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FIRST MOLECULAR DETECTION OF *LEUCOCYTOZOON* (HAEMOSPORIDA: LEUCOCYTOZOIDAE) IN THE ATLANTIC FOREST: IDENTIFICATION OF NEW LINEAGE BASED ON SEQUENCE OF THE MITOCHONDRIAL GENE CYTOCHROME B



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INTRODUCTION

Haemosporidian are the etiologic agent of avian malaria, an important disease of wild and captive birds, which is transmitted to their hosts by hematophagous diptera. Currently, 250 species of these parasites are recognized within the genera *Haemoproteus*, *Leucocytozoon* and *Plasmodium*. The taxonomy of these parasites is still very controversial, and many studies are based on the morphology of the sexual and asexual phases, morphometric analysis and the presence or absence of hemozoin as well. Recent studies using molecular biology techniques have generated gene sequences deposited in GenBank, many of them have been diagnosed only up to genus level and, in some cases in-depth taxonomic levels. Despite Brazil ranks the second country with bird diversity (1919 species), there are few studies describing the diversity, distribution and prevalence of haemosporidians across bird communities, especially for *Leucocytozoon*.

This study aimed to contribute to the identification of haemosporidians in samples of birds from Atlantic Forest biome.

METHODS

Birds were trapped with mist-nets in three different Brazilian states: Rio Grande do Sul (n = 188), São Paulo (n = 121) and Bahia (n = 41)(Fig. 1). Blood samples were collected in the brachial vein and preserved in FTA cards DNA was extracted from each blood sample using the Wizard® Genomic DNA Purification System kit and screened for the presence of *Plasmodium*, *Haemoproteus* and *Leucocytozoon* through a nested PCR targeting the mitochondrial cytochrome b (cytb) of the parasite. PCR products were examined by 1 % agarose gel electrophoresis and the ones with approximately 480 bp were considered positive. Positive samples for haemosporidians were sequenced using Big Dye Terminator v3.0 Cycle Sequencing Kit on ABI Genetic Analyzer. The sequences obtained were compared with sequences deposited in public databases such as NCBI and MalAvi to identify the species or genera (Fig. 2). Those absent in MalAvi database were considered as new and were named according to the MalAvi nomenclature.

