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Interspecific hybridization in *Tragelaphus*

A Pliocene hybridisation event reconciles incongruent mitochondrial and nuclear gene trees among spiral-horned antelopes

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ABSTRACT

The spiral-horned antelopes (Genus *Tragelaphus*) are among the most phenotypically diverse of all large mammals, and evolved in Africa during an adaptive radiation that began in the late Miocene, around 6 million years ago. *Tragelaphus* was able to exploit the habitat heterogeneity created by Plio-Pleistocene paleoclimatic fluctuations and tectonic processes to eventually occupy almost every habitat type in present day Sub-Saharan Africa. The smallest of the spiral-horned antelopes, the bushbuck (*T. scriptus*), is also widely distributed across Africa, but is genetically divided into unrelated *Scriptus* and *Sylvaticus* mitochondrial (mt)DNA superlineages that inhabit opposite halves of the continent – suggesting the convergent evolution of independent bushbuck species. In this study, we provide the first evidence showing that *Scriptus* and *Sylvaticus* are reciprocally monophyletic at nuclear DNA loci, and so the bushbuck comprise a single species across its continental range. Given that mtDNA will sort into species-specific lineages more quickly than nuclear DNA, only an ancient interspecific hybridization event – between a proto-nyala female and a proto-bushbuck male - can reconcile the cyto-nuclear incongruence. We dated this event to the early Pliocene about 4.5 -5 million years ago. This study adds to an increasing body of evidence that suggests interspecific hybridisation may be more common than previously thought. We suggest a potential role for interspecies gene flow in adaptive radiations.

Key words: adaptive radiation, interspecific hybridisation, paleoclimate, *Tragelaphus*, Sub-Saharan Africa.

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Speciation is at its most striking during an adaptive radiation, when specific combinations of ecological and genetic circumstances allow evolutionary forces to drive phenotypic divergence. The most recent adaptive radiations occurred during the Plio-Pleistocene (~5,400,000 – 12,000 years ago), and have been attributed to fluctuations in paleoclimate (Blois and Hadly 2009; Bobe and Behrensmeyer 2004; Vrba 1995) and tectonic uplift (Partridge et al. 1995; Pickford 1990). This period of massive faunal diversification and turnover (Vrba 1985, 1993) is sometimes referred to as the "golden age of mammals"(Estes 1990; Huxley 1965). One group of mammals in particular - the bovids - were able to adaptively exploit heterogeneity in the productive paleohabitats of the Plio-Pleistocene, radiating in several subgroups (or tribes) to become the most diverse and abundant of all herbivores (Du Toit and Cumming 1999), comprising 142 extant species (IUCN 2017).

The spiral-horned antelopes of the genus *Tragelaphus* (Tribe: Tragelaphini) comprise one of the more spectacular radiations within the Bovidae, occurring from the late Miocene-early Pliocene (Bibi 2013, Gentry 2010). Within that timeframe, spiral-horned antelopes populated almost every habitat type in sub-Saharan Africa, and with just nine extant species they currently exhibit substantial phenotypic variation in size, physiology, life history traits, sexual dimorphism and social systems (Table 1). More remarkable, however, is the level of convergent evolution within *Tragelaphus* (Fig. 1), the extent of which was only revealed through molecular paraphyly between morphologically sister species, such as the kudu (*T. strepciceros*) and lesser kudu (*T. imberbis*) (Hassanin and Douzery 1999; Matthee and Robinson 1999) and the nyala (*T. angasi*) and mountain nyala (*T. buxtoni*) (Willows-Munro et al. 2005). A similar relationship has also been shown for the bushbuck (*T. scriptus*), a highly diverse species complex inhabiting most of sub-Saharan Africa and whose paraphyletic (*Scriptus* and *Sylvaticus*) mitochondrial DNA super-lineages (Moodley et al. 2009; Hassanin et al. 2012) suggest convergence to a bushbuck-like phenotype (Moodley and Wronski 2009).

However, Bibi (2013) recently suggested that the bushbuck paraphyly may have also resulted from mitochondrial lineage introgression between non-sister taxa, rather than from phenotypic convergence. This view is supported by the ability of closely related bovid species, including spiral horned antelopes, to produce fertile hybrids in captivity (Boulineau 1933; Koulischer 1973), which suggests that reproductive isolation has lagged behind phenotypic divergence. Indeed Plio-Pleistocene hybridisation has already been demonstrated through nuclear and mitochondrial gene tree incongruence in the Caprini (Ropiquet and Hassanin 2005) and Bovini (Verkaar et al. 2004). Alternatively, paraphyly in the bushbuck could also conceivably have resulted from incomplete lineage sorting (ILS, Pamilo and Nei 1988), but until now, the nuclear DNA data required to test these hypothesises have not been available.

In this study, we obtained both nuclear and mitochondrial DNA sequences representing both *Scriptus* and *Sylvaticus* bushbuck super-lineages, combined with existing and newly generated data for all other con-generic species, in order to reconstruct the *Tragelaphus* species tree and test the hypothesis that the two bushbuck super-lineages *Scriptus* and *Sylvaticus* are independent species that arose via convergent evolution (Wronski and Moodley, 2009). The expectation is that convergent evolution would have affected both mitochondrial and nuclear genomes similarly, resulting in the same patterns of phylogenetic relatedness among spiral-horned antelope species for both molecular markers. Conversely, a cyto-nuclear genomic dissociation would suggest that either incomplete lineage sorting or introgression was responsible for the bushbuck's mitochondrial paraphyly.

MATERIALS AND METHODS

Samples.— Bushbuck individuals representing the genetic and geographical heterogeneity of both *Scriptus* and *Sylvaticus* mitochondrial superlineages were sampled (Table 2). We also sampled an eland (*T. oryx*), a sitatunga (*T. spekei*) and two kudu, which we used in addition to

sequences from 19 other *Tragelaphus* individuals published previously (Hassanin and Douzery 1999; Mattheeand Robinson 1999; Willows-Munro et al. 2005). All new samples were obtained from museum collections and taxidermists (Table 2). Our data set therefore contained the two potential bushbuck "species", the eight other *Tragelaphus* species, and with each species represented by one to seven individual multilocus sequence profiles (Table 1). We selected four outgroup taxa, each with a different level of relatedness to the ingroup and included a representative of the sister groups Boselaphini (*Boselaphus tragocamelus),* Bovinae (*Bos taurus* and *Syncerus caffer*) and Bovidae (*Apyceros melampus*) after Willows-Munro et al. (2005). The current study is in line with the guidelines of the American Society of Mammalogists.

