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Published in:
G3: Genes, Genomes, Genetics

DOI:
10.1093/g3journal/jkab002

Publication date:
2021

Document version
Publisher's PDF, also known as Version of record

Document license:
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Citation for published version (APA):
Gonçalves, M., Siegismund, H. R., Van Vuuren, B. J., Koepfli, K-P., Ferrand, N., & Godinho, R. (2021). De novo whole-genome assembly and resequencing resources for the roan (Hippotragus equinus), an iconic African antelope. G3: Genes, Genomes, Genetics, 11(2), [jkab002]. https://doi.org/10.1093/g3journal/jkab002
De novo whole-genome assembly and resequencing resources for the roan (Hippotragus equinus), an iconic African antelope

Margarida Gonçalves,1,2,* Hans R. Siegismund,3 Bettine Jansen van Vuuren,4 Klaus-Peter Koepfli,5,6 Nuno Ferrand,1,2,4 and Raquel Godinho1,2,4

1CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus de Vairão, 4485-661 Vairão, Portugal
2Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, 4169-007 Porto, Portugal
3Department of Biology, University of Copenhagen, 2200 Copenhagen, Denmark
4Department of Zoology, Centre for Ecological Genomics and Wildlife Conservation, University of Johannesburg, 2006 Johannesburg, South Africa
5Smithsonian-Mason School of Conservation, Front Royal, VA 22630, USA
6Smithsonian Conservation Biology Institute, Center for Species Survival, National Zoological Park, Washington, DC 20008, USA

*Corresponding authors: CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus de Vairão, 4485-661 Vairão, Portugal.
M.goncalves@cibio.up.pt (M.G.); rgodinho@cibio.up.pt (R.G.)

Abstract
Roan antelope (Hippotragus equinus) is the second-largest member of the Hippotraginae (Bovidae), and is widely distributed across sub-Saharan mesic woodlands. Despite being listed as “Least Concern” across its African range, population numbers are decreasing with many regional Red List statuses varying between Endangered and Locally Extinct. Although the roan antelope has become an economically-important game species in Southern Africa, the vast majority of wild populations are found only in fragmented protected areas, which is of conservation concern. Genomic information is crucial in devising optimal management plans. To this end, we report here the first de novo assembly and annotation of the whole-genome sequence of a male roan antelope from a captive-breeding program. Additionally, we uncover single-nucleotide variants (SNVs) through re-sequencing of five wild individuals representing five of the six described subspecies. We used 10X Genomics Chromium chemistry to produce a draft genome of 2.56 Gb consisting of 16,880 scaffolds with N50 = 8.42 Mb and a BUSCO completeness of 91.2%. The draft roan genome includes 1.1 Gbp (42.2%) repetitive sequences. De novo annotation identified 20,518 protein-coding genes. Genome synteny to the domestic cow showed an average identity of 92.7%. Re-sequencing of five wild individuals to an average sequencing depth of 9.8x resulted in the identification of a filtered set of 3.4x10^6 bi-allelic SNVs. The proportion of alternative homozygous SNVs for the individuals representing different subspecies, as well as differentiation as measured by PCA, were consistent with expected divergence from the reference genome and among samples. The roan antelope genome is a valuable resource for evolutionary and population genomic questions, as well as management and conservation actions.

Keywords: 10X Genomics Chromium; Bovidae; de novo genome assembly; Hippotragus equinus; genomic diversity; roan antelope

Introduction
Roan antelopes (Hippotragus equinus) are endemic to Africa, ranging throughout sub-Saharan habitats of mesic savannah woodlands. The species comprises six recognized subspecies, namely H. e. koba in the northwest, H. e. charicus and H. e. bakeri across central Africa, H. e. langheldi in the east, and H. e. cottoni and H. e. equinus in southern Africa. These subspecies were accepted by Ansell (1972) based on geographical range, but their validity has been challenged by both nuclear and mitochondrial data (Matthee and Robinson 1999; Alpers et al. 2004). To fully resolve the validity of the subspecies and regional genetic differentiation, more powerful genomic tools and sampling coverage is needed. Despite being listed as “Least Concern” by the IUCN Red List of Threatened Species, the roan antelope is severely threatened by different anthropogenic pressures, including habitat loss and epizootic diseases (IUCN SSC Antelope Specialist Group 2017). Currently, most wild populations are found only within protected areas, with decreasing population trends recorded over the past decades, some resulting in local extinctions across eastern and southern Africa (East and IUCN SSC Antelope Specialist Group 1999; Chardonnet and Crosmary 2013). Over parts of its range, roan antelope has become established as an economically-important game species, leading to intensive captive breeding of animals. To this end, appropriately planned and executed translocations of wild animals are becoming an important management tool (Jansen van Vuuren et al. 2017), whereas the availability of reference genomes and high-resolution genomic resources add significantly to the management and
conservation toolbox (Allendorf et al. 2010; Shafer et al. 2015; Dresser et al. 2017; Brandies et al. 2019).

