

# Unraveling the genomes of ancient Iberian *Canis*

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## INTRODUCTION

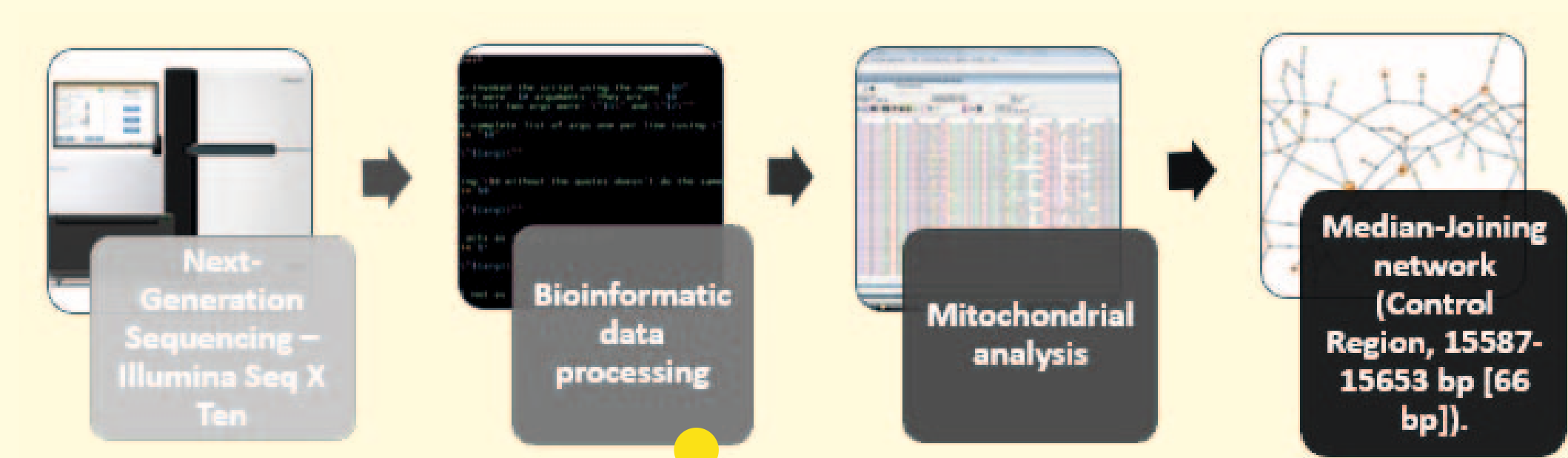
- Ancient DNA is in general of low quantity and highly degraded [8] and its reconstruction and study is very demanding.
- Bioinformatic tools have revealed new insights into genomics of ancient specimens [15].
- Domestic dogs exist in the Iberian Peninsula at least since ca. 16,000 BP [17].
- Genetic studies of Iberian extant dogs shown four mtDNA Haplogroups (Hg): A, B, C and D - haplogroup A and B are dominant, whereas clade D is the least represented [11,12].
- A recent study [13] focusing on the genetic composition of 6 Mesolithic Iberian dogs reported a high frequency (85%) of dog HgA in the pre-Neolithic period, contrasting with a previous study of other parts of Europe [3] that indicates that HgA is rare or absent in European *Canis* older than 3,000 years (cal BP).

