**Online Resources**

# Isolation and characterization of species-specific microsatellite markers for blue- and black wildebeest (*Connochaetes taurinus* and *C. gnou*)

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**Abstract:**

The blue wildebeest (*Connochaetes taurinus*) is distributed throughout southern and east Africa while the black wildebeest (*Connochaetes gnou*) is endemic to South Africa and was driven to near extinction in the early 1900s due to hunting pressure and disease outbreaks. Extensive translocation of both species throughout South Africa is threatening the genetic integrity of blue- and black wildebeest. In order to effectively manage these species, genetic tools that can be used to detect hybrid individuals, identify genetically unique subpopulations and determine levels of genetic diversity is required. In this study, 11 microsatellite markers for wildebeest were developed via next-generation sequencing. The microsatellite loci displayed 2.00 - 4.14 alleles, unbiased heterozygosity values ranging from 0.32 to 0.60 and observed heterozygosity values ranging from 0.26 to 0.52. The comparatively high level of polymorphism observed in the microsatellite markers indicates that these markers can contribute significantly to our knowledge of population genetic structure, relatedness, genetic diversity and hybridization in these species.

**Keywords:** blue wildebeest; *Connochaetes taurinus*; black wildebeest; *Connochaetes gnou*; microsatellite markers

**Introduction**

The blue wildebeest (*Connochaetes taurinus*; Burchell, 1823) and the black wildebeest (*Connochaetes gnou*; Zimmerman, 1780) belong to the tribe Alcelaphini. Fossil records indicate this tribe evolved in Africa (Gentry, 1978; Vrba, 1979), and provide evidence that the black wildebeest diverged from a blue wildebeest-like ancestor approximately one million years ago (Corbet and Robinson, 1991; Brink, 1993, 2005). Various studies have reported low levels of genetic differentiation between the two species (Corbet and Robinson, 1991; Corbet *et al.* 1994; Grobler and Van der Bank, 1995), in line with their recent separation. Corbet and Robinson (1991) conducted a karyotypic analysis and indicated an absence of species-specific G or C-banded chromosomal markers using mitotic chromosomes and mitochondrial DNA. The authors suggested that the karyotypes were invariant. Furthermore, Corbet *et al*. (1994) observed low genetic distance between the two species using allozymes and suggested that the two species appear to be at an early stage of evolutionary divergence. (Grobler and Van der Bank, 1995) showed that the two wildebeest species are more closely related than congeneric ungulate species, using allozymes.

The black wildebeest is endemic to South Africa where it was driven to near extinction in the early 1900s due to hunting pressure, disease outbreaks and habitat destruction (Von Richter, 1974). Conservation of the remaining genetic diversity following the bottleneck is therefore of high importance. The blue wildebeest is widely distributed and is reported to consist of five sub-species: *C. t. albojubatus* (Thomas, 1892), with a distribution range that extends from northern Tanzania to central Kenya; *C. t. cooksoni* (Blaine, 1914), with a restricted range to the Luangwa Valley in Zambia; *C. t. johnstoni* (Sclater, 1896), which is reported to occur from Mozambique to east-central Tanzania; *C. t. mearnsi* (Heller, 1913), with a range that extends from northern Tanzania to southern Kenya; and *C. t. taurinus*, which was historically distributed in southern Africa (Angola, Zambia, Namibia, Mozambique, Zimbabwe and South Africa). In South Africa, the ranges of the black wildebeest and blue wildebeest overlap slightly in the Gauteng, Free State, Northern Cape and North West Provinces. However, the species differ in terms of habitat preference, with black wildebeest showing a preference for open grassland plateaus while blue wildebeest are associated with savanna woodlands (Skinner and Chimimba, 2005). A number of morphological differences separate these species with the most recognizable being horn shape, coat colour and body size (Figure 1) (Skinner and Chimimba, 2005).