As part of the horse-like antelopes, roan antelope is a member of the subfamily Hippotraginae, a group of bovids that consists of three genera namely Hippotragus, which includes the roan and the sable antelope (H. niger) as well as the extinct bluebuck (H. leucophaurus); Addax, a single-species genus including only the addax (Addax nasomaculatus); and Oryx, which comprises four species, namely the beisa (O. beisa), the scimitar-horned oryx (O. dammah), the gembok (O. gazelle), and the Arabian oryx (O. leucoryx) (Fernández and Vrba 2005; Bíbi 2013). Recently, assembled genomes became available from three species within the Hippotraginae. Studies on gembok (Farré et al. 2019), the sable antelope (Koeppfli et al. 2019), and the scimitar-horned oryx have exemplified how management and conservation actions can be mended by the use of genome-wide resources. Additionally, assembled reference genomes and inherent annotation information may also be used to address important biological questions related to adaptation to ecological conditions, both in natural and human-managed environments (Ge et al. 2013; Kardos et al. 2016; Armstrong et al. 2018; Marchenko et al. 2018).

In this study, we used the 10X Genomics linked-reads platform to sequence and assemble the first genome for roan antelope. We tested its quality, comparing the roan genome assembly to other assembled genomes generated within the Hippotraginae, Bovidae, and also to other ruminant species. We also generated and used re-sequencing data of wild individuals, representing five out of the six currently recognized subspecies across the roan antelope’s native range, to assess general levels of intraspecific diversity.

Materials and methods
Sample collection, library preparation, and sequencing
For the reference genome, a tissue sample was collected from a roan antelope bull housed in the Lisbon Zoological Garden (Portugal; ID: 10954). This individual is fifth-generation captive-bred, with a genetic pool that represents an example of ex-situ conservation and a collaboration between several European Zoological Gardens. The animal descends from wild-caught individuals in Uganda and Zambia, which became the founders of the population in the Hannover Zoological Garden, Germany (Supplementary Figure S1). The sample was collected with a telebiopsy dart (Vario Syringes S300V, Telinject USA, Inc.), and snap-frozen in liquid nitrogen to guarantee DNA quality. The tissue sample was processed for DNA isolation, DNA library preparation, and sequencing by the Genomic Services Laboratory at the Hannover Zoological Gardens. The animal descends from wild-caught individuals in Uganda and Zambia, which became the founders of the population in the Hannover Zoological Garden, Germany (Supplementary Figure S1). The sample was collected with a telebiopsy dart (Vario Syringes S300V, Telinject USA, Inc.), and snap-frozen in liquid nitrogen to guarantee DNA quality. The tissue sample was processed for DNA isolation, DNA library preparation, and sequencing by the Genomic Services Laboratory at the Hannover Zoological Gardens. The animal descends from wild-caught individuals in Uganda and Zambia, which became the founders of the population in the Hannover Zoological Garden, Germany (Supplementary Figure S1). The sample was collected with a telebiopsy dart (Vario Syringes S300V, Telinject USA, Inc.), and snap-frozen in liquid nitrogen to guarantee DNA quality. The tissue sample was processed for DNA isolation, DNA library preparation, and sequencing by the Genomic Services Laboratory at the Hannover Zoological Gardens. The animal descends from wild-caught individuals in Uganda and Zambia, which became the founders of the population in the Hannover Zoological Garden, Germany (Supplementary Figure S1). The sample was collected with a telebiopsy dart (Vario Syringes S300V, Telinject USA, Inc.), and snap-frozen in liquid nitrogen to guarantee DNA quality.

