

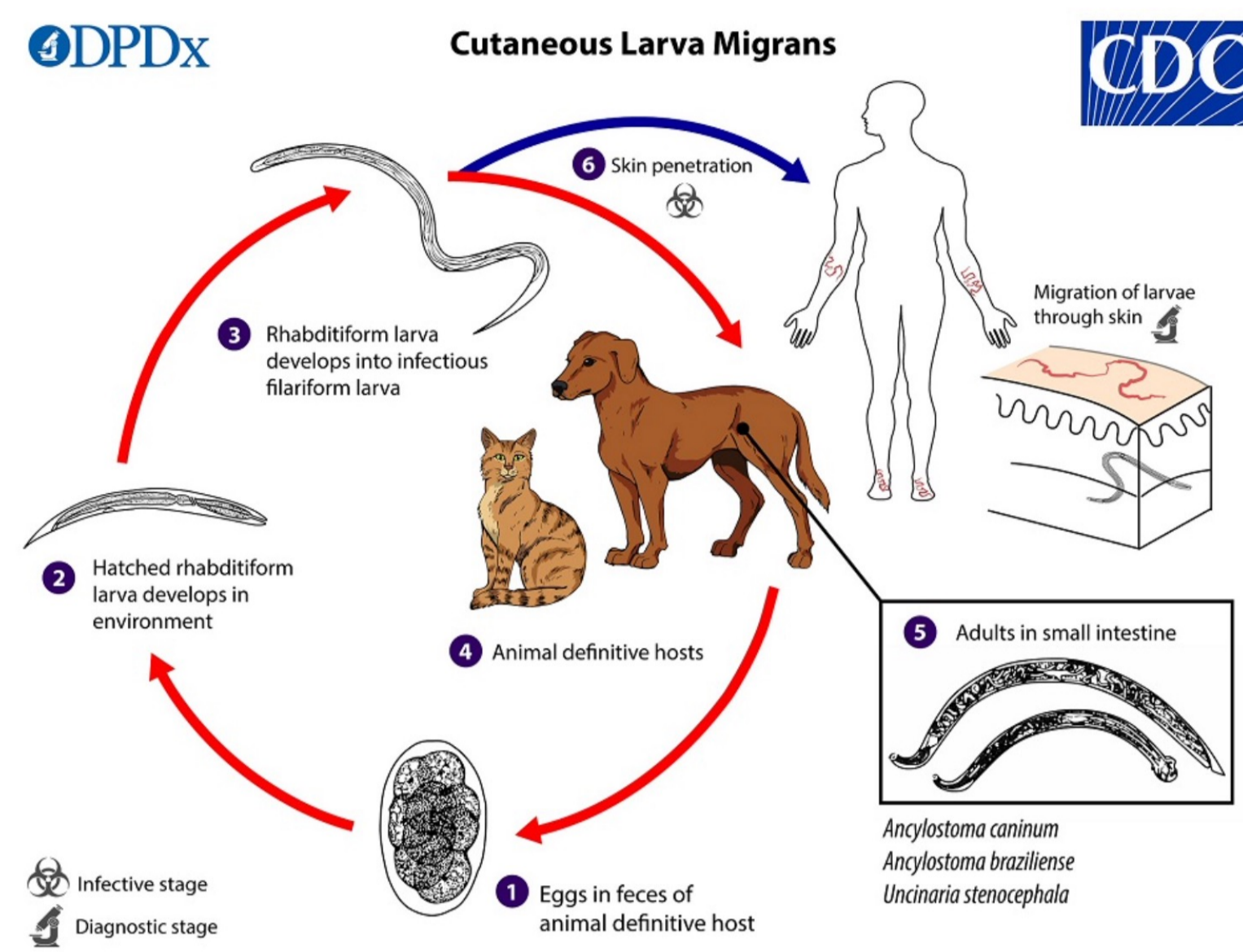
# A Molecular Genetic Investigation Into the Origins of Anthelmintic Drug Resistance in *Ancylostoma caninum* in Pet Dogs Across the USA

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## INTRODUCTION

- Most important gastrointestinal parasitic nematode of dogs in the USA.
- Infection can occur through percutaneous invasion or ingestion.
- Hookworms mature in the small intestine of dogs and can be passed on as eggs in the feces of infected dogs.