The wildlife industry in South Africa is unique, as game species may be privately owned which is in accordance with the Game Theft Act, Act No. 105 of 1991. This has led to the reintroduction of free roaming games species within or outside their natural distribution ranges to pursue economic and/or production objectives. Conservation concerns for wildlife species in South Africa includes; fragmentation, hybridization, loss of genetic diversity due to commercial breeding and loss of local adaptation. The genetic integrity of both pure black wildebeest and blue wildebeest populations are threatened by the possible existence of hybrid species. Local levels of genetic diversity may also be at risk due to the existence of small and isolated populations in both species. Research-informed management strategies are thus necessary to counter these threats posed by injudicious translocations, fragmentation and hybridization.

Hybridization was first reported between these species in the 1960s, following translocations of these species. Fabricius *et al*. (1988) investigated a hybrid wildebeest population on a private farm in the Northern Cape where all the pure animals died out. These authors determined that the hybrid offspring were fertile as they observed neonates that accompanied the females as well as some yearlings. Initial studies to detect species-specific alleles between these species were unsuccessful. A study conducted using protein-coding loci reported allele frequency differences as well as low frequency species-specific alleles, but fixed diagnostic species-specific alleles could not be detected (Grobler and Van der Bank, 1995). Grobler *et al*. (2005) conducted a study based on five cross-species microsatellite markers and identified potential species-specific alleles. However, a larger sample size including pure reference populations indicated that the potential species-specific alleles were shared between the two species (Grobler and Kotze, unpublished data).

This study aimed to assist future genetic studies on wildebeest by characterizing new microsatellites for both species, which can be combined with 17 microsatellite markers already developed from blue wildebeest (*Connochaetes taurinus*) in Tanzania (Røed *et al.* 2011), as well as five cross-species markers that have been shown to amplify well in wildebeest (Grobler *et al.* 2005). These microsatellite primers will allow researchers to potentially identify genetically unique subpopulations, determine levels of genetic diversity, identify hybrids and measure levels of genetic connectivity among subpopulations in order to ultimately secure pure populations.

**Materials and Methods**

A total of 45 black wildebeest and 18 blue wildebeest reference samples were collected from the Free State, Western Cape, North West and Mpumalanga Provinces in South Africa (Table 1). The samples were collected from populations with a known history of purity, based on discussion with South African conservation agencies. Blood was collected and stored in Ethylene Diamine Tetra-acetic Acid (EDTA) preservative (approximately 0.5 mL) and hair samples were collected and stored in envelopes. DNA extraction was conducted using the Qiagen DNeasy® Blood and Tissue Kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's protocols for blood and hair.

Microsatellite enrichment was achieved using the fast isolation by AFLP of sequencing containing repeats (FIASCO) protocols (Zane *et al.* 2002; Cortinas *et al.* 2006) with the following probes; $(AGGG)_4$, $(GTG)_5$, $(GTA)_5$, $(AC)_5$, $(AAAT)_5$. $(ATA)_5$, $(CT)_5$, and $(TGC)_5$. The microsatellite-enriched library was prepared by Inqaba Biotech (Pretoria, Gauteng, South Africa) using three samples of each species and was sequenced on the Ion Torrent PGM platform at the Central Analytical Facilities (CAF) at Stellenbosch University (Stellenbosch, Western Cape, South Africa). Analytical processing and quality filtering were performed at the CAF using the parameters of the PGM system. Reads ranging from 450–500 bp length for the black wildebeest (2,426 total reads) and the blue wildebeest (8,526 total reads) were obtained. MSATCOMMANDER version 1.0.8 (Faircloth, 2008) was used to search the resulting reads for microsatellite motifs of between 2 and 6 bp and with $\geq 8$ repeats in length. A total of 128 reads were identified containing these microsatellite repeats.