Genome assembly and completeness
Total raw-data were assembled using the 10X Genomics software Supernova v.2.1.1 (Weissenfeld et al. 2017). We used ca. 1.2 Gbp randomly selected reads to obtain a raw read coverage of 56x, as recommended by 10X Genomics. Subsequent fasta files were generated using Supernova mkoutput, with a kmer junction of k = 48, a minimum contig size of 1,000 bp, and using the raw style, where all edges are represented by a fasta record. Raw style was compared to two additional style outputs, where branches are selected according to coverage, gaps are merged with subsequent sequences, and no reverse complement sequences are represented: (1) megabubble style, where each arm is represented by a fasta record, and (2) pseudohap style, which extracts a single pseudohaplotype per scaffold, choosing arbitrarily between maternal and paternal alleles. Duplicated scaffolds were removed with GenomeTools sequin v.1.6.1 (Gremme et al. 2013).

Assembly completeness was assessed in Benchmarking Universal Single-Copy Orthologs (BUSCO) v.3.0.2 (Simão et al. 2015), using the Mammalia OrthoDB v9 gene set (Zdobnov et al. 2017) containing 4,104 genes. By comparing BUSCO results between different output assembly styles, we found that the pseudohaplotype fasta style had the highest completeness (Supplementary Figure S2). Therefore, only this assembly was retained for further analyses.
To analyze the repeat content of the roan antelope genome, we generated a de novo repeat library from the assembly using RepeatModeler v.2.0.1 (Zeng et al. 2018) which integrates both RECON v.1.08 (Bao and Eddy 2002) and RepeatScout v.1.0.6 (Price et al. 2005) to predominantly find transposable elements. De novo generated interspersed repeats were classified using the integrated script in RepeatClassifier (Zeng et al. 2018). Based on this custom library, the roan assembly was screened for repetitive elements using RepeatMasker v.4.0.9 (Smit et al. 2015) with the NCBI-RMBlast v.2.6.0þ search engine, including interspersed genomic repeats and low-complexity sequences. We used the -s option to increase sensitivity and -xsmall to produce a soft-masked output, with repeat regions indicated in lower case letters.

We used Augustus v.3.3.2 (Stanke et al. 2008) to identify candidate protein-coding genes in the masked-assembled genome. The software was launched disabling annotation of untranslated regions (–UTR=off), using the masked sequence as evidence against exons (–softmasking=1) and applying the human gene model for gene prediction (–species=human). Candidate genes were translated into protein sequences using the incorporated Augustus script getAnnoFasta and filtered using eggNOG-mapper (Huerta-Cepas et al. 2017). Annotation quality was assessed based on eggNOG v.4.5 orthology data (Huerta-Cepas et al. 2016), with a minimum query cover of 50% and an e-value cut-off of 1x10^{-4}. Finally, annotation quality of the roan genome was compared with two other Hippotraginae (sable antelope and scimitar-horned oryx) and the domestic cow assemblies for a set of common metrics using GenomeQC (Manchanda et al. 2020).

Roan genome assembly was aligned to the domestic cow genome (BosTaur—GenBank accession number: GCA_002263795.2; Zimin et al. 2009) using LAST v.0.874 (Kiełbasa et al. 2011). The domestic cow genome was first prepared for alignment using the lastdb command option, and the alignment was run using lastal and last-split commands. Then, we used the maf-swap incorporated script to change the order of the sequences into the resulting MAF-format alignment to obtain the best pairwise synteny blocks between genomes. Ordered scaffolds above 10 Kbp were used to visualize genome synteny with Circos v.0.69.6 (Krzywinski et al. 2009).

### Re-sequencing alignment

Read data from the five re-sequenced wild individuals were aligned to the roan antelope genome assembly using BWA-mem (Li 2013) with default settings, following adapter-trimming with Trimmomatic v.0.36 (Bolger et al. 2014) and retaining only reads >