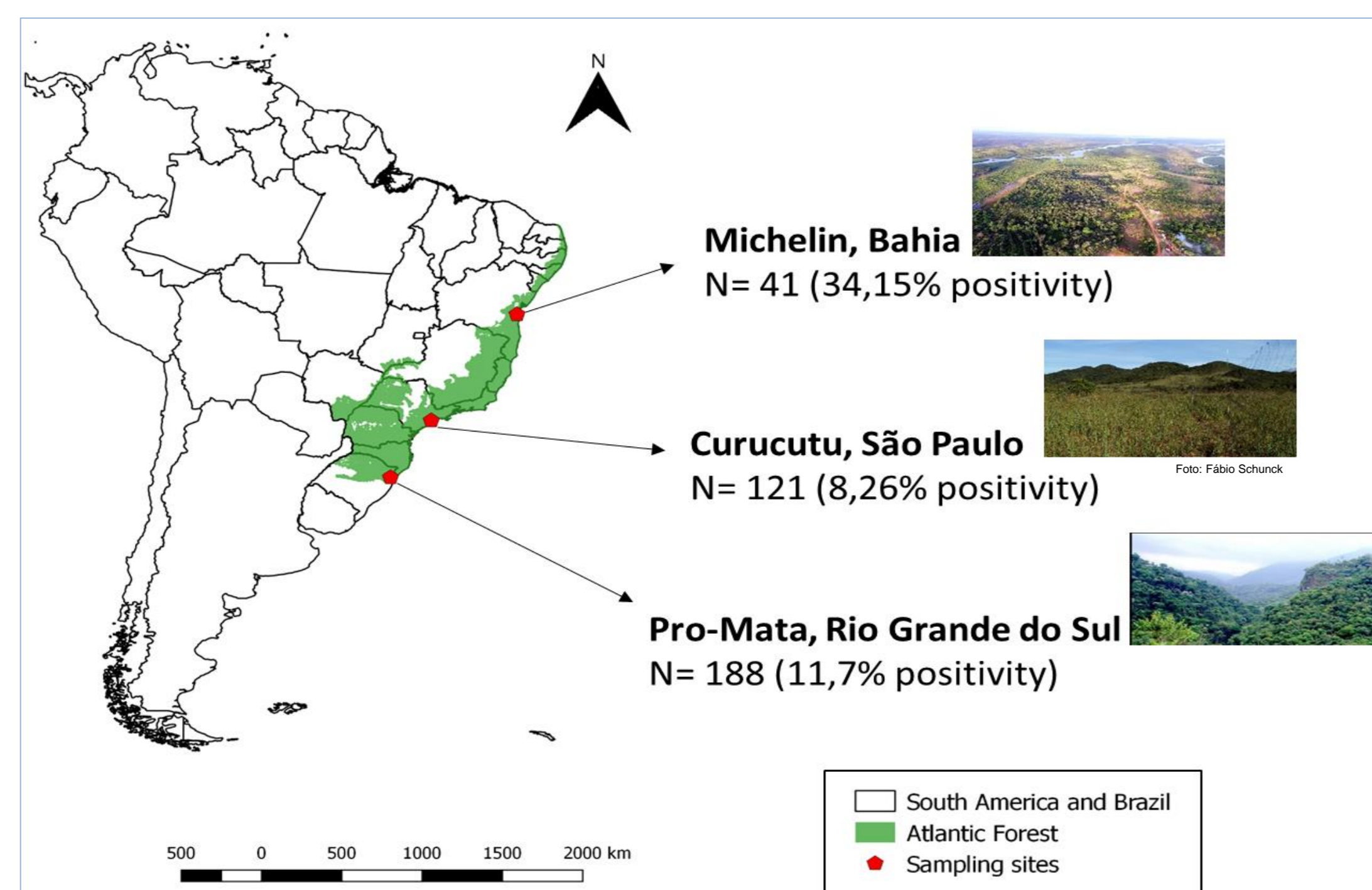


Figure 1 – Map depicting the sampling sites with the respective sample size and positivity to haemosporidians

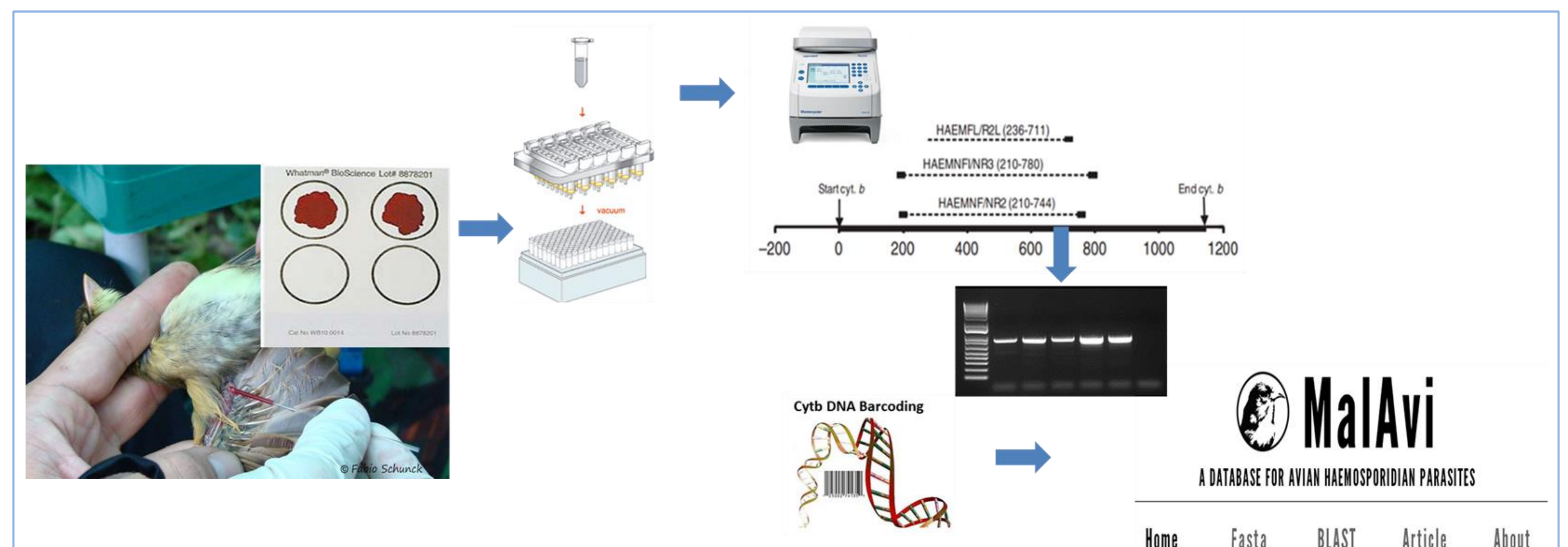


Figure 2 – Schematic representation of DNA processing and analysis of bird samples