Molecular methods.—DNA was extracted using a modified SDS-proteinase K/phenol-chloroform method (Moodley and Bruford 2007) and quantified by UV spectrophotometry. Using 25ng of template DNA, we amplified the following seven gene fragments mitochondrial genes 12SrRNA, 16SrRNA and cytochrome *b*; and nuclear intron fragments of the stem cell factor (MGF, Lyons et al. 1997), protein-kinase CI (PRKCI, Matthee et al. 2001), B-spectrin non-erythrocytic1 (SPTBN1, Venta et al. 1996) and thyrotropin (TH, Matthee et al. 2001) genes. PCR conditions were the same as in Matthee et al. (2001) and Matthee and Davis (2001). PCR products were sequenced using Big Dye terminators (Applied Biosystems) and analysed on an ABI Prism 3130 DNA sequencer. Sequence assembly, trimming (according to published reference sequences) and alignment were performed in BioEdit (Hall, 1999).

Genetic diversity and tests of neutrality.—Genetic diversity of ingroup taxa was estimated for each gene fragment. Nucleotide and haplotype diversities for nuclear intron and mitochondrial alignments were calculated in DNAsp (Librado and Rozas 2009). Nuclear introns were phased using the Phase algorithm (Stephens et al. 2001), consisting of 100,000

iterations with a burnin proportion of 10%. We tested each locus for neutrality using coalescent statistics Fs (Fu and Li 1993) and D (Tajima 1989).

Gene networks.—We reconstructed haplotype/allele networks for each locus to assess levels of interspecific allele sharing. Since loci located in the mitochondrial genome are tightly linked, we concatenated mtDNA sequences and reconstructed a single mitochondrial gene network. We used the median joining method in PopART (Bandelt et al. 1999; Leigh and Bryant, 2015) to calculate and draw the five resulting networks. We set the epsilon value to 0, gave equal weighting to transitions and transversions and used the correction cost algorithm.

Species tree reconstruction. —Gene and species trees were reconstructed in a fully Bayesian framework using *BEAST v 2.5.0 (Bouckaert et al. 2014). Sequence alignments of each of the seven gene fragments were parameterised in BEAUti (Drummond et al. 2012). The nucleotide substitution model for each gene partition (Table 2) was determined in jModelTest (Posada, 2008) using BIC, base frequencies were estimated from the data, and gamma distributions were imposed for substitution rate priors. All site and clock models were unlinked, but alternative lognormal and exponential clock models were tested against a strict clock for each partition. After one run of one 1,000,000,000 billion MCMC iterations, sampling every 100,000 steps and discarding 20% as burnin, the standard deviations of the posterior marginal distributions of both exponential and lognormal parameters included zero for all genes. Subsequent analyses were therefore carried out under a strict clock. Clock rate priors were uniform, with initial rates of 3.4×10^{-9} substitutions/site/year for 12SrRNA, 4.9×10^{-9} for 16SrRNA (Pesole et al. 1999), 9.8×10^{-8} for cytochrome *b* (Nabholz et al. 2008) and 1.9×10^{-9} for nuclear introns (Liu et al. 2006). Tree priors were linked to yield separate mitochondrial and nuclear gene trees. The species tree was reconstructed using a birth-death speciation prior, with a linear demographic prior, assuming that prehistoric population sizes

were constant. Ten independent MCMC analyses were carried out, each of which consisted of 1,000,000,000 iterations, logging parameters, gene and species trees every 100,000 iterations and discarding the first 20% of each run as burn-in. Each analysis was checked for convergence in Tracer (Rambaut et al. 2014), and in all cases effective sample size values were greater than 200, indicating a well-mixed Markov chain. Post-burnin posterior samples of gene and species trees of all ten analyses were combined in LogCombiner and maximum clade credibility gene and species trees were produced showing common ancestor heights and only nodes with a posterior probability of 1.

As with all molecular dating analyses, reliability of fossil calibration dates, and their implementation can affect the accuracy of divergence time inferences (Donoghue and Benton, 2007, Parham et al. 2012). Fortunately, Tragelaphine antelopes are relatively well represented in the Plio-Pleistocene fossil record (Gentry, 2010) and here we select the three relevant fossil calibration points of the 16 used by Bibi (2013) in his recent mitochondrial genome analysis of the Bovidae. To allow for the possibility of erroneous fossil dating, soft, rather than hard bounds were used on calibration dates (Yang and Rannala 2006). Calibration dates were therefore were parameterised as most recent common ancestor (MRCA) priors, with the minimum and maximum fossil dates adjusted to the 2.5% and 97.5% quantiles of a normal distribution respectively. These included 18 Ma (16 – 20 Ma) for the coalescence of all analysed bovid lineages in the analysis, 8.8 Ma (7-11 Ma) for the crown outgroup Bovini, 5.72 Ma (4.7 – 6.7 Ma) for the divergence of the Crown Tragelaphini, 3.4 Ma (3.4 - 4.5 Ma) for stem *T. euryceros* and for stem *T. strepciceros* (Bibi 2013 and references therein). Monophyly was not invoked for any of the calibration points, and all resulting trees were unconstrained.

RESULTS

Diversity and selection.—The complete data set consisted of 4680 bp of DNA sequence data (2080 bp mtDNA, 2,600 bp nuclear introns) for 28 individuals representing nine ingroup species and four outgroup species (Table 3). Mitochondrial DNA diversity was high, ranging in nucleotide diversity among loci from 3.7 to 9.3 % (Table 3). In comparison, nuclear loci were generally less diverse. PRKCI and TH were the least diverse loci, but nucleotide diversities of just over 1% were observed for MGF and SPTBNI. Although several D and Fs values were negative among all loci, none were significant, suggesting loci evolve neutrally, without past demographic change.

Gene networks.—All three mtDNA gene networks were well sorted into species specific lineages, and with no haplotype sharing between species (Supplementary Data S1 A-C). High mtDNA diversity was also reflected in low haplotype sharing, even within species. Identical sequences occurred in the mountain nyala and the kudu for all three mtDNA genes, but also in the Eland at cytochome *b*. All seven bushbuck individuals possessed unique haplotypes, but with *Scriptus* and *Sylvaticus* (light green and light blue haplotypes, Supplementary Data S1 A-C) clustering separately, each more closely allied to other species than to each other. Nuclear introns, although less diverse, were also structured into species specific groups, with some allele sharing among species (Supplementary Data S1 D-G). At PRKCI, the eland and giant eland shared an allele, whereas at TH, a single allele was shared by a bongo, a sitatunga and three *Scriptus* bushbuck. Importantly, all other nuclear loci linked *Scriptus* and *Sylvaticus* bushbuck into a single species-specific group, and with allele sharing between the two evident at loci SPTBNI and PRKCI.

