Ph.D. THESIS

Ecology and epidemiology of bat specialist ectoparasites in South-Eastern Europe

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Summary

In recent years there was a rising scientific attention towards the role of bats in pathogen transmission. More and more studies are surveying how bats contribute to the distribution and evolution of different groups of pathogens (WIBBELT ET AL., 2010). The currently ongoing intense globalization process will give a chance for more contact for humans with wildlife, including bats. Globalization also disrupts the natural balance of bat populations and their associated pathogens, causing spill-overs more frequently. These spill-over events may pose a real threat to human health (as we experienced with the COVID-19 epidemic), and may affect livestock, or wild animals too (MORATELLI AND CALISHER, 2015). In the spread of these pathogens, ectoparasites have a major role as reservoirs or vectors (MCCOY ET AL., 2013). Besides mites, fleas, or cimicids (bed bugs) we consider ticks and bat flies the epidemiologically most important groups of bat ectoparasites. In the case of several pathogens (*Bartonella* spp., *Borrelia* spp., *Babesia* spp., *Rickettsia* spp. or malaria-related *Poluchromophilus* spp.) it was already proven that ticks and bat flies act as vectors (LVOV ET AL., 1973; HOOGSTRAAL, 1985; HUBBARD ET AL., 1998; EVANS ET AL., 2009; SOCOLOVSCHI ET AL., 2012; HORNOK ET AL., 2017).

Bat ectoparasites usually are highly host-specific (mono- or oligoxenous parasites), having one or just a few bat species as hosts. However, there are some ectoparasite species (the soft tick species *Argas vespertilionis* for ex.) that are more generalists and sometimes infect other mammals, too (ROMN ET AL., 2012). Other more bat-specific tick species in Europe are, from the family of hard ticks (Ixodidae) *Ixodes vespertilionis, I. simplex, I. ariadnae*, and the second, less frequent soft tick (Argasidae) species *A. transgariepinus*.

On the other hand, all bat fly species are exclusive parasites of bats, with ten bat fly species reported from Romania and 14 from Europe. Bat fly species show important differences in host preference (SZENTIVÁNYI ET AL., 2016), presenting an interesting model system to survey host specificity.

Even though more and more studies are focusing on the epidemiology of bats and even the role of bat ectoparasites in the transmission of pathogens, we still lack important and, in some cases, basic information. With this study I tried to contribute to filling these scientific gaps, therefore the aims of this work are the following:

- Evaluate the ecology of bat specific ticks and bat flies (their seasonality and host specificity) in South-Eastern Europe
- Evaluate the host selection preferences and strategies of bat specialist ectoparasites

- Evaluate the most important factors determining their reproductive cycles

- To clarify their geographical distribution and factors influencing it
- To provide new data regarding their biology and ecology
- To evaluate the epidemiological aspects (prevalence, infestation levels) of bat-bat ectoparasite systems
- To evaluate factors that influence the parasitism of bats by ectoparasites
- To evaluate the role of bat ectoparasites in the transmission of malaria-related haemosporidian parasites

In the first part of this thesis (I. Introduction), I summarized the information from literature regarding bats, their parasites and the diseases associated with them and also on the ecology and epidemiology of bat specialist ectoparasite species.
The second part (2. Original research), which contains a total of 9 original manuscripts is divided again into two parts. The first part covers my studies on the bat specialist ticks (four manuscripts) and the second part is on bat flies (five manuscripts).
At the end of the thesis, I summarized my conclusions and at last, the references listed from the mentioned studies.

In the first chapter of the second part of this thesis (2.1.1.), I present my studies on host selection and distribution of tick species and development stages on different bat species. I also tested if tick species occurring on bats show reproductive synchronization with their host's reproduction (as it was shown in several other bat-ectoparasite systems). Since ectoparasites with different modes of transmission may respond in different ways to challenges posed by their hosts, I expected differences in infestation patterns between tick species with high host-specialization and more generalist bat ticks. My studies showed that bat specialist ticks show different adaptations to their hosts, in concordance with their primary hosts' ecology, life history, and social organization. These adaptations may be morphological and behavioral (long vs. short legs, seasonality in abundance) or just behavioral (seasonality in occurrence). As there are no major morphological differences between two of the bat specialist tick species (*I. ariadnae* vs. *I vespertilionis*) to reduce interspecific competition, these ticks target different groups of host species in the same geographical space (inside the same underground shelters) with differently timing their activity peaks, thus showing geographical sympatry, but temporal allopatry in their activity.

In the second chapter on bat specialist ticks (2.1.2.) I collated the published records on five soft tick species (Argasidae) in the Western Palearctic, looking for data on their geographical distribution, host-parasite relationships, and vectorial importance and also raising awareness on future challenges posed by some of these species to human health. I was also looking for the abiotic (climate-linked) and biotic (host distribution linked) factors regulating the distribution of these soft ticks in the Western Palearctic. Based on the literature survey (and 894 distinct georeferenced records), I present the geographical range, host selection, and vectorial potential for soft ticks occurring on bats in this region. *A. vespertilionis* shows the largest distribution range and was found on most host species, being ubiquitous wherever crevice-roosting bat species occur. All the other species were located only in the Mediterranean climate areas, with *A. boueti*, *A. confusus*, and *O. salahi* missing entirely from Europe. These species have a host palette of bats roosting primarily in caves, while *A. transgariepinus* is attacking also crevice-roosting species. All but one of the soft ticks are known to feed on humans and may be vectors of important disease agents (like *Rickettsia* spp., *Borrelia* spp., *Bartonella* spp., *Ehrlichia* spp., *Babesia* spp., as well several flaviviruses). Several crevice-roosting bat species show a continuous adaptation to human-altered areas, with certain species becoming common city-dwellers in Western Palearctic. Therefore, the study of bat specialist soft ticks is becoming important in the region not only from evolutionary, but also from epidemiological perspectives, too.