Primer pairs flanking repeat regions were designed using Primer3 software (Rozen and Skaletsky, 2000) for 32 unique loci. The current species-specific marker set provided here were additionally compared with nine markers developed by Røed et al. (2011) for wildebeest from East Africa (CT-02, CT-03, CT-10, CT-17, CT-18, CT-19, CT-25, CT-27 and CT-30) as well as loci from the StockMarks® cattle genotyping kit (Thermo Fisher Scientific, Inc.) that were previously shown to amplify in wildebeest species (ETH10, TGLA53, TGLA122, BM1824 and OARCP26). Polymerase Chain Reaction (PCR) amplification was conducted in a 12.5 µl reaction volume consisting of KAPA2G Robust HotStart ReadyMix, forward and reverse primers (0.5 µM each), and 50 ng genomic DNA template. The conditions for PCR amplification were as follows: 3 min at 95°C denaturation, 35 cycles for 15 sec at 95°C, 15 sec at 55-66°C and 15 sec at 72°C, followed by extension at 72°C for 10 min in a T100™ Thermal Cycler (Bio-Rad Laboratories, Inc. Hercules, CA, USA). PCR products were pooled and run against a Genescan™ 500 LIZ™ internal size standard on an ABI 3130 Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA, USA). Samples were genotyped using GeneMapper v. 4.0 software (Applied Biosystems, Inc., Foster City, CA, USA). MICRO-CHECKER (van Oosterhout *et al.*, 2004) was used to detect possible genotyping errors, allele dropout, and null alleles. The number of alleles per locus (A), allelic richness (AR), observed heterozygosity ($H_o$), and unbiased heterozygosity ($H_z$) was calculated with MS Toolkit (Park, 2001) and GenAlEx 6.5 (Peakall and Smouse, 2006, 2012), while Arlequin 3.5 (Excoffier and Lischer, 2010) was used to test for deviations from expected Hardy-Weinberg (HW) proportions of genotypes and to evaluate loci for gametic disequilibrium. Associated probability values were corrected for multiple comparisons using a modified false discovery rate (B-Y FDR) (Narum, 2006) for a significance level of 0.05.

**Results and Discussion**

*Species-specific microsatellite markers*

A total of 11 out of 32 loci tested showed allelic polymorphism and amplified consistently between the two species, with the average number of alleles ranging from 2.0 to 2.3 in the black wildebeest populations and with 4.1 in the single blue wildebeest population. Primer sequences, expected PCR product sizes, repeat unit, and fluorescent labels are listed in Table 2. A summary of localities, sample sizes, and genetic diversity statistics is shown in Table 1. The average allelic richness was higher in the blue wildebeest population (3.958) compared to the black wildebeest populations (1.716 – 1.881) (Table 1). The mean $H_o$ ranged from 0.26 to 0.45 in the black wildebeest populations and was 0.52 in the blue wildebeest population, while the mean $H_z$ varied from 0.32 to 0.43 in black wildebeest and was 0.60 in the blue wildebeest population (Table 1). A significant deviation from expected Hardy-Weinberg equilibrium of genotypes ($P = 0.00040$) was observed at one locus (BLUW8) in one black wildebeest population following B-Y FDR. The same locus indicated possible null alleles in the same population (Geluk Farm, Free State, Table 1). Linkage disequilibrium was only observed in the Western Cape black wildebeest population for the following marker pairs: BLAWB7 and BLAWB13; BLAWB7 and BLAWB16; BLAWB13 and BLAWB16; plus, BLUW6 and BLUW10 following B-Y FDR.

*Comparison of markers sets for wildebeest*

A comparison of the two published microsatellite marker sets and the markers provided here, is shown in Table 3. The amplification success for the microsatellite markers developed by Røed *et al*. (2011) ranged from 88 – 100% and 89 – 100% in the black wildebeest and blue wildebeest respectively. This in accordance with results

