

Dylan Yaffy<sup>1</sup>, Becki Lawson<sup>2</sup>, Edmund Flach<sup>3</sup>, Henny Martineau<sup>1</sup>, Damer Blake<sup>1</sup>

<sup>1</sup>Department of Pathobiology and Population Sciences, Royal Veterinary College, Hatfield AL9 7TA, UK, <sup>2</sup>Institute of Zoology, Zoological Society of London, Regents Park, London NW1 4RY, United Kingdom, <sup>3</sup>Wildlife Health Services, Zoological Society of London, Regents Park, London NW1 4RY, United Kingdom

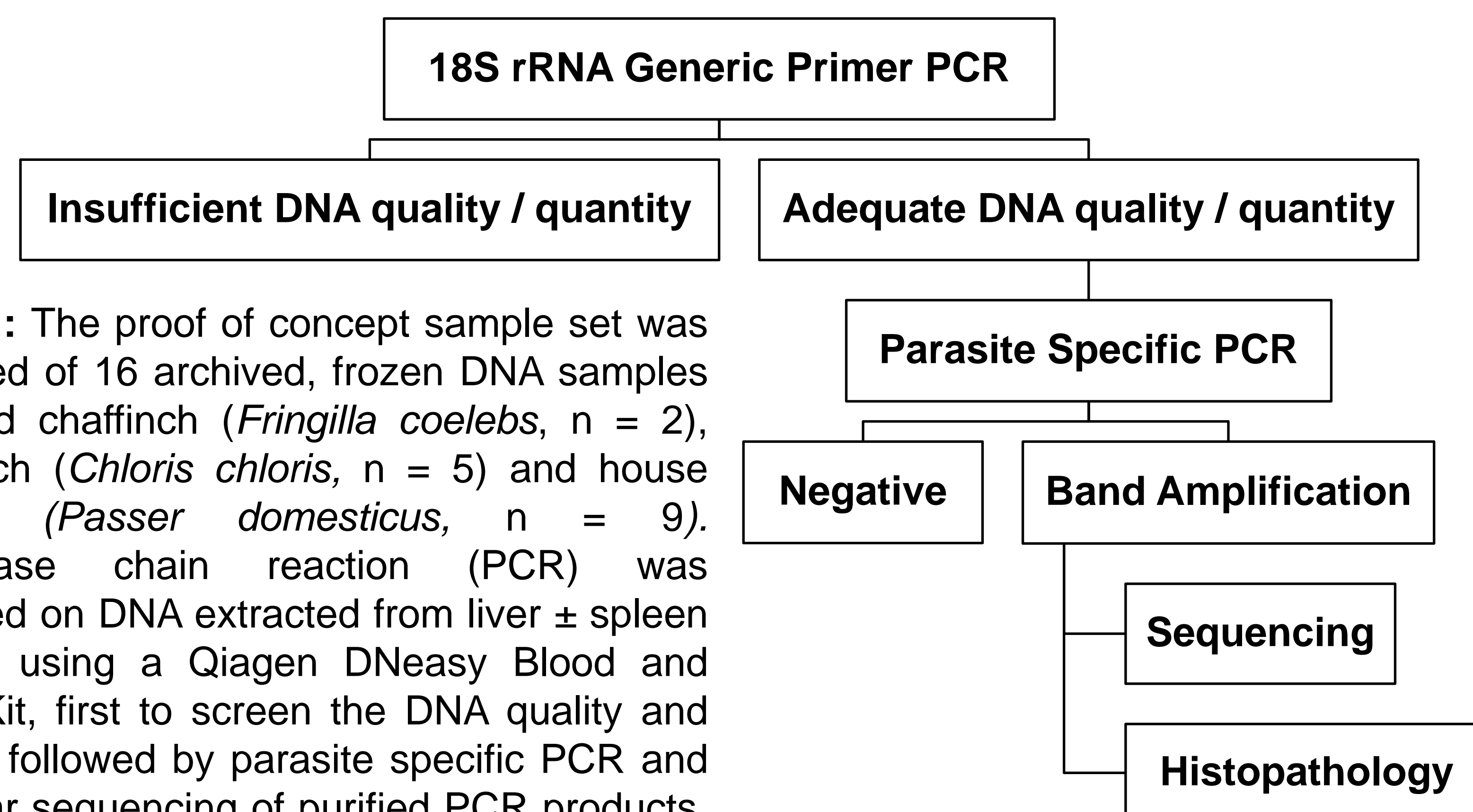
## Introduction

Systemic infections with *Isospora* protozoa (formerly *Atoxoplasma*) in both wild and captive passerine birds has been documented worldwide. Due to host-parasite co-evolution, infections in passerine birds are commonly subclinical but cross-infection between passerine species can result in severe clinical disease and increased mortality in captive populations (1). This poses a significant risk for conservation and reintroduction programs, particularly captive breeding colonies. The aim of this study is to investigate the occurrence and pathogenicity of systemic isosporosis in British garden birds.