RESULTS AND DISCUSSION

A total of 350 blood samples of birds belonging to 7 orders, 28 families and 102 species were sampled (Fig. 3). We detected 12.3% (43) positive samples in 22 host species belonging to the families Conopophagidae, Formicariidae, Furnariidae, Parulidae, Passerellidae, Thamnophilidae, Thraupidae, Trochilidae, Turdidae, Tyrannidae, Vireonidae and Vireonidae respectively. Being 7.7% (27) *Plasmodium*, 2.9% (10) *Haemoproteus*, 1.4% (5) *Plasmodium/Haemoproteus*, and 0.3% (1) *Leucocytozoon*. 23 genetic sequences were identified: 14 lineages of *Plasmodium*, 8 of *Haemoproteus* and one of *Leucocytozoon*. Some of these lineages have been described in Brazil and others are new descriptions. The *Leucocytozoon* lineage (ELACHI01) is the first description of haemosporidians in *Elaenia albiceps chilensis* (Chilean Elaenia)(Fig. 4), in the Atlantic Forest. Chilean Elaenia is a migratory passerine of the family Tyrannidae, which travels between the southern region of South America, where it reproduces, to the northeast and central regions of Brazil, where it winter. ELACHI01 has 95% identity with *Leucocytozoon californicus* (the morphospecies closest to the lineage SETAUD30 described recently in Brazilian Amazon) being closer (97% identity) to *Leucocytozoon quynzae* (Fig. 5). The two Brazilian lineages (from Atlantic Forest and Amazon) are genetically different, remaining in distinct clades in the phylogenetic tree. We suggest that different migratory routes may determine the distributions of lineages across South America, where the Amazon receives lineages from North and Central America and the Atlantic Forest has more influence of the austral migratory routes.