obtained by Miller *et al.*, (2016)which provides details of amplification success of these markers in South African blue wildebeest. The loci from the StockMarks® cattle genotyping kit had lower amplification success in blue wildebeest (39 – 94%) compared to black wildebeest (88 – 100%). The four species-specific markers (BLAWB6, BLAWB7, BLAWB13 and BLAWB16) developed for black wildebeest and the seven species-specific markers (BLUW5, BLUW6, BLUW7, BLUW8, BLUW10, BLUW11 and BLUW15) developed for blue wildebeest displayed varying amplification success per population (0 – 100%). The black wildebeest species-specific markers had a higher amplification success in the black wildebeest populations compared to the blue wildebeest. One species-specific marker (BLAWB16) developed for black wildebeest did not amplify in the black wildebeest population from the North West Province which may be attributed to sample quality. Only one black wildebeest species-specific marker (BLAWB6) had a 100% amplification success in the blue wildebeest population. Two blue wildebeest species-specific markers (BLUW5 and BLUW15) did not amplify in the blue wildebeest. However, the amplification success for the blue wildebeest species-specific markers that amplified was higher overall compared to the black wildebeest species-specific markers ranging from 82 – 100% and 94 – 100% in the black wildebeest and blue wildebeest respectively.

One marker (CT-17) was monomorphic in all three black wildebeest populations. In addition, CT-10, CT-17, CT-25, OARCP26, ETH10, BLAWB13, BLUW8, BLUW10 and BLUW11 (Table 3) where monomorphic in some of the black wildebeest populations. No markers where monomorphic in the blue wildebeest populations. The average number of alleles (as determined following removal of monomorphic alleles and those that did not amplify) ranged from 2 - 5 and 2 – 9 while the allelic richness ranged from 1.06 – 3.81 and 1.41 – 6.24 in black- and blue wildebeest respectively (Table 3). Observed heterozygosity (0.06 – 1) and unbiased heterozygosity (0.06 – 0.76) in the black wildebeest populations was lower compared to $H_o$ (0.22 – 1) and $H_z$ (0.30 – 0.84) in the blue wildebeest population (Table 3). A significant deviation from expected Hardy-Weinberg equilibrium (following B-Y FDR) was observed in the following three markers in three different populations; BLUW8 (P = 0.0004) in the Geluk Farm population that indicated possible null alleles as discussed above, CT-02 (P = 0.009) in the Groote Schuur population and TGLA53 (P = 0.0009) in the Kruger National Park population. The overall lower levels of genetic diversity observed in the black wildebeest populations may be attributed to a population bottleneck described in the early 1900s due to hunting pressure, disease outbreaks, and habitat destruction (Von Richter, 1974). In regards to possible hybrid detection, all three sets of microsatellite markers harbour private and shared alleles in both species (Table 4). However, the blue wildebeest population (56) displayed more private alleles compared to the black wildebeest populations (44). In conclusion, the 11 microsatellite loci presented here in combination with previously reported marker sets can contribute to estimates of genetic diversity, population genetic structure, relatedness and hybridization in blue and black wildebeest.



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**Compliance with ethical standards**

Black wildebeest samples were sanctioned under TOPS permit 036006 (University of the Free State) and a standing permit 03309 (National Zoological Gardens of South Africa). Samples from the Free State Provinces were collected under permit no. 01/30307 issued by DESTEA. Ethical clearance from the respective Institutional Research Ethics Committees was also obtained; UFS-AED2015/0067 (University of the Free State) and P7/12 (National Zoological Gardens of South Africa).

**Conflict of interest**

The authors declare that they have no conflict of interest.

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Table 1: Summary of origin, sample sizes and levels of genetic diversity in black wildebeest (*Connochaetes gnou*) and blue wildebeest (*Connochaetes taurinus*). A = mean number of alleles; Allelic Richness (AR); $H_o$ = observed heterozygosity; $H_z$ = unbiased heterozygosity.