## Objectives

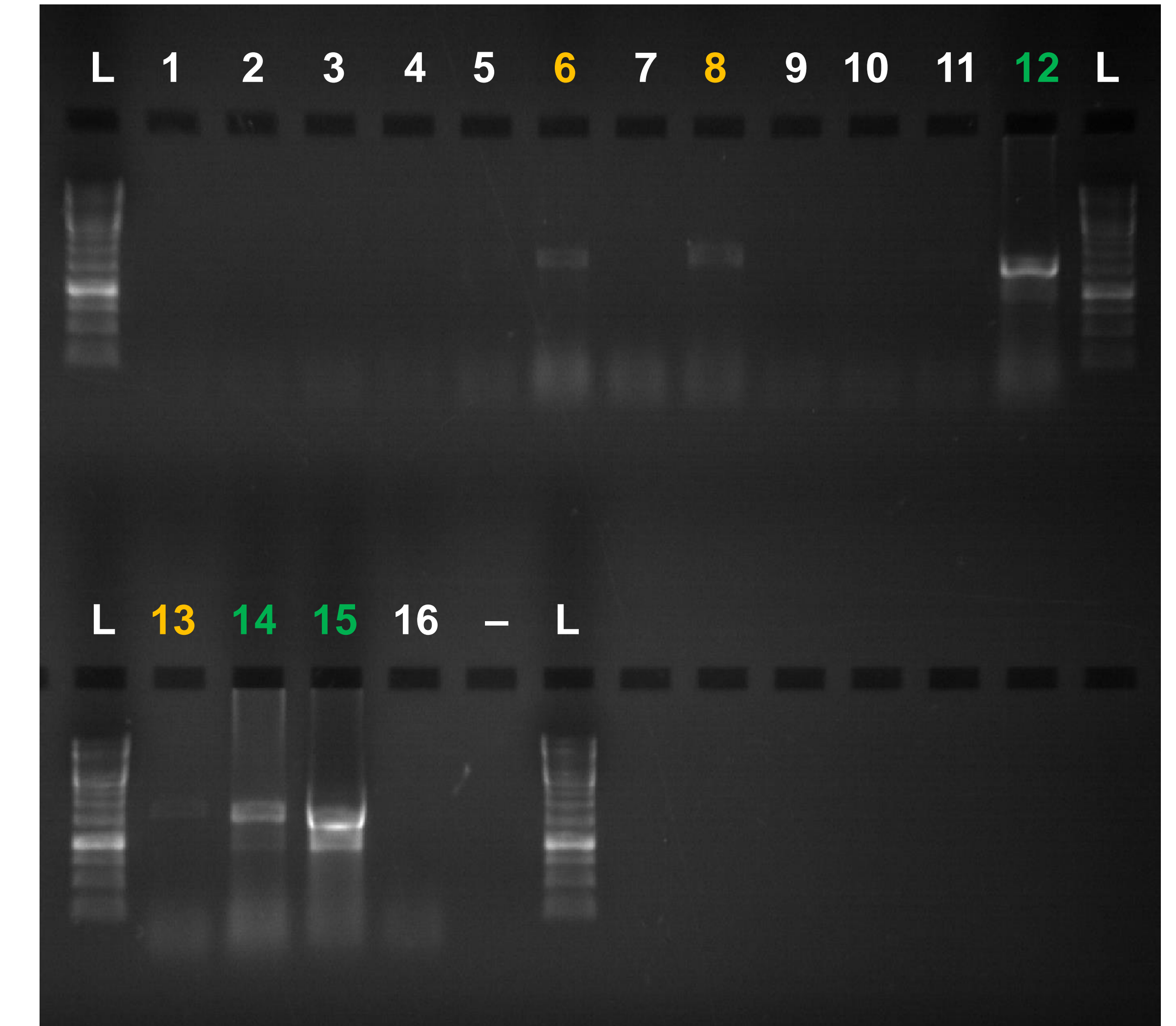
- 1) Use molecular detection to document systemic isosporosis in multiple species of British garden birds
- 2) Identify *Isospora* genotypes through sequence characterisation
- 3) Perform histopathology to investigate the clinical significance of infection
- 4) Compare *Isospora* detected in wild and captive bird species and assess population structure

## Methods



**Figure 1:** The proof of concept sample set was comprised of 16 archived, frozen DNA samples from wild chaffinch (*Fringilla coelebs*, n = 2), greenfinch (*Chloris chloris*, n = 5) and house sparrow (*Passer domesticus*, n = 9). Polymerase chain reaction (PCR) was performed on DNA extracted from liver ± spleen samples using a Qiagen DNeasy Blood and Tissue Kit, first to screen the DNA quality and quantity, followed by parasite specific PCR and molecular sequencing of purified PCR products. When liver ± splenic tissue is available, histopathological examination will be performed.

