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Review of the PhD entitled 'Contribution to the taxonomy and biodiversity of crustose lichens from the family Teloschistaceae' by Ivan Frolov

Overall assessment

The thesis focuses on different aspects of the taxonomy and diversity of microlichens from the family Teloschistaceae, with a major focus on the diversity of the family in Central Europe, and especially, in Russia.

The grounds of the thesis are clear and sounded, in a highly diverse family of lichen-forming fungi such as the Teloschistaceae, there exist numerous problems regarding the taxonomy and systematics of different groups and there is scarcity of knowledge on the diversity of many problematic or poorly known group, species and geographic areas. Thus, the different chapters address these problems from different points of view.

The thesis counts on six different chapters and a solid introduction in which the topic of lichen-forming fungi taxonomy is reviewed as well as the topics of species recognition and delimitation and the problematic of the taxonomy in the family Teloschistaceae. The introduction and all chapters are well documented and include the state-of-the-art literature in each case. All chapters have been already published in peer-reviewed journals so they have past the filter of qualified reviews by expert colleagues (journals: *Journal of Sytematics and Evolution, The Lichenologist (x3), Chornomorsky botanishniy zhurnal, Annales Botanici Fennici*). Follows, I summarize the chapters:

I. The first chapter is the more ambitious. It analyses the phylogenetic relationships within the genus *Pyrenodesmia*, a lineage of Teloschistaceae characterized by the lack of red anthraquinones as well as the systematic position of other groups lacking anthraquinones in the whole thallus or part of it. In this chapter the genus *Kuettlinderia* is resurrected and *Sanguineodiscus* is described. The dataset utilized is large and results are solid and highly interesting. The study covers a large range of species and includes sophisticated analyses of the molecular data.

II. The second chapter deals with the taxonomic characters used in the study of the Teloschistaceae. The chapter makes a thorough review on the characters traditionally used, the concepts of some of them and the way they should be measured for a proper taxonomic praxis. This is a rare and very useful chapter. Although general floras usually include dictionary of terms, it is not easy to find this information for a certain group. I think this chapter is extremely useful for future researchers and students who want to work in the family Teloschistaceae.

III. This chapter describes three new species for science of the *Pyrenodesmia* group. New species have a molecular and phenotypic ground, and although they could be characterized as almost cryptic, some characters are given that allow distinguishing them. A key for the group is provided.

IV. This chapter describes three new species and report two rarely collected species not known from Central Europe. New species are described based on thorough anatomical examinations as well as using molecular methods to inspect their phylogenetic affinities.

V. This chapter shows extensions in the geographic ranges of several poorly known species, especially concerning the Russian territory. Further, using ITS phylogenies, several new phylogenetic relationships in the genera *Calogaya*, *Caloplaca*, *Flavoplaca* and *Gyalolechia* are shown.

VI. Three new species of *Caloplaca* s. lat are described, one belonging to *Caloplaca* s. str., one in the recently described genus *Lendemeriella* and other in *Orientophila*. Likewise in previous chapters, species are described based on thorough anatomical, chemical and morphological studies and confirmed using molecular methods.

To sum up, I think that the PhD thesis presented by Ivan Frolov fully meets the high standards for a doctoral thesis at the University of South Bohemia in České Budějovice, and I have no hesitation to recommend Ivan for a doctorate award.

Since all chapters have been already published I do not have clear objections or problems regarding methodological aspects, and my questions are rather general, mostly personal interests on the taxonomy and systematics of the family and general questions on the delimitation of lichen-forming fungi, especially in such a diverse group:

1. The introduction covers a large number of issues regarding species delimitation in lichen forming fungi, with the corresponding problems, however I have missed one point that I consider extremely useful for delimiting species: time. Could you please explain, how using dated phylogenies may contribute to species delimitation? and how do you think this information could help in the taxonomy of the Teloschistaceae?

2. As a simple user of Teloschistaceae generic concepts, I am astonished with the inflation of generic names in the family in the last decade. However, it seems not everybody is accepting all new names. I would like to know your opinion about the current split of Caloplaca *s. lat. in putatively more natural units and also, what should be the rules for describing new genera in order to avoid spurious descriptions.*

3. You have focused a considerable part of your research studying the family Teloschistaceae in Russia, according to your current knowledge do you consider the region well-known? Which are the worst known regions? Very briefly, if you had the funding to do it, how would you design a project to increase the knowledge on the diversity of the family in the area?

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THE NEW YORK BOTANICAL GARDEN

14 May 2021

Dear Dr. Kucera,

Thank you again for the opportunity to serve an external reviewer and member of the defense committee for Ivan Frolov. It was a pleasure to read the dissertation entitled *Contribution to the taxonomy and biodiversity of crustose lichens in the family Teloschistaceae*. The dissertation focuses on taxonomy, systematics and biogeography of selected lineages within the highly speciose lichen family Teloschistaceae. All but one of the chapters of the dissertation have already been published, and the final chapter is currently accepted for publication.

