interviewed farmers acknowledged that visitors, particularly the farm-gate buyers, entered the premises to purchase pigs, increasing the risk of ASFV dissemination, as described also in other regions of Mozambique (Penrith *et al.*, 2007). Networks and linkages in the pork value chain that link production system in the GD, markets and consumers are well

described (Penrith *et al.,* 2007). This entails a "trade network" that provides numerous opportunities for ASFV transmission between different stakeholders along the pig value chain. Two alternative solutions have been suggested to reduce the risks associated with the marketing system of pigs from GD to the Beira Corridor and other regions:

- The construction of an official slaughterhouse for pigs in Gorongosa, to enable resident pig producers to sell their pigs locally for production of frozen or processed pork. An immediate advantage will be the avoidance of intermediaries between the producer and the consumers; it will restrict access to the farm premises, limiting live pig trade and discouragement of illegal animal slaughtering. It would also lead to official pre and post-mortem examination of carcasses to ensure that no unsuitable products reach the consumer. This would allow keeping frozen carcasses that can be sold in different markets of Beira Corridor, covering the pig meat needs for the main towns, without potentially incurring the risk of spreading ASFV through the movement of infected animals and the sale of meat of uncertain origin for public consumption.
- Setting up of a small pig-meat processing plant, in addition to the slaughterhouse, for the production of sausages and other pork products. This could bring an added values to these products that could subsequently reach more distant markets than those located in the Beira Corridor. Further, it could be a way of pre-treating and converting pork and pork products coming from GD into a more commercially-oriented and branded product from GD.

These solutions must be complemented by regular disease monitoring systems and implementation of good farm-to-fork traceability plans at the abattoir level to rapidly detect and destroy infected animals and their carcasses. In view of the above, the different role players in the animal industry (veterinarians, epidemiologists, ecologists and wildlife conservationists) and the public health officials in Sofala Province in particular, and the country in general, will need to cooperate in using the scarce government resources to achieve a jointly-operated efficient surveillance and monitoring system. The proposed construction of a formal slaughterhouse and the improvement of the current veterinary inspection systems will enable periodic epidemiological surveillance of pigs to avoid introducing ASFV infected pigs in the pig meat value chain (Sanchez-Vizcaino & Mur, 2013). Additionally, the national veterinary services in

partnership with the private sector and NGOs must devise means of securing sufficient funds to achieve this goal.

African swine fever in Mozambique still remains under-reported due to various reasons including but not limited to - the lack of veterinary infrastructure, farmers' awareness and well equipped field personnel. Efforts to improve the situation are being implemented. The Regional Veterinary Laboratory (RVL) in Chimoio (Central Mozambique), located approximately 130 Km from the GD, was recently equipped and laboratory personnel have received training in Blocking *p72* ELISA for ASFV antibody detection, direct immunofluorescence for ASFV and PCR as well as histopathology to diagnose ASF and other diseases. In addition, the National Directorate of Veterinary Service (NDVS) and RVL are jointly implementing a project on infectious disease control system including ASF and other transboundary diseases. Moreover, the molecular epidemiological data presented in the Chapter 3 as well as sero-epidemiological tools can be used to trace the origin of the disease and the circulation of ASFV in a particular area (Penrith *et al*., 2007, 2013) and would give support to the formulation of appropriate strategies for disease control (Boshoff *et al*., 2007).

The outcomes of this body of work warrants the need for more research in Mozambique in order to identify potential areas at risk of emergence of ASF. These areas include the northeastern district of Tete Province (Central Mozambique) that shares the border with southern Malawi where the first outbreak of ASF was confirmed in the mid 50's as well as Caia and Marromeu Districts (north of Sofala Province), the Morrumbala District in Zambezia Province (Central Mozambique) within Zambeze River Basin and the area neighboring the transfrontier conservation areas (TFCA) in the south of Mozambique where there are very little data available. Surveillance and control efforts targeted to those areas would ensure that the most commonly circulating ASFV strains are known.

In summary, the results of this study have shown for the first time that the epidemiology of ASF in Mozambique is as complex as in many other countries in eastern and southern Africa where a sylvatic and a domestic cycle of ASF co-exist. The management of ASF in the domestic pig population is not only dependent on the production systems and trade patterns, but also on the interconnection with the sylvatic cycle (i.e. wild hosts and soft ticks) and many socio-economical, cultural and management factors. All the key components of the sylvatic as well as

domestic cycle and the concomitant risk factors are present in the study area, which certainly contribute to the maintenance of the disease and pose challenges for control at farm level and on a broader scale (perhaps at regional level). Additionally, the fact that pigs in the area show a relatively low survival rate during outbreaks (as suggested by the low level seropositive animals) complicates sero-surveillance efforts, added to the probable incursion of new ASFV strains spilling over from sylvatic cycle to the domestic cycle. This means that sero-surveillance in such areas provides only limited information about the current presence and spread of the disease. Thus, the approach of combining targetted questionnaire (to various stakeholders) with pig sampling was assumed to be of great importance particularly in areas where information is scarce. It enabled more in-depth knowledge of the complex epidemiology of ASFV as well as the identification of factors that are likely to contribute to the risk of maintenance and dissemination of the disease. It may shed light on the socio-economic and socio-cultural dynamics that complicates this epidemiology. Therefore, these are considered important tools for appropriate disease control strategies within the pig production value chain (Penrith *et al*., 2013).

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APPENDIX I

A. Nucleotide alignment of *p72* gene the 52 sequences in this study (in bold) and in previous studies. Dots indicate nucleotide sites identical to that of the master sequence (Mal/1/02)

AY494553 MAL/1/02	C	A	G	C	C	T	A	C	T	C	A	C	C	A	C	G	C	A	G	A	G	A	T	A	A	G	C	T	T	T	C	A	G	G	A	T	A	G	A	40
AY494552 TAN/1/01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	40
AY494551 TAN/2/03	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
AY351555 NYA/12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
AY351543 MZI/1/92	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
AY351542 SUM/1411	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
AY351522 KAB/62	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
AY351518 MOZ/2/02	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
AF449475 UGA/1/95	.	.	.	.	.	.	.	.	C	.	.	.	.	.	?	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
AF302818 RSA/1/98	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
AF301541 Tengani/60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
AF270712 MOZ/8/94	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
AF270711 MOZ/1/94	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
AF270710 SPEC265	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
AF270709 Mozambique/1979	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
AF270708 Mozambique/1960	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
AF270706 MAD/1/98	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
AF270705 MOZ/1/98	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
AF449463 BUR/1/84	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
AF302816 BEN/1/97	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
AF301542 ANG/70	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
AF301539 Lisbon/60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
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Moz 11/2006	.	.	.	.	.	C	T	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
Moz 13/2006	.	C	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
Moz 12/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
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AY494553 MAL/1/02	G	A	T	A	C	A	G	C	T	C	T	T	C	C	A	G	A	C	G	C	A	T	G	T	T	C	A	T	C	T	A	T	A	T	C	T	G	A	T	80
AY494552 TAN/1/01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
AY494551 TAN/2/03	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
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AY351543 MZI/1/92	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
AY351542 SUM/1411	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
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AY351518 MOZ/2/02	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
AF449475 UGA/1/95	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	80
AF302818 RSA/1/98	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
AF301541 Tengani/60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80

AF270712 MOZ/8/94	. . . .	80
AF270711 MOZ/1/94	. . . .	80
AF270710 SPEC265	. . . .	80
AF270709 Mozambique/1979	. . . .	80
AF270708 Mozambique/1960	. . . .	80
AF270706 MAD/1/98	. . . .	80
AF270705 MOZ/1/98	. . . .	80
AF449463 BUR/1/84	. . . . T . . . . C . . . . . A . . .	80
AF302816 BEN/1/97	. . . . G . . .	80
AF301542 ANG/70	. . . . G . . .	80
AF301539 Lisbon/60	. . . . G . . .	80
SPEC/245	. . . .	80
SPEC/257	. . . .	80
ZIM/1/92	. . . . G . . .	80
NAM/1/95	. . . .	80
RSA/1/96	. . . .	80
RSA/3/96	. . . .	80
RSA/1/99/Thab	. . . . G . . .	80
SPEC/205	. . . . G . . .	80
UGA/1B/03	. . . . T . . . . C	80
Lillie	. . . .	80
MAU/2007/1	. . . .	80
Moz 1/2006	. . . .	80
Moz 2/2006	. . . .	80
Moz 3/2006	. . . .	80
Moz 4/2006	. . . .	80
Moz 5/2006	. . . .	80
Moz 6/2006	. . . . A . . . . T	80
Moz 7/2006	. . . . C . . . . T	80
Moz 8/2006	. . . .	80
Moz 9/2006	. . . .	80
Moz 10/2006	. . . .	80
Moz 11/2006	. . . .	80
Moz 13/2006	. . . .	80
Moz 12/2006	. . . .	80

Moz 14/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
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AY494553 MAL/1/02	A	T	T	A	G	C	C	C	C	G	T	C	A	C	G	T	A	T	C	C	G	A	T	C	A	C	A	T	T	A	C	C	T	A	T	T	A	T	T	120
AY494552 TAN/1/01	.	.	.	.	A	T	.	.	.	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
AY494551 TAN/2/03	.	.	.	.	A	.	.	.	.	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
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AY351543 MZI/1/92	.	.	.	.	A	.	.	.	.	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
AY351542 SUM/1411	.	.	.	.	A	.	.	.	.	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
AY351522 KAB/62	.	.	.	.	A	.	.	.	.	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
AY351518 MOZ/2/02	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
AF449475 UGA/1/95	.	.	.	.	C	.	.	.	.	A	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
AF302818 RSA/1/98	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
AF301541 Tengani/60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
AF270712 MOZ/8/94	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
AF270711 MOZ/1/94	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
AF270710 SPEC265	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
AF270709 Mozambique/1979	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
AF270708 Mozambique/1960	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
AF270706 MAD/1/98	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
AF270705 MOZ/1/98	.	.	.	.	A	.	.	.	.	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
AF449463 BUR/1/84	.	.	.	T	C	.	.	.	.	A	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	120
AF302816 BEN/1/97	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
AF301542 ANG/70	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
AF301539 Lisbon/60	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
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ZIM/1/92	.	.	.	.	.	.	.	.	.	A	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
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RSA/1/96	.	.	.	.	.	.	.	.	.	A	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
RSA/3/96	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	120

RSA/1/99/Thab	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
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UGA/1B/03	.	.	.	.	C	.	.	.	.	A	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
Lillie	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	120
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AY494553 MAL/1/02	A	A	A	A	A	C	A	T	T	T	C	C	G	T	A	A	C	T	G	C	T	C	A	T	G	G	T	A	T	C	A	A	T	C	T	T	A	T	C	160
AY494552 TAN/1/01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	160
AY494551 TAN/2/03	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	160
AY351555 NYA/12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	160
AY351543 MZI/1/92	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	160
AY351542 SUM/1411	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	160
AY351522 KAB/62	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	160
AY351518 MOZ/2/02	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	160
AF449475 UGA/1/95	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	160
AF302818 RSA/1/98	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	160
AF301541 Tengani/60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	160

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AF270709 Mozambique/1979	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	160
AF270708 Mozambique/1960	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	160
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AF449463 BUR/1/84	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	160
AF302816 BEN/1/97	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	160
AF301542 ANG/70	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	160
AF301539 Lisbon/60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	160
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AY494553 MAL/1/02	G	A	T	A	A	A	T	T	T	C	C	A	T	C	A	A	A	G	T	T	C	T	G	C	A	G	C	T	C	T	T	A	C	A	T	A	C	C	T	200
AY494552 TAN/1/01	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	200
AY494551 TAN/2/03	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	200
AY351555 NYA/12	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	200
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AY351542 SUM/1411	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	200
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AY351518 MOZ/2/02	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	200
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AF301541 Tengani/60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	200
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AF270709 Mozambique/1979	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	200
AF270708 Mozambique/1960	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	200
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AF449463 BUR/1/84	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	200
AF302816 BEN/1/97	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	200
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AY494553 MAL/1/02	T	T	C	C	A	C	T	A	C	G	G	A	G	G	C	A	A	T	G	C	G	A	T	T	A	A	A	A	C	C	C	C	C	G	A	T	G	A	T	240
AY494552 TAN/1/01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	T	.	.	.	.	.	.	240
AY494551 TAN/2/03	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	T	.	.	C	.	.	.	240
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AY351543 MZI/1/92	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	240
AY351542 SUM/1411	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	240
AY351522 KAB/62	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	240
AY351518 MOZ/2/02	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	240
AF449475 UGA/1/95	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	240
AF302818 RSA/1/98	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	.	.	240
AF301541 Tengani/60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	240