Hookworm infections are usually treated by Benzimidazoles\*, Macrocytic lactones, or Pyrantel.

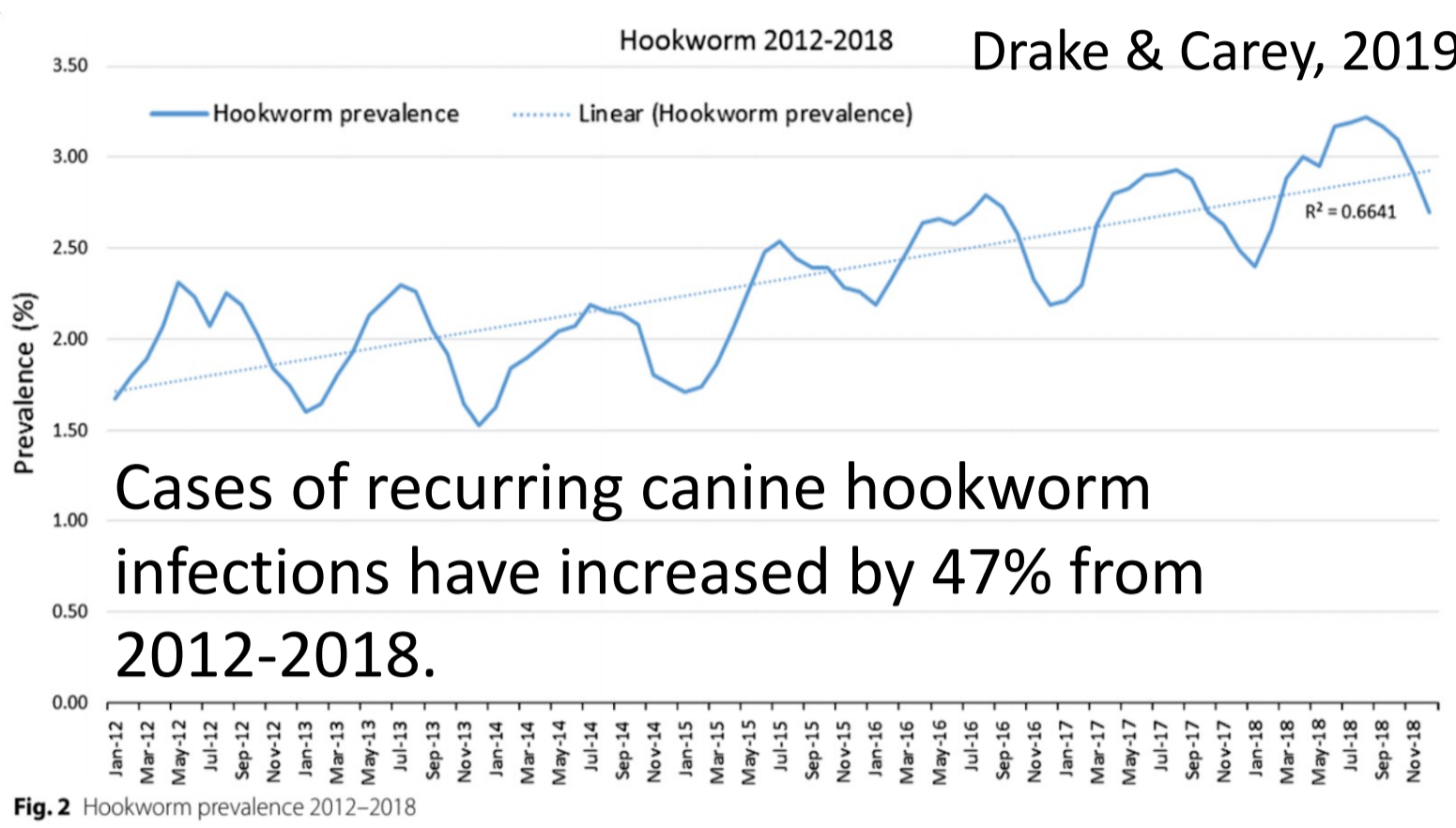
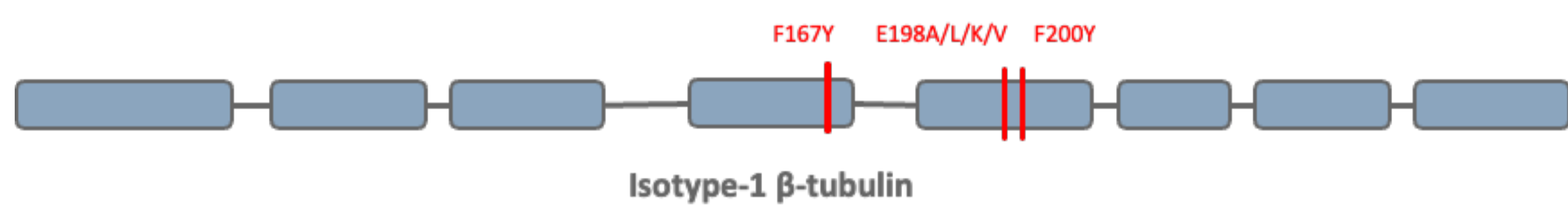
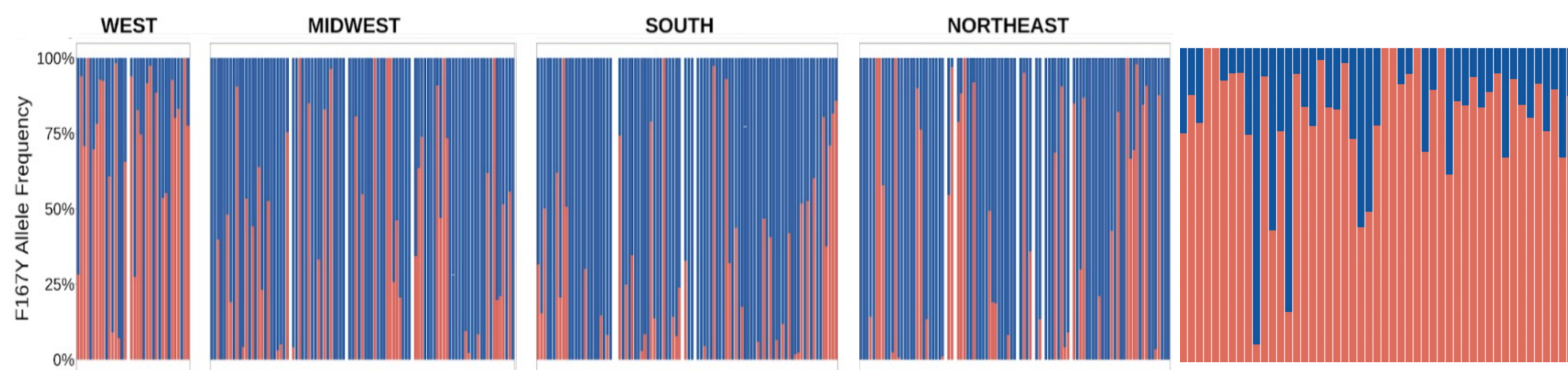