| Province | Sample size | Classification | Mean number of alleles per locus (A) | Mean Aalelic richness (AR) | Unbiased Heterozygosity ($H_z$) | Observed Heterozygosity ($H_o$) | Null alleles present |
|---|---|---|---|---|---|---|---|
| Geluk Farm, Free State | 20 | Pure black wildebeest | 2.18 | 1.881 | 0.3204 | 0.2603 | Yes (BLUW8) |
| Groote Schuur Estate, Western Cape | 17 | Pure black wildebeest | 2.00 | 1.716 | 0.3632 | 0.3615 | No |
| SA Lombard Nature Reserve, North West | 8 | Pure black wildebeest | 2.30 | 1.767 | 0.4325 | 0.4500 | No |
| Kruger National Park, Mpumalanga | 18 | Pure blue wildebeest | 4.14 | 3.958 | 0.5996 | 0.5187 | No |

Table 2: Characterization of eleven microsatellite loci for *Connochaetes gnou* and *Connochaetes taurinus*. F = forward primer; R = reverse primer; bp = base pairs; No = locus number. GenBank accession numbers are MF197522 – MF197532.

| No | Name | Species | Sequence (5'-3') | Fluorescent dye label | Product size in bp | Microsatellite repeat |
|---|---|---|---|---|---|---|
| 1 | BLAWB6F | *Connochaetes gnou* | aacggcattagagaggcttg | NED | 272 | $(AC)_{14}$ |
| | BLAWB6R | | aagaatctgcccgtcaatgc | | | |
| 2 | BLAWB7F | *Connochaetes gnou* | tgggccttttatggagatgc | FAM | 300 | $(AC)_{13}$ |
| | BLAWB7R | | gggcacagagaaagagaagc | | | |
| 3 | BLAWB13F | *Connochaetes gnou* | aatgttcctaggggttggca | FAM | 242 | $(AC)_{14}$ |
| | BLAWB13R | | ccttgatcttgctcgccatt | | | |
| 4 | BLAWB16F | *Connochaetes gnou* | tgggtagattcatattgctgcag | NED | 222 | $(AC)_{17}$ |
| | BLAWB16R | | aaaagacacatgcagcccag | | | |
| 5 | BLUW5F | *Connochaetes taurinus* | aagtccgactacagcaaggg | NED | 333 | $(ACTC)_{7}$ |
| | BLUW5R | | agtaagctgttgggaccagg | | | |
| 6 | BLUW6F | *Connochaetes taurinus* | ctggggttcaatctctgggt | FAM | 238 | $(ACTC)_{6}$ |
| | BLUW6R | | agttcttccggacatcctgg | | | |
| 7 | BLUW7F | *Connochaetes taurinus* | ccagctaccaattttcgggg | PET | 288 | $(AGAT)_{6}$ |
| | BLUW7R | | tctaagtctggggcagcaaa | | | |
| 8 | BLUW8F | *Connochaetes taurinus* | tcctgctcagttatggtcctg | VIC | 230 | $(AGAT)_{7}$ |
| | BLUW8R | | tggacagtggagcttgatgg | | | |
| 9 | BLUW10F | *Connochaetes taurinus* | cgcccatcagaggacctaag | FAM | 398 | $(AGC)_{11}$ |
| | BLUW10R | | actgcctggttgttgattcc | | | |
| 10 | BLUW11F | *Connochaetes taurinus* | ggccaagaccagagaccttt | PET | 222 | $(AGC)_{8}$ |

| No | Name | Species | Sequence (5’-3’) | Fluorescent dye label | Product size in bp | Microsatellite repeat |
|---|---|---|---|---|---|---|
| | BLUW11R | | acacgactgagcgacttcat | | | |
| 11 | BLUW15F | *Connochaetes taurinus* | tcttacctctcctgcgttgg | VIC | 146 | $(AGC)_9$ |
| | BLUW15R | | ctatggcgttgcacagagtc | | | |