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AF270711 MOZ/1/94	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	240
AF270710 SPEC265	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	240
AF270709 Mozambique/1979	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	240
AF270708 Mozambique/1960	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	240
AF270706 MAD/1/98	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	240
AF270705 MOZ/1/98	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	240
AF449463 BUR/1/84	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	240
AF302816 BEN/1/97	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	240
AF301542 ANG/70	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	240
AF301539 Lisbon/60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	240
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AY494553 MAL/1/02	C	C	G	G	G	T	G	C	G	A	T	G	A	T	G	A	T	T	A	C	C	T	T	T	G	C	C	T	T	G	A	A	G	C	C	A	C	G	G	280
AY494552 TAN/1/01	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	280
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AF301541 Tengani/60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	280
AF270712 MOZ/8/94	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	280
AF270711 MOZ/1/94	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	280
AF270710 SPEC265	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	280
AF270709 Mozambique/1979	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	280
AF270708 Mozambique/1960	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	280
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AF301539 Lisbon/60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	280
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AY494552 TAN/1/01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	320
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AY351543 MZI/1/92	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	320
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AF301539 Lisbon/60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	320
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Moz 17/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	320
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AY494553 MAL/1/02	G	C	A	A	G	A	G	A	A	T	T	T	T	A	T	A	T	T	A	G	T	T	G	G	G	A	T	A	C	G	G	A	T	T	A	C	G	T	G	360
AY494552 TAN/1/01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.	.	.	360
AY494551 TAN/2/03	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.	.	.	360
AY351555 NYA/12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.	.	.	360
AY351543 MZI/1/92	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.	.	.	360
AY351542 SUM/1411	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.	.	.	360
AY351522 KAB/62	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.	.	.	360
AY351518 MOZ/2/02	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	360
AF449475 UGA/1/95	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	A	.	.	.	.	.	T	.	.	.	360
AF302818 RSA/1/98	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.	.	.	360
AF301541 Tengani/60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	360
AF270712 MOZ/8/94	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	360
AF270711 MOZ/1/94	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	360
AF270710 SPEC265	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	360
AF270709 Mozambique/1979	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	360
AF270708 Mozambique/1960	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	360
AF270706 MAD/1/98	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	360
AF270705 MOZ/1/98	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.	.	.	360
AF449463 BUR/1/84	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	A	.	.	.	.	.	T	.	.	.	360
AF302816 BEN/1/97	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	360
AF301542 ANG/70	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	360
AF301539 Lisbon/60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	360
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UGA/1B/03	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	A	.	.	.	.	.	T	.	.	.	360
Lillie	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	360
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Moz 4/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	360
Moz 5/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	360
Moz 6/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	360
Moz 7/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	360
Moz 8/2006	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	360
Moz 9/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	360
Moz 10/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	360
Moz 11/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	360
Moz 13/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	360
Moz 12/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	360
Moz 14/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	360
Moz 16/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	360
Moz 17/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	360
Moz 18/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	360
Moz 19/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	360
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AY494553 MAL/1/02	G	G	G	T	C	T	A	T	C	A	C	T	A	C	G	G	C	T	G	A	T	C	T	T	G	T	G	G	T	A	T	C	A	G	C	A	T	C	T	400
AY494552 TAN/1/01	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
AY494551 TAN/2/03	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
AY351555 NYA/12	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
AY351543 MZI/1/92	.	.	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
AY351542 SUM/1411	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	C	.	.	G	C	.	G	.	.	.	.	400
AY351522 KAB/62	.	.	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	400
AY351518 MOZ/2/02	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
AF449475 UGA/1/95	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	400
AF302818 RSA/1/98	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
AF301541 Tengani/60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	400

AF270712 MOZ/8/94	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
AF270711 MOZ/1/94	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
AF270710 SPEC265	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
AF270709 Mozambique/1979	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	400
AF270708 Mozambique/1960	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
AF270706 MAD/1/98	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
AF270705 MOZ/1/98	.	.	A	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	400
AF449463 BUR/1/84	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	400
AF302816 BEN/1/97	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
AF301542 ANG/70	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
AF301539 Lisbon/60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
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SPEC/257	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
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RSA/3/96	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
RSA/1/99/Thab	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
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UGA/1B/03	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	400
Lillie	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
MAU/2007/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
Moz 1/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
Moz 2/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
Moz 3/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
Moz 4/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
Moz 5/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
Moz 6/2006	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
Moz 7/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	400
Moz 8/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
Moz 9/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
Moz 10/2006	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
Moz 11/2006	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400
Moz 13/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	?	.	.	.	.	.	.	G	.	.	.	.	.	.	400
Moz 12/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	400

Moz 14/2006	.	.	.	.	.	.	.	.	G	400
Moz 16/2006	.	.	.	.	.	C	.	.	G	400
Moz 17/2006	.	.	.	.	.	.	.	.	G	400
Moz 18/2006	.	.	.	.	.	C	.	.	G	400
Moz 19/2006	.	.	.	.	.	C	.	.	G	400
Moz 15/2006	.	.	.	.	.	.	.	.	G	400

AY494553 MAL/1/02	G	C	T	A	T	T	A	A	C	410
AY494552 TAN/1/01	.	.	.	.	.	.	.	.	.	410
AY494551 TAN/2/03	.	.	.	.	.	.	.	.	.	410
AY351555 NYA/12	.	.	C	.	.	.	.	.	.	410
AY351543 MZI/1/92	.	.	.	.	.	.	.	.	.	410
AY351542 SUM/1411	.	.	.	.	.	.	.	.	.	410
AY351522 KAB/62	.	.	.	.	.	.	.	.	.	410
AY351518 MOZ/2/02	.	.	.	.	.	.	.	.	.	410
AF449475 UGA/1/95	.	.	.	.	.	.	.	.	.	410
AF302818 RSA/1/98	.	.	.	.	.	.	.	.	.	410
AF301541 Tengani/60	.	.	.	.	.	.	.	.	.	410
AF270712 MOZ/8/94	.	.	.	.	.	.	.	.	.	410
AF270711 MOZ/1/94	.	.	.	.	.	.	.	.	.	410
AF270710 SPEC265	.	.	.	.	.	.	.	.	.	410
AF270709 Mozambique/1979	.	.	.	.	.	.	.	.	.	410
AF270708 Mozambique/1960	.	.	.	.	.	.	.	.	.	410
AF270706 MAD/1/98	.	.	.	.	.	.	.	.	.	410
AF270705 MOZ/1/98	.	.	.	.	.	.	.	.	.	410
AF449463 BUR/1/84	.	.	.	.	.	.	.	.	.	410
AF302816 BEN/1/97	.	.	.	.	.	.	.	.	.	410
AF301542 ANG/70	.	.	.	.	.	.	.	.	.	410
AF301539 Lisbon/60	.	.	.	.	.	.	.	.	.	410
SPEC/245	.	.	.	.	.	.	.	.	.	410
SPEC/257	.	.	.	.	.	.	.	.	.	410
ZIM/1/92	.	.	.	.	.	.	.	.	.	410
NAM/1/95	.	.	.	.	.	.	.	.	.	410
RSA/1/96	.	.	.	.	.	.	.	.	.	410
RSA/3/96	.	.	.	.	.	.	.	.	.	410

B. Nucleotide alignment of *p30* gene the 44 sequences in this study (in bold) and in previous studies. Dots indicate nucleotide sites identical to that of the master sequence (TAN/03/2)

TAN/03/2	A	T	G	A	A	A	A	T	G	G	A	G	G	T	C	A	T	C	T	T	C	A	A	A	A	C	G	G	A	T	T	T	A	A	G	A	T	C	A	40
MOZ/94/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
SPEC/265	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
SPEC/257	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
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MOZ/02/2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
MOZ/94/8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
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MZI/92/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
KAB/62	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
BEN/97/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
TENGANI/62	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
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UGA/95/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
NYA1/2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	G	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	40
TAN/01/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
SPEC/205	G	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
LILLIE	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
RSA/99/1/W	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
MOZ/1960	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
MOZ/1979	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
MAL/02/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
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MUR/07/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
Moz 16/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
Moz 17/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
Moz 14/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
Moz 15/2006	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
Moz 10/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40

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Moz 19/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
Moz 18/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
Moz 8/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	40
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Moz 7/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
Moz 6/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
Moz 5/2006	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
Moz 4/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
Moz 3/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
Moz 2/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
Moz 12/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
Moz 9/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
TAN/03/2	T	C	T	T	C	A	C	A	A	G	T	C	G	T	G	T	T	T	C	A	T	G	C	A	G	G	T	A	G	C	C	T	G	T	A	T	A	A	T	80
MOZ/94/1	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
SPEC/265	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
SPEC/257	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	80
ANG/70	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	80
LIS/60	.	.	.	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	80
MOZ/02/2	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
MOZ/94/8	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
RSA/96/3	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	80
SUM/1411	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	80
MZI/92/1	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	80
KAB/62	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
BEN/97/1	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	80
TENGANI/62	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
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UGA/95/1	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	C	C	80
NYA1/2	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
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LILLIE	.	.	C	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
RSA/99/1/W	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	80
MOZ/1960	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80

MOZ/1979	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
MAL/02/1	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	80
RSA/98/1	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	80
MUR/07/1	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
Moz 16/2006	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
Moz 17/2006	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
Moz 14/2006	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
Moz 15/2006	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
Moz 10/2006	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
Moz 13/2006	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
Moz 19/2006	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
Moz 18/2006	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
Moz 8/2006	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
Moz 1/2006	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
Moz 7/2006	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
Moz 6/2006	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
Moz 5/2006	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
Moz 4/2006	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
Moz 3/2006	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
Moz 2/2006	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
Moz 12/2006	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80
Moz 9/2006	.	.	.	.	.	.	.	C	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	80

TAN/03/2	T	G	G	T	T	T	T	C	C	A	T	T	G	A	G	A	T	T	A	T	T	A	A	T	A	G	T	G	G	T	A	G	A	A	T	T	G	T	T	120
MOZ/94/1	.	.	.	.	.	.	.	.	T	G	.	.	T	C	C	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
SPEC/265	.	.	.	.	.	.	.	.	T	G	.	.	.	T	C	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
SPEC/257	.	.	.	.	.	.	.	.	T	G	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
ANG/70	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
LIS/60	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
MOZ/02/2	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
MOZ/94/8	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
RSA/96/3	.	.	.	.	.	.	.	.	T	G	.	.	C	.	.	.	.	.	.	.	C	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	120
SUM/1411	.	.	.	.	.	.	.	.	.	T	.	C	C	C	C	.	.	.	.	.	.	.	.	G	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	120
MZI/92/1	.	.	.	.	.	.	.	.	.	T	.	.	C	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
KAB/62	.	.	.	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
BEN/97/1	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120

TENGANI/62	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
BUR/84/1	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
UGA/95/1	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
NYA1/2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
TAN/01/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
SPEC/205	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
LILLIE	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
RSA/99/1/W	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
MOZ/1960	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
MOZ/1979	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
MAL/02/1	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
RSA/98/1	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
MUR/07/1	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
Moz 16/2006	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
Moz 17/2006	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
Moz 14/2006	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
Moz 15/2006	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
Moz 10/2006	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
Moz 13/2006	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
Moz 19/2006	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
Moz 18/2006	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
Moz 8/2006	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
Moz 1/2006	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
Moz 7/2006	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
Moz 6/2006	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
Moz 5/2006	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
Moz 4/2006	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
Moz 3/2006	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
Moz 2/2006	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
Moz 12/2006	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
Moz 9/2006	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	120
TAN/03/2	A	C	T	A	C	C	G	C	T	A	T	A	A	A	A	A	C	A	T	T	G	C	T	C	A	A	T	A	C	T	G	T	T	A	A	G	T	A	T	160
MOZ/94/1	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	160
SPEC/265	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	160
SPEC/257	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	160