As an expert on asexually reproducing crustose lichens, and someone with a general interest in temperate Northern Hemisphere lichen diversity, I have followed this research as it has been published. Despite the fact that Teloschistaceae are among the most conspicuous and diverse groups of lichens, they remain remarkably understudied and poorly known. Names seem to have been very broadly applied in the past, and morphological concepts have been inconsistent. It has become increasingly clear that a combination of detailed molecular sampling, extensive fieldwork, and careful morphological study are needed to accurately circumscribe taxa in this group. It is also clear that there are far more taxa than most workers previously thought.

From my perspective this dissertation presents an excellent series of studies that form a body of work that has propelled understanding of Teloschistaceae forward, especially at the species level. In many ways it reminds me of what I attempted to do with *Lepraria*, especially chapter 2 which attempts to [finally!] bring introduce a clear standardization to descriptive morphology in this group. Taken together, this work clearly constitutes a significant, original scientific contribution. Below are a series of questions that were raised during my reading of the dissertation:

General comment

i) Only three of six chapters were first authored by the person who is defending their dissertation. There is a breakdown of author contributions presented, and in the other three chapters the contribution of the defending student is listed as 20%. In my experience at U.S. institutions it is not allowed to include material not lead authored by the student because other authorship positions imply that the person was not primarily responsible for the work. My understanding is that this is acceptable at your institution, but I do want to highlight this and ask for a little bit more explanation of how substantive the author's role in these three chapters was.

1) Introduction

Comment: The introduction presents a general overview of lichens, phylogenetics and species delimitation. Overall it is a reasonable summary of this topic, I am curious as to how the following questions would be answered given that they relate so closely to the published thesis chapters:

1a) There is increasing evidence of the role of the bacterial microbiome in lichens, and much attention has recently been drawn to the finding of basidiomycete yeasts on the thalli of lichens. In the introduction it is stated that because there was a correlation between the presence of yeasts and phenotypic characters (in this case abundance of a chemical pigment) that is evidence the yeasts function as the third primary member of the symbiosis. Can you please elaborate on this and explain why apparently influencing the

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concentration/production of a pigment is evidence of a functional role in a symbiosis? If the lichen symbiosis is obligate, does this serve as evidence that the lichen cannot survive without the yeast? Are there examples from other groups of organisms.
1b) In the species concepts section there is heavy citation of several general review papers. While I understand the temptation to cite these sorts of review papers, I think it's important to acknowledge that these are not quantified meta-analyses. In fact lichenology is remarkably devoid of such studies and reviews such as those cited have a way of advancing perspectives using selected illustrative examples rather actually quantifying all the studies on a subject in an objective way.

1c) Of the statement "Lichens display few taxonomically useful characters and many of them are widely variable; the homology of character states within and between groups is difficult to assess (Printzen 2010). Consequently, molecular genetic data has gained great influence in the species delimitation of lichens (Grube and Winka 2002, Printzen 2010, Lumbsch and Leavitt 2011, Leavitt 2015)." Do you really believe, given all the phenotypic characters used in the published studies, that lichens have "few taxonomically useful characters and many of them are widely variable"? And do you believe that molecular data have become widely employed because of this? This effectively states that liches have no morphology and molecular data are the only way to effectively delimit species. Does that agree with the published chapters?

1d) The introduction states that molecular data can be used to delimit species, and that a voucher of extracted DNA can be the type, but that these taxa are not accepted. An example from the *Code* is cited (reproduced below). Does this support the statement? Perhaps see Tripp & Lendemer 2014 (https://www.jstor.org/stable/taxon.63.5.969) and Renner 2016 (https://academic.oup.com/sysbio/article/65/6/1085/2281632) neither are cited.

From Turland et al. 2018:

40.5. For the purpose of Art. 40.1, the type of a name of a new species or infraspecific taxon of microscopic algae or microfungi (fossils excepted: see Art. 8.5) may be an effectively published illustration if there are technical difficulties of specimen preservation or if it is impossible to preserve a specimen that would show the features attributed to the taxon by the author of the name.

Ex. 6. Lücking & Moncada (in Fungal Diversity 84: 119–138. 2017) introduced *"Lawreymyces"* and seven intended microfungal species names using representations of diagnostic sequences of bases of DNA from the Internal Transcribed Spacer (ITS) region as intended types. These representations are not illustrations under Art. 6.1 footnote because they are not depictions of features of the organisms, and consequently the intended names were not validly published.

1d) Some elaboration on species pairs and reproductive modes is needed. The introduction states that these pairs consist of a sexual species and a vegetative species. This implies there is a strict dichotomy between sexual and asexual reproduction. But in the vast majority of lichens is this true? How many asexually reproducing species are strictly asexually reproducing? What are the implications of this for the species pair concept in lichens? (For some discussion see Lendemer et al. 2016: https://www.tandfonline.com/doi/abs/10.3852/14-263?journalCode=umyc20).

