

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

Final report

1. Team

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2. Project focus

The initiative lasted from July 1st till December 31st 2024. The project thematically built on and extended the project “Studying the lichen-mite interactions on the example of *Cladonia norvegica*” carried out in 2023 in cooperation between Charles University and the University of Graz. In both projects, 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 rhodocladonic 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.

In 2024, we built on and expanded the activities carried out in the previous year. We focused on finalising the phylogeny of the mycobiont of *Cladonia norvegica*. We also analysed the photobiont diversity associated with this species. Other thematic areas were the complementation of mite-feeding experiments and the whole genome sequencing of *C. norvegica*.

3. Objectives and aims

In the project proposal, we defined the following goals:

1. To include more material of *Cladonia norvegica* and genes studied for the lichen-taxonomical study so that the results can be used for the preparation of the scientific paper
2. To publish the results of the lichen-taxonomic study in a peer-reviewed scientific journal (e.g. Lichenologist)
3. To sequence the COI-2 and 18S regions of the nymphs present in the lichen thalli since they cannot be identified morphologically (in contrast to adult mites)
4. To study the effect of seasonality on the species composition of the mite communities in the lichens
5. To sequence and assemble the whole genome of *Cladonia norvegica* and other *Cladonia* taxa

Here we describe how we managed to achieve them:

1. We included more material of *Cladonia norvegica* (specimens from Central Europe, Estonia, Norway, Canada and the United Kingdom). We also sequenced two photobiont markers: algal ITS rDNA and actin locus I.
2. We worked on the manuscript describing the new lichen species (title “*Cladonia rubrotincta*, a new species distinguished from *C. norvegica*”). We decided to add HPLC and LC-HRMS analyses of the red pigment which were not planned originally. This led to a delay of approximately

three months. We plan to submit the manuscript to the *Lichenologist* journal in March/April. The manuscript draft is attached to the final report, and AKTION is acknowledged.

3. We sequenced and identified the nymphs present in the lichens as planned. Results are summarized in the diploma theses of Maria Liebmann-Reindl and Věra Vtípilová (attached).
4. Not implemented. Instead, we focused on expanding the feeding mite experiments so that the results could be published in a peer-reviewed scientific journal. This was accomplished during the stay of Heda Ghlimová at the University of Graz in December 2024.
5. We sequenced the genome of *Cladonia norvegica* on the Illumina platform. We were not able to perform the long-read sequencing on the Oxford Nanopore Technology MinION because we didn't have a sufficient amount of fresh lichen material. *Cladonia norvegica* s.str. only grows in Central and Northern Norway, so we were unfortunately unable to collect the fresh material. However, the results of Illumina sequencing are sufficient for answering the questions we defined.

4. Outcomes and results

We successfully strengthened and deepened the collaboration between the lichenology and acarology research groups at Charles University and the University of Graz. As part of this initiative, two diploma theses were defended in 2024:

- Maria Liebmann-Reindl: "*Biologie, Ökologie und Entwicklung der mit der Flechte Cladonia norvegica assoziierten Hornmilben*"
- Věra Vtípilová: "*Mites and lichens interactions on the example of Cladonia norvegica*"

The students collaborated closely, comparing and discussing their results. Věra Vtípilová visited the University of Graz in May 2024, where she conducted the final set of experiments for her master's thesis and analyzed the results obtained so far.

Additionally, we made significant progress on a joint publication describing a new lichen species, which is scheduled for submission to *The Lichenologist* in March/April. The manuscript is based on data from Věra Vtípilová's thesis, supplemented with a study on associated photobionts. We also decided to conduct an additional chemical analysis of the red pigment to confirm its identity. This was done in collaboration with colleagues from the Microbiological Institute in Prague. The manuscript is co-authored by Dr. Einar Timdal from the Natural History Museum in Oslo, who worked on *Cladonia norvegica* over 30 years ago and facilitated the collaboration between Charles University and the University of Graz.

Further data for another publication on feeding experiments were collected throughout 2024, primarily by Heda Ghlimová during her one-month stay at the University of Graz. Additionally, she collected lichen material for her PhD thesis on serpentine rock outcrops near Kirchdorf (close to Bruck an der Mur). In 2025, she plans to return and continue this collaboration.

In late October, Jana Steinová visited Graz, where she and Philipp Resl focused primarily on the joint publication's progress. We also identified additional research directions, as we consider our collaboration highly successful, beneficial, and mutually enriching for both institutions. To support further cooperation, we applied for another *Aktion* bilateral cooperation grant for 2025. Additionally, Dr. Philipp Resl submitted an FWF project proposal with Czech partners as collaborators.

The entire team regularly discusses progress and future plans through email and online meetings. Unfortunately, the planned research stays of Dr. Tobias Pfingstl and Dr. Philipp Resl could not take place due to increased teaching responsibilities. Moreover, Dr. Pfingstl recently secured a large research project requiring his full presence in Graz. Nevertheless, he actively contributed to the initiative remotely.