ANG/70	. . G . . . . G	160
LIS/60	. . G . . . . G	160
MOZ/02/2	. . G . . . . T . G	160
MOZ/94/8	. . G . . . . G	160
RSA/96/3	. . G . . . . G	160
SUM/1411	. . G	160
MZI/92/1	. . G	160
KAB/62	. . G	160
BEN/97/1	. . G . . . . G	160
TENGANI/62	. . G . . . . G	160
BUR/84/1	. . A . . . . C . . . . . . G	160
UGA/95/1	. . A . . . . C . . . . . . G	160
NYA1/2	. . G	160
TAN/01/1	. . . .	160
SPEC/205	. . G	160
LILLIE	. . G . . . . G	160
RSA/99/1/W	. . G . . . . G	160
MOZ/1960	. . G . . . . G	160
MOZ/1979	. . G . . . . G	160
MAL/02/1	. . G . . . . G	160
RSA/98/1	. . G . . . . G	160
MUR/07/1	. . G . . . . T . G	160
Moz 16/2006	. . G . . . . G	160
Moz 17/2006	. . G . . . . G	160
Moz 14/2006	. . G . . . . G	160
Moz 15/2006	. . G . . . . G	160
Moz 10/2006	. . G . . . . G	160
Moz 13/2006	. . G . . . . T . G	160
Moz 19/2006	. . G . . . . G	160
Moz 18/2006	. . G . . . . G	160
Moz 8/2006	. . G . . . . T . G	160
Moz 1/2006	. . G . . . . T . G	160
Moz 7/2006	. . G . . . . T . G	160
Moz 6/2006	. . G . . . . T . G	160
Moz 5/2006	. . G . . . . T . G	160
Moz 4/2006	. . G . . . . T . G	160

Moz 3/2006	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	160
Moz 2/2006	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	160
Moz 12/2006	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	160
Moz 9/2006	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	160

TAN/03/2	G	A	T	A	T	T	G	T	G	A	G	A	T	C	T	G	C	G	C	G	T	G	T	A	T	A	C	G	C	A	G	G	G	C	A	G	G	G	A	200
MOZ/94/1	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	T	.	.	.	.	.	A	.	.	A	.	.	G	200
SPEC/265	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	T	.	.	.	.	.	A	.	.	A	.	.	G	200
SPEC/257	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	G	200
ANG/70	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	T	.	A	.	A	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	G	200
LIS/60	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	T	.	A	.	A	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	G	200
MOZ/02/2	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	G	200
MOZ/94/8	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	G	200
RSA/96/3	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	T	.	.	.	C	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	G	200
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TENGANI/62	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	T	.	.	.	.	.	A	.	.	A	.	.	G	200
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UGA/95/1	.	.	.	.	.	.	.	.	.	.	.	.	A	A	.	.	.	T	.	.	C	A	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	200
NYA1/2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	200
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MOZ/1979	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	T	.	.	.	.	.	A	.	.	A	.	.	G	200
MAL/02/1	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	G	200
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Moz 14/2006	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	T	.	.	.	.	.	A	.	.	A	.	.	G	200
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Moz 8/2006	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	G	200
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Moz 5/2006	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	G	200
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Moz 2/2006	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	G	200
Moz 12/2006	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	G	200
Moz 9/2006	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	G	200

TAN/03/2	T	A	T	A	C	T	G	A	A	C	A	G	C	A	G	G	C	T	C	A	A	G	A	A	G	A	A	T	G	G	A	A	C	A	T	G	A	T	T	240
MOZ/94/1	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	240
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NYA1/2	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	240
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MUR/07/1	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	240
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TAN/03/2	C	T	G	C	A	C	G	T	G	C	T	G	T	T	T	G	A	A	G	A	G	G	A	G	A	T	G	G	A	A	T	C	C	T	C	A	-	-	-	280
MOZ/94/1	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
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MOZ/02/2	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
MOZ/94/8	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
RSA/96/3	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
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MZI/92/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	C	A	.	.	.	.	.	.	.	.	.	-	-	-	280
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BEN/97/1	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	.	.	.	.	.	.	.	.	.	-	-	-	280

TENGANI/62	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
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UGA/95/1	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	A	G	.	.	-	-	-	280
NYA1/2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
TAN/01/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	.	.	.	.	.	.	.	.	.	-	-	-	280
SPEC/205	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
LILLIE	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
RSA/99/1/W	.	.	.	.	.	T	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
MOZ/1960	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
MOZ/1979	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
MAL/02/1	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
RSA/98/1	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
MUR/07/1	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
Moz 16/2006	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
Moz 17/2006	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
Moz 14/2006	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
Moz 15/2006	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
Moz 10/2006	.	.	A	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
Moz 13/2006	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
Moz 19/2006	.	.	A	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
Moz 18/2006	.	.	A	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
Moz 8/2006	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
Moz 1/2006	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
Moz 7/2006	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
Moz 6/2006	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
Moz 5/2006	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
Moz 4/2006	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
Moz 3/2006	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
Moz 2/2006	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
Moz 12/2006	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280
Moz 9/2006	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	-	-	-	280

TAN/03/2	-	-	-	G	G	A	T	C	A	T	T	G	G	A	A	A	G	T	A	T	T	C	A	T	G	A	A	A	A	A	A	A	T	-	-	-	-	-	-	320
MOZ/94/1	-	-	-	.	C	.	.	.	.	.	C	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
SPEC/265	-	-	-	.	C	.	.	.	.	.	C	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
SPEC/257	-	-	-	.	C	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320

ANG/70	-	-	-	.	C	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
LIS/60	-	-	-	.	C	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
MOZ/02/2	-	-	-	.	C	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
MOZ/94/8	-	-	-	.	C	.	.	.	.	.	C	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
RSA/96/3	-	-	-	.	C	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
SUM/1411	-	-	-	.	C	.	.	.	C	.	.	A	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
MZI/92/1	-	-	-	.	C	.	.	.	C	.	.	A	.	.	G	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
KAB/62	-	-	-	.	C	.	.	.	C	.	.	A	.	.	G	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
BEN/97/1	-	-	-	.	C	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
TENGANI/62	-	-	-	.	C	.	.	.	.	.	C	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
BUR/84/1	-	-	-	-	-	-	.	.	C	.	.	A	.	.	G	.	.	.	.	A	.	.	.	.	.	.	.	.	C	T	.	.	.	G	G	T	C	A	T	320
UGA/95/1	-	-	-	-	-	-	.	.	C	.	.	A	.	.	G	.	.	C	.	A	.	.	.	.	.	.	.	.	C	T	.	.	.	G	G	T	C	A	T	320
NYA1/2	-	-	-	.	C	.	.	.	C	.	.	A	.	.	G	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
TAN/01/1	-	-	-	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
SPEC/205	-	-	-	.	.	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	A	T	C	A	T	320
LILLIE	-	-	-	.	C	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
RSA/99/1/W	-	-	-	.	C	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
MOZ/1960	-	-	-	.	C	.	.	.	.	.	C	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
MOZ/1979	-	-	-	.	C	.	.	.	.	.	C	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
MAL/02/1	-	-	-	.	C	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
RSA/98/1	-	-	-	.	C	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
MUR/07/1	-	-	-	.	C	.	.	.	T	.	C	.	.	.	G	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
Moz 16/2006	-	-	-	.	C	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
Moz 17/2006	-	-	-	.	C	.	.	.	.	.	C	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
Moz 14/2006	-	-	-	.	C	.	.	.	.	.	C	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
Moz 15/2006	-	-	-	.	C	.	.	.	.	.	C	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
Moz 10/2006	-	-	-	.	C	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
Moz 13/2006	-	-	-	.	C	.	.	.	T	.	C	.	.	.	G	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
Moz 19/2006	-	-	-	.	C	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
Moz 18/2006	-	-	-	.	C	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
Moz 8/2006	-	-	-	.	C	.	.	.	T	.	C	.	.	.	G	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
Moz 1/2006	-	-	-	.	C	.	.	.	T	.	C	.	.	.	G	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
Moz 7/2006	-	-	-	.	C	.	.	.	T	.	C	.	.	.	G	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
Moz 6/2006	-	-	-	.	C	.	.	.	T	.	C	.	.	.	G	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
Moz 5/2006	-	-	-	.	C	.	.	.	T	.	C	.	.	.	G	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
Moz 4/2006	-	-	-	.	C	.	.	.	T	.	C	.	.	.	G	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320

Moz 3/2006	-	-	-	.	C	.	.	.	T	.	C	.	.	.	G	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
Moz 2/2006	-	-	-	.	C	.	.	.	T	.	C	.	.	.	G	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
Moz 12/2006	-	-	-	.	C	.	.	.	T	.	C	.	.	.	G	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
Moz 9/2006	-	-	-	.	C	.	.	.	T	.	C	.	.	.	G	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	320
TAN/03/2	-	-	-	-	-	-	-	-	-	G	G	T	C	A	T	G	A	A	G	C	T	A	A	T	G	A	A	T	G	C	A	C	A	T	C	A	T	C	T	360
MOZ/94/1	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
SPEC/265	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
SPEC/257	-	-	-	-	-	-	-	-	-	.	A	.	A	G	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
ANG/70	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
LIS/60	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
MOZ/02/2	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
MOZ/94/8	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	C	360
RSA/96/3	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
SUM/1411	-	-	-	-	-	-	-	-	-	.	A	.	.	.	.	.	.	.	A	T	A	G	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	360
MZI/92/1	-	-	-	-	-	-	-	-	-	.	A	.	.	.	.	.	.	.	A	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	360
KAB/62	-	-	-	-	-	-	-	-	-	.	A	.	.	.	.	.	.	.	A	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	360
BEN/97/1	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
TENGANI/62	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
BUR/84/1	A	A	A	G	C	G	G	A	T	.	.	C	.	.	.	.	.	.	A	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	360
UGA/95/1	A	A	A	G	C	G	G	A	T	.	.	C	.	.	.	.	.	.	A	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
NYA1/2	-	-	-	-	-	-	-	-	-	.	A	.	.	.	.	.	.	.	A	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	360
TAN/01/1	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	360
SPEC/205	G	A	A	A	A	A	A	A	T	.	.	.	G	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	360
LILLIE	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	G	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
RSA/99/1/W	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
MOZ/1960	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
MOZ/1979	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
MAL/02/1	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
RSA/98/1	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
MUR/07/1	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
Moz 16/2006	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	C	360
Moz 17/2006	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
Moz 14/2006	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
Moz 15/2006	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
Moz 10/2006	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	C	360