Mitochondrial vs nuclear trees.—A multilocus Bayesian framework was used to reconstruction the interspecific relationships among spiral-horned antelopes. Tree priors were linked across loci to produce mitochondrial and nuclear trees (Fig. 2). Nodal posterior probabilities were high (>0.99) for almost all interspecies ingroup nodes, although placement

of the nilgai (*B. tragocamelus*) relative to other outgroup taxa was less reliable for both sets of markers. Among ingroup taxa, mtDNA resolved the nodes placing the kudu sister to the mountain nyala-sitatunga-bongo-bushbuck (*Sylvaticus*), and bongo sister to the bushbuck (*Sylvaticus*) less reliably that other nodes (Fig. 2A), whereas nuclear DNA could not fully resolve the node splitting the bongo-sitatunga from the bushbuck (Fig. 2B). The mitochondrial tree (Fig. 2A) did not differ markedly from the mtDNA phylogenies produced by Moodley et al. (2009), Hassanin et al. (2012) and Bibi (2013), although it should be noted that the latter two studies did not include a representative of the mountain nyala (*T. buxtoni*). Therefore, the well-known paraphyly among the bushbuck, where the *Scriptus* lineage is sister to the nyala (*T. angasi*) and the *Sylvaticus* sister to the bongo-sitatunga clade, appears characteristic of the mitochondrial genome as a whole (Fig. 2A). Both Hassanin et al. (2012) and Bibi (2013) use mtDNA to place the kudu sister to the eland-giant eland, nuclear DNA places the kudu sister to the mountain nyala-sitatunga-bongo-bushbuck (*Sylvaticus*), as observed by Willows-Munro et al. (2005).

In contrast to mtDNA, nuclear DNA resolved bushbuck to be fully monophyletic (Fig. 2B), with *Scriptus* and *Sylvaticus* lineages coalescing in a species level bushbuck clade. Although both markers show that the bushbuck forms a tight monophyletic clade with the sitatunga, bongo and mountain nyala, the relationships among these four species is not fully resolved by the nuclear markers used here.

Species Tree.—A single consensus species tree was reconstructed from five independent MCMC runs displaying all nodes supported with a posterior probability of 1. Despite their mtDNA paraphyly, the species tree resolved a monophyletic sister relationship between *Scriptus* and *Sylvaticus* bushbuck lineages. These formed part of a clade consisting of the bongo-sitatunga sister group and the mountain nyala. The eland and giant eland were also placed as sister species, corroborating the majority of their gene trees. Notwithstanding

gene tree inconsistencies, the kudu was found to be more similar to the bushbuck-bongo-sitatunga-mountan nyala clade, than to the eland-giant eland clade, and the nyala and lesser kudu were the most basal tragelaphines. The species tree shows clearly that phenotypically similar taxa such as the kudu and lesser kudu, and nyala and mountain nyala, are not the most closely related genetically.

Divergence.—The divergence of species tree nodes were determined using three relevant, soft bounded bovid fossil calibration points. The ingroup coalesced to a common ancestor 5.43 Ma during the late Miocene. A series of middle Pliocene diversification events is inferred to have occurred approximately between 3 and 5 Ma (Fig. 3), differentiating the nyala, both eland species, kudu and mountain nyala. The monophyletic group containing sitatunga, bongo and bushbuck arose from a common ancestor that lived about 2.3 Ma. Within this group, the bushbuck divergence of *Scriptus* from *Sylvaticus* occurred about 1.8 Ma, and the bongo and sitatunga divergence from a common ancestor 1.9. Ma.

DISCUSSION

High genetic diversity at the three mtDNA loci is consistent with very high control region diversity of 11.7% (Moodley and Bruford 2007). The observed cytochrome *b* diversity (9.3 %, Table 3) is higher than most mammal species (Alpers et al. 2004; Nyakaana and Arctander ,1999; Simonsen, 1997; Arctander et al. 1996a, 1996b). This exceptionally high diversity at mitochondrial loci stems from the much deeper Pliocene common ancestry of *Scriptus* and *Sylvaticus* bushbuck lineages (Fig. 3), which is not reflected in the nuclear DNA.

The Influence of Paleoclimate and Geomorphology on Divergence.—The first spiral-horned antelope to appear in the fossil record was a nyala-like species in the late Miocene-early (about 6 Ma, Gentry 2010). Following an initial divergence of the lesser kudu in the late Miocene, the next series of interspecies divergence events differentiating the common ancestors of the nyala, kudu and eland species occurred during the middle Pliocene, followed

by late Pliocene-early Pleistocene emergence of all other species. The Pliocene was generally a warm period, with summer temperatures 5-3 Ma approximately 3C warmer than the present (Haywood and Valdes, 2004), suiting more arid adapted species, potentially like the lesser kudu. At around 4 Ma, the paleoclimate became progressively colder with intensive glaciations in the Northern Hemisphere reaching their maximum by 2.7 Ma (Bartoli et al. 2011). The emergence of the savanna-adapted kudu and eland, as well as the closed thicket nyala may have been influenced by the expansion of grasslands during this cooler time, and the recession of closed canopy forest. This trend of cooling became more cyclical during the Pleistocene and, together with an upsurge in tectonic activity along the Albertine and Gregory Rifts in East Africa (Pickford 1990; Partridge et al. 1995), resulted in a period of major climate-driven faunal turnover in Africa (Bobe and Eck 2001; Hernández Fernández and Vrba 2006). This is the period during which the later events of specialisation is inferred to have occurred in *Tragelaphus*. The mountain nyala is likely to have adapted to the colder glacial periods, specialising to feed on the Afromontane forests and grasslands to which it is restricted today. The common ancestor of the bushbuck, bongo and sitatunga also diverged during the early Pleisticene, with *Scriptus* and *Sylvaticus* bushbuck lineages geographically isolated in the north-western and south-eastern halves of Sub-Saharan Africa. It is also possible that vicariance due to tectonic uplift during the early Pleistocene (Pickford 1990; Partridge et al. 1995) could have separated the two bushbuck lineages. Perhaps the most spectacular of all tragelaphine adaptations occurred relatively recently, after the divergence of bongo from the sitatunga about 2 Ma. Although closely related sister taxa, the bongo is a large forest specialist restricted to those parts of Africa with remaining closed canopy forest, whereas the sitatunga is a small-medium sized tragelaphine, with specially adapted hooves and fur that allow it a semi-amphibious existence along most inland waterways and swamps

in Sub-Saharan Africa. Their close evolutionary relationship points to rapid ecological specialisation during the middle to late Pleistocene.