---

**Table 1** Roan antelope native samples used for whole-genome re-sequencing

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Original label ID</th>
<th>Subspecies</th>
<th>Year</th>
<th>Sex</th>
<th>Locality</th>
<th>N PCR cycles</th>
<th>Mapping (%)</th>
<th>Mean coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>He80</td>
<td>7324</td>
<td>H. e. koba</td>
<td>1998</td>
<td>F</td>
<td>Kablima, Ghana</td>
<td>8</td>
<td>99.8</td>
<td>5.2x</td>
</tr>
<tr>
<td>HeNI226</td>
<td>NN.252</td>
<td>H. e. charicus</td>
<td>1925</td>
<td>M</td>
<td>Nana Barya Reserve, CAR</td>
<td>9</td>
<td>99.7</td>
<td>5.9x</td>
</tr>
<tr>
<td>He108</td>
<td>9293</td>
<td>H. e. bakeri</td>
<td>-</td>
<td>M</td>
<td>Akobe septum, Ethiopia</td>
<td>8</td>
<td>99.8</td>
<td>11.7x</td>
</tr>
<tr>
<td>He95</td>
<td>8250</td>
<td>H. e. langfieldi</td>
<td>1997</td>
<td>M</td>
<td>Maswa Game Reserve, Tanzania</td>
<td>10</td>
<td>99.7</td>
<td>12.2x</td>
</tr>
<tr>
<td>HeNI207</td>
<td>SWA.74</td>
<td>H. e. cottoni</td>
<td>1937</td>
<td>F</td>
<td>Cubango river, Angola</td>
<td>7</td>
<td>99.7</td>
<td>14.1x</td>
</tr>
</tbody>
</table>

*Contemporary samples from tissue collection of the University of Copenhagen, Department of Biology (responsible: H.R. Siegismund).

Historic samples donated by the Powell-Cotton Museum, Kent, UK; CAR—Central African Republic.*
50 bp. Duplicated reads were marked with Picard v.2.21.4 (http://
broadinstitute.github.io/picard/) and local realignment around indels was improved using GATK v.3.8 IndelRealigner (McKenna
et al. 2010). Final mapping quality was assessed with QualiMap
v.2.2.2 (Okonechnikov et al. 2016).

**SNV calling and filtering**

Filtered alignments were used in GATK v.3.8 HaplotypeCaller
(van der Auwera et al. 2013) to separately call potential single-
nucleotide variants (SNVs) for each of the five individuals. GATK
GenotypeGVCFs was then used for joint genotyping based on in-
dividual genomic VCFs. Joint genotyping on cohorts improves var-
iant calling by preventing base uncertainty errors. Using both
BCFtools v.1.9 (Li et al. 2009) and VCFtools v.0.1.16 (Danecek et
al. 2011), variant data were filtered to retain (1) only bi-allelic SNVs,
(2) SNVs without missing data, (3) no indels, (4) SNVs covered by
more than 5 reads, and (5) SNVs with a quality score > 50. The fi-
nal set of filtered SNVs was used for principal component analy-
sis, with the gdsfmt v.1.8.3 and SNPRelate v.1.6.4 R packages
(Zheng et al. 2012).

**Data availability**

Genomic resources of *H. equinus* (whole-genome assembly,
Illumina paired-end reads from five individuals and samples)
have been deposited at NCBI databases under BioProject
PRJNA682311. The whole-genome of *H. equinus* as DDBJ/ENA/
GenBank is under the accession JAEFBM000000000. The version
described in this paper is version JAEFBM010000000. Supplemen-
tary material is available at figshare: https://doi.org/
10.25387/g3.13378799.

**Results and discussion**

**Genome assembly and completeness**

The genome sequencing of roan individual ID: 10954 using 10X
Genomics Chromium generated approximately 1.2×10^9 paired-
end reads (Supplementary Table S1). The raw and effective (i.e.
the number of reads retained after filtering) read coverage recom-
manded by 10X Genomics is 56x and 42x, respectively. The as-
sembly summary statistics confirm the obtained values as close as
to the recommended ones (60.7x raw and 39.7x effective cover-
age, Supplementary Table S1). This genome has a contig N50 of
239.6 Kb, assembled into 16,880 scaffolds with an N50 of 8.42 Mb
(Supplementary Table S1). Overall assembly quality is reflected
in the high number of scaffolds (>2,200) more than 10 Kb in
length and in the low percentage (4.1%) of missing bases from
such scaffolds (Supplementary Table S1). Total genome assembly
size was 2.56 Gb, which is close to the genome size estimated from
the k-mer distribution of 3.01 Gb (Supplementary Table S1). The
Supernova v.2.1.1 software estimated a genome-wide G+C content
of 41.9%, and an overall heterozygosity of 0.0015 (one heterozygous
position every 636 bp).

