Iberian Chalcolithic Canis: a genomic approach to know them better

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INTRODUCTION

- DNA extracted from tissues such as bones and/or teeth is in general of low quantity and highly degraded [9].
- Bioinformatic tools have revealed new insights into genomics of ancient specimen [16].
- Domestic dogs exist in the Iberian Peninsula at least since the Upper Late Paleolithic, dated to 16,000 BP years old [18].
- Genetic studies of Iberian extant dogs revealed four haplogroups in mtDNA: A, B, C, D - haplogroup A and B are dominant, whereas clade D is the least represented [12,13].
- A recent study [14] focusing in 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).
- Archaeological remains from Iberian dogs dated to the Chalcolithic period already exhibited a large phenotypic variability – indicating that human driven selection acted towards the appearance of different dog types for the performance of various tasks (e.g. hunting, livestock guarding, defense) [1,11].

AIMS

- To amplify the genome of 5 Chalcolithic Iberian Canis (Table 1) - dogs (n=4) and wolf (n=1).
- To assign mtDNA sequences to the major dog and wolf haplogroups;
- To describe the genetics basis of their phenotypic traits;
- To identify their sex;
- To provide data to understand the origin and diversity of the Chalcolithic dogs.

METHODS

Bioinformatic data processing

- We carried out Next-Generation Sequencing - Illumina HiSeq X Ten, and used the most up-to-date bioinformatic tools (Fig. 3) to recover and analyze mitochondrial (Control Region, 15587-15693 bp [107 bp]) and nuclear ancient DNA.
- Evolutionary relationships between Chalcolithic data and reference data sequences, such as: Camfam3.1 genome, extant Iberian dogs and wolves [12, 15, 5, 6, 2] and ancient Iberian dogs and wolves [14] were infered through a Median-Joining network.
- Due to a low coverage of the region of interest, the sample LYEP27 (wolf) was merged with data obtained by a 1st generation NGS method – 454 from a previous study [14].

Sex determination of samples

- The reference CanFam3.1 genome (a female dog) lacks the Y chromosome, therefore an available partial reference sequence of the MSY chromosome (male-specific Y chromosome, ~2,5 Mbp) were used (Accession number KP081776).
- Additionally, we used a read depth based method - as in [3], comparing the ratio of reads/Mbp over all chromosomes in order to check the read dosage on chromosome X and Y compared with the autosome chromosomes.
- For the wolf sample, it was not possible to map against a wolf reference nuclear genome, because the only de novo assembly available until date [4] is consisted of unplaced scaffolds. Thus, assignment of LYEP27 sex was carried out using dog nuclear reference CanFam3.1 genome and the Y chromosome reference sequence mentioned prior.

Phenotypic Traits

- The software Integrative Genomic View (IGV) was used to search for variants within specific nuclear genes related to coat color (ASIP, CBD103 and MC1R), coat texture (RSPO2, FGF5, KRT71), body size (IGF1, GHR) to infer phenotypic traits of ancient dogs.