Distinguishing ILS from Gene Flow.—Both mitochondrial and nuclear gene trees showed high, species specific structuring (Fig. 2 and Supplementary Data S1), indicating that lineages appeared to have effectively sorted into monophyly, with little evidence of recently shared haplotypes through interspecific gene flow. The only instance of allele sharing was among closely related species of the bushbuck-sitatunga-bongo clade at the least diverse nuclear locus in our data set (TH). This, together with a low evolutionary rate (Table 3) suggests ILS at the TH locus, rather than interspecific gene flow, as all other bushbuck, sitatunga and bongo alleles at TH were within a single mutation of the shared allele.

On the other hand, we can rule out ILS as the cause of the mtDNA paraphyly of the *Scriptus* and *Sylvaticus* bushbuck lineages since lineage sorting is dependent on population size, with the effective size of a mtDNA population sample being approximately four times lower than an equivalent nuclear DNA sample. Therefore, mtDNA is expected to sort faster than nuclear DNA. However, since unlinked nuclear introns sort *Scriptus* and *Sylvaticus* lineages into a monophyletic species clade (Supplementary Data S1 D-F, Fig. 2B), it is impossible that mtDNA lineages could be paraphyletic due to ILS. Only an ancient interspecific hybridization event – between a proto-nyala female and a proto-bushbuck male in the early stages of the *Tragelaphus* radiation during the early Pliocene (about 4.5 -5Ma red asterisk Fig. 3), can reconcile mitochondrial and nuclear gene trees. This hybridization event resulted in the initial split between *Scriptus* and *Sylvaticus* mtDNA lineages because all bushbuck in the north-western half of sub-Saharan Africa possess nyala-like mtDNA. It also suggests that this split may have been due to vicariance, since there is no evidence for haplotype sharing between the mtDNA lineages. This analysis also hints at a much wider paleo-distribution for the nyala (or proto-nyala), since this species is presently isolated to a

small part of south eastern Africa, within the large range of *Sylvaticus* bushbuck, but thousands of kilometres away from the nearest *Scriptus* with which they are mitochondrial sister taxa.

A role for interspecific gene flow in adaptive radiations.—Hybridization between species or speciating lineages may be a key process within the adaptive radiation of Tragelaphus. This is because reproductive isolation mechanisms appear not to be fully developed in this tribe as several species produce fertile hybrids in captivity (Boulineau 1933, Koulischer 1973, van Gelder 1977). Furthermore, mitochondrial lineage introgression has been demonstrated in two other bovid radiations (Gilbert, Ropiquet and Hassanin, 2006; Verkaar et al. 2004), suggesting a potential role for this phenomenon in the speciation process.

Under the classical view of speciation through divergence, gene flow could only follow hybridisation if reproductive isolation was incomplete (Hewitt 2001), since any disruption to species specific characteristics via recombination was thought to result in a reduction of fitness and selection against hybrids (Mayr 1966, Coyne and Orr, 2004). However, the potential for this classical view of speciation in facilitating adaptive rations is unlikely for two reasons. Firstly, cyclic paleoclimatic fluctuation would also have meant periodic disruption to the establishment of reproductive isolation once environmental conditions changed to favour expansion, secondary contact and the restoration of homogenising gene flow between previously isolated populations. Secondly, the combined effect of directional selection and genetic drift during rapid radiations would have severely reduced the genetic diversity of small and isolated refugial populations, with potentially detrimental consequences. Gene flow via hybridisation is one potential explanation for both problems. If gene flow is able to retard the onset of reproductive isolation among radiating lineages, the inevitable loss of genetic diversity during rapid differentiation may be offset by

312 the influx of genetic variation from hybrids. In a scenario where certain traits evolved or
313 acquired by one (incipient) species are of adaptive value to another on secondary contact
314 (Mallet 2007), selection would be expected to favour the introgression of adaptive traits via
315 hybridization, leading to phenotypic convergence (Dasmahapatra et al. 2012, Nadeau et al.
316 2012, Barbato et al. 2017). Plants obtain significant adaptive advantages and often speciate
317 through polyploid hybridization (Grant 1981; Whitney et al. 2006), but hybridization without
318 a change in chromosome number (homoploid hybridization) is considered rare in nature
319 (Reiseberg 1997). Nevertheless, mounting empirical evidence from butterflies (Mavárez et al,
320 Gompert et al. 2006), fruitflies (Noor, 1995; Machado et al. 2002), fish (Nolte et al. 2005),
321 amphibians (Sequeira et al. 2005) and mammals (Gilbert, Ropiquet and Hassanin, 2006;
322 Verkaar et al. 2004) suggest that interspecific hybridisation and gene flow may be more
323 common than previously thought. Interestingly, almost all evidence on introgression between
324 animal species is associated with adaptive radiations, concordant with the idea originally
325 proposed by Mallet (2008). Therefore, the frequency of convergent evolution we observe in
326 *Tragelaphus*, suggests a role for adaptive introgression as some phenotypes appear to be more
327 advantageous than others. The selective forces and molecular mechanisms underpinning this
328 convergent evolution, however, are not clear and would require genome-level data to unravel.
329 We provide the first evidence that the *Scriptus* and *Sylvaticus* bushbuck lineages are
330 reciprocally monophyletic, sharing a relatively recent common ancestor in the early-middle
331 Pleistocene. This observation rules out the hypothesis of convergent evolution of the
332 bushbuck phenotype put forward by several authors (Moodley and Wron ski, Hassanin et al,
333 Bibi) on the basis of mtDNA paraphyly. One the other hand, a Pliocene interspecific
334 hybridisation event reconciles mitochondrial with nuclear phylogenies, and given the high
335 levels of phenotypic convergence we observe within the spiral-horned antelopes, it seems
336 reasonable that highly similar phenotypes may have evolved through adaptive introgression.

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SUPPLEMENTARY DATA

Supplementary Data S1. —Gene networks showing lineage sorting within the spiral-horded antelopes (*Tragelaphus* spp).