The assembly size and the G+C content of our roan antelope
genome is comparable to other available genomes for Hippotraginae,
as well as to genomes of other members of the Bovidae family and
other ruminant species. For example, the assembly genome size for the European bison and the sable antelope
are 2.58 Gb and 2.60 Gb, respectively (Wang et al. 2017;
Koepfli et al. 2019), whereas estimates of G+C content for the sa-
bale antelope and the scimitar-horned oryx are 41.8% (Koepfli et
al. 2019; Humble et al. 2020). However, the 10X Genomics Chromium
library system implemented for the roan genome assembly
allowed us to considerably improve the scaffold N50 (8.42 Mb) in
comparison with other strategies used for the sable antelope
(4.59 Mb; Koepfli et al. 2019), the blue wildebeest (3.5 Mb; Chen
et al. 2019), the Tibetan antelope (2.76 Mb; Ge et al. 2013), or for
the African buffalo (2.32 Mb; Chen et al. 2013). Such improve-
ments probably reflect the unique 10X Genomics library prepara-
ion protocol, which was specifically created to obtain long-range
anchored information (van Dijk et al. 2018). The mean heterozy-
gosity of 0.0015 was in line with estimates within mammal spe-
cies and is higher than the value observed for Hippotraginae of
critical conservation concern (scimitar-horned oryx; Humble et
al. 2020).

BUSCO evaluation of gene completeness retrieved 91.2% com-
plete genes (3,743 out of 4,104 genes; Table 2). This value is simi-
lar to the one observed for the Thomson’s gazelle (91.1%) by
Chen et al. (2019), but lower than the values retrieved for the
gemsbok (above 92%) and the sable antelope (94.8%) by Farré et
al. (2019) and Koepfli et al. (2019), respectively.

**Genome annotation and synteny**

An estimated 42.2% (about 1.1 Gbp) of the roan genome was com-
posed of repetitive sequences, based on short- and long-
interspersed nuclear elements (SINEs and LINEs), long-
interspersed retrotransposons (LTR), DNA elements, small RNAs,
and simple and low complexity tandem repeats (Table 3). LINEs
were the most common repetitive element, representing 30.4% of
the overall content, followed by LTR elements, which represented
4.0%. We also obtained a low percentage of unclassified repeats
(2.5%) that did not correspond to any familiar element in the
RepeatModeler specific library. Transposable elements exist in el-
lemental families and comprise a significant fraction of eukary-
otic genomes (Biscotti et al. 2015). Each family is derived from
the multiplication of a particular element in the genome of a com-
non ancestor and is thus a proxy for shared evolutionary history
across species (Schibler et al. 2006; Platt et al. 2018; Qi et al. 2018).
Thus, a similar percentage of the repetitive content among as-
semblies of roan antelope and other members of its evolutionary
lineage were expected (e.g., Ou et al. 2018). Accordingly, the 42.2%
of repetitive sequences observed for the roan assembly is consis-
tent with the genome assemblies of other Hippotraginae, such as
the sable antelope (46.97%), the gemsbok (41.27%), and the
scimitar-horned oryx (47.8%) (Farré et al. 2019; Koepfli et al. 2019;
Humble et al. 2020), and also other bovids as the domestic cow
(45.28%) and the European bison (47.3%) (Zimin et al. 2009; Wang
et al. 2017). Additionally, longinterspersed repeats (LINEs) were
the most common element found in our assembly, which is also in
agreement with general knowledge for the bovine genome
(Adelson et al. 2009). However, identification of transposable ele-
ments can be achieved through numerous available tools, and
discrepancies observed across different genomes may be related

<table>
<thead>
<tr>
<th>BUSCOs</th>
<th>Total number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>3,743</td>
<td>91.2</td>
</tr>
<tr>
<td>Complete and single-copy</td>
<td>3,677</td>
<td>89.6</td>
</tr>
<tr>
<td>Complete and duplicated</td>
<td>66</td>
<td>1.6</td>
</tr>
<tr>
<td>Fragmented</td>
<td>194</td>
<td>4.7</td>
</tr>
<tr>
<td>Missing</td>
<td>167</td>
<td>4.1</td>
</tr>
</tbody>
</table>

BUSCO scores using the Mammalia OrthoDB v9 gene set, containing 4,104 genes.