Moz 13/2006	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
Moz 19/2006	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	C	360
Moz 18/2006	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	C	360
Moz 8/2006	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
Moz 1/2006	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
Moz 7/2006	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
Moz 6/2006	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
Moz 5/2006	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
Moz 4/2006	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
Moz 3/2006	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
Moz 2/2006	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
Moz 12/2006	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
Moz 9/2006	-	-	-	-	-	-	-	-	-	.	A	.	A	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	360
TAN/03/2	T	T	T	G	A	A	A	C	T	C	T	A	T	T	T	G	A	A	C	A	A	G	A	G	C	C	C	T	C	T	T	C	A	A	C	A	G	A	G	400
MOZ/94/1	.	.	.	.	.	.	.	.	A	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	.	A	.	.	.	-	-	-	.	.	T	400
SPEC/265	.	.	.	.	.	.	.	.	A	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	.	A	.	.	.	-	-	-	.	.	T	400
SPEC/257	.	.	.	.	.	.	.	.	A	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	G	-	-	-	.	.	.	400
ANG/70	.	.	.	.	.	.	.	.	A	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	-	-	-	.	.	.	400
LIS/60	.	.	.	.	.	.	.	.	A	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	-	-	-	.	.	.	400
MOZ/02/2	.	.	.	.	.	.	.	.	G	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	G	-	-	-	.	.	.	400
MOZ/94/8	.	.	.	.	.	.	.	.	A	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	.	A	.	.	.	-	-	-	.	.	T	400
RSA/96/3	.	.	.	.	.	.	.	.	A	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	G	-	-	-	.	.	.	400
SUM/1411	.	.	.	.	.	.	.	.	C	.	.	G	.	.	.	.	.	G	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	400
MZI/92/1	.	.	.	.	.	.	.	.	C	.	.	G	.	.	.	.	.	G	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	400
KAB/62	.	.	.	.	.	.	.	.	C	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	400
BEN/97/1	.	.	.	.	.	.	.	.	A	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	-	-	-	.	.	.	400
TENGANI/62	.	.	.	.	.	.	.	.	A	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	.	A	.	.	.	-	-	-	.	.	T	400
BUR/84/1	.	.	.	.	.	.	.	.	G	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	-	-	-	.	.	.	400
UGA/95/1	.	.	.	.	.	.	.	.	G	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	-	-	-	.	.	.	400
NYA1/2	.	.	.	.	.	.	.	.	C	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	400
TAN/01/1	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	400
SPEC/205	.	.	.	.	.	.	.	.	C	.	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	400
LILLIE	.	.	.	.	.	.	.	.	A	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	G	-	-	-	.	.	.	400
RSA/99/1/W	.	.	.	.	.	.	.	.	A	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	.	A	.	.	G	-	-	-	.	.	.	400
MOZ/1960	.	.	.	.	.	.	.	.	A	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	.	A	.	.	.	-	-	-	.	.	T	400

MOZ/1979	.	.	.	.	.	.	.	.	A	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	.	A	.	.	.	-	-	-	.	.	T	400
MAL/02/1	.	.	.	.	.	.	.	.	A	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	G	-	-	-	.	.	.	400
RSA/98/1	.	.	.	.	.	.	.	.	A	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	G	-	-	-	.	.	.	400
MUR/07/1	.	.	.	.	.	.	.	.	G	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	G	-	-	-	.	.	.	400
Moz 16/2006	.	.	.	.	.	.	.	.	C	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	G	-	-	-	.	.	.	400
Moz 17/2006	.	.	.	.	.	.	.	.	A	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	.	A	.	.	.	-	-	-	.	.	T	400
Moz 14/2006	.	.	.	.	.	.	.	.	A	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	.	A	.	.	.	-	-	-	.	.	T	400
Moz 15/2006	.	.	.	.	.	.	.	.	A	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	.	A	.	.	.	-	-	-	.	.	T	400
Moz 10/2006	.	.	.	.	.	.	.	.	C	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	G	-	-	-	.	.	.	400
Moz 13/2006	.	.	.	.	.	.	.	.	G	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	G	-	-	-	.	.	.	400
Moz 19/2006	.	.	.	.	.	.	.	.	C	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	G	-	-	-	.	.	.	400
Moz 18/2006	.	.	.	.	.	.	.	.	C	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	G	-	-	-	.	.	.	400
Moz 8/2006	.	.	.	.	.	.	.	.	G	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	G	-	-	-	.	.	.	400
Moz 1/2006	.	.	.	.	.	.	.	.	G	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	.	A	.	.	G	-	-	-	.	.	.	400
Moz 7/2006	.	.	.	.	.	.	.	.	G	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	G	-	-	-	.	.	.	400
Moz 6/2006	.	.	.	.	.	.	.	.	G	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	G	-	-	-	.	.	.	400
Moz 5/2006	.	.	.	.	.	.	.	.	G	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	G	-	-	-	.	.	.	400
Moz 4/2006	.	.	.	.	.	.	.	.	G	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	G	-	-	-	.	.	.	400
Moz 3/2006	.	.	.	.	.	.	.	.	G	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	G	-	-	-	.	.	.	400
Moz 2/2006	.	.	.	.	.	.	.	.	G	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	G	-	-	-	.	.	.	400
Moz 12/2006	.	.	.	.	.	.	.	.	G	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	G	-	-	-	.	.	.	400
Moz 9/2006	.	.	.	.	.	.	.	.	G	T	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	G	-	-	-	.	.	.	400

TAN/03/2	A	C	G	C	C	C	A	A	A	G	A	C	G	C	C	A	A	A	C	T	G	T	A	T	G	C	A	C	T	T	G	C	A	C	A	A	A	A	G	440
MOZ/94/1	G	.	A	.	.	T	.	.	.	.	.	.	T	.	.	.	C	G	.	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	440
SPEC/265	G	.	A	.	.	T	.	.	.	.	.	.	T	.	.	.	C	G	.	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	440
SPEC/257	G	T	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	440
ANG/70	G	A	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	440
LIS/60	G	A	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	440
MOZ/02/2	G	T	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	440
MOZ/94/8	G	.	A	.	.	T	.	.	.	.	.	.	T	.	.	.	C	G	.	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	440
RSA/96/3	G	T	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	440
SUM/1411	.	.	A	T	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	440
MZI/92/1	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	440
KAB/62	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	440
BEN/97/1	G	A	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	440

TENGANI/62	G	.	A	.	.	T	.	.	.	.	.	.	T	.	.	.	C	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
BUR/84/1	.	.	A	.	T	.	.	.	G	A	.	.	A	.	.	.	.	G	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	440
UGA/95/1	.	T	A	.	T	.	.	.	G	A	.	.	T	.	.	.	.	G	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
NYA1/2	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	440
TAN/01/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	440
SPEC/205	.	.	A	.	.	.	.	.	.	.	.	T	A	.	.	.	.	G	.	.	A	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	440
LILLIE	G	T	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	A	T	G	T	.	.	.	.	.	.	.	.	.	.	.	440
RSA/99/1/W	G	T	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
MOZ/1960	G	.	A	.	.	T	.	.	.	.	.	.	T	.	.	.	C	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
MOZ/1979	G	.	A	.	.	T	.	.	.	.	.	.	T	.	.	.	C	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
MAL/02/1	G	T	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
RSA/98/1	G	T	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
MUR/07/1	G	T	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
Moz 16/2006	G	T	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
Moz 17/2006	G	.	A	.	.	T	.	.	.	.	.	.	T	.	.	.	C	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
Moz 14/2006	G	.	A	.	.	T	.	.	.	.	.	.	T	.	.	.	C	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
Moz 15/2006	G	.	A	.	.	T	.	.	.	.	.	.	T	.	.	.	C	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
Moz 10/2006	G	T	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
Moz 13/2006	G	T	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
Moz 19/2006	G	T	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
Moz 18/2006	G	T	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
Moz 8/2006	G	T	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
Moz 1/2006	G	T	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
Moz 7/2006	G	T	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
Moz 6/2006	G	T	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
Moz 5/2006	G	T	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
Moz 4/2006	G	T	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
Moz 3/2006	G	T	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
Moz 2/2006	G	T	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
Moz 12/2006	G	T	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
Moz 9/2006	G	T	A	.	.	T	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	A	T	G	.	.	.	.	.	.	.	.	.	.	.	.	440
TAN/03/2	A	C	T	G	T	G	C	A	A	C	A	C	A	T	T	G	A	A	C	A	G	T	A	T	G	G	A	A	A	G	G	C	A	C	C	T	G	A	T	480
MOZ/94/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	480
SPEC/265	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	480
SPEC/257	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	480

ANG/70		T	A	480
LIS/60		T	A	480
MOZ/02/2		T	A	480
MOZ/94/8			A	480
RSA/96/3			A	480
SUM/1411				480
MZI/92/1				480
KAB/62				480
BEN/97/1		T	A	480
TENGANI/62			A	480
BUR/84/1	G			480
UGA/95/1	G	T		480
NYA1/2				480
TAN/01/1				480
SPEC/205				480
LILLIE		T	A	480
RSA/99/1/W		T	A	480
MOZ/1960			A	480
MOZ/1979			A	480
MAL/02/1			A	480
RSA/98/1			A	480
MUR/07/1		T	A	480
Moz 16/2006		T	A	480
Moz 17/2006			A	480
Moz 14/2006			A	480
Moz 15/2006			A	480
Moz 10/2006		T	A	480
Moz 13/2006		T	A	480
Moz 19/2006		T	A	480
Moz 18/2006		T	A	480
Moz 8/2006		T	A	480
Moz 1/2006		T	A	480
Moz 7/2006		T	A	480
Moz 6/2006		T	A	480
Moz 5/2006		T	A	480
Moz 4/2006		T	A	480

Moz 3/2006	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	480
Moz 2/2006	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	480
Moz 12/2006	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	480
Moz 9/2006	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	480
TAN/03/2	T	T	T	A	A	C	A	A	G	A	T	C	A	T	T	A	G	A	G	C	C	C	A	T	A	A	C	T	T	T	A	T	T	C	A	A	A	C	A	520
MOZ/94/1	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
SPEC/265	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
SPEC/257	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
ANG/70	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
LIS/60	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
MOZ/02/2	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
MOZ/94/8	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
RSA/96/3	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
SUM/1411	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
MZI/92/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
KAB/62	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	520
BEN/97/1	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
TENGANI/62	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
BUR/84/1	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	520
UGA/95/1	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	520
NYA1/2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	520
TAN/01/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	520
SPEC/205	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
LILLIE	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
RSA/99/1/W	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
MOZ/1960	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
MOZ/1979	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
MAL/02/1	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
RSA/98/1	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
MUR/07/1	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	C	520
Moz 16/2006	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
Moz 17/2006	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
Moz 14/2006	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
Moz 15/2006	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
Moz 10/2006	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520

Moz 13/2006	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	C	520
Moz 19/2006	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
Moz 18/2006	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	520
Moz 8/2006	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	C	520
Moz 1/2006	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	C	520
Moz 7/2006	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	C	520
Moz 6/2006	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	C	520
Moz 5/2006	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	C	520
Moz 4/2006	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	C	520
Moz 3/2006	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	C	520
Moz 2/2006	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	C	520
Moz 12/2006	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	C	520
Moz 9/2006	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	C	520

TAN/03/2	A	T	T	T	A	T	G	G	A	A	C	C	C	C	C	C	T	A	A	A	G	G	A	G	G	A	A	G	A	A	A	A	A	G	A	G	G	A	G	560
MOZ/94/1	.	.	.	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	560
SPEC/265	.	.	.	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	560
SPEC/257	.	.	.	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	560
ANG/70	.	.	.	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	560
LIS/60	.	.	.	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	560
MOZ/02/2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	560
MOZ/94/8	.	.	.	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	560
RSA/96/3	.	.	.	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	560
SUM/1411	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	560
MZI/92/1	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	560
KAB/62	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	560
BEN/97/1	.	.	.	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	560
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TAN/03/2	G	T	A	A	G	A	C	T	C	A	T	G	G	T	C	A	T	T	A	A	A	C	T	T	T	T	A	A	A	A	A	A	A	A	A	A	T	A	A	600
MOZ/94/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	600
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C. Nucleotide alignment of *p54* gene the 44 sequences in this study (in bold) and in previous studies. Dots indicate nucleotide sites identical to that of the master sequence (TENGANI/60)

TENGANI/60	A	T	G	G	A	T	T	C	T	G	A	A	T	T	T	T	T	T	C	A	A	C	C	G	G	T	T	T	A	T	C	C	G	C	G	G	C	A	T	40
BEN/97/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40
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TENGANI/60	T	A	T	G	G	T	G	A	G	T	G	T	T	T	G	T	C	A	C	C	A	G	T	C	T	C	T	A	C	A	C	C	A	A	G	C	T	T	C	80
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MAL/02/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
RSA/98/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
RSA/96/3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
MOZ/02/2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
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MAUR/07/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	120
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TENGANI/60	G	T	C	T	T	A	G	T	C	A	T	T	A	T	T	A	T	C	A	T	C	G	T	T	C	T	A	A	T	T	T	A	T	C	T	A	T	T	T	160
BEN/97/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	160
LIS/60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	160
ANG/70	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	160