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551

552 ## FIGURE LEGENDS

553 **Fig. 1.—Convergent evolution in *Tragelaphus*.** Highly similar, but independently evolved,
554 nyala and kudu phenotypes. Red lineages on each tree show the two species in picture,
555 whereas black lineages represent other species within the genus. Phylogenies are identical and
556 redrawn after Fig. 2 in Willows-Munro et al. (2005).
557
558 **Fig. 2.—Mitochondrial vs nuclear phylogenetic trees representing the diversity of the**
559 ***Tragalaphus* radiation. A.** Combined mtDNA tree showing the paraphyletic *Scriptus*
560 (yellow) and *Sylvaticus* (cyan) bushbuck lineages. **B.** Combined nuclear DNA tree showing
561 monophyletic bushbuck lineages. Branches are coloured by posterior probability.
562
563 **Fig. 3.—Species tree reconstruction and divergence times among the spiral-horned**
564 **antelopes (*Tragelaphus* spp.).** Thick black lines show the *Tragelaphus* species tree, on which
565 thick blue lines indicate the 95% HPD for nodal divergence times. The mitochondrial DNA
566 tree (light grey) is overlaid onto the species tree for direct comparison. The red lineage is the
567 *Scriptus* bushbuck mtDNA lineage. The red asterisk marks the Pliocene hybridization event
568 between a proto-nyala female and proto-*Scriptus* bushbuck male that reconciles the two trees.
569

570 **Tables**

571 Table 1.—Geographic, habitat and feeding heterogeneity among the spiral-horned antelopes (*Tragelaphus* spp.*)*

Species	**Common name**	**Taxonomic Reference**	**N**	**Distribution**	**Habitat**	**Feeding strategy**	**SD***	**Shoulder height (cm)**	**Weight (kg)**
Tragelaphus scriptus	Bushbuck	Pallas, 1766	7	Sub-Saharan Africa	Rainforest, forest edge, gallery forest, thickets, bush, sub-desert.	mainly browsing	yes	61-100	24-80
Tragelaphus angasi	Nyala	Angas, 1848	2	South-east Africa	Dense thickets, forest, and open-thicket woodland mosaic.	mixed	yes	80-121	62-140
Tragelaphus buxtoni	Mountain Nyala	Lydekker, 1910	2	Ethiopian Highlands	East African Afromontane forest and grassland.	mixed	yes	90-135	150-300
Tragelaphus imberbis	Lesser Kudu	Blyth, 1869	1	North-East Africa	Semi-arid Acacia-*Commiphora* thornbush.	mainly browsing	yes	90-110	56-108

Tragelaphus euryceros	Bongo	Ogilby, 1837	2	Central Africa	Congolian Rainforest, East African Afromontane forest.	mainly browsing	no	110-130	210-405
Tragelaphus spekei	Sitatunga	Speke, 1863	2	West, Central and East Africa	Waterways and swamps.	mixed	yes	75-125	40-130
Tragelaphus strepciceros	Kudu	Pallas, 1766	4	Central, East and southern Africa	Mixed scrub woodland, *Acacia* and *Mopane* bush.	mainly browsing	yes	100-150	120-315
Tragelaphus oryx	Eland	Pallas, 1766	3	East and Southern sub-Saharan Africa	*Acacia* savanna, miombo woodland, sub-desert	mainly browsing	no	125-178	300-942
Tragelaphus derbanius	Giant Eland	Gray, 1847	1	West and Northern sub-Saharan Africa	Sudanian and Guinean savannas	mainly browsing	no	140-176	300-907

572

573

574 Table 2.—Spiral-horned antelope (*Tragelaphus* spp.) sequence data generated for this study

Species	**Lineage (haplogroup*)**	**Common name**	**Voucher**	**Source**	**Country/Region**
Tragelaphus scriptus	Scriptus (*phaleratus*)	Bushbuck	17820	Royal Museum for Central Africa, Tervuren	Democratic Republic of Congo
Tragelaphus scriptus	Scriptus (*decula*)	Bushbuck	DDF1	Travel Ethiopia, Addis Abeba	Ethiopia
Tragelaphus scriptus	Scriptus (*scriptus*)	Bushbuck	SL01	O'Donoghue Collection	Sierra Leone
Tragelaphus scriptus	Sylvaticus (*ornatus*)	Bushbuck	Zimbabwe07	Bromley Game Skin Tannery, Harare	Zimbabwe
Tragelaphus scriptus	Sylvaticus (zambezi)	Bushbuck	Zimbabwe18	Taxidermy Enterprises, Bulawayo	Zimbabwe
Tragelaphus oryx	N/A	Eland	E01	Nico van Rooyen Taxidermy, Rosslyn	South Africa
Tragelaphus spekei	N/A	Sitatunga	S01	Nico van Rooyen Taxidermy, Rosslyn	Unknown
Tragelaphus strepciceros	N/A	Greater Kudu	K01	Nico van Rooyen Taxidermy, Rosslyn	South Africa
Tragelaphus	N/A	Greater	K02	Nico van Rooyen	South Africa

strepciceros	Kudu	Taxidermy, Rosslyn

575 *mtDNA haplogroups after Moodley et al. 2007

576

577

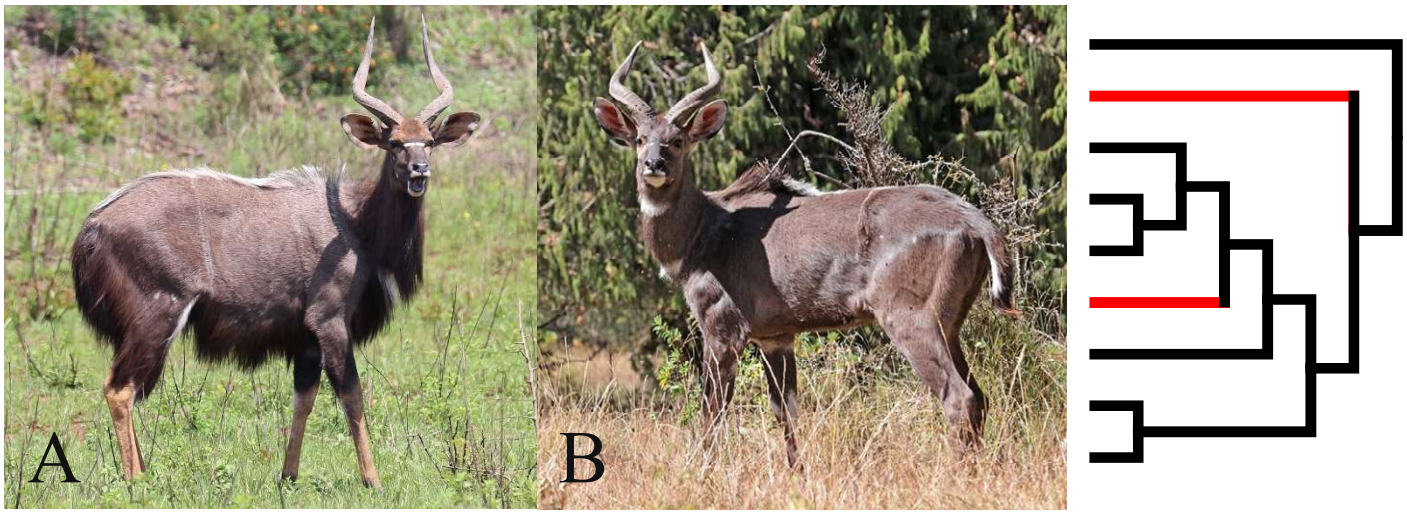
578 Table 3.—Diversity, selection and model settings for seven gene fragments among spiral-
579 horned antelopes (*Tragelaphus* spp).