Table 2 Assembly statistics based on gene completeness scores by BUSCO v.3.0.2 for the whole-genome assembly of roan antelope
to genome assembly quality and different challenges during the identification process (Saha et al. 2008; Makalowski et al. 2019).

De novo genome annotation with Augustus identified a total of 30,622 candidate protein-coding genes (PCGs), of which 20,518 matched common gene names, after filtering with eggNOG-mapper. Direct comparisons of these values with other assemblies could be biased, since annotation and the final number of estimated PCGs can be influenced by the assembly method and quality (Florea et al. 2011). We avoided this possible bias using the recently available web framework GenomeQC that enables comparison across multiple assemblies and assembly types. The final number of PCGs estimated for our roan assembly is consistent with estimates for the domestic cow (30,589; Zimin et al. 2004), with slightly higher than those estimated for both the sable antelope (24,031) and the scimitar-horned oryx (28,559) genome annotations (Koepfli et al. 2019; Humble et al. 2020). Such estimates can be used as proxy for the quality of roan antelope genome (Supplementary Table S2).

Roan scaffolds alignment against the domestic cow reference genome resulted in 85.6% synteny alignment (ca. 2.25 Gbp), with an average identity of 92.7%. Long stretches of synteny blocks covered more than 95% of each of the 29 bovine autosomal chromosomes, as well as the X chromosome (Figure 2; Supplementary Tables S3 and S4).

Table 3 Summary of the repetitive content found in roan antelope genome assembly, using RepeatMasker

<table>
<thead>
<tr>
<th>Number</th>
<th>Length occupied (bp)</th>
<th>Percent masked</th>
</tr>
</thead>
<tbody>
<tr>
<td>SINEs</td>
<td>222,376</td>
<td>27,885,908</td>
</tr>
<tr>
<td>LINEs</td>
<td>2,731,480</td>
<td>799,087,834</td>
</tr>
<tr>
<td>LTR elements</td>
<td>496,668</td>
<td>106,731,053</td>
</tr>
<tr>
<td>DNA elements</td>
<td>377,402</td>
<td>54,829,227</td>
</tr>
<tr>
<td>Unclassified</td>
<td>413,329</td>
<td>65,638,505</td>
</tr>
<tr>
<td>Total interspersed repeats</td>
<td>1,104,172,527</td>
<td>1,106,510,938</td>
</tr>
<tr>
<td>Small RNA</td>
<td>298,364</td>
<td>38,764,415</td>
</tr>
<tr>
<td>Simple repeats</td>
<td>470,855</td>
<td>18,232,272</td>
</tr>
<tr>
<td>Low complexity</td>
<td>80,149</td>
<td>3,946,798</td>
</tr>
</tbody>
</table>

### Intraspecific diversity

Alignment of the five re-sequenced wild individuals to the roan antelope genome assembly resulted in 99.7% and 99.8% of mapped reads (Table 1). The average depth of coverage was 9.8x, ranging between 5.2x and 14.1x for Ghana and Angolan individuals, respectively (Table 1). Our intraspecific evaluation of the roan antelope genome allows the first assessment of genomic diversity patterns of the species across its native distribution range. From the alignment of the re-sequenced individuals, we found a total of 21,127,946 SNVs. Among these, 20,896,201 were bi-allelic which, after quality filtering, resulted in a final set of 3,401,741 SNVs across the five roan individuals. Overall transition/transversion ratio across SNVs was 2.07 (2,294,647/1,107,094). The number of alternative homozygous SNVs varied between 949,845 and 577,765 SNVs for individuals from Ghana and Tanzania, respectively. The number of heterozygous SNVs ranged from 1,043,928 in He108 from Ethiopia to 711,962 in HeNI207 from Angola (Figure 3A). Results for historic samples did not show any obvious bias for low coverage, reduced alignment efficiency or low number of heterozygous SNVs, which can be an important source of errors for degraded and chemically modified DNA samples (Rowe et al. 2011; Ewart et al. 2019). However, sampling size in our study hampers further interpretations.