## AIMS

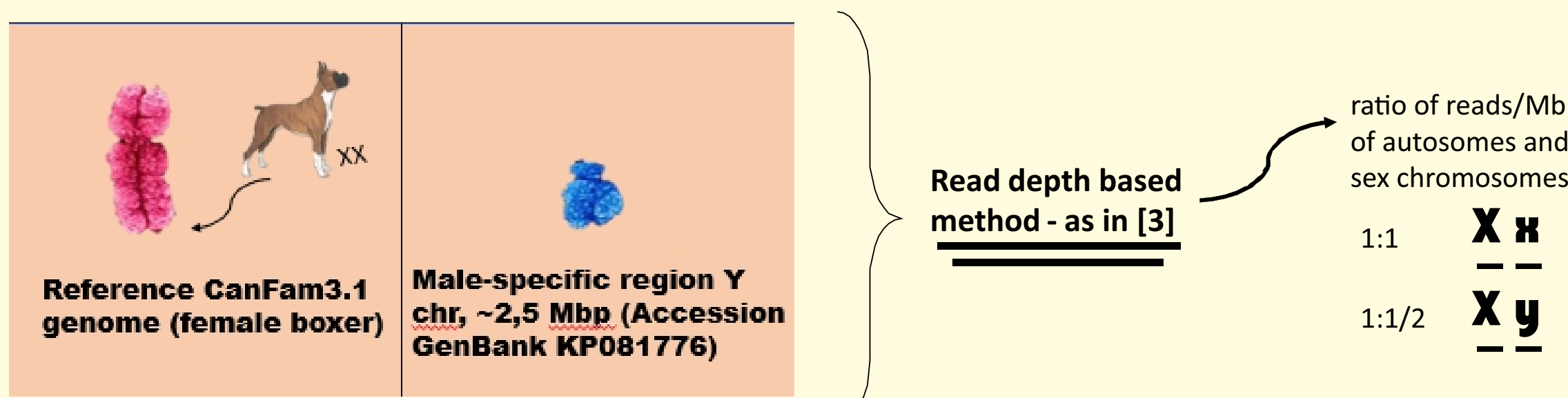
- Generate whole-genome data for 5 Chalcolithic Iberian *Canis* (Table 1) - dogs (n=4) and wolf (n=1);
- Assign mtDNA sequences to the major dog and wolf Hgs;
- Describe the genetic basis of some of their phenotypic traits;
- Identify their sex;
- Provide data to understand the origin and diversity of the Chalcolithic dogs.

