

Summary

Molecular studies of the specificity of avian malaria parasites (Haemosporida) to the vectors of the genus *Culicoides* Latreille, 1809 (Diptera: Ceratopogonidae)

Aneliya Borisova Bobeva

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Institute of Biodiversity and Ecosystem Research – Bulgarian Academy of Sciences, 2
Gagarin Street, 1113 Sofia, Bulgaria

Supervisors: Prof. Boyko Georgiev, PhD, DSc

Assoc. Prof. Pavel Zehindjiev, PhD

The aim of the present study was to identify the biting midges of the genus *Culicoides* in North-East Bulgaria and to determine their feeding preferences in order to reveal their potential role in the transmission of avian haemosporidians. Eleven *Culicoides* species were recorded during the course of the study; two of them were reported for the first time for the Bulgarian fauna.

By applying two nested-PCR assays, one targeting the avian *cyt b* gene and another targeting the COI gene of a wide range of vertebrates, we identified the blood hosts of six biting midge species: *C. circumscriptus*, *C. festivipennis*, *C. punctatus*, *C. pictipennis*, *C. alazanicus* and *C. griseidorsum*. Bird DNA was found in 50.36% of 95 investigated bloodmeals whereas mammalian DNA was identified in 13.7%. Two *Culicoides* species were found feeding both on birds and mammals. There was a remarkable diversity of avian hosts: blood of 23 species of four orders was identified in the abdomens of four *Culicoides* species. The most common bird species identified was magpie, *Pica pica* (N=7) registered in all four ornithophilic biting midge species. Six bloodmeals from the great tit, *Parus major*, were recorded only in *C. alazanicus*. None of the studied species of *Culicoides* appeared to be strictly specialized to a single avian host.

PCR-based technique amplifying part of mitochondrial cytochrome *b* gene of haemosporidian parasites was applied on naturally infected biting midges. Totally, 870 parous individuals of 11 species and 95 blood-fed individuals of 6 species of *Culicoides* were examined for the presence of DNA of haemosporidians. Haemosporidian genetic lineages

were identified in individuals of 4 insect species: *C. alazanicus* (12 lineages, 9 lineages of *Haemoproteus* and 3 lineages of *Plasmodium*), *C. circumscriptus* and *C. festivipennis* (with 3 and 2 lineages of *Haemoproteus*, respectively) and *C. pictipennis* (with one *Haemoproteus* lineage). Two genetic lineages of *Haemoproteus* were recorded in more than one vector species. These results demonstrate variations in the specificity of *Haemoproteus* genetic lineages to their potential vectors, since some lineages are recorded in a single vector species and others occur in two or more vector species.