RESULTS AND DISCUSSION

- Chalcolithic dogs carried mtDNA variants that segregate within dog mtDNA haplogroups A and C (Fig. 4) .
- The Chalcolithic wolf sequence segregate together with extant wolf sequence within the wolf mtDNA haplogroup H1. Although few samples (n wolf=1; n dog=18) were analysed we suspect that, by the Chalcolithic time period, Iberian dogs and wolves were already structured populations based on their mtDNA genetic composition, as happens for current populations.
- The only two Paleolithic Iberian wolves * segregate within dog haplogroup, suggesting that Mesolithic dogs kept the genetic signature of ancient Iberian wolves, transmiting it up to present-days dogs.
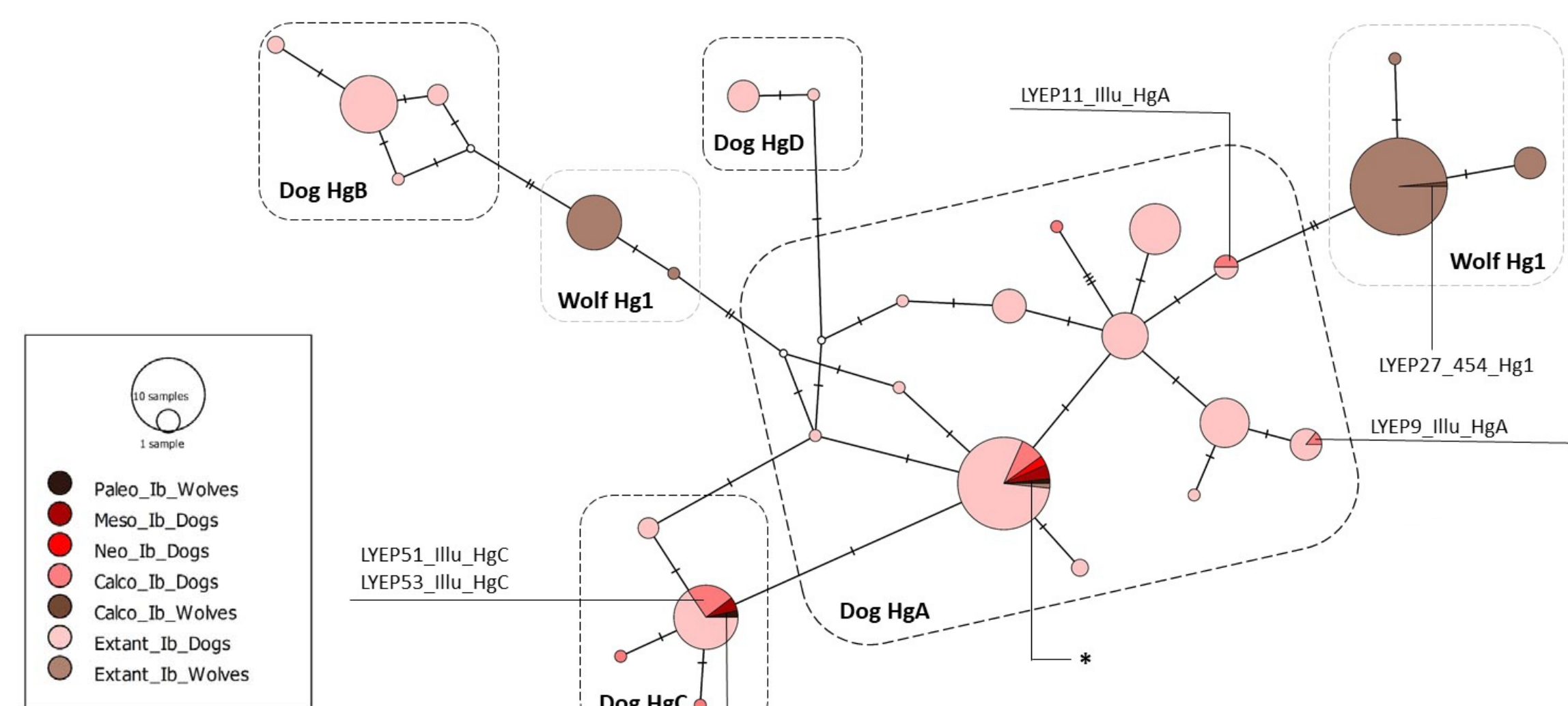

