Molecular detection and characterisation of an African swine fever *p72* genotype XXV virus in *Ornithodoros* ticks from the Kruger National Park, South Africa

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Introduction

African swine fever (ASF) virus is maintained in an ancient sylvatic cycle involving long-lived, eyeless soft ticks of the genus *Ornithodoros* and common warthogs, *Phacochoerus africanus*. ASF viruses from the sylvatic cycle are under-represented in reference databases despite the fact that infected tick populations represent a permanent source of infection for susceptible domestic suids. In this retrospective study of *Ornithodoros moubata* species complex ticks sampled from 163 warthog burrows throughout the Kruger National Park (KNP), we confirm the presence of a novel genotype (XXV) through *p72* genotyping and multi-locus sequence analysis.

Materials and Methods

- Warthog burrows were sampled across a 300 km latitudinal gradient in the Kruger National Park (Fig. 1), in both the wet season (n=85 burrows) and the dry season (n=78 burrows).
- Homogenates were prepared for individual ticks and pooled prior to DNA extraction.
- Tick pools were screened using a duplex PCR assay that targets the C-terminal *p72* gene region of the ASF virus (ASFV) genome and the 16S rRNA gene of the tick genome (Bastos et al. 2009).
- DNA was extracted from individual tick homogenates of all ASFV-positive pools and rescreened with the same duplex PCR assay.
- Individual ASF-positive tick extracts were typed by PCR amplification and sequencing of four virus genome regions (*p72, p54,* CVR and *TK*).
- The effect of season on burrow infestation rates, tick developmental stage, sex of adult ticks present as well as tick infection rate was investigated using generalised linear models.

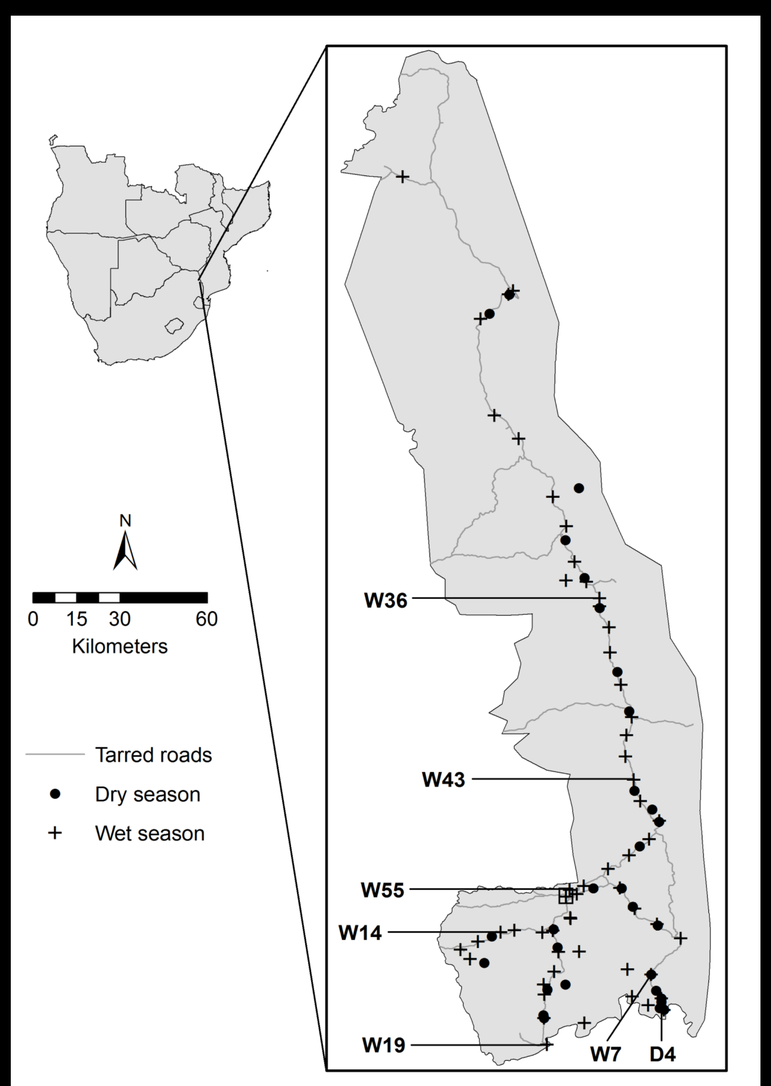

Fig. 1. Location of infested warthog burrows sampled in the wet (2003, n=56) and dry (2002, n=31) seasons, in the Kruger National Park (KNP), South Africa. Burrow numbers preceded with a W (wet season) and D (dry season) correspond to the seven burrows containing ticks found to be infected with African swine fever virus.