This project has allowed us to expand the thematic scope of our future collaboration. We plan to focus on lichen (phylo)genomics, particularly studying problematic groups within the genus *Cladonia* and exploring the functional implications of genomic differences in these species. Additionally, a Master's student in Graz will continue to work on the juvenile mite stages found in *C. norvegica*. All

these topics hold significant publication potential and are of great interest to the lichenological and acarological community.

5. Photos

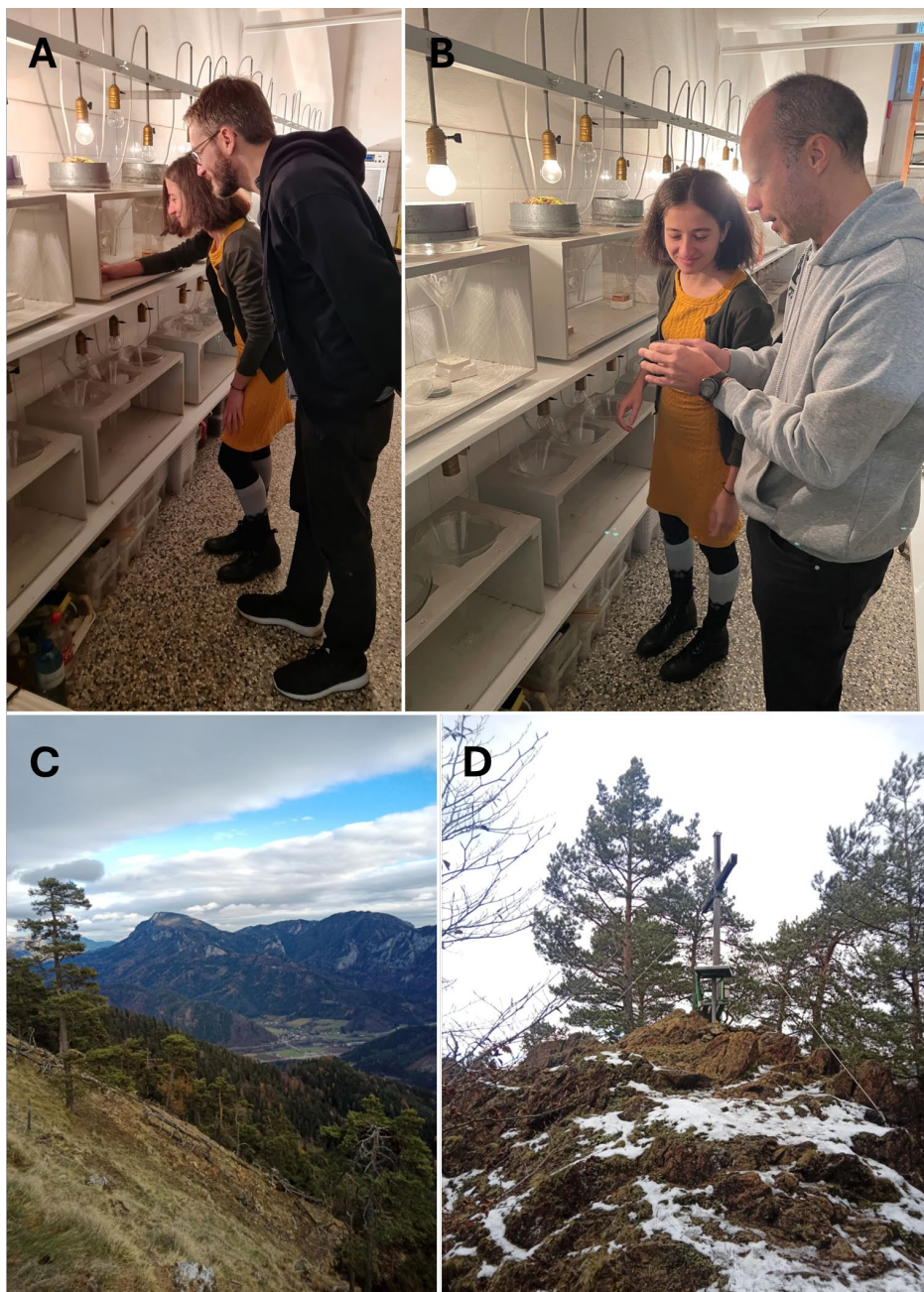


Figure 1: A, B: setting of the mite-feeding experiments (Heda Ghlimová, Philipp Resl and Tobias Pfingstl), C, D: serpentine locality close to Kirchberg where Heda Ghlimová collected lichen material for her PhD thesis

In Prague



Mgr. Jana Steinová, Ph.D.

In Graz



Dr. Philipp Resl