Fig 4. Median-joining network depicting the relationship between 61 extant dogs, 23 extant wolves, 25 ancient dogs and 3 ancient wolves - Iberian samples only. * These haplotypes belong to Paleolithic Iberian wolves [14] that segregate within wolf Hg 2 (wolf haplogroup as defined in [10]).
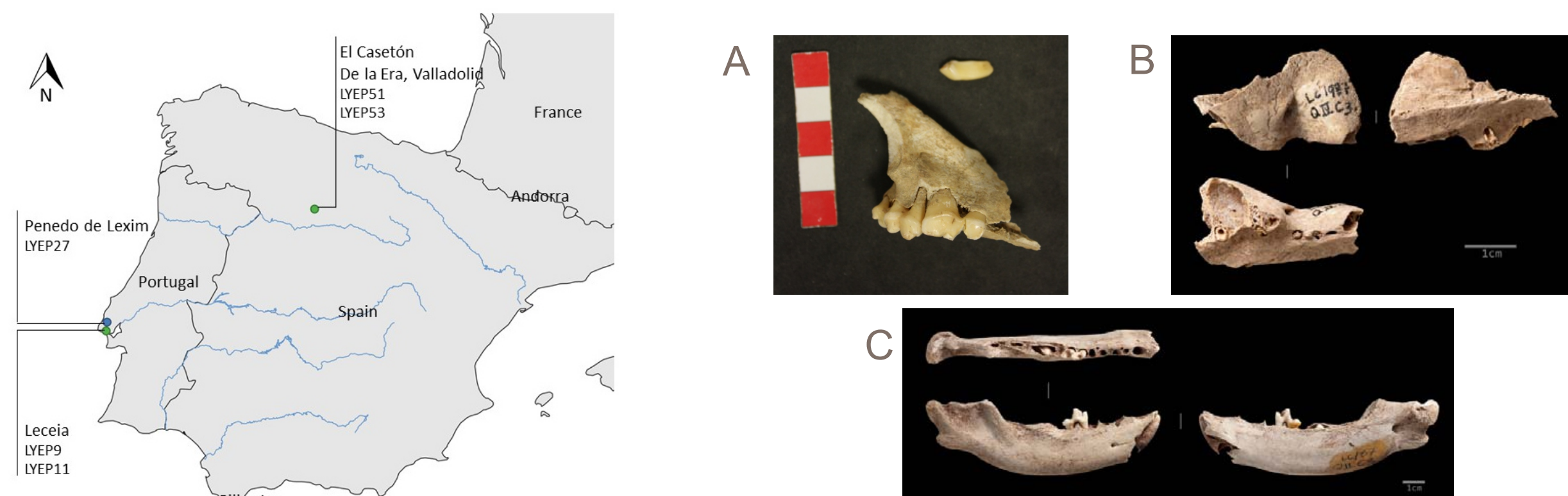
MATERIALS

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 Fernandez-Rodrigues (remains from Spain) and José Paulo Ruas (remains from Portugal).

Table 1. Samples information analysed in this study.

Ancient Canis sample ID	Scientific name/ Common name	Skeletal element recovered	Origin	Chronology	Reference
LYEP9	Canis lupus familiaris/ domestic dog	Mandible	Leceia (PT)	ca. 5,000–4,300* BP	11,14
LYEP11	Canis lupus familiaris/ domestic dog	Maxilla	Leceia (PT)	ca. 5,000–4,300 BP*	11,14
LYEP51	Canis lupus familiaris/ domestic dog	Maxilla	El Casetón de la Era, Valladolid (ES)	ca. 4,000 BP*	1, 14
LYEP53	Canis lupus familiaris/ domestic dog	Tooth (3rd Incisor)	El Casetón de la Era, Valladolid (ES)	ca. 4,000 BP*	1, 14
LYEP27	Canis lupus signatus/ Iberian wolf	Tooth (1st Lower molar)	Penedo de Lexim, Mafra (PT)	4,085–3,856 cal BP**	14, 17