Table 3: Comparison of amplification success, average number of alleles (A), allelic richness (AR), observed heterozygosity ($H_o$) and unbiased heterozygosity ($H_z$) for each of the populations

| **Geluk Farm. Free State** | | | | | |
|---|---|---|---|---|---|
| **Locus** | **Amplification success (%)** | **A** | **AR** | **$H_o$** | **$H_z$** |
| CT - 02 | 100 | 3.000 | 1.361 | 0.300 | 0.272 |
| CT - 03 | 100 | 3.000 | 2.694 | 0.800 | 0.645 |
| CT - 30 | 100 | 2.000 | 1.923 | 0.600 | 0.492 |
| CT - 10 | 100 | 2.000 | 1.663 | 0.450 | 0.409 |
| CT - 25 | 100 | 4.000 | 2.847 | 0.550 | 0.665 |
| CT - 27 | 100 | 3.000 | 1.946 | 0.500 | 0.499 |
| CT - 19 | 90 | 5.000 | 2.769 | 0.778 | 0.657 |
| CT - 17 | 100 | 1.000 | 1.000 | 0.000 | 0.000 |
| TGLA53 | 100 | 4.000 | 3.463 | 0.800 | 0.729 |
| ETH10 | 100 | 2.000 | 1.105 | 0.100 | 0.097 |
| TGLA122 | 100 | 4.000 | 2.847 | 0.750 | 0.665 |
| BM1824 | 100 | 5.000 | 2.168 | 0.550 | 0.553 |
| OARCP26 | 100 | 1.000 | 1.000 | 0.000 | 0.000 |
| BLAWB6 | 100 | 3.000 | 2.930 | 0.550 | 0.676 |
| BLAWB7 | 100 | 4.000 | 2.367 | 0.650 | 0.592 |
| BLAWB13 | 95 | 1.000 | 1.000 | 0.000 | 0.000 |
| BLAWB16 | 100 | 2.000 | 1.105 | 0.100 | 0.097 |
| BLUW5 | 100 | 2.000 | 1.342 | 0.200 | 0.262 |
| BLUW6 | 100 | 2.000 | 1.956 | 0.250 | 0.501 |
| BLUW7 | 100 | 3.000 | 1.578 | 0.450 | 0.376 |
| BLUW8 | 95 | 3.000 | 2.352 | 0.263 | 0.590 |
| BLUW10 | 100 | 1.000 | 1.000 | 0.000 | 0.000 |
| BLUW11 | 100 | 1.000 | 1.000 | 0.000 | 0.000 |
| BLUW15 | 100 | 2.000 | 1.724 | 0.400 | 0.431 |
| **Groote Schuur Estate. Western Cape** | | | | | |
| **Locus** | **Amplification success (%)** | **A** | **AR** | **$H_o$** | **$H_z$** |
| CT - 02 | 100 | 4.000 | 2.072 | 0.412 | 0.533 |
| CT - 03 | 100 | 3.000 | 1.270 | 0.176 | 0.219 |
| CT - 30 | 100 | 3.000 | 2.639 | 0.765 | 0.640 |
| CT - 10 | 94 | 1.000 | 1.000 | 0.000 | 0.000 |
| CT - 25 | 88 | 3.000 | 2.018 | 0.400 | 0.522 |
| CT - 27 | 100 | 3.000 | 1.509 | 0.353 | 0.348 |
| CT - 19 | 100 | 3.000 | 2.232 | 0.412 | 0.569 |
| CT - 17 | 88 | 1.000 | 1.000 | 0.000 | 0.000 |

| | | | | | |
|---|---|---|---|---|---|
| TGLA53 | 94 | 2.000 | 1.600 | 0.500 | 0.387 |
| ETH10 | 94 | 1.000 | 1.000 | 0.000 | 0.000 |
| TGLA122 | 88 | 4.000 | 3.814 | 0.667 | 0.763 |
| BM1824 | 94 | 4.000 | 2.008 | 0.688 | 0.518 |
| OARCP26 | 100 | 2.000 | 1.061 | 0.059 | 0.059 |
| BLAWB6 | 100 | 2.000 | 1.940 | 0.353 | 0.499 |
| BLAWB7 | 24 | 2.000 | 2.000 | 0.500 | 0.571 |
| BLAWB13 | 24 | 2.000 | 1.882 | 0.750 | 0.536 |
| BLAWB16 | 35 | 2.000 | 1.946 | 0.500 | 0.530 |
| BLUW5 | 82 | 2.000 | 1.508 | 0.429 | 0.349 |
| BLUW6 | 94 | 3.000 | 2.073 | 0.375 | 0.534 |
| BLUW7 | 100 | 2.000 | 1.486 | 0.412 | 0.337 |
| BLUW8 | 94 | 1.000 | 1.000 | 0.000 | 0.000 |
| BLUW10 | 94 | 3.000 | 1.135 | 0.125 | 0.123 |
| BLUW11 | 94 | 1.000 | 1.000 | 0.000 | 0.000 |
| BLUW15 | 88 | 2.000 | 1.991 | 0.533 | 0.515 |