## METHODS

### Bioinformatics



### Sex determination



## MATERIALS

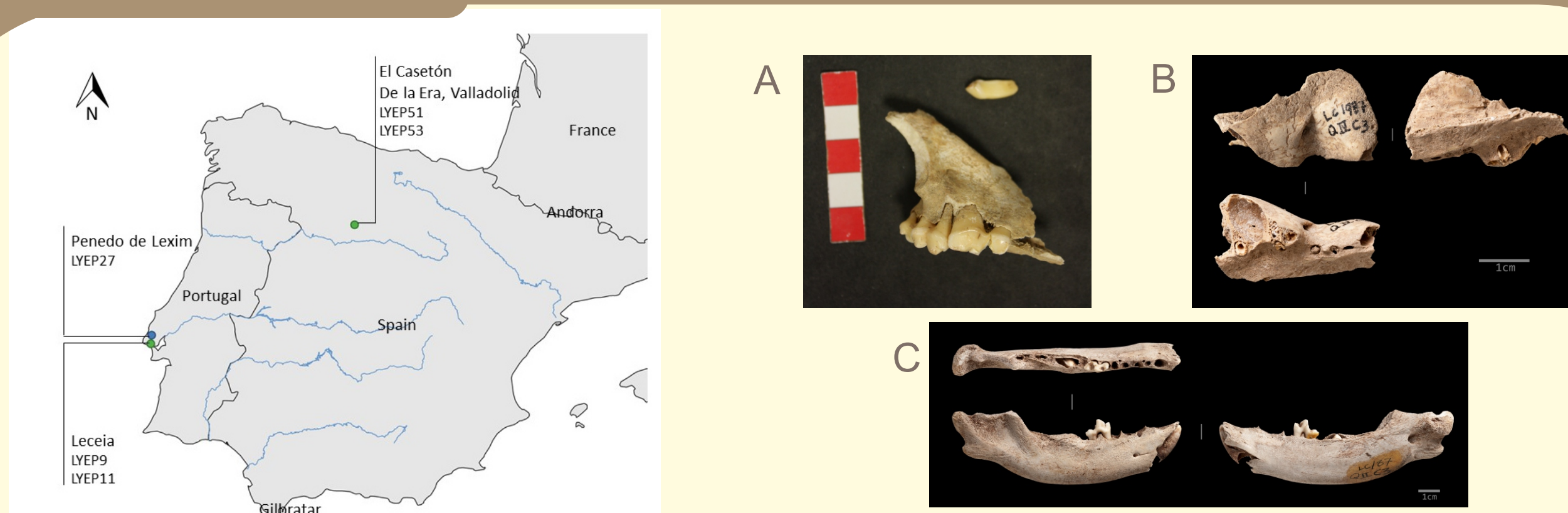


Fig. 1. Location of the Iberian Chalcolithic archaeological sites that provided the samples.

Fig. 2. A. sample LYEP51; B. sample LYEP11; C. sample LYEP9. Note: no picture is available for sample LYEP53, nor LYEP27 (wolf). Photos by Carlos Fernández-Rodríguez (remains from Spain) and José Paulo Ruas (remains from Portugal).

Table 1. Samples information analysed in this study.

Ancient <i>Canis</i> sample ID	Scientific name/ Common name	Skeletal element recovered	Origin	Chronology	Reference	Mean Coverage (Nuclearmt)	DNA covered >2X (Nuclearmt)	Endogenous DNA (Nuclearmt)
LYEP9	<i>Canis lupus familiaris</i> domestic dog	Mandible	Lecela (PT)	ca. 5,000-4,300 BP	10, 13	0.264x/68x	5.17%/99.88%	3.9%/0.011%
LYEP11	<i>Canis lupus familiaris</i> domestic dog	Maxilla	Lecela (PT)	ca. 5,000-4,300 BP*	10, 13	0.0092x/36x	0.02%/99.79%	0.60%/0.012%
LYEP51	<i>Canis lupus familiaris</i> domestic dog	Maxilla	El Casetón de la Era, Valladolid (ES)	ca. 4,000 BP*	1, 13	0.0258x/10x	0.05%/98.72%	1.01%/0.002%
LYEP53	<i>Canis lupus familiaris</i> domestic dog	Tooth (3rd Incisor)	El Casetón de la Era, Valladolid (ES)	ca. 4,000 BP*	1, 13	0.0015x/16x	0.00%/98.59%	0.09%/0.005%
LYEP27	<i>Canis lupus signatus</i> Iberian wolf	Tooth (1st Lower molar)	Penedo de Lexim, Mafra (PT)	4,085-3,856 cal BP**	13, 16	0.0017x/3x	0.00%/77.58%	0.62%/0.005%

\*Dated by archaeological context  
\*\*Indirect radiocarbon date for a specimen of *Sus* from the same stratigraphic unit.