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Moz 10/2006	. . . .	160
Moz 11/2006	. . . .	160
MoZ 5/2006	. . . . C . . . . C C	160

Moz 4/2006	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	160
Moz 3/2006	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	160
Moz 8/2006	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	160
Moz 9/2006	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	160
TENGANI/60	T	C	T	T	C	A	A	G	A	A	A	G	A	A	A	A	A	A	-	-	-	G	C	T	G	C	T	G	C	C	G	C	T	A	T	T	G	A	A	200
BEN/97/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	200
LIS/60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	200
ANG/70	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	200
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NYA/1/2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	200
KAB/62	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	200
SPEC/257	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	200
RSA/96/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	200
LILLIE	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	200
MOZ/94/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	200
MOZ/94/8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	200
SPEC/265	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	200
ZIM/92/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	200
TAN/02/3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	200
TAN/01/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	200
SUM/1411	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	200
MZI/94/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	200
UGA/95/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	C	T	.	.	.	.	.	C	C	.	.	.	.	.	.	.	.	.	.	G	200
BUR/84/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	C	T	.	.	.	.	.	C	C	.	.	.	.	.	.	.	.	.	.	G	200
RSA/99/1/W	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	200
MOZ/1960	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	200
MOZ/1979	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	200
MAL/02/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	200
RSA/98/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	200
RSA/96/3	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	200
MOZ/02/2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	.	.	.	.	.	T	.	.	.	.	.	.	.	.	G	200
SPEC/245	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	200
MAUR/07/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	G	200
Moz 14/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	200
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Moz 16/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	200
Moz 17/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	200
Moz 13/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	G	200
Moz 1/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	C	T	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	G	200
Moz 19/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	200
Moz 18/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	200
Moz 10/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	200
Moz 11/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	200
MoZ 5/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	G	200
Moz 4/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	G	200
Moz 3/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	G	200
Moz 8/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	G	200
Moz 9/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	200
TENGANI/60	G	A	G	G	A	A	G	A	T	A	T	A	C	A	G	T	T	T	A	T	A	A	A	T	C	C	T	T	A	T	C	A	A	G	A	T	C	A	G	240
BEN/97/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	240
LIS/60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	240
ANG/70	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	240
SPEC/205	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	240
NYA/1/2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	240
KAB/62	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	240
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TENGANI/60	C	A	G	T	G	G	G	T	A	G	A	A	G	T	C	A	C	T	C	C	A	C	A	A	C	C	A	G	G	T	A	C	C	T	C	T	A	A	A	280
BEN/97/1	.	.	A	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	280
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TENGANI/60	C	C	G	G	C	T	G	G	A	G	T	G	A	C	T	A	C	A	G	C	A	A	G	T	G	T	A	G	G	C	A	A	G	C	C	A	G	T	C	320
BEN/97/1	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	A	.	.	.	.	.	.	320
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TAN/01/1	.	.	.	.	.	.	.	.	.	.	C	A	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	320
SUM/1411	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	G	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	320
MZI/94/1	.	.	A	.	.	.	.	.	.	.	C	A	T	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	320
UGA/95/1	.	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.	.	G	C	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	T	320
BUR/84/1	.	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.	.	G	C	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	T	320
RSA/99/1/W	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	320
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MAL/02/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	320
RSA/98/1	.	T	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	G	.	-	-	-	-	-	-	.	.	G	.	.	A	.	.	.	.	.	.	320
RSA/96/3	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	G	.	-	-	-	-	-	-	.	.	G	.	.	A	.	.	.	.	.	.	320
MOZ/02/2	.	.	A	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	320
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MAUR/07/1	.	.	A	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	320
Moz 14/2006	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	320
Moz 15/2006	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	320
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Moz 1/2006	.	.	.	.	.	.	.	.	.	.	C	C	T	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	320
Moz 19/2006	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	320
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Moz 10/2006	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	320
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Moz 4/2006	.	.	A	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	320
Moz 3/2006	.	.	A	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	320
Moz 8/2006	.	.	A	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	320
Moz 9/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	320
TENGANI/60	A	C	G	G	G	C	A	G	G	C	C	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	360
BEN/97/1	.	.	.	.	.	.	.	.	A	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	360
LIS/60	.	.	.	.	.	.	.	.	A	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	360
ANG/70	.	.	.	.	.	.	.	.	A	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	360
SPEC/205	.	T	.	.	.	.	.	.	A	.	.	A	G	T	T	A	C	G	A	A	C	A	A	G	C	C	A	A	T	T	A	C	G	A	A	C	A	A	G	360
NYA/1/2	.	T	.	.	.	.	.	.	A	.	.	A	G	T	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	C	G	A	A	C	A	A	G	360
KAB/62	.	T	.	.	.	.	.	.	A	.	.	A	G	T	T	A	C	G	A	A	C	A	A	G	C	C	A	A	T	T	A	C	G	A	A	C	A	A	G	360
SPEC/257	.	.	.	.	A	.	.	.	A	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	360
RSA/96/1	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	360
LILLIE	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	360
MOZ/94/1	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	360
MOZ/94/8	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	360
SPEC/265	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	360
ZIM/92/1	.	.	.	.	.	.	.	.	A	.	.	.	G	C	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	C	A	A	A	C	A	G	A	360
TAN/02/3	.	.	.	C	A	.	.	.	A	.	.	A	G	T	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	C	G	A	A	C	A	A	C	360
TAN/01/1	.	.	.	C	A	.	.	A	A	.	.	A	G	T	C	A	C	G	A	A	C	A	A	C	C	C	A	G	T	T	A	T	G	A	A	C	A	A	T	360
SUM/1411	.	T	.	.	.	.	.	.	A	.	.	A	G	T	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	C	G	A	A	C	A	A	G	360
MZI/94/1	.	T	.	.	.	.	.	.	A	.	.	A	G	T	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	C	G	A	A	C	A	A	G	360
UGA/95/1	.	.	A	.	A	.	.	.	.	.	.	A	G	T	T	G	C	C	A	T	G	-	-	-	-	-	-	-	-	-	-	-	-	A	A	T	A	G	G	360
BUR/84/1	.	.	A	.	A	.	.	.	.	.	.	A	G	T	T	G	C	C	A	T	G	-	-	-	-	-	-	-	-	-	-	-	-	A	A	T	A	G	G	360
RSA/99/1/W	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	360
MOZ/1960	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	360
MOZ/1979	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	360
MAL/02/1	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	360
RSA/98/1	.	.	.	.	A	.	.	.	A	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	360
RSA/96/3	.	.	.	.	A	.	.	.	A	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	360
MOZ/02/2	.	.	.	.	.	.	.	.	A	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	360
SPEC/245	.	.	.	.	A	.	.	.	A	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	360
MAUR/07/1	.	.	.	.	.	.	.	.	A	.	.	.	G	C	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	C	A	A	A	C	A	G	A	360
Moz 14/2006	.	.	.	.	.	.	.	.	.	.	.	.	G	C	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	C	A	A	A	C	A	G	A	360
Moz 15/2006	.	.	.	.	.	.	.	.	.	.	.	.	G	C	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	C	A	A	A	C	A	G	A	360

Moz 16/2006	.	.	.	.	.	.	.	.	.	.	.	.	G	C	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	C	A	A	A	C	A	G	A	360
Moz 17/2006	.	.	.	.	.	.	.	.	.	.	.	.	G	C	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	C	A	A	A	C	A	G	A	360
Moz 13/2006	.	.	.	.	.	.	.	.	A	.	.	.	G	C	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	C	A	A	A	C	A	G	A	360
Moz 1/2006	T	A	.	.	.	.	.	.	.	.	.	.	G	C	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	C	A	A	A	C	A	G	A	360
Moz 19/2006	.	.	.	.	.	.	.	.	.	.	.	.	G	C	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	C	A	A	A	C	A	G	A	360
Moz 18/2006	.	.	.	.	.	.	.	.	.	.	.	.	G	C	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	C	A	A	A	C	A	G	A	360
Moz 10/2006	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	C	A	A	A	C	A	G	A	360
Moz 11/2006	.	.	.	.	.	.	.	.	.	.	.	.	G	C	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	C	A	A	A	C	A	G	A	360
MoZ 5/2006	.	.	.	.	.	.	.	.	A	.	.	.	G	C	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	C	A	A	A	C	A	G	A	360
Moz 4/2006	.	.	.	.	.	.	.	.	A	.	.	.	G	C	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	C	A	A	A	C	A	G	A	360
Moz 3/2006	.	.	.	.	.	.	.	.	A	.	.	.	G	C	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	C	A	A	A	C	A	G	A	360
Moz 8/2006	.	.	.	.	.	.	.	.	A	.	.	.	G	C	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	C	A	A	A	C	A	G	A	360
Moz 9/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	360
TENGANI/60	-	-	-	G	C	A	A	C	A	A	A	C	A	G	A	C	C	A	G	T	T	A	C	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	400
BEN/97/1	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	.	.	A	A	A	C	A	A	A	C	C	A	G	T	C	A	C	G	400
LIS/60	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	.	.	A	A	A	C	A	A	A	C	C	A	G	T	C	A	C	G	400
ANG/70	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	.	.	A	A	A	C	A	A	A	C	C	A	G	T	C	A	C	G	400
SPEC/205	C	C	A	.	T	T	.	.	.	.	.	.	.	A	C	.	.	G	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	400
NYA/1/2	C	C	A	.	T	T	.	.	G	.	.	.	.	A	C	.	.	G	A	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	400
KAB/62	C	C	A	.	T	T	.	.	.	.	.	.	.	A	C	.	.	G	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	400
SPEC/257	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	.	G	A	T	A	G	A	C	C	A	G	C	A	A	C	A	400
RSA/96/1	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	A	T	A	G	A	C	C	A	G	C	A	A	C	A	400
LILLIE	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	A	T	A	G	A	C	C	A	G	C	A	A	C	A	400
MOZ/94/1	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	400
MOZ/94/8	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	400
SPEC/265	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	400
ZIM/92/1	C	C	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	400
TAN/02/3	C	C	A	.	T	T	.	.	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	A	A	C	A	A	C	C	C	A	G	T	T	A	C	G	400
TAN/01/1	C	C	A	.	T	T	.	.	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	A	A	C	A	A	C	C	C	A	G	T	T	A	C	G	400
SUM/1411	C	C	A	.	T	T	.	.	G	.	.	.	.	A	G	.	.	.	.	.	.	.	.	A	A	A	C	A	A	T	C	C	A	G	T	T	A	C	G	400
MZI/94/1	C	C	A	.	T	T	.	.	G	.	.	T	.	A	G	.	.	.	.	.	.	.	.	.	A	A	C	A	A	T	C	C	G	G	T	T	A	C	G	400
UGA/95/1	C	C	A	.	T	T	.	.	G	.	.	.	.	.	C	T	.	G	.	.	C	G	.	.	G	A	C	A	G	G	C	C	A	G	T	T	A	T	G	400
BUR/84/1	C	C	A	.	T	T	.	.	G	.	.	.	.	.	C	T	.	G	.	.	C	G	.	.	G	A	C	A	G	G	C	C	A	G	T	T	A	T	G	400
RSA/99/1/W	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	A	T	A	G	A	C	C	A	G	C	A	A	C	A	400
MOZ/1960	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	400