**SA Lombard Nature Reserve. North West**

| Locus | Amplification success (%) | A | AR | $H_o$ | $H_z$ |
|---|---|---|---|---|---|
| CT - 02 | 100 | 2.000 | 1.133 | 0.125 | 0.125 |
| CT - 03 | 100 | 2.000 | 2.000 | 0.750 | 0.533 |
| CT - 30 | 100 | 3.000 | 2.246 | 0.625 | 0.592 |
| CT - 10 | 100 | 2.000 | 1.280 | 0.250 | 0.233 |
| CT - 25 | 100 | 1.000 | 1.000 | 0.000 | 0.000 |
| CT - 27 | 100 | 3.000 | 2.844 | 0.750 | 0.692 |
| CT - 19 | 100 | 3.000 | 2.723 | 0.625 | 0.675 |
| CT - 17 | 100 | 1.000 | 1.000 | 0.000 | 0.000 |
| TGLA53 | 100 | 4.000 | 3.459 | 0.875 | 0.758 |
| ETH10 | 100 | 1.000 | 1.000 | 0.000 | 0.000 |
| TGLA122 | 100 | 4.000 | 2.723 | 0.625 | 0.675 |
| BM1824 | 100 | 2.000 | 1.969 | 0.375 | 0.525 |
| OARCP26 | 88 | 1.000 | 1.000 | 0.000 | 0.000 |
| BLAWB6 | 100 | 2.000 | 1.600 | 0.500 | 0.400 |
| BLAWB7 | 100 | 3.000 | 1.662 | 0.125 | 0.425 |
| BLAWB13 | 100 | 2.000 | 2.000 | 0.500 | 0.533 |
| BLAWB16 | 0 | 0.000 | 0.000 | 0.000 | 0.000 |
| BLUW5 | 100 | 2.000 | 1.280 | 0.250 | 0.233 |
| BLUW6 | 100 | 3.000 | 2.667 | 0.500 | 0.667 |
| BLUW7 | 100 | 3.000 | 2.612 | 1.000 | 0.658 |

| | | | | | |
|---|---|---|---|---|---|
| BLUW8 | 100 | 2.000 | 1.438 | 0.375 | 0.325 |
| BLUW10 | 100 | 2.000 | 1.133 | 0.125 | 0.125 |
| BLUW11 | 100 | 2.000 | 1.882 | 0.500 | 0.500 |
| BLUW15 | 100 | 2.000 | 1.753 | 0.625 | 0.458 |