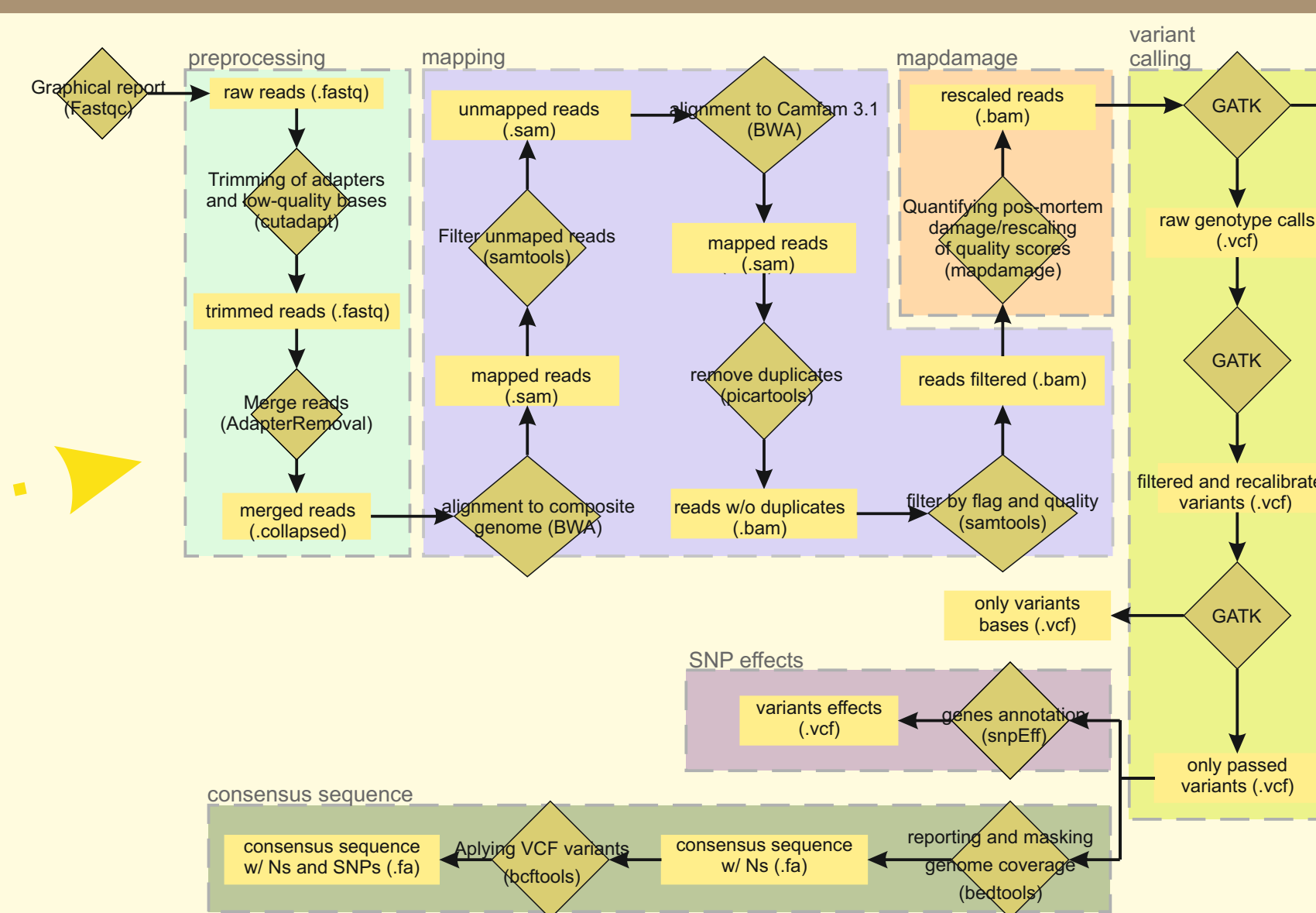


Fig. 3. Schematic representation of the pipeline.

## RESULTS AND DISCUSSION

- The mitogenomes recovered are the only available worldwide for this chronology. A small fragment of the Control Region (CR) was used (Fig. 4) since there are not other contemporaneous mitogenomes available for comparison.
- Chalcolithic dogs carried mtDNA variants that segregate within dog mtDNA Hg A and C (Fig. 4).
- The Chalcolithic wolf sequence segregates together with extant wolf sequence within the wolf mtDNA Hg H1. Although few ancient samples (wolf=1; dog=18) were analysed we suspect that, by the Chalcolithic time period, Iberian dogs and wolves were already structured populations based on their mtDNA haplotypes, as in extant populations.
- The two Paleolithic Iberian wolves (\*) segregate within extant Iberian dogs Hg A and C together with Mesolithic dogs. The latter kept the genetic signature of ancient Iberian wolves, transmitting it up to present-days dogs.