## Results



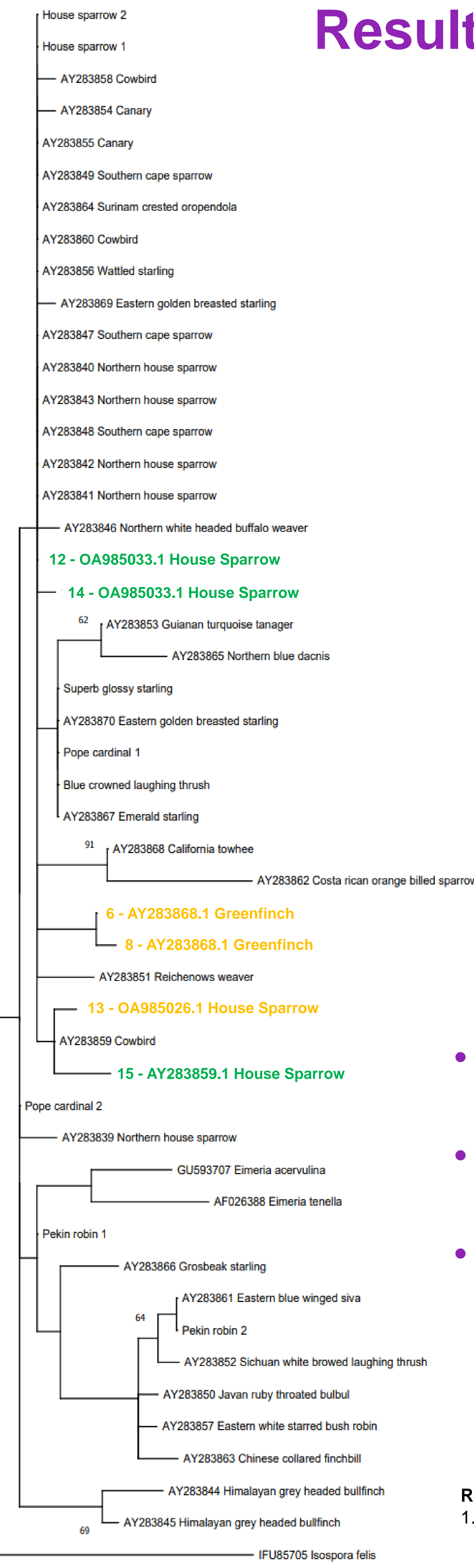
**Figure 2:** Amplification of *Isospora* genomic DNA by polymerase chain reaction using parasite specific primers targeting the 23S rRNA locus (2). Two greenfinch and four house sparrow (samples 6, 8 & 12, 13, 14, 15 respectively) demonstrated multiple *Isospora* genotypes. Yellow and green samples represent faint and moderate to strong parasite specific PCR band amplification respectively. L = 1kb gene ruler. – = negative control.

## Conclusion & Next Steps

- Successful PCR detection of multiple *Isospora* genotypes in wild greenfinch and house sparrow (Fig 2).
- Phylogenetic analysis reveals similarities in the parasite DNA sequences within the same passerine species (Fig 3).
- Future work: continuing molecular testing with additional greenfinch and house sparrow, in addition to other British wild bird species, supported by histopathology when feasible, to address the study objectives.

### References:

1. Flach, E., Dodhia, H., Guthrie, A. and Blake, D., 2022. Systemic Isosporiasis (Atoxoplasmosis) In Passerine Birds At The Zoological Society Of London, London Zoo. *Journal of Zoo and Wildlife Medicine*, 53(1), pp.70-82.
2. Schrenzel, M., Maalouf, G., Gaffney, P., Tokarz, D., Keener, L., McClure, D., Griffey, S., McAloose, D. and Rideout, B., 2005. Molecular Characterization Of Isosporid Coccidia (*Isospora* And *Atoxoplasma* Spp.) In Passerine Birds. *Journal of Parasitology*, 91(3), pp.635-647.



**Figure 3:** Optimal Maximum Likelihood (ML) tree inferred using the partial *Isospora* 23S rRNA locus. Support for each node is presented, indicating outcomes from ML when more than 60% of replicate trees presented the same relationship or the partition probability exceeded 0.6.

0.01