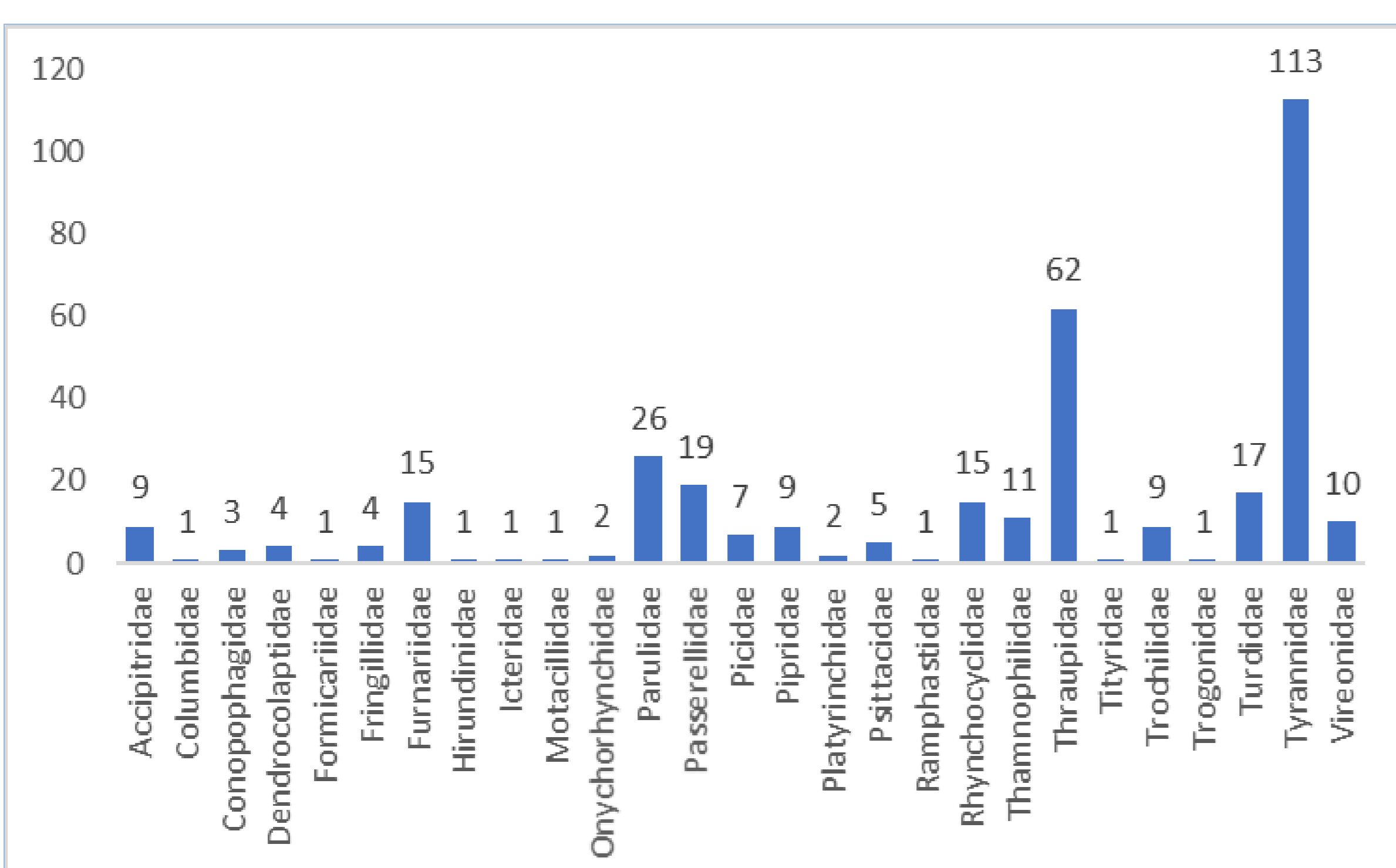


Figure 3 – Number of species sampled per family



Figure 4 – Chilean Elaenia

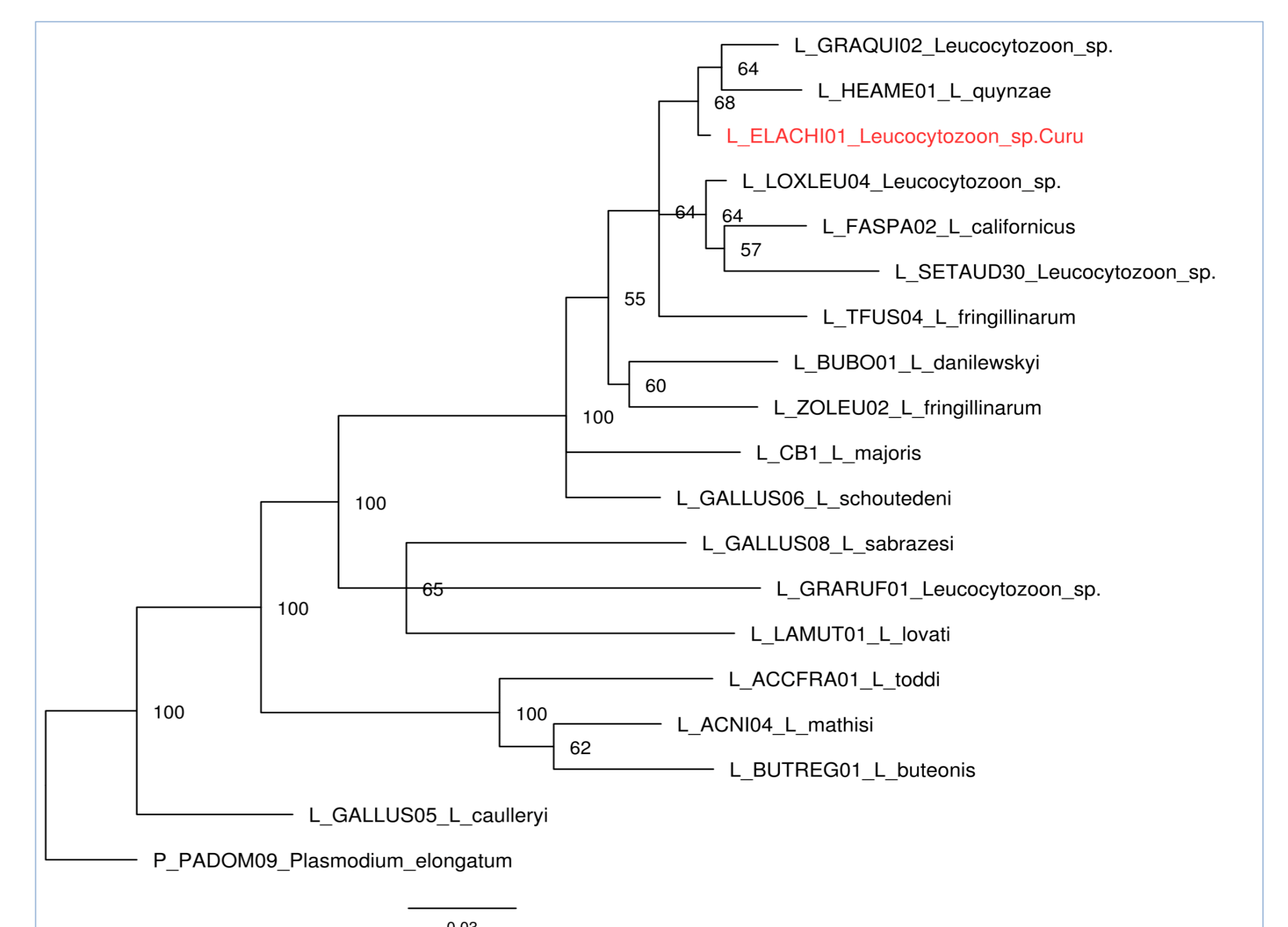


Figure 5 – Bayesian phylogeny of cytb gene lineages of *Leucocytozoon* species of avian hemosporean parasites showing the new Brazilian line ELACHI01 (in red)