		Diversity				Selection		Reconstruction		
	Size (bp)	**P**	**H**	**HD**	**π**	**D**	**Fs**	**Model**	**shape**	**posterior clock rate**
Mitochondrial										
12SrRNA	593	87	22	0.993	0.03647	-0.58082	-5.2151	HKY +G	0.072	6.8×10^{-3}
16SrRNA	347	64	20	0.982	0.0470	-0.68065	-3.9721	HKY +G	0.194	9.0×10^{-3}
cytochrome *b*	1140	364	22	0.993	0.09303	-0.18439	0.3781	HKY +G	0.193	1.8×10^{-2}
Nuclear Intron										
MGF	671	44	21	0.962	0.01110	-0.96585	-3.2605	GTR +G	0.558	1.6×10^{-3}
PRKCI	498	24	14	0.897	0.00876	-0.91712	-1.7387	HKY	N/A	9.9×10^{-4}
SPTBN1	765	47	16	0.924	0.01242	-0.62365	0.8428	GTR +G	0.427	1.7×10^{-3}
TH	666	26	16	0.926	0.00729	-0.58101	-2.2164	GTR +G	0.298	1.1×10^{-3}

580 P, polymorphic sites; H, haplotypes/alleles; HD, haplotype diversity; D, Tajima's D; Fs, Fu's
581 Fs.

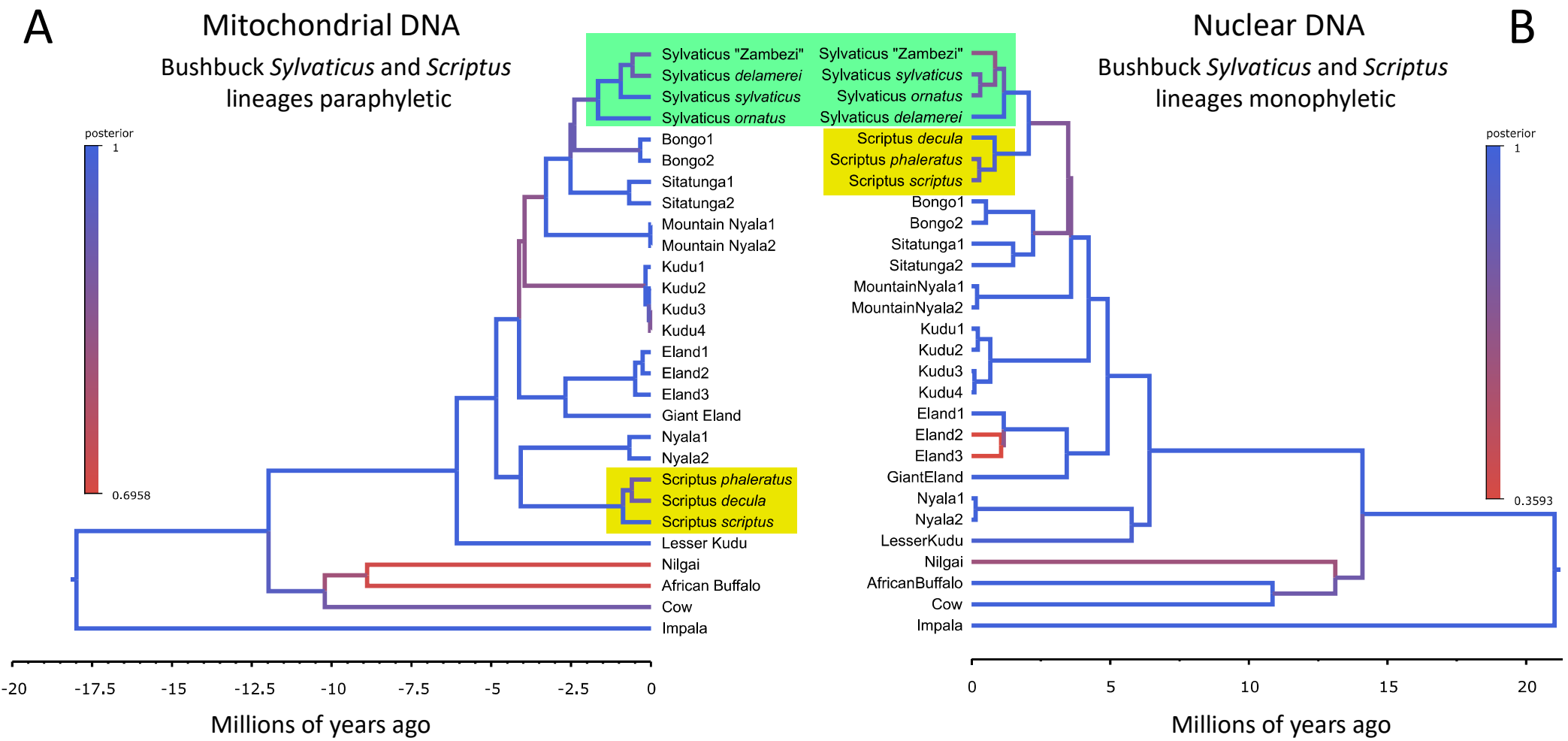
582

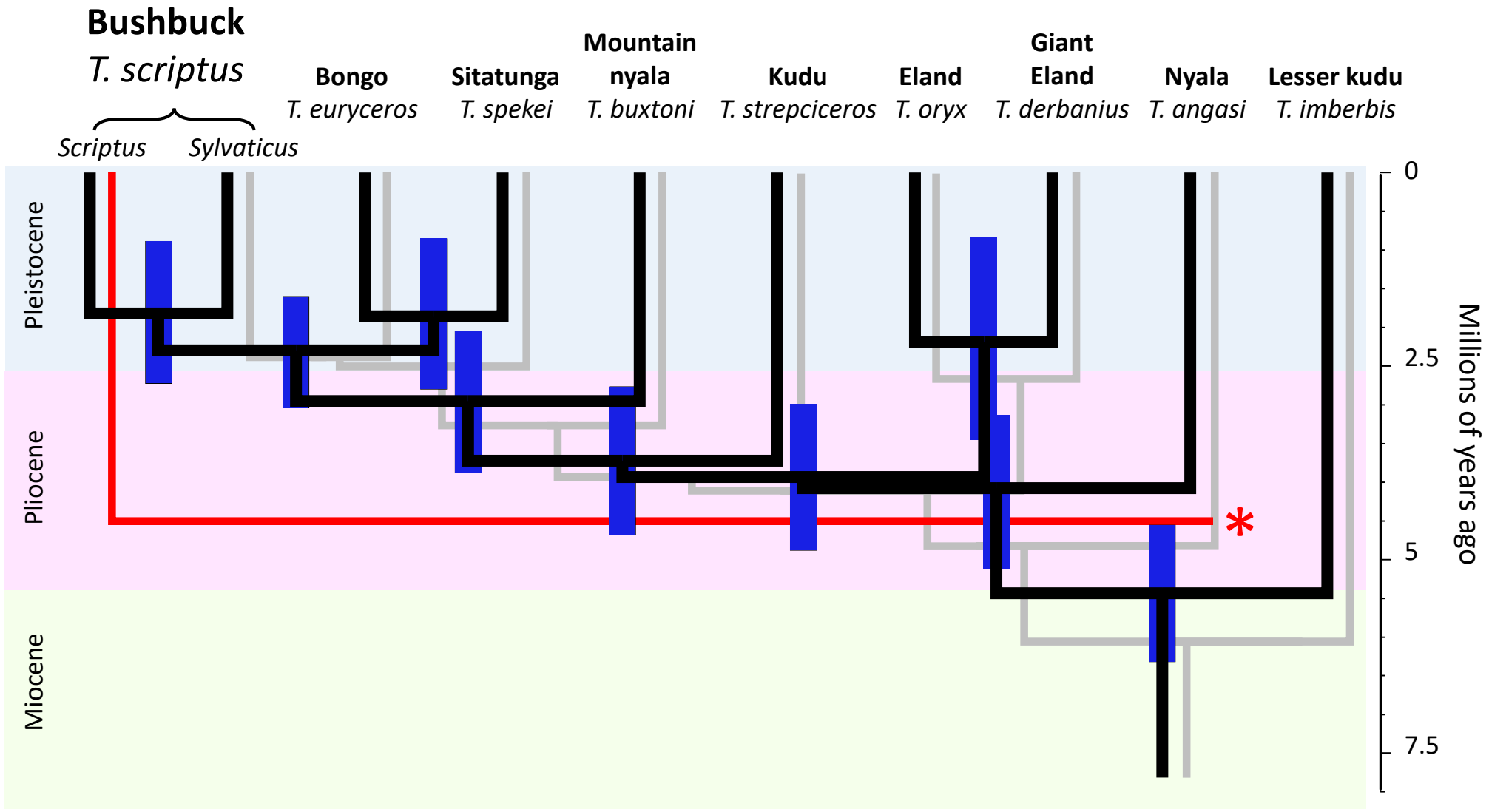
583



Nyala (*T. angasi*) Mountain Nyala (*T. buxtoni*)

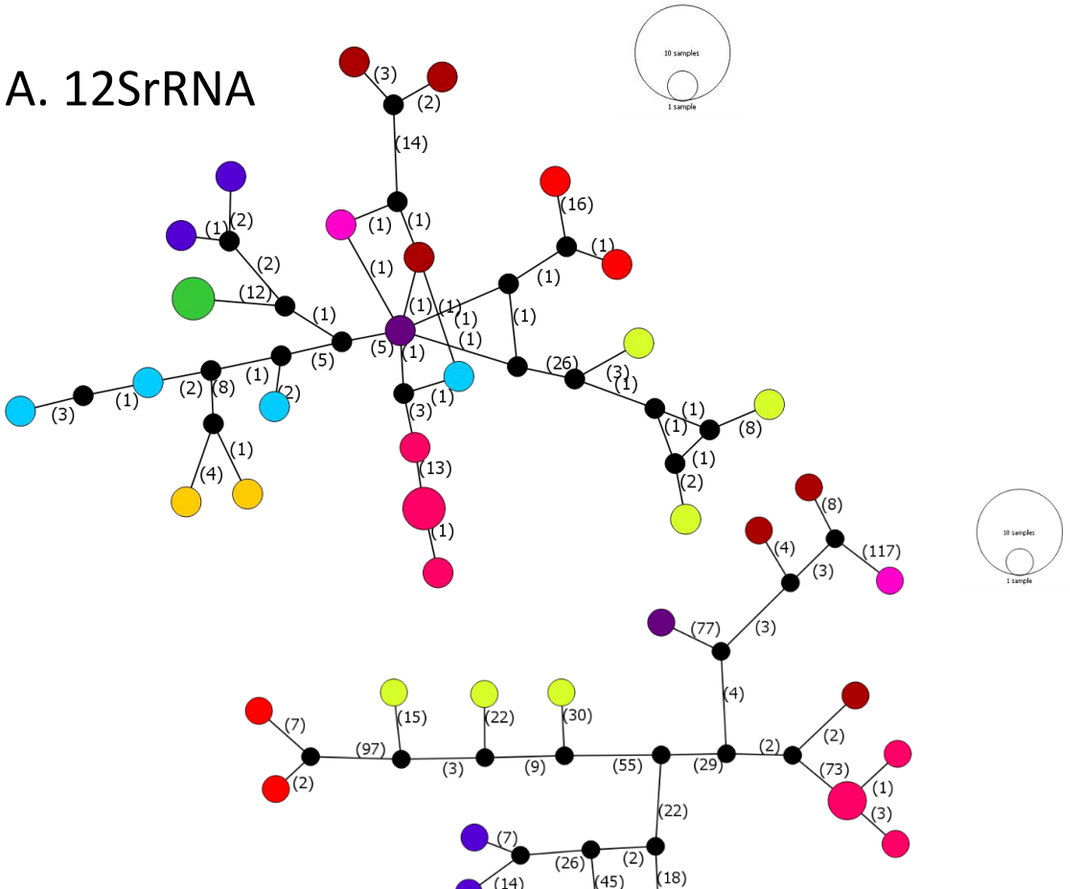