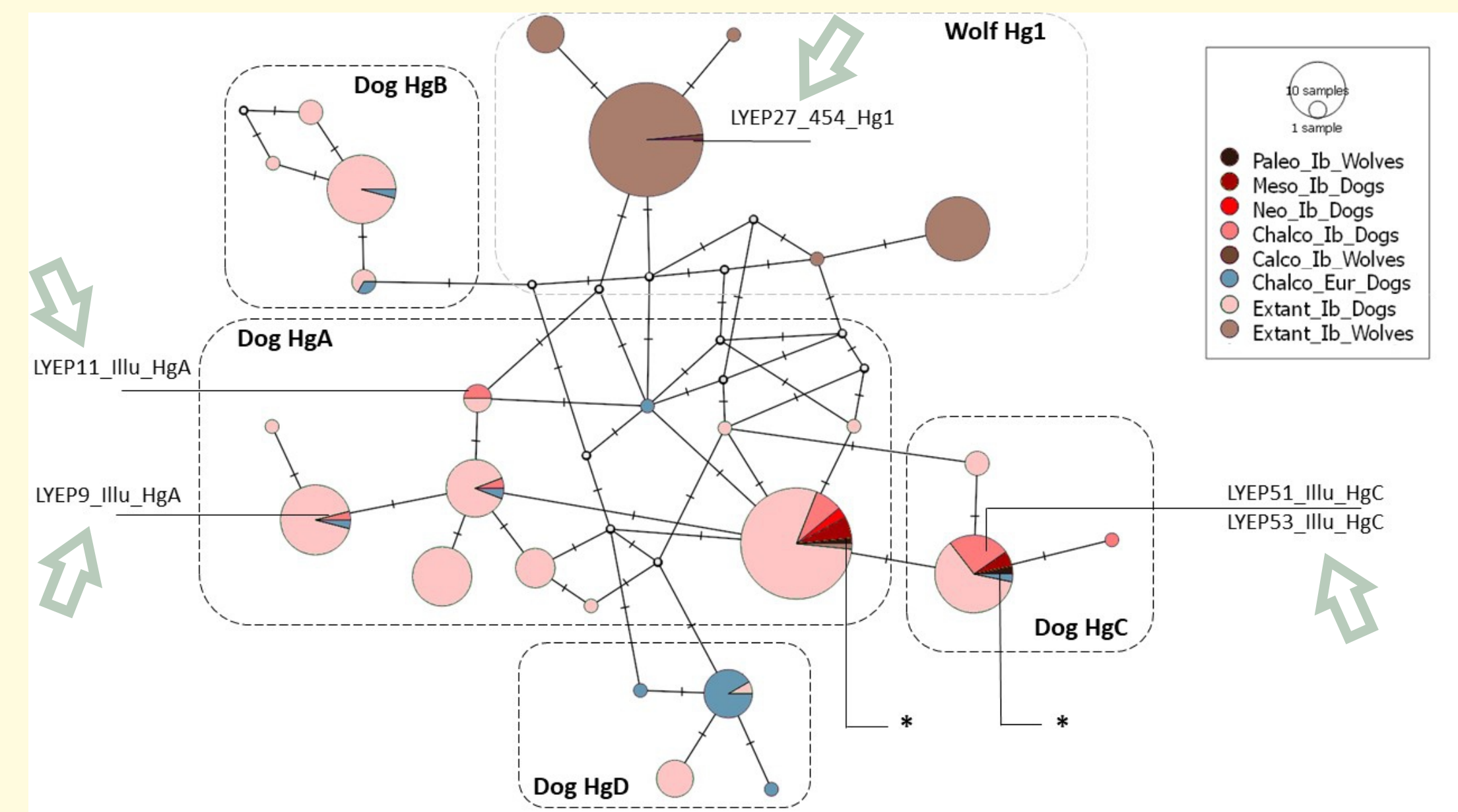


Fig. 4. Median-Joining network (based on a partial fragment of 66 bp of the CR) depicting the relationship between Chalcolithic data and reference data sequences: 61 extant dogs and 23 extant wolves [11, 14, 4, 5, 2]; 25 ancient dogs and 3 ancient wolves [13] - Iberian samples only. Due to a low coverage of the region of interest, the sample LYEP27 (wolf) was merged with data obtained by a 2<sup>nd</sup> generation NGS method - 454 from a previous study [13]. \* These haplotypes belong to Paleolithic Iberian wolves [13] that segregate within wolf Hg2 (wolf Hgs as defined in [9] [data not shown]).