Fig. 2 Hookworm prevalence 2012-2018

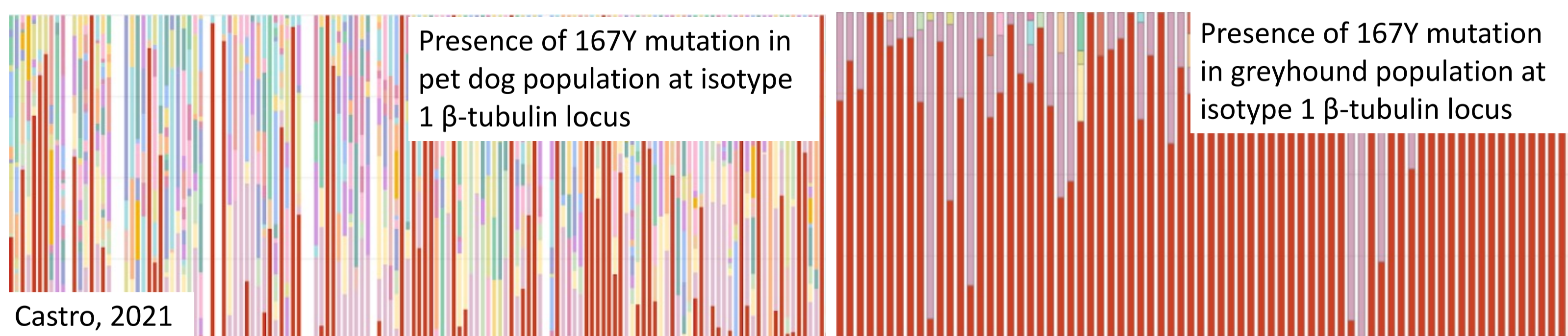
- The isotype 1  $\beta$ -tubulin locus is associated with Benzimidazole\* resistance.
- The three most common mutations associated with resistance at this locus are the mutations at codons 167Y, 198L/V, and 200Y.
- Red bars indicate the predominant 167Y mutation.



Presence of the 167Y mutation in *A. caninum* in pet dogs and greyhounds across the USA



Genetic diversity at the isotype 1  $\beta$ -tubulin locus in *A. caninum* in greyhounds and pet dogs



- Colored bars are Amplicon Sequence Variants and represent the overall diversity.
- The 167Y mutation is present at a much higher frequency in the greyhound population.
- Genetic diversity in the greyhound population has lower diversity compared to pet dogs at the isotype 1  $\beta$ -tubulin locus.
- Greyhounds are often exposed to environments that are ideal for hookworm survival and growth, and as a result, are often subject to intense deworming protocols that could select for resistance.
- The rehoming of greyhounds across the USA could result in the spread of resistance to pet dogs.

## RESEARCH OBJECTIVE

### Hypothesis

There is more intense selection for benzimidazole resistance happening at the isotype 1  $\beta$ -tubulin locus in *A. caninum* infecting greyhounds than pet dogs.

### Objective

Sequence two neutral mitochondrial markers at depth - COX 1 and ND1 to compare the genetic diversity of *A. caninum* in greyhounds versus pet dogs and determine if there is drug selection pressure in *A. caninum* infecting greyhounds at the isotype 1  $\beta$ -tubulin marker.

### Understanding the Hypothesis

Case 1: Similar *A. caninum* diversity between the greyhound and pet dog samples in the mitochondrial markers.