The principal component analysis provided a visualization of genetic distances among re-sequenced individuals, which correlates with their geographical distribution (Figure 3B). The two first axes explain 60% of the total variance observed among the genome of these individuals. The first axis (PC1) explains 36.6% of the variance and separates representatives in the northern distribution of the range, namely from Ghana and Central African Republic, from those in the central and southern range of the species, namely Ethiopia, Tanzania, and Angola. The second axis (PC2), with an explained variance of 23.3%, retrieved the individual dispersion along the west-east geographical axis. Therefore, levels of intraspecific differentiation translate into a clear separation between north-south and west-east geographical axes. Although based on only a few individuals, this result supports the currently recognized subspecies and previous results on roan antelope population genetics, including the two proposed Evolutionary Significant Units (ESUs) (Ansell 1972; Alpers et al. 2004). Interestingly, we found a higher number of heterozygous SNVs for Ethiopian (~1044) and Tanzanian (~946) individuals among the five wild individuals, indicative of a higher genetic diversity. Increased intraspecific genetic diversity may be, among other factors, a signature of the presence of a contact zone, following population diversification in isolation and range expansions (Hewitt 2011). Roan antelope populations in Ethiopia and Tanzania are located in a possible contact zone between the proposed ESUs, which would extend towards west and south of those regions, respectively. Additionally, the higher proportion of reference alleles in these two individuals also indicates a closer genetic similarity to the assembled genome. This may also be explained by the evidence that the sample used to build the genome assembly is from a five-generation captive-bred individual with a maternal ancestry of wild-caught individuals from Uganda and Zambia (Supplementary Figure S1). Both countries are geographically closer to Ethiopia and Tanzania, and therefore, higher genetic similarity to the reference genome may reflect geographical proximity of these populations. Studying genome-wide diversity constitutes an important application of SNVs towards species management in both ex- and in-situ programs, as it is being applied for the sable antelope (Gooley et al. 2020).

### Future prospects

Non-model mammal species are difficult to sample in the wild due to cost and/or logistical constraints (Etherington et al. 2020). Ex-situ management programs, such as those carried out by zoological gardens, can become important donors of unique genomic resources (notably fresh tissue or non-invasive samples for DNA) for threatened and non-threatened species (Clarke 2009; Norman et al. 2019). From a sample collected at the Lisbon Zoological Garden, we successfully assembled the first genome
for the roan antelope, one of the most iconic dwellers of the African savannah. This draft genome assembly represents a valuable genomic resource that may provide input, among others, on phylogenetic relationships, demographic history, and evolution of adaptive traits, such as headgear, multichambered stomach, and adaptation to extreme environments (Elsik et al. 2009; Reese et al. 2010; Bao et al. 2019; Chen et al. 2019). Across the three genera that comprise Hippotraginae, both Oryx and Addax species are arid-adapted, whereas Hippotragus species dwell in more mesic savannah habitats. The adaptation to different environments is an example of a future research topic leading to important biological discoveries among this subfamily.

Furthermore, the availability of the roan antelope’s reference genome and the additional genomic resources included in this work may prove highly valuable on management and conservation decisions (Dresser et al. 2017; Supple and Shapiro 2018; Brandies et al. 2019). Improvements to the roan’s draft genome can be accomplished by proximity ligation sequencing (Hi-C) to generate chromosome-length scaffolds (Dudchenko et al. 2018).

**Acknowledgments**

We thank the Lisbon Zoological Garden for donating the roan antelope tissue sample used to produce the assembled genome.
particularly to Dr. Teresa Lobo Fernandes and Dr. Rui Bernardino for supervision of sample collection. We thank the Powell-Cotton Museum (UK) for donating samples used for re-sequencing. We are grateful to Miguel Carneiro and Stephen Sabatino for helpful input regarding genome sequencing and assembly, and two anonymous reviewers for providing suggestions that improved our work. We thank the CIBIO-CTM technical staff for lab assistance.

**Funding**

This work was supported by the Portuguese Foundation for Science and Technology, FCT (PD/BD/114032/2015 and contract under DL57/2016, respectively, to MG and RG) and National Funds through FCT in the scope of the project UIDB/50027/2020.

**Conflicts of interest:** None declared.

**Literature cited**


**Figure 3** Intraspecific variation in roan antelope. (A) Bar chart comparing the number of quality-filtered alternative homozygous and heterozygous single-nucleotide variants (millions of SNVs) among the five re-sequenced wild individuals. (B) Plot of principal component analysis (PCA) for the same individuals. PC1 and PC2 show the first and second axes with corresponding percentage of explained variance. Circles identify each individual and colors correspond to the respective subspecies, following Figure 1.


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Communicating editor: A. Whitehead