Results and Discussion

- 14 of the 1079 ticks (1.31%) were ASFV-positive. This infection rate is comparable to the 1.4% infection rate reported in a prior survey (1979-1981) in the Kruger National Park (Thomson et al. 1983).
- ASFV-infection rates differed by season (1.08 % in the dry season and 1.37% in the wet season), however, these differences were not significant.
- Season had a significant effect on burrow infestation rate (higher in the wet season), tick developmental stage (more nymphs present in the dry season) and sex of the adult ticks present (more males collected during the dry season).
- Phylogenetic analyses confirmed the presence of six ASF genotypes (I, VII, XVII, XX, XXI XXV) in ticks from the Kruger National Park, based on *p72* and *p54* gene sequencing. Genotype XXV represents a novel *p72* genotype (Fig. 2).

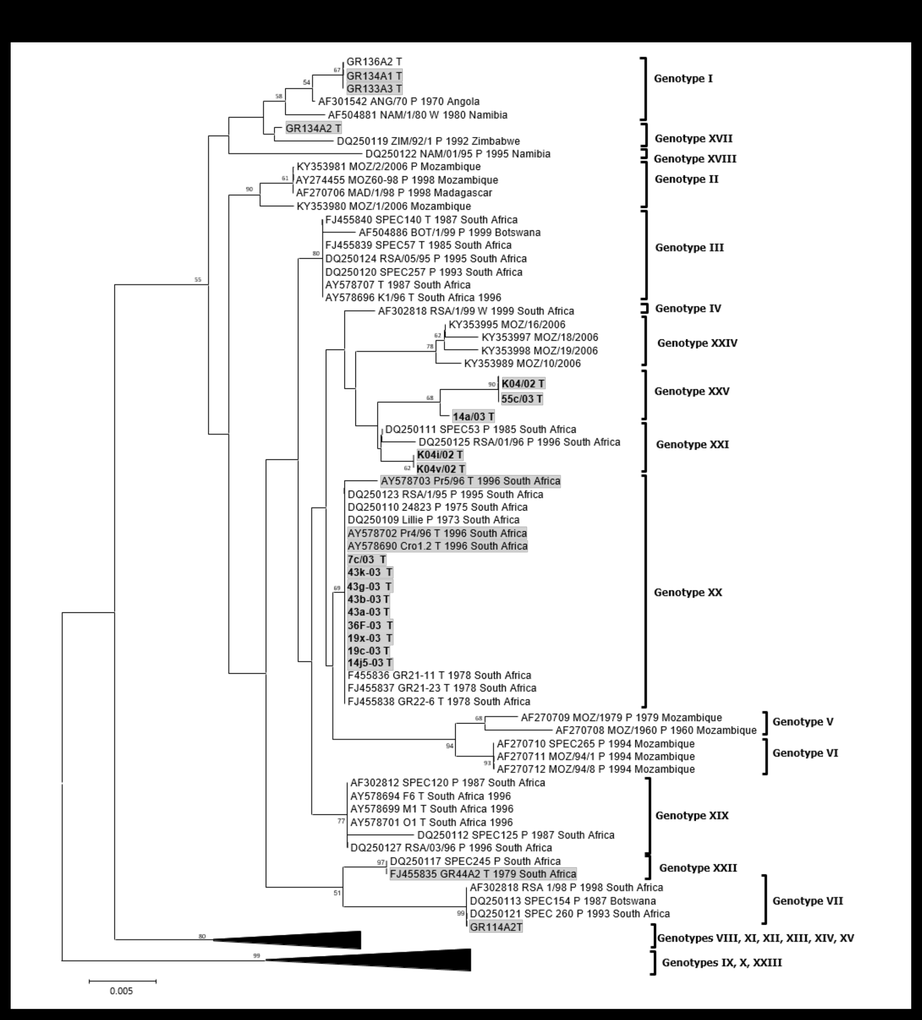

Fig. 2. *p72* gene tree depicting the 24 African swine fever virus genotypes (I-XXIV) that have been described to date, and a novel genotype (XXV) detected in *Ornithodoros* ticks sampled from warthog burrows in the Kruger National Park (KNP), South Africa**.** ASF viruses from KNP are indicated with grey shading, whilst those characterised in this study are indicated in bold. Taxon names include Genbank accession number, isolate name, host origin (P=pig, T=tick, W=warthog), year of isolation and country of origin.

Conclusions

- ASFV variation is high in sylvatic cycle *Ornithodoros* ticks; six genotypes occur within a single wildlife reserve (KNP) in South Africa, including a novel *p72* genotype (genotype XXV).
- Intensified sampling and characterisation of ASF viruses from sylvatic cycle ticks is likely to result in the identification of additional novel genotypes.

References

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