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MOZ/1979          ---...................---------------GATAGACAAGCAAGTTACG 400
MAL/02/1          ---...................---------------GATAGACAAGCAAGTTACG 400
RSA/98/1          ---...........T.....G..GATAGACCAGCAAGCAACA 400
RSA/96/3          ---...........T.....G..GATAGACCAGCAAGCAACA 400
MOZ/02/2          ---.........CA..A AAACAAAACCAGTTACG 400
SPEC/245          ---...........T.....G..GATAGACCAGCAAGCAACA 400
MAUR/07/1         CCA.....A.....GACAAACCCAGTTACG 400
Moz 14/2006       CCA.....GACAAACCCAGTTACG 400
Moz 15/2006       CCA.....GACAAACCCAGTTACG 400
Moz 16/2006       CCA.....GACAAACCCAGTTACG 400
Moz 17/2006       CCA.....GACAAACCCAGTTACG 400
Moz 13/2006       CCA.....A.....GACAAACCCAGTTACG 400
Moz 1/2006        CCA.TC.AC....A.....C.....GACAAACCcaGTTACG 400
Moz 19/2006       CCA.....GACAAACCCAGTTACG 400
Moz 18/2006       CCA.....GACAAACCCAGTTACG 400
Moz 10/2006       CCA.....GACAAACCCAGTTACG 400
Moz 11/2006       CCA.....GACAAACCCAGTTACG 400
MoZ 5/2006        CCA.....A.....GACAAACCCAGTTACG 400
Moz 4/2006        CCA.....A.....GACAAACCCAGTTACG 400
Moz 3/2006        CCA.....A.....GACAAACCCAGTTACG 400
Moz 8/2006        CCA.....A.....GACAAACCCAGTTACG 400
Moz 9/2006        ---...................---------------------------- 400

TENGANI/60        ---...................---------------GACAGGCTA 440
BEN/97/1          GACAACCCAGTTACG-----------.....A... 440
LIS/60            GACAACCCAGTTACG-----------.....A... 440
ANG/70            GACAACCCAGTTACG-----------.....A... 440
SPEC/205          ---...................---------------.....A... 440
NYA/1/2           ---...................---------------.....A... 440
KAB/62            ---...................---------------.....A... 440
SPEC/257          AACAGACCAGTTACG-----------....AC.C. 440
RSA/96/1          AACAACCCAGTTACG-----------.....A... 440
LILLIE            AACAACCCAGTTACG-----------.....A... 440
MOZ/94/1          ---...................---------------........ 440
MOZ/94/8          ---...................---------------........ 440
SPEC/265          ---...................---------------........ 440
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ZIM/92/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	C	A	G	T	T	A	C	G	.	.	.	.	.	A	.	.	.	440
TAN/02/3	A	A	C	A	A	T	C	C	A	G	T	T	A	C	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	A	.	.	.	440
TAN/01/1	A	A	C	A	A	T	C	C	A	G	T	T	A	C	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	A	.	.	.	440
SUM/1411	A	A	C	A	A	T	C	C	A	G	T	G	A	T	A	A	A	C	A	A	C	T	C	A	G	T	T	A	C	G	.	.	.	.	.	A	.	.	.	440
MZI/94/1	A	A	C	A	A	C	C	C	G	G	T	T	A	C	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	A	.	.	.	440
UGA/95/1	A	A	C	A	A	C	C	C	A	G	T	T	A	C	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	A	.	.	.	440
BUR/84/1	A	A	C	A	A	C	C	C	A	G	T	T	A	C	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	A	.	.	.	440
RSA/99/1/W	A	A	C	A	A	C	C	C	A	G	T	T	A	C	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	A	.	.	.	440
MOZ/1960	-	-	-	-	-	-	C	C	A	G	T	T	A	C	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	440
MOZ/1979	-	-	-	-	-	-	C	C	A	G	T	T	A	C	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	440
MAL/02/1	-	-	-	-	-	-	C	C	A	G	T	T	A	C	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	440
RSA/98/1	A	A	C	A	G	A	C	C	A	G	T	T	A	T	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	A	C	.	C	.	440
RSA/96/3	A	A	C	A	G	A	C	C	A	G	T	T	A	T	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	A	C	.	C	.	440
MOZ/02/2	G	A	C	A	A	C	C	C	A	G	T	T	A	C	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	A	.	.	.	440
SPEC/245	A	A	C	A	G	A	C	C	A	G	T	T	A	T	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	A	C	.	C	.	440
MAUR/07/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	A	.	.	.	440
Moz 14/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	440
Moz 15/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	440
Moz 16/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	440
Moz 17/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	440
Moz 13/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	A	.	.	.	440
Moz 1/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	A	G	C	.	440
Moz 19/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	440
Moz 18/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	440
Moz 10/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	440
Moz 11/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	440
MoZ 5/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	A	.	.	.	440
Moz 4/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	A	.	.	.	440
Moz 3/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	A	.	.	.	440
Moz 8/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	A	.	.	.	440
Moz 9/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	440

TENGANI/60	G	T	C	A	T	G	G	C	A	A	C	T	G	G	C	G	G	G	C	C	A	G	C	G	G	C	C	G	C	A	A	G	T	G	C	G	G	C	C	480
BEN/97/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.	.	480
LIS/60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.	.	480
ANG/70	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.	.	480

SPEC/205	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.	.	480
NYA/1/2	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.	.	480
KAB/62	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.	.	480
SPEC/257	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	480
RSA/96/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	480
LILLIE	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	480
MOZ/94/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	480
MOZ/94/8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	480
SPEC/265	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	480
ZIM/92/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	-	-	-	-	-	-	-	-	-	480
TAN/02/3	.	G	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	480
TAN/01/1	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	480
SUM/1411	.	G	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	480
MZI/94/1	.	G	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	480
UGA/95/1	A	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	480
BUR/84/1	A	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	480
RSA/99/1/W	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	480
MOZ/1960	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	480
MOZ/1979	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	480
MAL/02/1	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	480
RSA/98/1	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	480
RSA/96/3	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	480
MOZ/02/2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.	.	480
SPEC/245	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	480
MAUR/07/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	-	-	-	480
Moz 14/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	480
Moz 15/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	480
Moz 16/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	.	.	.	480
Moz 17/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	480
Moz 13/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.	.	480
Moz 1/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.	.	480
Moz 19/2006	.	.	.	.	A	.	.	G	C	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	480
Moz 18/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	480
Moz 10/2006	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	.	.	.	480
Moz 11/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	.	.	.	480
MoZ 5/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.	.	480

Moz 4/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.	.	480
Moz 3/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.	.	480
Moz 8/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.	.	480
Moz 9/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	480

TENGANI/60	G	C	A	A	G	T	G	C	G	G	C	C	G	C	A	A	G	T	G	C	G	G	C	C	G	C	A	A	G	T	G	C	G	G	C	T	G	C	G	520
BEN/97/1	.	.	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
LIS/60	.	.	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
ANG/70	.	.	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
SPEC/205	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
NYA/1/2	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
KAB/62	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
SPEC/257	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.	.	.	.	A	.	.	T	.	.	G	.	.	.	.	.	A	.	.	C	.	.	.	520
RSA/96/1	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	A	.	.	.	A	.	.	T	.	.	G	.	.	.	.	.	A	.	.	C	.	.	.	520
LILLIE	.	.	.	.	.	.	.	.	A	.	.	.	.	.	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
MOZ/94/1	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
MOZ/94/8	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
SPEC/265	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
ZIM/92/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
TAN/02/3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
TAN/01/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
SUM/1411	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
MZI/94/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
UGA/95/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
BUR/84/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
RSA/99/1/W	.	.	G	.	.	.	.	.	A	.	.	T	.	.	G	.	.	.	.	.	A	.	.	.	.	.	G	-	-	-	-	-	-	-	-	-	-	-	-	520
MOZ/1960	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	A	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
MOZ/1979	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	A	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
MAL/02/1	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	A	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
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RSA/96/3	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.	.	.	.	A	.	.	T	.	.	G	.	.	.	.	.	A	.	.	C	.	.	.	520
MOZ/02/2	.	.	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
SPEC/245	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.	.	.	.	A	.	.	T	.	.	G	-	-	-	-	-	-	-	-	-	-	-	-	520
MAUR/07/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
Moz 14/2006	.	.	.	.	.	.	.	.	.	.	.	.	A	.	G	.	.	.	.	.	A	.	.	T	.	.	G	.	.	.	-	-	-	-	-	-	-	-	-	520
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Moz 13/2006	.	.	G	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
Moz 1/2006	.	.	G	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
Moz 19/2006	.	.	.	.	.	.	.	.	.	.	.	.	A	.	G	.	.	.	.	.	A	.	.	T	.	.	G	.	.	.	-	-	-	-	-	-	-	-	-	520
Moz 18/2006	.	.	.	.	.	.	.	.	.	.	.	.	A	.	G	.	.	.	.	.	A	.	.	T	.	.	G	.	.	.	-	-	-	-	-	-	-	-	-	520
Moz 10/2006	.	.	.	.	.	.	.	.	.	.	.	.	A	.	G	.	.	.	.	.	A	.	.	T	.	.	G	.	.	.	-	-	-	-	-	-	-	-	-	520
Moz 11/2006	.	.	.	.	.	.	.	.	.	.	.	.	A	.	G	.	.	.	.	.	A	.	.	T	.	.	G	.	.	.	-	-	-	-	-	-	-	-	-	520
MoZ 5/2006	.	.	G	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
Moz 4/2006	.	.	G	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
Moz 3/2006	.	.	G	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
Moz 8/2006	.	.	G	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520
Moz 9/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	520

TENGANI/60	A	A	T	G	C	G	G	C	C	A	C	G	A	A	T	A	C	A	G	C	T	G	C	G	A	G	T	G	C	A	G	C	C	G	C	G	-	-	-	560
BEN/97/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
LIS/60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
ANG/70	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
SPEC/205	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
NYA/1/2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
KAB/62	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
SPEC/257	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
RSA/96/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
LILLIE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
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MOZ/94/8	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	G	T	560
SPEC/265	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	G	T	560
ZIM/92/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	T	.	.	.	A	G	T	560
TAN/02/3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
TAN/01/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
SUM/1411	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
MZI/94/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
UGA/95/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
BUR/84/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
RSA/99/1/W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
MOZ/1960	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	560

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MAL/02/1	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	560
RSA/98/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
RSA/96/3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
MOZ/02/2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
SPEC/245	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
MAUR/07/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
Moz 14/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	A	G	T	560
Moz 15/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	A	G	T	560
Moz 16/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	A	G	T	560
Moz 17/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	A	G	T	560
Moz 13/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
Moz 1/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
Moz 19/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
Moz 18/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
Moz 10/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
Moz 11/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
MoZ 5/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
Moz 4/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
Moz 3/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
Moz 8/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560
Moz 9/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	560

TENGANI/60	-	-	-	-	-	-	-	-	-	A	G	T	G	C	T	C	C	T	G	C	T	C	A	T	C	C	G	G	C	T	G	A	G	C	C	T	T	A	T	600
BEN/97/1	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	-	-	-	-	-	-	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	C	600
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NYA/1/2	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	T	.	.	.	.	600
KAB/62	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	600
SPEC/257	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	600
RSA/96/1	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	600
LILLIE	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	600
MOZ/94/1	G	C	A	G	C	C	G	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	600
MOZ/94/8	G	C	A	G	C	C	G	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	600
SPEC/265	G	C	A	G	C	C	G	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	600

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TAN/02/3	-	-	-	-	-	-	-	-	-	.	.	.	.	.	C	.	.	.	.	.	.	.	.	A	T	.	.	.	.	.	.	.	.	.	T	.	.	.	.	600
TAN/01/1	-	-	-	-	-	-	-	-	-	.	.	.	.	.	C	.	.	.	.	.	.	.	.	A	T	.	.	.	.	.	.	.	.	.	T	.	.	.	.	600
SUM/1411	-	-	-	-	-	-	-	-	-	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	T	.	.	.	.	600
MZI/94/1	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	600
UGA/95/1	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	T	.	.	.	A	.	.	.	.	.	T	.	.	.	.	600
BUR/84/1	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	600
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MAL/02/1	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	600
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MAUR/07/1	-	-	-	G	C	C	G	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	600
Moz 14/2006	G	C	A	G	C	C	G	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	600
Moz 15/2006	G	C	A	G	C	C	G	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	600
Moz 16/2006	G	C	A	G	C	C	G	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	600
Moz 17/2006	G	C	A	G	C	C	G	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	600
Moz 13/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	600
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Moz 19/2006	G	C	A	G	C	C	G	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	600
Moz 18/2006	G	C	A	G	C	C	G	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	600
Moz 10/2006	G	C	A	G	C	C	G	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	600
Moz 11/2006	G	C	A	G	C	C	G	C	G	.	.	.	.	.	.	.	.	.	.	G	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	600
MoZ 5/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	600
Moz 4/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	600
Moz 3/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	600
Moz 8/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	600
Moz 9/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	600

