WRITTEN ASSESSMENT OF THE THESIS

The study of photobionts in the lichenized genus *Lepraria*

by

ONDREJ PEKSA
Charles University in Prague

Made by:
Prof. EVA BARRENO
University of Valencia, Spain

Evaluation of specific objectives and their relevance.

This study is an interesting and original approach to understand the taxonomical characteristics (morphological and DNA sequencing, the biodiversity and the specificity of lichenized microalgae (phycobionts) in relation to their fungal partnerships (mycobionts) and/or their ecological relationships.

To achieve these objectives the author has investigated more intensively the species included in the genus *Asterochloris* and only two of the genus *Trebouxia*. He mainly has worked in three ways:

1. Analyzing the chloroplast structures and their changes in relation with different culture conditions.
2. Analyzing the microalgae growing in lichens colonizing heavy metals substrata.
3. Analyzing the biodiversity, the specificity in relation to mycobionts and the ecological conditions of the thalli collected in different areas and habitats.

All this objectives are very relevant in terms of actual scientific knowledge.

The thesis is structured in one broad General Introduction, followed by clearly defined objectives, aims and outline of the thesis. Several chapters on which specific hypothesis/ questions are tested with a specific body of experimental research included in each of the papers published. It finalizes with a chapter of general conclusions, two appendixes, a C.V. and the publications of the author.

I suggest, if possible, that the conclusions should include a broad view of all the thesis achievements and future work more than a sum of published papers

conclusions. The index of contents is difficult to understand through Paper 1, Paper 2, etc., other indications should clarify which papers are to the readers.
The amount of work and the quality of the content is adequate for a PhD thesis. Moreover most of the work was already published showing the relevance of this work.

QUESTIONS TO THE CANDIDATE:

1. You described in paper 1, your methodology to isolate and propagate the phycobionts from lichen thalli. I consider this type of isolation may produce lots of contaminants and it is nearly impossible to obtain cultures from only one cell, to be sure you have only one taxon or only one genotype of microalgae. How are you sure that in the small populations you transfer to BBM 3N and to Trebouxia medium, only one species of microalgae is propagated?

2. Do you consider that HOMOLOGY and SIMILARITY are equivalent concepts? (see page 53)

3. In paper 2, p. 72: "2.6. sequences of ITS variants were reconstructed from the sequences containing ambiguities, etc. Those containing a single ambiguous position were resolved as two variants differing at the single position, etc.". Do you know the paper by Nieto Feliner and Roselló 2007: MPE 44: 911-919?. Just there, they propose interesting guidelines for insightful utilization of nrDNA ITS in species-level evolutionary studies. With some of the results you obtained in this work, may you discuss if the optimal way to resolve these ambiguities could be through the verification of the polymorphic sites and cloning of the PCR products?

4. What do you consider that "multiple photobionts in a single thallus" means?. Have you found different species of algae in a thallus?

5. Why you only use ITS regions and actin markers to identify photobiont species?. Why you never use chloroplast markers?. Have you designed new molecular markers to clarify important problems in the species identification in lichen algae?

6. In paper 3, you found that three clades (A2, A12 and A13·) achieved low levels of statistical support, however, they were statistically supported in other previous paper. Other weakly supported lineages were inferred with a high statistical support in previous studies by you and other authors. Do you have a realistic explanation for these results?. Why you never tried to use the TCS programme (Posada) to identify the algal haplotypes?