



Studying lichen-mite interactions on the example of *Cladonia norvegica* Final report

1. Team

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2. Project focus

The initiative lasted from July 1st till December 31st 2023. We focused on the lichen-mite interactions and studied *Cladonia norvegica*, a lichen species characterised (apart from other characters) by red spots on the thallus. These red spots are caused by rhodoclanic acid (otherwise known from the fruiting bodies of red-fruited *Cladonia* species), and it is thought that the lichen produces this substance in response to mite infection and feeding.

This topic was previously rather neglected, but two master's theses began to address it independently in Austria and Czechia in 2022 (Věra Vtípilová in Prague, Maria Liebmann-Reindl in Graz). The focus of the theses overlapped in some ways and differed in others, which made it a very good starting point for collaboration between the two institutes.

3. Programme and schedule

We planned the following activities:

1. Joint field trip – kick-off meeting, lichen-mite excursion, fresh samples collection – August 2023, 5 days
2. Identification of collected material (following 1-2 months)
3. One-month stay of Věra Vtípilová at the University of Graz – fieldwork, molecular identification of the mite material, work in GZU herbarium, knowledge exchange with Maria Liebmann-Reindl and Tobias Pfingstl about cafeteria experiments – October 2023
4. Visit of Jana Steinová and Jan Mourek at the University of Graz – lecture, work in herbarium, discussing further cooperation – October 2023, 5 days
5. Visit of Philipp Resl and Tobias Pfingstl at the Charles University – lecture at the Seminars of the Departments of Botany and Zoology, discussing further cooperation – November 2023, 5 days

We managed to implement all planned activities except the last one. The kick-off meeting was held between August 7th and 11th. Apart from an excursion to the Handalm area, where we collected *Cladonia norvegica* and other lichen species, we observed the collected material and studied mites present in the collected thalli. Interestingly, the first record of the mite *Tricheremaeus abnobensis* for Styria was made. Furthermore, we summarised the results produced by both teams and planned the next steps. We also discussed methodologies used by Czech and Austrian teams.

Věra Vtípilová stayed from October 14th to November 14th at the University of Graz, where she cooperated closely primarily with Tobias Pfingstl and Maria Liebmann-Reindl. First, she collected a fresh material of *Cladonia norvegica*, which she later used for cafeteria experiments. She also learned



how to extract DNA from mites and sequenced the mite material from Austria, the Czech Republic and Norway. These results will be used in her master's thesis.

Jan Mourek visited the University of Graz from October 17th to 20th and worked together with Tobias Pfungstl, Věra Vtípilová and Sylvia Schäffer, a molecular taxonomist, who has extensive experience with molecular taxonomy and phylogenetics of oribatid mites. The main aim of the visit was to design feeding preference tests with three species of oribatid mites (*Carabodes areolatus*, *Carabodes marginatus* and *Mycobates carli*): to compare: (i) feeding on different parts of the thalus of the lichen *Cladonia norvegica* and (ii) feeding on two closely related species of *Cladonia*. Further, DNA isolation and sequencing of selected gene loci were performed in order to identify the species of oribatid juveniles feeding on *Cladonia norvegica*. The main benefit was that we learned the methods of DNA isolation from individual mite specimens.

Jana Steinová visited Graz in November (from the 6th to the 10th). She discussed with Austrian partners and Věra Vtípilová results achieved so far. Together with Philipp Resl and Věra Vtípilová, she worked on the phylogeny of *Cladonia norvegica*, and they planned the joint publication (description of the new *Cladonia* species planned for the *Lichenologist* journal). Together with all members of the team, we planned future cooperation.

The visit of Austrian team members was not implemented due to teaching-related obligations and because Jana Steinová was sick.

4. Objectives and aims

In the project proposal, we defined the following questions to be answered as the main aims of the project:

1. Is *Cladonia norvegica* a well-defined and monophyletic taxon? If not, how many species are included in this complex?
2. Which group of *Cladonia* lichens does this species belong to (as defined by Stenroos et al., 2019)?
3. Which mite species cause the red reaction on *C. norvegica*?
4. Do mite species from the material collected in Austria differ from those collected in Czechia?
5. Do the mites breed on the lichen?
6. On what parts and what layers of the lichen do the mites preferably feed?

We managed to answer all questions defined:

1. By sequencing three molecular markers of tens of *Cladonia norvegica* specimens collected in different parts of Europe (Czech Republic, Austria, Norway, Scotland, Estonia), we found out that there are two species differing by the presence/absence of the red spots and geography. One of these two species is undescribed. The description of this new species will be one of the goals for our cooperation in 2024.
2. Both species belong to the clade Ochroleucae.
3. The mites causing the red reaction are *Carabodes areolatus*, *Carabodes labyrinthicus*, *Carabodes marginatus* and *Mycobates carli*. We did not observe that the feeding of the adult caused the red staining, but the juvenile developing within the lichen tissue clearly caused red staining.
4. The most abundant mite species present in the thalli of *Cladonia norvegica* from Austria and Czechia did not differ.
5. Yes, the mites breed in the lichen. Adults somehow deposit their eggs into the lichen, and the immature stages dwell in the lichen (and feed on it) until the adult stage is reached. Then they leave the lichen tissue.
6. We could not identify any preference for certain parts of the lichen yet. The mites feed on podetia and squamules, but we need further data to clarify if there are any preferences. The mite preference for green or red parts of the thalli was not observed.

5. Outcomes and results

We managed to establish cooperation between the lichenology and acarology research groups of Charles University and the University of Graz. We are working on the joint publication based on the data obtained so far, which will be finished in spring/summer 2024. We also applied for another Aktion bilateral cooperation for the year 2024. Dr. Philipp Resl applied for the FWF project with Czech partners as collaborators.

The members of both teams exchanged knowledge in different areas (e.g. DNA extraction from the mite material, design of cafeteria experiments, mite identification, *Cladonia* identification etc.).

Věra Vtípišlová and Maria Liebmann-Reindl worked together on their master theses, which will be defended in 2024. The students keep discussing their results.

The whole team regularly discusses the results achieved and plans further progress. This is done either through regular email communications or online meetings (with a frequency of approximately once a month).

6. Photos



Figure 1: A, B: field trip and material collecting, C: presenting results and discussions, D: mite identification

In Prague



Mgr. Jana Steinová, Ph.D.

In Graz



Doz. Mag. Tobias Pflingstl, Ph.D.