TENGANI/60	A	C	G	A	C	A	G	T	C	A	C	T	A	C	T	C	A	G	A	A	C	A	C	T	G	C	T	T	C	C	C	A	A	A	C	A	A	T	G	640
BEN/97/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	640
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KAB/62	.	.	.	.	.	.	.	C	.	.	.	G	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	640
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LILLIE	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	640
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TAN/01/1	.	.	.	.	.	.	.	C	.	.	.	G	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	640
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UGA/95/1	.	.	A	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	640
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RSA/99/1/W	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	640
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MAL/02/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	640
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TENGANI/60	T	C	G	G	C	T	A	T	T	G	A	A	A	A	T	C	T	A	C	G	G	C	A	A	A	G	A	A	G	C	A	C	C	T	A	T	A	C	G	680
BEN/97/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	680
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NYA/1/2	C	.	A	.	.	.	G	A	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	A	680
KAB/62	A	.	.	.	.	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	A	680
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MOZ/94/8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	680
SPEC/265	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	680
ZIM/92/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	680
TAN/02/3	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	G	.	.	.	G	.	.	.	.	.	.	.	680
TAN/01/1	.	.	.	A	.	C	.	.	.	.	G	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	680
SUM/1411	C	.	.	.	.	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	T	.	.	.	.	.	.	A	680
MZI/94/1	.	.	.	.	.	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	A	680
UGA/95/1	C	.	.	.	.	.	G	.	.	.	.	G	G	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	680
BUR/84/1	C	.	.	.	.	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	A	680
RSA/99/1/W	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	680
MOZ/1960	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	680
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MAL/02/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	680
RSA/98/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	680
RSA/96/3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	680
MOZ/02/2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	680
SPEC/245	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	680
MAUR/07/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	680
Moz 14/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	680
Moz 15/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	680

Moz 16/2006	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	680
Moz 17/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	680
Moz 13/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	680
Moz 1/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	680
Moz 19/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	680
Moz 18/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	680
Moz 10/2006	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	680
Moz 11/2006	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	680
MoZ 5/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	680
Moz 4/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	680
Moz 3/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	T	.	.	680
Moz 8/2006	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	680
Moz 9/2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	.	.	.	.	.	.	.	.	.	680

TENGANI/60	C	A	T	A	A	A	G	A	C	C	T	A	G	A	A	A	A	C	T	C	C	T	T	G	T	A	A	707
BEN/97/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	707
LIS/60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	707
ANG/70	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	707
SPEC/205	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	707
NYA/1/2	.	.	.	.	.	G	A	C	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	707
KAB/62	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	707
SPEC/257	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	707
RSA/96/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	707
LILLIE	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	707
MOZ/94/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	707
MOZ/94/8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	707
SPEC/265	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	707
ZIM/92/1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	707
TAN/02/3	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	707
TAN/01/1	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	707
SUM/1411	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	707
MZI/94/1	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	707
UGA/95/1	.	.	C	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	707
BUR/84/1	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	707
RSA/99/1/W	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	707
MOZ/1960	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	707

MOZ/1979			707
MAL/02/1			707
RSA/98/1	T		707
RSA/96/3			707
MOZ/02/2			707
SPEC/245			707
MAUR/07/1			707
Moz 14/2006			707
Moz 15/2006			707
Moz 16/2006			707
Moz 17/2006			707
Moz 13/2006			707
Moz 1/2006			707
Moz 19/2006			707
Moz 18/2006			707
Moz 10/2006			707
Moz 11/2006			707
MoZ 5/2006			707
Moz 4/2006			707
Moz 3/2006			707
Moz 8/2006	T		707
Moz 9/2006	T		707

APPENDIX II

A. COATING BUFFER ASFV ANTIGEN FOR ELISA

Carbonate/bicarbonate buffer 0.05M (pH 9.6)

Na_2CO_3 (Merck 1.06392)	1.59 g
$NaHCO_3$ (Merck 6329)	3.88 g
Distilled water to	1000ml

Store at 4ºC. Check the pH before use.

B. WASHING SOLUTION FOR ELISA

PBS 1x pH 7.2 - 0.1% Tween-20 (PBST)

NaCl (Merck Ref. 1.06404)	8.0 g
KH_2PO_4 (Merck Ref. 1.04873)	0.2 g
Na_2HPO_4 12 H2O (Merck Ref. 1.06586)	2.9 g
KCl (Merck Ref. 1.04936)	0.2 g
Tween-20 (Merck Ref. 8.22184)	1 ml
Distilled water to	1000 ml

Check the pH before use it. Store at + 4ºC.

C. BLOCKING BUFFER FOR ELISA

2% (w/v) Bovine Serum Albumin (Sigma, Missouri, USA) in PBS 1x pH 7.2 - 0.1% Tween-20

D. SUBSTRATE:

2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS)

Buffer solution 1x: Citric acid 35 mM

Na2HPO4 67 mM in pH 5.0

1mM ABTS in buffer pH 5.0 (dissolved immediately before being used) and 0.015% H_2O_2

Example: 15 ml buffer 1x + 6.75 µl H_2O_2 (al 33%) + 8.2 mg ABTS.

E. STOPPING SOLUTION

1.25M H_2SO_4

H2SO4 (98%, Unilab)	67ml
Water with crushed ice	933ml

To prepare stopping solution: add the concentrated acid very slowly to the water

F. PBS-CASEIN pH 7.2 -7.4

To prepare 2% stock solution:

Casein (Unilab)	40.0g
PBS	2.0 litre

Store in 80ml aliquots at -20oC

To prepare 0.5% casein for use:

Thaw 2% casein stock in 37oC waterbath.

Add 80ml of the above to 240ml PBS to obtain a final concentration of 0.5% casein.

APPENDIX III

Serum samples used for serological test comparisons

No	Location code	Animal ID	I-ELISA	Blocking *p72* ELISA	*rp30* ELISA	Immuno-blotting
1	MOGTP	B003	Negative	Negative	Negative	Negative
2	MOGTP	B004	Negative	Negative	Positive	Negative
3	MOGTP	B007	Negative	Negative	Negative	Negative
4	MOGTP	B008	Negative	Negative	Negative	Negative
5	MOGTP	B010	Negative	Negative	Negative	Negative
6	MOGTP	B011	Negative	Negative	Negative	Negative
7	MOGTP	B016	Negative	Negative	Negative	Negative
8	MOGTP	B018	Negative	Negative	Negative	Negative
9	MOGTP	B019	Negative	Negative	Negative	Negative
10	MOGTP	B020	Negative	Negative	Negative	Negative
11	MOGTP	B021	Negative	Negative	Negative	Negative
12	MOGTP	B022	Negative	Negative	Negative	Negative
13	MOGTP	B023	Negative	Negative	Negative	Negative
14	MOGTP	B025	Negative	Negative	Negative	Negative
15	MOGTP	B026	Negative	Negative	Negative	Negative
16	MOGTP	B028	Positive	Positive	Positive	Positive
17	MOGTP	B029	Negative	Negative	Positive	Negative
18	MOGTP	B033	Negative	Negative	Positive	Negative
19	MOGTP	B035	Positive	Negative	Positive	Positive
20	MOGTP	B038	Negative	Negative	Negative	Negative
21	MOGTP	B039	Negative	Negative	Negative	Negative
22	MOGCP	B003	Negative	Negative	Negative	Negative
23	MOGCP	B006	Negative	Negative	Negative	Negative

24	MOGCP	B016	Negative	Negative	Negative	Negative
25	MOGCP	B017	Negative	Negative	Negative	Negative
26	MOGCP	B020	Negative	Negative	Negative	Negative
27	MOGCP	B022	Negative	Negative	Negative	Negative
28	MOGCP	B024	Negative	Negative	Negative	Negative
29	MOGCP	B025	Negative	Negative	Negative	Negative
30	MOGCP	B027	Negative	Negative	Negative	Negative
31	MOGCP	B029	Negative	Negative	Negative	Negative
32	MOGCP	B032	Negative	Negative	Negative	Negative
33	MOGKP	B001	Positive	Negative	Negative	Positive
34	MOGKP	B002	Negative	Negative	Negative	Negative
35	MOGKP	B003	Negative	Negative	Negative	Negative
36	MOGKP	B004	Negative	Negative	Negative	Negative
37	MOGKP	B005	Negative	Negative	Negative	Negative
38	MOGKP	B006	Negative	Negative	Negative	Negative
39	MOGKP	B007	Negative	Negative	Negative	Positive
40	MOGKP	B008	Negative	Negative	Negative	Negative
41	MOGKP	B009	Negative	Negative	Negative	Negative
42	MOGKP	B010	Negative	Negative	Negative	Negative
43	MOGKP	B011	Negative	Negative	Negative	Negative
44	MOGKP	B012	Negative	Negative	Negative	Negative
45	MOGKP	B013	Negative	Negative	Negative	Negative
46	MOGKP	B014	Negative	Negative	Negative	Negative
47	MOGKP	B015	Negative	Negative	Negative	Negative
48	MOGKP	B016	Negative	Negative	Negative	Negative
49	MOGKP	B017	Negative	Negative	Negative	Negative
50	MOGKP	B018	Negative	Negative	Negative	Negative
51	MOGKP	B019	Positive	Negative	Negative	Positive

52	MOGKP	B020	Negative	Negative	Negative	Negative
53	MOGKP	B021	Negative	Negative	Negative	Negative
54	MOGKP	B022	Negative	Negative	Negative	Negative
55	MOGKP	B023	Negative	Negative	Negative	Negative
56	MOGKP	B024	Negative	Negative	Negative	Negative
57	MOGKP	B025	Negative	Negative	Negative	Negative
58	MOGKP	B026	Negative	Negative	Negative	Negative
59	MOGKP	B027	Negative	Negative	Negative	Negative
60	MOGKP	B028	Negative	Negative	Negative	Negative
61	MOGKP	B029	Positive	Negative	Negative	Negative
62	MOGKP	B030	Positive	Negative	Negative	Positive
63	MOGKP	B031	Positive	Negative	Negative	Positive
64	MOGKP	B032	Positive	Negative	Negative	Positive
65	MOGKP	B033	Positive	Negative	Negative	Negative
66	MOGKP	B034	Positive	Negative	Negative	Positive
67	MOGKP	B035	Positive	Negative	Negative	Positive
68	MOGKP	B036	Positive	Negative	Negative	Negative
69	MOGKP	B037	Positive	Negative	Negative	Positive
70	MOGKP	B038	Positive	Negative	Negative	Positive
71	MOGKP	B039	Negative	Negative	Negative	Negative
72	MOGKP	B040	Negative	Negative	Negative	Negative
73	MOGKP	B041	Negative	Negative	Negative	Negative
74	MOGKP	B042	Negative	Negative	Negative	Negative
75	MOPPP	B001	Negative	Negative	Negative	Negative
76	MOPPP	B002	Negative	Negative	Negative	Negative
77	MOPPP	B003	Positive	Negative	Negative	Positive
78	MOPPP	B004	Positive	Negative	Negative	Positive
79	MOPPP	B005	Negative	Negative	Negative	Negative