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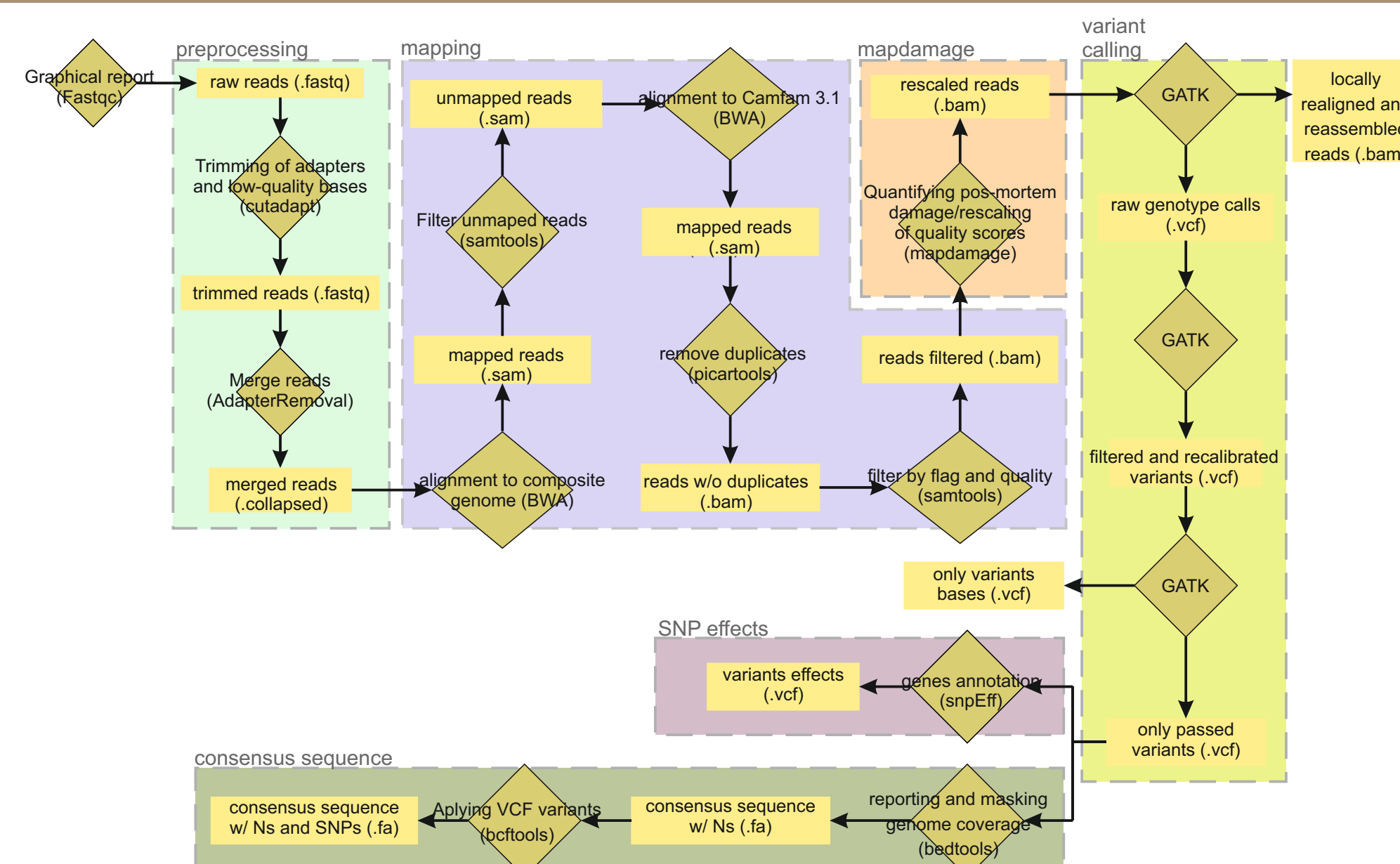

Fig. 3. Schematic representation of the pipeline.