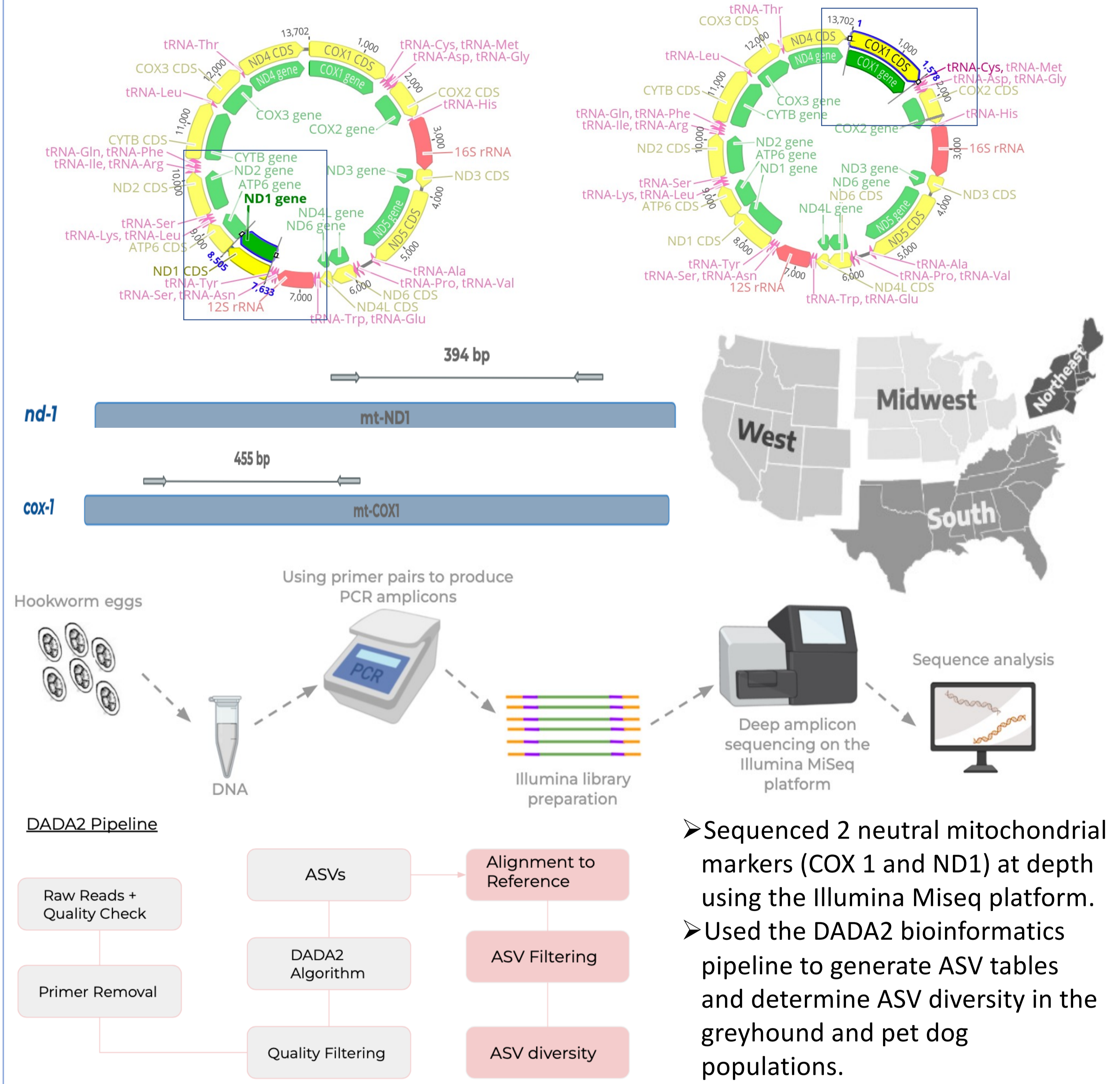
Interpretation: There is **more** intense selection at the isotype 1  $\beta$ -tubulin locus since mitochondrial markers are neutral and are not associated with resistance.

Case 2: **Lower** *A. caninum* diversity in the greyhound samples than the pet dog samples in the mitochondrial markers.

Interpretation: The **overall** hookworm population infecting greyhounds has low genetic diversity, which indicates that the population could have experienced a bottleneck.

\* Higher *A. caninum* diversity in the greyhound samples compared to the pet dog samples is a highly unlikely outcome for this study and will not be discussed.