80	MOPPP	B006	Negative	Negative	Negative	Negative
81	MOPPP	B007	Negative	Negative	Negative	Negative
82	MOPPP	B008	Negative	Negative	Negative	Negative
83	MOPPP	B009	Negative	Negative	Negative	Negative
84	MOPPP	B010	Negative	Negative	Negative	Negative
85	MOPPP	B011	Negative	Negative	Negative	Negative
86	MOPPP	B012	Negative	Negative	Negative	Negative
87	MOPPP	B013	Negative	Negative	Negative	Negative
88	MOPPP	B014	Negative	Negative	Negative	Negative
89	MOPPP	B015	Negative	Negative	Negative	Negative
90	MOPPP	B016	Negative	Negative	Negative	Negative
91	MOPPP	B017	Negative	Negative	Negative	Negative
92	MOPPP	B018	Negative	Negative	Negative	Negative
93	MOPPP	B019	Positive	Positive	Negative	Positive
94	MOPPP	B020	Positive	Positive	Negative	Negative
95	MOPPP	B021	Negative	Negative	Negative	Negative
96	MOPPP	B022	Negative	Negative	Negative	Negative
97	MOPPP	B023	Negative	Negative	Negative	Negative
98	MOPPP	B024	Negative	Negative	Negative	Negative
99	MOPPP	B025	Negative	Negative	Negative	Negative
100	MOPPP	B026	Negative	Negative	Negative	Negative
101	MOPPP	B027	Negative	Negative	Negative	Negative
102	MOPPP	B028	Negative	Negative	Negative	Negative
103	MOPPP	B029	Negative	Negative	Negative	Negative
104	MOPPP	B030	Negative	Negative	Negative	Negative
105	MOGSP	B001	Negative	Negative	Negative	Negative
106	MOGSP	B002	Negative	Negative	Negative	Negative
107	MOGSP	B003	Negative	Negative	Negative	Negative

108	MOGSP	B004	Negative	Negative	Negative	Negative
109	MOGSP	B005	Negative	Negative	Negative	Negative
110	MOGSP	B006	Negative	Negative	Negative	Negative
111	MOGSP	B007	Negative	Negative	Negative	Negative
112	MOGSP	B008	Negative	Negative	Negative	Negative
113	MOGSP	B009	Negative	Negative	Negative	Negative
114	MOGSP	B010	Negative	Negative	Negative	Negative
115	MOGSP	B011	Negative	Negative	Negative	Negative
116	MOGSP	B012	Negative	Negative	Negative	Negative
117	MOGSP	B013	Negative	Negative	Negative	Negative
118	MOGSP	B015	Negative	Negative	Negative	Negative
119	MOGSP	B016	Negative	Negative	Negative	Negative
120	MOGSP	B018	Negative	Negative	Negative	Negative
121	MOGSP	B020	Negative	Negative	Negative	Negative
122	MOGSP	B021	Negative	Negative	Negative	Negative
123	MOGSP	B022	Negative	Negative	Negative	Negative
124	MOGSP	B023	Negative	Negative	Negative	Negative
125	MOGSP	B024	Negative	Negative	Negative	Negative
126	MOGSP	B025	Negative	Negative	Negative	Negative
127	MOGSP	B026	Negative	Negative	Negative	Negative
128	MOGSP	B019	Positive	Negative	Negative	Positive
129	MOGSP	B027	Negative	Negative	Negative	Negative
130	MOPMP	B001	Negative	Negative	Negative	Negative
131	MOPMP	B002	Negative	Negative	Negative	Negative
132	MOPMP	B003	Negative	Negative	Negative	Negative
133	MOPMP	B004	Negative	Negative	Negative	Negative
134	MOPMP	B005	Negative	Negative	Negative	Negative
135	MOPMP	B006	Negative	Positive	Negative	Negative

136	MOPMP	B007	Negative	Negative	Negative	Negative
137	MOPMP	B008	Negative	Negative	Negative	Negative
138	MOPMP	B009	Negative	Negative	Negative	Negative
139	MOPMP	B010	Positive	Negative	Negative	Positive
140	MOPMP	B011	Negative	Negative	Negative	Negative
141	MOPMP	B012	Negative	Negative	Negative	Negative
142	MOPMP	B013	Negative	Negative	Negative	Negative
143	MOPMP	B014	Negative	Negative	Negative	Negative
144	MOPMP	B015	Negative	Negative	Negative	Negative
145	MOPMP	B016	Negative	Negative	Negative	Negative
146	MOPMP	B017	Negative	Negative	Negative	Negative
147	MOPMP	B018	Negative	Negative	Negative	Negative
148	MOPMP	B019	Negative	Negative	Negative	Negative
149	MOPMP	B020	Negative	Negative	Negative	Negative
150	MOPMP	B021	Negative	Positive	Negative	Negative
151	MOPMP	B022	Negative	Negative	Negative	Negative
152	MOPMP	B023	Negative	Negative	Negative	Negative
153	MOPMP	B024	Negative	Negative	Negative	Negative
154	MOPMP	B025	Negative	Negative	Negative	Negative
155	MOPMP	B026	Negative	Negative	Negative	Negative
156	MOPMP	B027	Negative	Negative	Negative	Negative
157	MOPMP	B028	Negative	Negative	Negative	Negative
158	MOPMP	B029	Negative	Negative	Negative	Negative
159	MOPMP	B030	Negative	Negative	Negative	Negative
160	MOPMP	B031	Negative	Negative	Negative	Negative
161	MOPMP	B032	Negative	Negative	Negative	Negative
162	MOPMP	B033	Negative	Negative	Negative	Negative
163	MOGMP	B001	Negative	Negative	Negative	Negative

164	MOGMP	B002	Negative	Negative	Negative	Negative
165	MOGMP	B003	Negative	Negative	Negative	Negative
166	MOGMP	B004	Negative	Negative	Negative	Negative
167	MOGMP	B005	Negative	Negative	Negative	Negative
168	MOGMP	B007	Negative	Negative	Negative	Negative
169	MOGMP	B008	Negative	Negative	Negative	Negative
170	MOGMP	B009	Positive	Positive	Negative	Positive
171	MOGMP	B010	Positive	Positive	Negative	Positive
172	MOGMP	B011	Positive	Positive	Positive	Positive
173	MOGMP	B012	Positive	Positive	Negative	Positive
174	MOGMP	B013	Positive	Positive	Positive	Positive
175	MOGMP	B016	Negative	Negative	Negative	Negative
176	MOGMP	B017	Negative	Negative	Negative	Negative
177	MOGMP	B018	Negative	Negative	Negative	Negative
178	MOGMP	B019	Negative	Negative	Negative	Negative
179	MOGMP	B020	Negative	Negative	Negative	Negative
180	MOGMP	B021	Negative	Negative	Negative	Negative
181	MOGMP	B022	Positive	Positive	Positive	Positive
182	MOGMP	B023	Negative	Negative	Negative	Negative
183	MOGMP	B024	Negative	Negative	Negative	Negative
184	MOGMP	B026	Positive	Positive	Positive	Positive
185	MOGMP	B027	Positive	Positive	Positive	Positive
186	MOGMP	B029	Positive	Positive	Negative	Positive
187	MOGAP	B001	Negative	Negative	Negative	Negative
188	MOGAP	B002	Negative	Negative	Negative	Negative
189	MOGAP	B003	Negative	Negative	Negative	Negative
190	MOGAP	B004	Negative	Negative	Negative	Negative
191	MOGAP	B005	Positive	Negative	Negative	Negative

192	MOGAP	B006	Negative	Negative	Negative	Negative
193	MOGAP	B007	Negative	Negative	Negative	Negative
194	MOGAP	B009	Negative	Negative	Negative	Negative
195	MOGAP	B010	Negative	Negative	Negative	Negative
196	MOGAP	B011	Negative	Positive	Negative	Positive
197	MOGAP	B012	Negative	Negative	Negative	Negative
198	MOGAP	B013	Negative	Negative	Negative	Negative
199	MOGAP	B015	Positive	Negative	Negative	Negative
200	MOGAP	B016	Negative	Negative	Negative	Negative
201	MOGAP	B018	Negative	Negative	Negative	Negative
202	MOGAP	B023	Negative	Negative	Negative	Negative
203	MOGAP	B024	Negative	Negative	Negative	Negative
204	MOGAP	B025	Negative	Negative	Negative	Negative
205	MOGAP	B026	Negative	Negative	Negative	Negative
206	MOGAP	B027	Negative	Negative	Negative	Negative
207	MOGAP	B028	Negative	Negative	Negative	Negative
208	MOGAP	B029	Negative	Negative	Negative	Negative
209	MOGAP	B030	Negative	Negative	Negative	Negative
210	MOGAP	B031	Negative	Negative	Negative	Negative
211	MOGAP	B032	Negative	Negative	Negative	Negative
212	MOGAP	B033	Negative	Negative	Negative	Negative
213	MOGAP	B034	Negative	Negative	Negative	Negative
214	MOGAP	B035	Negative	Negative	Negative	Negative
215	MOGAP	B036	Negative	Negative	Negative	Negative
216	MOGAP	B037	Negative	Negative	Negative	Negative
217	MOGAP	B038	Negative	Negative	Negative	Negative
218	MOGAP	B039	Negative	Negative	Negative	Negative
219	MOGAP	B040	Negative	Negative	Negative	Negative

220	MOGAP	B041	Negative	Negative	Negative	Negative
221	MOGAP	B042	Negative	Negative	Negative	Negative
222	MOGAP	B043	Negative	Negative	Negative	Negative
223	MOGAP	B044	Negative	Negative	Negative	Negative
224	MOGAP	B045	Negative	Negative	Negative	Negative
225	MOGAP	B046	Negative	Negative	Negative	Negative
226	MOGAP	B047	Negative	Negative	Negative	Negative
227	MOGAP	B048	Negative	Negative	Negative	Negative
228	MOGAP	B049	Negative	Negative	Negative	Positive
229	MOGAP	B050	Negative	Negative	Negative	Negative
230	MOGAP	B051	Negative	Negative	Negative	Negative
231	MOGAP	B052	Negative	Negative	Negative	Negative
232	MOGAP	B053	Negative	Negative	Negative	Negative
233	MOGAP	B054	Negative	Negative	Negative	Negative
234	MOGAP	B055	Negative	Negative	Negative	Negative
235	MOGAP	B056	Negative	Negative	Negative	Negative
236	MOGAP	B057	Negative	Negative	Negative	Negative
237	MOGAP	B058	Negative	Negative	Negative	Negative
238	MOGWP	B001	Positive	Negative	Negative	Positive
239	MOGWP	B003	Negative	Negative	Positive	Negative
240	MOGWP	B004	Negative	Negative	Negative	Negative
241	MOGWP	B012	Negative	Negative	Negative	Negative
242	MOGWP	B013	Negative	Negative	Negative	Negative
243	MOGWP	B014	Negative	Negative	Negative	Negative
244	MOGWP	B016	Negative	Negative	Negative	Negative
245	MOGWP	B017	Negative	Negative	Negative	Negative
246	MOGWP	B018	Negative	Negative	Positive	Negative
247	MOGWP	B019	Negative	Negative	Positive	Negative

248	MOGWP	B020	Negative	Negative	Positive	Negative
249	MOGWP	B021	Negative	Negative	Positive	Negative
250	MOGWP	B022	Positive	Positive	Negative	Positive
251	MOGWP	B023	Positive	Positive	Negative	Positive
252	MOGWP	B024	Negative	Positive	Negative	Positive
253	MOGWP	B025	Positive	Positive	Positive	Positive
254	MOGWP	B027	Positive	Positive	Positive	Positive
255	MOGVP	B001	Negative	Negative	Negative	Negative
256	MOGVP	B003	Negative	Negative	Negative	Negative
257	MOGVP	B004	Negative	Negative	Negative	Negative
258	MOGVP	B005	Negative	Negative	Negative	Negative
259	MOGVP	B006	Negative	Negative	Negative	Negative
260	MOGVP	B007	Negative	Positive	Negative	Negative
261	MOGVP	B008	Negative	Negative	Negative	Negative
262	MOGVP	B010	Negative	Negative	Negative	Negative
263	MOGVP	B012	Negative	Negative	Negative	Negative
264	MOGVP	B014	Negative	Negative	Negative	Negative
265	MOGVP	B016	Negative	Negative	Negative	Negative
266	MOGVP	B018	Negative	Negative	Negative	Negative
267	MOPTP	B002	Negative	Negative	Negative	Negative
268	MOPTP	B003	Negative	Negative	Negative	Negative
269	MOPTP	B004	Negative	Negative	Negative	Negative
270	MOPTP	B007	Negative	Negative	Negative	Negative
271	MOPTP	B008	Negative	Negative	Negative	Negative
272	MOPTP	B011	Negative	Negative	Negative	Negative
273	MOPTP	B012	Negative	Negative	Negative	Negative