Kudu *(T. strepciceros)* Lesser Kudu *(T. imberbis)*


A
Mitochondrial DNA
Bushbuck *Sylvaticus* and *Scriptus* lineages paraphyletic
posterior
1
0.6958
Sylvaticus "Zambezi"
Sylvaticus *delamerei*
Sylvaticus *sylvaticus*
Sylvaticus *ornatus*
Bongo1
Bongo2
Sitatunga1
Sitatunga2
Mountain Nyala1
Mountain Nyala2
Kudu1
Kudu2
Kudu3
Kudu4
Eland1
Eland2
Eland3
Giant Eland
Nyala1
Nyala2
Scriptus *phaleratus*
Scriptus *decula*
Scriptus *scriptus*
Lesser Kudu
Nilgai
African Buffalo
Cow
Impala
-20
-17.5
-15
-12.5
-10
-7.5
-5
-2.5
0
Millions of years ago
Nuclear DNA
B
Bushbuck *Sylvaticus* and *Scriptus* lineages monophyletic
posterior
1
0.3593
Sylvaticus "Zambezi"
Sylvaticus *sylvaticus*
Sylvaticus *ornatus*
Sylvaticus *delamerei*
Scriptus *decula*
Scriptus *phaleratus*
Scriptus *scriptus*
Bongo1
Bongo2
Sitatunga1
Sitatunga2
MountainNyala1
MountainNyala2
Kudu1
Kudu2
Kudu3
Kudu4
Eland1
Eland2
Eland3
GiantEland
Nyala1
Nyala2
LesserKudu
Nilgai
AfricanBuffalo
Cow
Impala
0
5
10
15
20
Millions of years ago