- Due to the endogenous DNA poor preservation (Table 1), variants within specific nuclear genes related to coat color, coat texture or body size could not be recovered (very low coverage). A comparison between the nuclear genome - recovered from NGS analysis - and the reference dog genome revealed SNPs of possible high impact, which are being analysed.
- Regarding sex determination, comparing the proportion of reads/Mbp (Fig. 5) for each chr, we observed that: 1) for LYEP9, LYEP11, LYEP53 and LYEP27 only half of the reads/Mbp aligned with the X chr, in contrast to the proportion observed for the autosomes. Fewer reads aligned against Y chr because the reference used here is only a part of the dog Y chr (its real size is not known, but is expected to be ~20Mbp long); 2) LYEP51 has the same proportion of reads/Mbp aligning along all the autosomal and X chr.

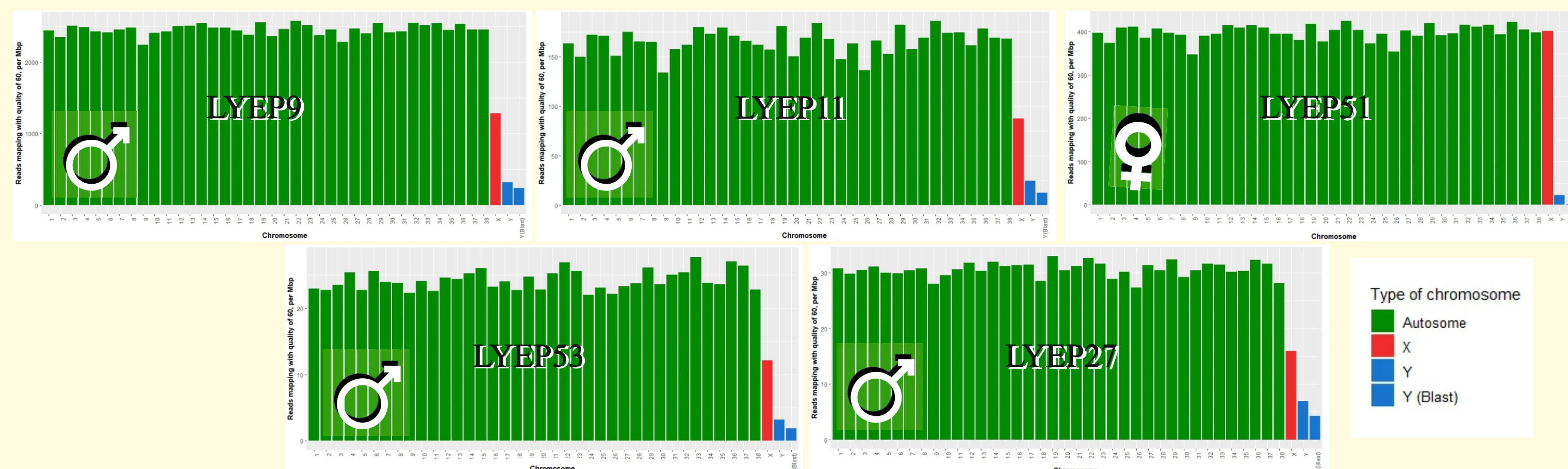


Fig. 5. Histograms representing the proportions of reads mapping each chromosome and the subsequent sex determination. \*Y (Blast) bar represents specific reads that matched only to dog MSY.

## CONCLUSIONS

- We successfully applied NGS methods to recover ancient DNA - mitogenomes from Iberian dogs and wolves remains.
- Chalcolithic Iberian dogs harboured haplotypes segregating within haplogroups A and C Their European counterparts exhibited more diversity (Hg A, B, C and D were presented).
- The distinct genetic composition of Iberian dogs is under investigation.
- The coverage of nuclear genome needs to be improved in order to study some genes related with phenotype.
- NGS Illumina applied on ancient DNA has potential to provide data for further investigations concerning domestication and evolutionary trajectories of dogs in Iberian.

## ACKNOWLEDGMENT

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