- Unfortunately, due to the endogenous DNA poor preservation, variants within specific nuclear genes related to coat color, coat texture or body size could not be recovered (no coverage). A comparison between the nuclear genome - recovered from NGS analysis - and the reference dog genome revealed SNPsof possibly high impact, which are being assigned to genes.
- Regarding sex determination, comparing the proportion reads/Mbp (Fig. 5) for each chromosome we observed that: 1) for LYEP9, LYEP11, LYEP53 and LYEP27 only half of the reads/Mbp aligned with the X chromosome, in contrast to the proportion observed for the autosomal chromosomes. Fewer reads aligned against Y chromosome because the reference used here is only a part of the dog Y chromosome (its real size is not know, but is expected to be ~20Mbp long); 2) LYEP51 has the same proportion of reads/Mbp aligning along all the chromosomes, including the X chromosome.
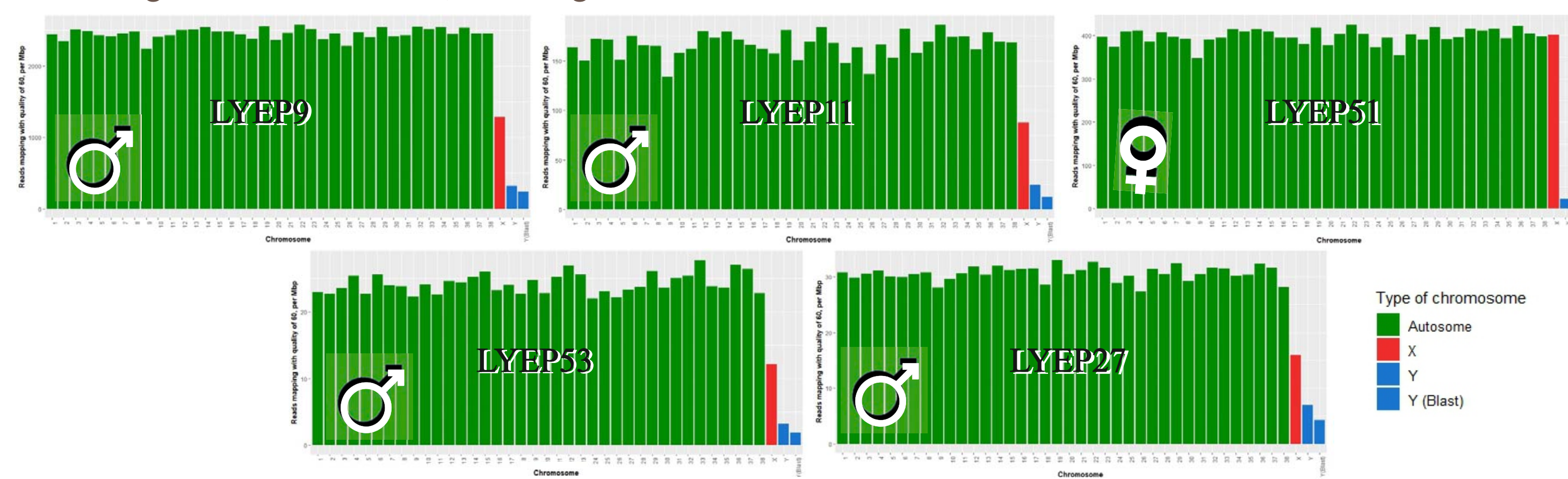

Fig. 5. Histograms representing the proportions of sequencing reads mapping each chromosome. 'Y (Blast)' bar represents specific reads that matched only to dog male-specific region.

CONCLUSIONS

- To our knowledge, for Iberian ancient dogs and wolves, this is the first attempt to successfully apply NGS methods.
- Chalcolithic Iberian dogs were represented by Hg A and C, contrary to their contemporaneous from other regions of Europe that were predominantely represented by Hg C.
- Ancient Iberian dogs had a distinct genetic composition, probably due to a particular event of domestication from the Iberian wolf
- Regarding nuclear genes, the results for the Chalcolithic Canis samples show that DNA is poorly preserved for some genes of interest related to coat color, coat texture and body size.
- NGS Illumina applied on ancient DNA has potential to provide data for further investigations concerning domestication and evolutionary trajectories of dogs.

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