In the third chapter (2.1.3.) I provide the re-description and detailed information on the morphological traits of the males of the bat specialist *I. simplex*. The male of this tick species was originally described in 1962 but on a single long-preserved and dried specimen. There were no drawings, while the description of the morphological characters was scanty. With the new detailed description and the attached photos, I hope that the future identification of *I. simplex* males will be a straightforward task.

In the last chapter on the bat specialist ticks (2.1.4.) I report on the first European case of *I. simplex* found attached to a human, completing with a review of human-related cases for bat specialist ticks known to occur on European bats. Ticks, in general, are known to be competent vectors for several vector-borne diseases, but the proven vectorial capacity of *I. simplex* for zoonotic pathogens is yet unknown. However, DNA of several unicellular and bacterial vector-borne pathogens was already identified in individuals of this species. Thus, *I. simplex* may be suspected to possess vector role for some of these microbes. Although the occurrence of this tick species (and other cave-dwelling bat specialist ticks) on humans is merely accidental (together with its associated potential zoonotic risk), we are not able to rule out potential future cases with the continuous increase in cave-tourism and associated human activities near bat colonies.

The first manuscript in the second part of the original research (2.2.1) is about the seasonality and infestation levels of local (South-Eastern region of Europe)

bat species with bat flies and the host specificity of these bat fly species. This evaluation reports data for the first time in this region. My main aim was also to determine the factors regulating the level of specificity of bat flies. My results show that the local presence of the multi-species community of bat hosts favors the occurrence of bat flies on non-primary hosts.
As the main conclusion of this manuscript, I suggest that the host specificity and host choice of bat fly species is determined by the season, the intensity of infestation, and by the species composition of the local bat population (i.e., the absence or presence of multiple secondary hosts). Choosing non-primary hosts in the mating period of temperate bats may be an adaptive choice for bat flies, thus increasing the dispersing ability of individual bat fly species.

The second manuscript on bat flies (2.2.2.) is a checklist of Romanian bat fly species and their host associations. Recently, several studies targeting bats and their parasites were initiated, with high numbers of bat flies collected. Using published and own unpublished data, I present the first annotated checklist of bat flies and their hosts from Romania. This checklist covers 11 bat fly species recorded on the territory of Romania and also data on host-parasite relationships of these. Altogether 71 different host-parasite relations are presented for the 11 bat fly species, with 11 new host-parasite associations never recorded before.

The third manuscript on bat flies (2.2.3.) reports for the first observation of a rare bat fly species, *Basilia italica* in Romania. On two separate trapping occasions at the same location in Apuseni mountains (IC Ponor) single bat flies were collected from adult Whiskered bats (*Myotis mystacinus*), turning out to be the first country record of this rare species. With this record, the number of Romanian bat fly species increased to 11. This is a novel and geographically distant record from the known distribution of this species, which also represents the easternmost occurrence of *B. italica*, therefore greatly enlarging its previously known geographical range.

In the fourth manuscript on bat flies (2.2.4.), I aimed to identify factors that may influence the incidence of the hyperparasitic Laboulbeniales fungi on nycteribiid bat flies in southeast Europe (Bulgaria and Romania) by analyzing a dataset of a large collection of bat flies. Laboulbeniales (Ascomycota: Laboulbeniomycetes) are parasitic fungi living on the outer surface of arthropods, forming their multicellular structure called thallus, with ascospore producing body pars (perithecia and antheridia) and the haustorium, the rootlike system with which they penetrate the host's tissues and absorb nutrients.
I found that Laboulbeniales infections are more common in bat flies that are infecting bat species with dense and long-lasting colonies (*Miniopterus shreibersii, My. myotis, My. blythii*), roosting primarily in underground sites. Inside these sites, environmental and biotic conditions (constant temperature

and humidity) may enhance the spread and development of fungal infections by Laboulbeniales. Gender-related differences in bat hosts' behavior also may affect fungal infection risk, with densely roosting female bats harboring more Laboulbeniales infected bat flies.

In the last manuscript (2.2.5.) I investigated the distribution of the bat-related, malaria-causing pathogens, the *Polychromophilus* spp. in Eastern Europe by sampling a diverse range of bat species at different locations. In this study, I also tested whether there are differences in *Polychromophilus* spp. infection between cave-dwelling and crevice-roosting bat species. Two malaria-related haemosporidian parasite species, *P. murinus* and *P. melanipherus* were identified in the samples, showing wide distribution among bats and their ectoparasites in SE Europe. High genetic diversity is reported for both *Polychromophilus* species, with diverse genetic variants present even at the same location, suggesting that the simultaneous presence of diverse host- and vector assemblages may enhance malaria-like parasite diversity, too.

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