**Kruger National Park. Mpumalanga**

| Locus | Amplification success (%) | A | AR | $H_o$ | $H_z$ |
|---|---|---|---|---|---|
| CT - 02 | 94 | 7.000 | 5.667 | 0.882 | 0.848 |
| CT - 03 | 94 | 2.000 | 1.486 | 0.412 | 0.337 |
| CT - 30 | 94 | 2.000 | 1.410 | 0.235 | 0.299 |
| CT - 10 | 100 | 9.000 | 5.400 | 0.778 | 0.838 |
| CT - 25 | 89 | 5.000 | 2.547 | 0.750 | 0.627 |
| CT - 27 | 89 | 9.000 | 6.244 | 0.688 | 0.867 |
| CT - 19 | 100 | 5.000 | 3.580 | 0.889 | 0.741 |
| CT - 17 | 94 | 5.000 | 2.481 | 0.647 | 0.615 |
| TGLA53 | 78 | 7.000 | 4.215 | 0.286 | 0.791 |
| ETH10 | 94 | 2.000 | 1.486 | 0.412 | 0.337 |
| TGLA122 | 94 | 5.000 | 3.380 | 0.824 | 0.725 |
| BM1824 | 39 | 5.000 | 2.882 | 0.857 | 0.703 |
| OARCP26 | 83 | 3.000 | 2.866 | 0.667 | 0.674 |
| BLAWB6 | 100 | 7.000 | 5.143 | 0.611 | 0.829 |
| BLAWB7 | 0 | 0.000 | 0.000 | 0.000 | 0.000 |
| BLAWB13 | 0 | 0.000 | 0.000 | 0.000 | 0.000 |
| BLAWB16 | 11 | 4.000 | 4.000 | 1.000 | 1.000 |
| BLUW5 | 0 | 0.000 | 0.000 | 0.000 | 0.000 |
| BLUW6 | 100 | 2.000 | 1.670 | 0.222 | 0.413 |
| BLUW7 | 100 | 3.000 | 2.197 | 0.444 | 0.560 |
| BLUW8 | 94 | 4.000 | 1.441 | 0.353 | 0.316 |
| BLUW10 | 94 | 6.000 | 3.905 | 0.765 | 0.766 |
| BLUW11 | 94 | 3.000 | 1.438 | 0.235 | 0.314 |
| BLUW15 | 0 | 0.000 | 0.000 | 0.000 | 0.000 |

Table 4: Private and shared alleles for blue wildebeest and black wildebeest and the total number (#) of alleles at each locus

| Locus | Blue wildebeest alleles | Black wildebeest alleles | Shared alleles | Total # of alleles |
|---|---|---|---|---|
| CT - 02 | 6 | 3 | 1 | 10 |
| CT - 03 | 1 | 2 | 1 | 4 |
| CT - 30 | 1 | 2 | 1 | 4 |
| CT - 10 | 7 | 1 | 2 | 10 |
| CT - 25 | 3 | 2 | 2 | 7 |
| CT - 27 | 6 | 2 | 3 | 11 |
| CT - 19 | 1 | 1 | 4 | 6 |
| CT - 17 | 4 | 0 | 1 | 5 |
| TGLA53 | 2 | 0 | 5 | 7 |
| ETH10 | 2 | 2 | 0 | 4 |
| TGLA 122 | 2 | 2 | 3 | 7 |
| BM1824 | 4 | 4 | 1 | 9 |
| BLAWB6 | 5 | 2 | 2 | 9 |
| BLAWB7 | 0 | 5 | 0 | 5 |
| BLAWB13 | 0 | 2 | 0 | 2 |
| BLAWB16 | 3 | 2 | 1 | 6 |
| BLUW5 | 0 | 2 | 0 | 2 |
| BLUW6 | 0 | 1 | 2 | 3 |
| BLUW7 | 0 | 2 | 3 | 5 |
| BLUW8 | 2 | 3 | 2 | 7 |
| BLUW10 | 4 | 1 | 2 | 7 |
| BLUW11 | 2 | 1 | 1 | 4 |
| BLUW15 | 0 | 2 | 0 | 2 |
| Oarcp26 | 1 | 0 | 2 | 3 |
| **Total** | **56** | **44** | **39** | **139** |

Figure 1: (A) Map indicating provinces and the natural distribution ranges of the blue wildebeest (*Connochaetes taurinus*) and black wildebeest (*C. gnou*) in South Africa; as well as overlapping areas (map from Briss et al., 2015 Unpublished). Inserts: Photographic representation of (B) a pure black wildebeest (photo courtesy of Elma Kuyler) and (C) a pure blue wildebeest (photo courtesy of Kobus Raath). Note differences relating to horn shape, colour and hair tufts.