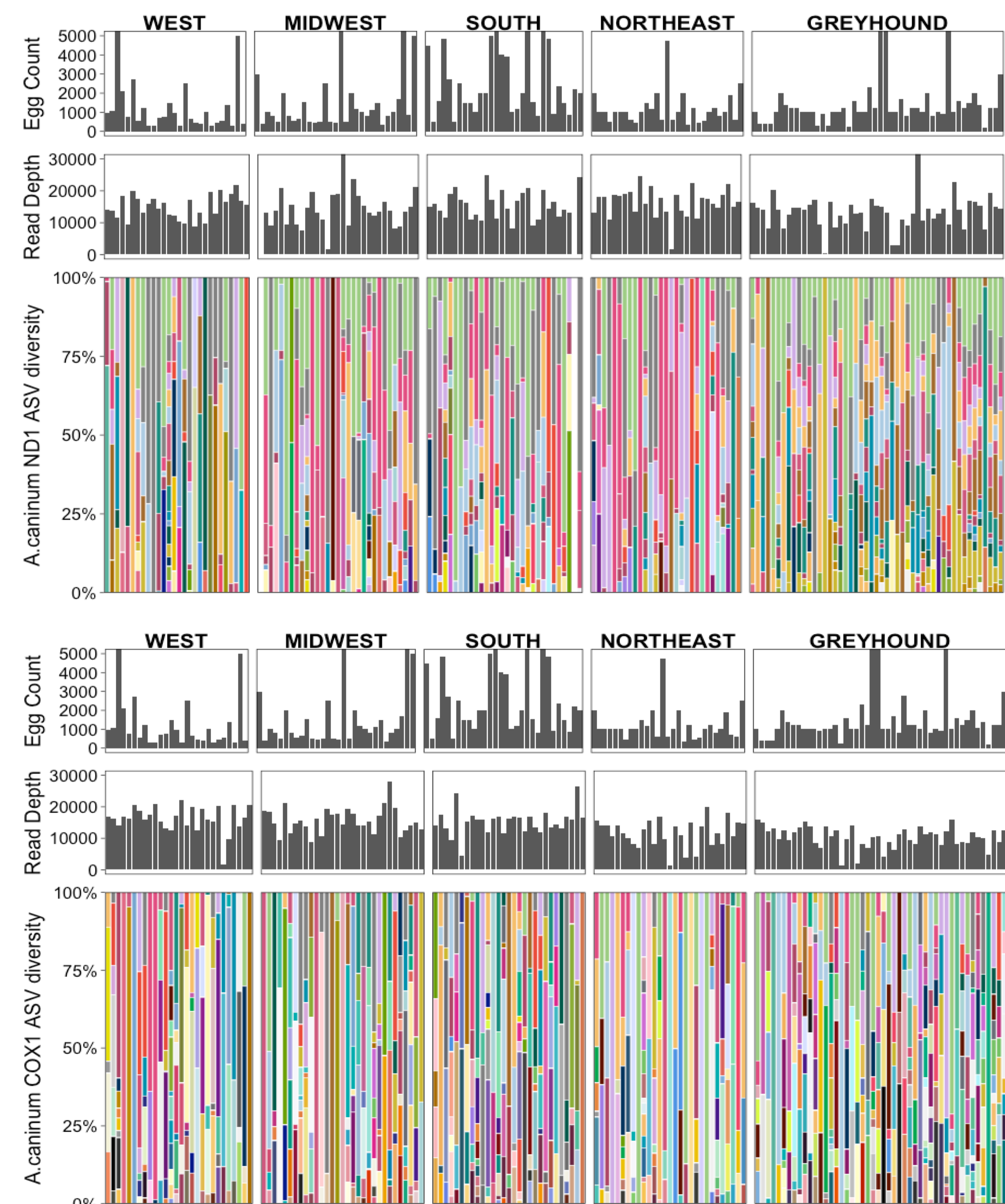
## METHODS



- Sequenced 2 neutral mitochondrial markers (COX 1 and ND1) at depth using the Illumina MiSeq platform.
- Used the DADA2 bioinformatics pipeline to generate ASV tables and determine ASV diversity in the greyhound and pet dog populations.

## RESULTS AND CONCLUSION

Genetic diversity in the ND1 and COX1 mitochondrial genes in *A. caninum* in pet dogs and greyhounds



Sequenced 50 greyhound samples and 120 pet dog samples from four different regions in the USA.

Both greyhound and pet dog samples have similar and high ASV diversity in the COX1 and ND1 plots.

Diversity plots show that *A. caninum* in greyhounds is undergoing more intense selection for resistance at the isotype 1  $\beta$ -tubulin marker in greyhounds than pet dogs.

Diversity stats in greyhounds and pet dogs in isotype 1  $\beta$ -tubulin and mitochondrial markers

Sample groups	$\beta$ -tubulin				COX1		ND1	
	$H_d$	$\pi$	D	p	$H_d$	$\pi$	$H_d$	$\pi$
Greyhounds	0.36	0.008	-2.70	0.006**	0.98	0.010	0.92	0.012
Pet dogs (overall)	0.89	0.017	-1.85	0.06	0.98	0.008	0.91	0.010

Diversity statistics prove that there is higher selection for resistance at the isotype 1  $\beta$ -tubulin marker in greyhounds than pet dogs.

$H_d$  = Gene diversity;  $\pi$  = Nucleotide diversity; D = Tajima D's test statistic, p = p-value for statistically significant departure from neutrality (p<0.05\*, p<0.01\*\*, p<0.005\*\*\*)