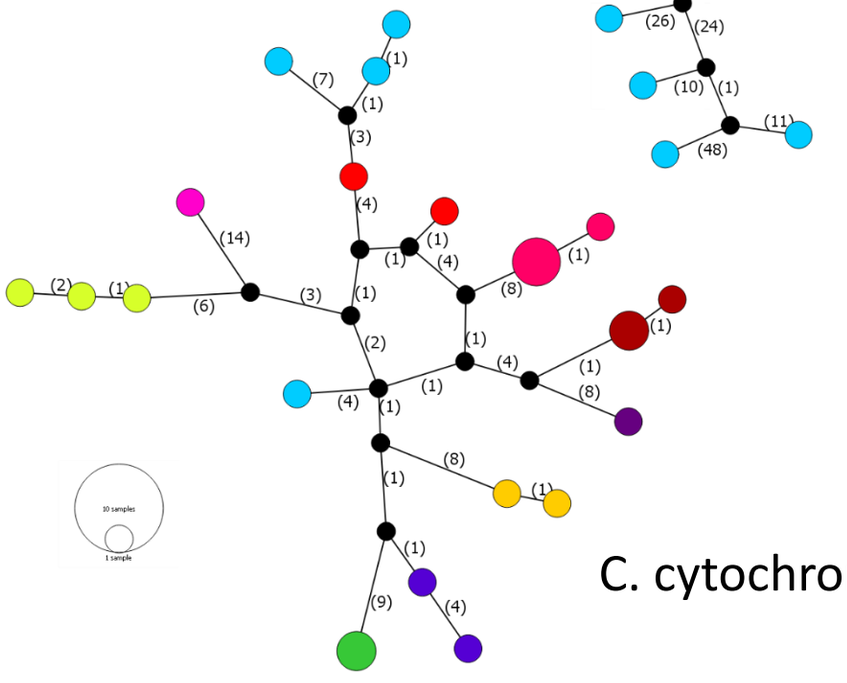

Bushbuck
T. scriptus
Scriptus
Sylvaticus
Bongo
T. euryceros
Sitatunga
T. spekei
Mountain nyala
T. buxtoni
Kudu
T. strepciceros
Eland
T. oryx
Giant Eland
T. derbanius
Nyala
T. angasi
Lesser kudu
T. imberbis
Pleistocene
Pliocene
Miocene
*
0
2.5
5
7.5
Millions of years ago

Mitochondrial genes

A. 12SrRNA



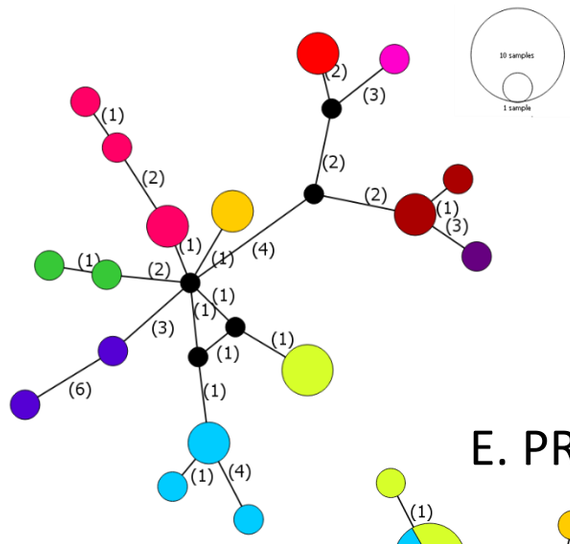
B. 16SrRNA



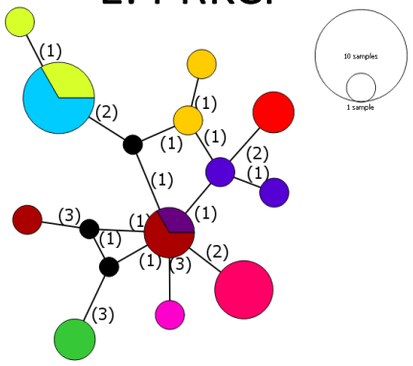
C. cytochrome *b*

Nuclear genes

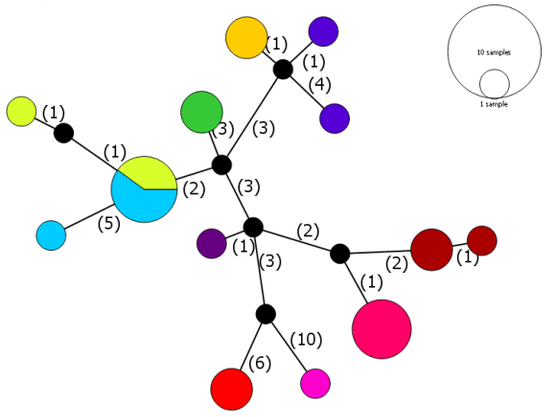
D. MGF



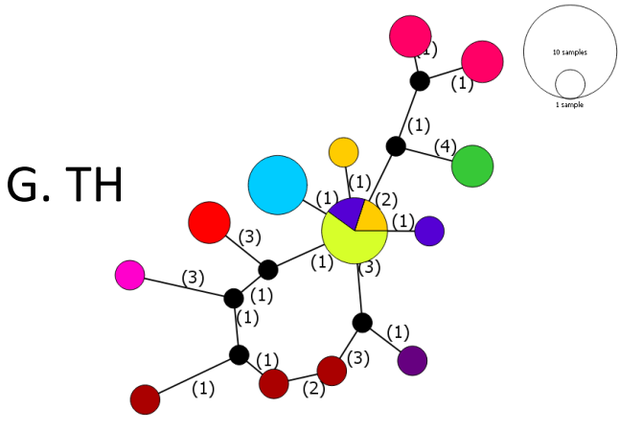
E. PRKCI



F. SPTBN1



G. TH



10 individuals

1 individual

- Nyala
- Mountain Nyala
- Giant Eland
- Bongo
- Lesser Kudu
- Eland
- Sitatunga
- Kudu
- Scriptus
- Sylvaticus