

When we are watching birds pecking seeds in the feeder and looking at their colorful feathers, most of us know that plumage protects birds against cold, heat, water, that attracts the attention of partners, masks, and of course allow flying. But only few of us know that the lower, tube-shaped part of the feathers, which is inside the bird's skin (quill) can serve as a warm and comfortable 'apartment' for the group of absolutely amazing (and little scary) tiny, eight-legged arthropods, which are called quill mites. Quill mites are obligatory, permanent bird parasites infecting one or a few closely related bird species (mono- or stenoxenous). The whole life cycle of these organisms occurs inside the quills of feathers, where they live, reproduce and feed (pierce quill walls using dagger-like structures called chelicerae and suck surrounding soft tissue). Only females disperse (move into quills when the host is forming a new feather or on nestlings during hatching, or occasionally by horizontal transfer), whereas the males never leave the quill. Mites are very 'feminist', the sex ratio in most species is strongly female biased (several females per 1 male) in some others, males have never been found. Why is that? Quill mites are birds parasites but simultaneously they are also hosts for another intriguing microorganisms, for bacteria from the genus *Wolbachia*. This is maternally (transovarially) inherited bacterium, which in arthropods is mainly known as a factor causing a range of reproductive disorders leading to increased numbers of females, such as: parthenogenesis (development of embryos without fertilization), feminization (changing genetic males into females), cytoplasmic incompatibility (phenomenon that results in sperm and eggs being unable to form viable offspring) and male-killing. However, some other ectoparasitic arthropods (bed bugs or bat flies) rely on *Wolbachia* for nutrient provisioning. We still do not know exactly what effect bacterium causes in quill mites but based on sex ratio observations and feeding, it seems that both reproductive disorders and providing nutrients can be induced.

As can be seen, we are dealing here with a tripartite system (bird host–quill mites–*Wolbachia*). This system thus enables testing predictions about horizontal and/or vertical transmission of *Wolbachia*, and about *Wolbachia*-host cophylogeny (parallel evolutionary development). What do we want to know is whether the isolated life style of quill mites has an impact on the scheme of cophylogenetic relationships with bacterium. If *Wolbachia* strains are transmitted vertically, it should persist within a host lineage over considerable timescales, resulting in evolutionary congruence with a host (what would be unusual case, since in most arthropods the lack of phylogenetic congruence with *Wolbachia* is observed, probably because of horizontal transmission or evolving of resistance, resulting in loss of the infection). On the other hand, ecology and evolutionary history of birds (which can be a place for exchange of bacteria between mites) may also have an additional impact on the phylogenetic relationships of the *Wolbachia*-mites system. How do you want to investigate it with such small organisms? And how much time would we need to be a witness of macro-evolutionary processes if human life is too short to observe 'by eyes' evolutionary changes?

To examine our model, we will use DNA fragments instead of 'eyes'. On the basis of genetic data we will be able to reconstruct the evolutionary history both of mites and bacteria. In the laboratory we will extract DNA from single specimens of mites (in which we have genetic information from mites and *Wolbachia* strains), then amplify a sets of genes dedicated to each of these groups (and allowing organisms identification and comparison with others) by PCR reactions (technology in molecular biology used to amplify a fragment of DNA) and finally we will sequence it (just read it to make our data useful for next analysis). Molecular data will be further analyzed with bioinformatic tools (calculating methods based on sophisticated mathematical algorithms) to reconstruct phylogenies of both *Wolbachia* strains and mites. At the end, analysis of the evolutionary congruence between quill mites and bacteria and tracing the bacteria movements across the quill mites phylogeny will be conducted. This will allow to assess if phylogenetic histories of *Wolbachia* and quill mites are linked. Next, we will determine quill mite taxonomy, taxonomy of quill mite bird hosts, geographic origin of quill mites and code these features as traits of the identified *Wolbachia* lineages. We will assess if these traits are statistically significantly associated with the *Wolbachia* phylogeny. If yes, this would argue for an impact of the tested trait onto the distribution of *Wolbachia* strains in quill mites and might also hint at potential pathways of *Wolbachia* transmission.

Why should we investigate this problem? Mainly because it will significantly advance our knowledge of the actual diversity of undoubtedly intriguing endosymbiont as *Wolbachia* is, possible spreading strategies and evolution of this bacterium. But that is not the whole truth. We also do this because we know that things are never as they seem (birds pecking seeds in the feeder are not only birds)... They are always much more fascinating!