

# IDENTIFICATION OF NEW HAEMOSPORIDIANS IN GRIFFON VULTURES (*Gyps fulvus*)

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## Introduction

**Haemosporidians** are a wide group of vector-borne protozoan parasites which infect the blood cells and other organs' tissues of most avian species worldwide and are transmitted via blood-sucking dipteran insects. Species of the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon* are the most important regarding wildlife and the most widely studied (Valkiūnas, 2005). They are reported to have a detrimental effect on birds' health, especially to debilitated hosts (Chakarov et al., 2021).

**Eurasian griffon vultures** (*Gyps fulvus*) are obligate avian scavengers of the Old World, constituting the most numerous and gregarious European vulture species (Espunyes et al., 2022; VCF). In Greece they are considered as critically endangered (International Union for Conservation of Nature). The scavenger birds play a vital role in the ecosystems and despite being estimated to have a low occurrence of blood parasites due to their potent immune system, their parasitism by haemosporidians has barely been studied (Chakarov et al., 2021).

The aim of this study was to assess, for the first time, the **prevalence** and the **genetic diversity** of infection by haemosporidians in Eurasian griffon vultures in Greece.

## Materials and Methods

**Twenty-four** Eurasian griffon vultures that were admitted for treatment to "ANIMA, Association for Wildlife Care and Protection" in Athens were involved in this study. All of these birds were originated from Heraklion and Chania, two departments of **Crete** island, and they were all young individuals presented with low body condition score and dehydrated.

**Blood samples** (100 µL) were collected from each vulture which were preserved in **SET buffer** (0.015M NaCl, 0.05M Tris, 0.001M ethylenediaminetetraacetic acid, pH 8, 1:10). DNA was extracted following the Quick-DNATM Miniprep Kit by Zymo Research. Samples were screened following a modification of the **nested PCR protocol** (Waldenström et al., 2004) by Perez-Rodriguez et al. (2013), which amplifies a fragment of the mitochondrial cytochrome b gene of all three haemosporidian genera. This protocol consists of a first PCR step using the primers Plas1F (5'-GAGAATTATGGAGTGGATGGTG-3'; Duval et al., 2007) and HaemNR3 (5'-ATAGAAAGATAAGAAATACCATTC-3'; Hellgren et al., 2004), and afterwards a nested PCR step with the internal primers 3760F (5'-GAGTGGATGGTGTGTTTATAGAT-3'; Beadell et al., 2004) and the HaemJR4 (5'-GAAATACCATTCTGGAACAATATG-3'). The positive samples were subjected to a second nested PCR using initially the primers HaemNFI (5'-CATATATTAAGAGAAITATGGAG-3') and HaemNR3 (5'-ATAGAAAGATAAGAAATACCATTC-3') to amplify parasite mtDNA and then HaemF (5'-ATGGTGCTTTTCGATATATGCATG-3') and HaemR2 (5'-GCATTATCTGGATGTGATAATGGT-3') primers (Bensch et al., 2000) for *Plasmodium* spp. and *Haemoproteus* spp. (Hellgren et al., 2004). Second PCR products were sequenced using Big Dye Terminator V3.1 Cycle **Sequencing** Kit and ABI PRISMTM 3100 capillary sequencing robot (Applied Biosystems, Foster City, CA, USA).

## Results

Three out of the twenty four Eurasian griffon vultures tested positive for haemosporidian infection (**3/24; 12.5%**). The first griffon vulture originated from Heraklion and was found positive for a mixed infection by different *Leucocytozoon* spp. lineages. The second one came from Chania and it was infected by the genetic lineage **LK03** of *Haemoproteus brachiatus*. The third vulture also originated from Chania and it was infected by a **new genetic lineage** of *Leucocytozoon* sp. that is recorded for the first time. Its sequence is the following:

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CTACCGGTGCATCATTGTATTATTCTAACATATCTACATATACTAAAAGGATTAATTATTCATATCTTACTT  
ACCATTATCATGGATTACTGGAGTTATGATATTTCTAATATCTATAGTAAGTGCCTTCTTAGGTTATGTATTACCA  
TGGGGTCAAATGAGTTTCTGGGGCGCAACTGTAATTAATTAATTAATTTATTCCTGGACTAATTTTCATG  
GGTCTGTGGTGGATATGCTGTAGGCGATCTAACCTTAAAGAGATTCTTTGATTACACTTTATCTTCCCATTCG  
TAGCTTTAGCTATGGTATTATACATATATTCTTTCTACATTTACAAGGTAGCAGTAATCCTTTAGGATACGATA  
CACCTTTAAAGATACCCTTCTATCCAAATCTATTATGTCTAGATATTAAGGATTAACATAATATTAATCTTATT  
CTTAGCTCAAAGCTTCTTTGGAAATTT
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## Discussion / Conclusion

The results of the present study support the interesting hypothesis that the potency of the vultures' immunity seems to protect them from blood parasites, hence they present a **low prevalence** of infection (12.5%), compared to other bird species. This is in accordance with a recent study which detected 3.1% prevalence of haemosporidian infection in griffon vultures in Spain (Chakarov et al., 2021). In that study all the blood parasites isolated belonged to the *Leucocytozoon* genus.

The low prevalence can be explained by the fact that the **cliff-nesting** habits of the griffon vultures, reduces their contact with the vectors, which look for the birds close to water bodies. This contact is also reduced because of the **early breeding** of griffon vultures which results in discrepancy between them and the vectors (Chakarov et al., 2021).

The sex and the exact age of the vultures that took part in the current study was not determined. Nevertheless, no effect of bird's **sex** or **age** on the prevalence of haemosporidian infection has been detected for griffon vultures (Chakarov et al., 2021).

This was the first time that *Haemoproteus brachiatus* was isolated from a vulture species (Yabsley et al., 2018).

The **0% prevalence of Plasmodium spp.** infection detected in the present study is not possible to be a result of the unavailability of avian malaria's vectors in Crete, as *Culicidae* have been recorded on this island (Beleri et al., 2023).

The results of the current study, in addition with the recent re-evaluation of the placement of *Haemoproteus catharti* - a blood parasite recently isolated from New World vultures - in the genus *Haemoproteus*, highlight the need for further investigations of the genetic lineages of Haemosporida. They will contribute to redetermine the borderlines of the genera of these parasites, as well as the relationship between haemosporidians of all animal species (Yabsley et al., 2018).

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