1e) There is also an entire section about how species in species pairs have not been recovered as reciprocally monophyletic and almost all the examples in this section are more than a decade old. From my perspective this does not accurately characterize the literature. For instance, *Porpidia* is often cited as a case where molecular data have demonstrated that species pairs do not exist. Yet when studied in detail with comparative sampling, *Porpidia albocaerulescens* and *P. degelii* were found to be a species pair.

The topic is discussed in Lendemer & Harris 2016 (https://bioone.org/journals/castanea/volume-79/issue-2/14-006/Studies-in-Lichens-and-Lichenicolous-FungiNo-18--Resolution-of/10.2179/14-006.short). As the citation of the RADseq study of *Usnea* that is cited in the introduction suggests, is it not possible that the molecular loci commonly used in lichens may simply be insufficient to address this question? Lichenologists routinely use single loci to infer population structure and population genetics statistics, even though this is a violation of basic assumptions of the methods. Could this not also be the case with species pairs?

1f) I feel the need to point out that for some reason in discussion of molecular data and genomic methods, the first population genomics study of lichens is not cited, and it was published by one of my students. (Allen et al. 2018; https://bsapubs.onlinelibrary.wiley.com/doi/pdf/10.1002/ajb2.1150). That worker's research group continues to be at the forefront of molecular advances in lichens (McKenzie et al. 2020, https://www.sciencedirect.com/science/article/abs/pii/S0888754320304614?via%3Dihub). Also in discussing the use of molecular data...and types of molecular data...there is a meta-analysis on this subject (Hoffman & Lendemer 2018; https://bioone.org/journals/the-bryologist/volume-121/issue-2/0007-2745-121.2.133/A-meta-analysis-of-trends-in-the-application-of-Sanger/10.1639/0007-2745-121.2.133.short).

2) Phylogenetic relationships in *Pyrenodesmia*
Comment: As someone whom has been confronted with gray, Sedifolia-gray only, species of *Caloplaca* s.l. this was exactly the study that I have been waiting for to finally accept *Pyrenodesmia* as a genus. Deposition of the molecular alignments is greatly appreciated, for some reason this is still not considered a basic requirement in lichenology. The authors deserve credit for doing such careful work and making it repeatable.

2a) In this chapter (and I suspect throughout the chapters) names described before the type concept are listed as having holotypes (e.g., *L. albolutescens* Nyl., *L. atroflava* Taylor). Do these protologues actually meet the standard for indication of a holotype currently applied in the botanical literature wherein a single specimen in single herbarium must be explicitly cited? Or are these cases where it is not clear from the protologue and names actually need to be lectotypified?

2b) In the description of *Sanguineodiscus* I am curious why the authors opted for a short descriptive diagnosis (common in lichenology for some reason) rather than a differential diagnosis (not common in lichenology). Either is acceptable, but why is a differential comparative diagnosis not preferred?

3) Methods in phenotypic evaluation
Comments: This is a great contribution and something I wholeheartedly support.

3a) I have to take issue with the definition of "granule" used here. If a granule is physically connected to the surface of thallus structure how is it not an isidium? When a thallus is formed entirely of granules then these are not connected to anything and such, have a very clearly different ontogeny as the form the basic unit of the thallus. See the extensive discussion by Lendemer 2013 (https://www.cambridge.org/core/services/aop-cambridge-core/content/view/S0024282911000326).

3b) I think "pycnidial tops" is not what was intended.

3c) The use of "suppressed apothecia" is awkward to me because the word suppressed implies that they are kept down and do not form. In America we have voter suppression and that means that people are prevented from voting. Apothecial suppression would mean preventing apothecia from becoming apothecia.

4) Three new, seemingly cryptic species
Comments: This is a nice integrative study of molecular and phenotypic data to address taxonomy. It's a good example of modern lichen taxonomy at the species-level. The key is well-constructed and I greatly appreciate the use of strictly contrasting couplets.

4a) Where are the datasets for this study deposited? Forgive me if I missed it.

4b) I am a little curious as to why there are no distribution maps presented.

5) New crustose Teloschistaceae in Central Europe
Comments: This is another good study combining molecular data and phenotypic data to address taxonomy of these lichens in one of the best-studied areas of the world. Inclusion of the distribution maps is greatly appreciated!

5a) A general comment that I have is that there is no consistency in how synonymies are presented. If synonyms are listed, then types should be listed, and names should be arranged in paragraphs of homotypic synonyms clearly separated by identity (≡). I understand that for instance, *Caloplaca interfulgens* is just getting some new reports added here, but the authors list only the current name and the basionym (not with ≡). I don't understand why lichenologists list only the current name and the basionym as though there are not other homotypic synonyms which should be listed (in the case of *L. interfulgens* the name has been combined in *Placodium*, *Gyalolechia* and *Xanthocarpia* as well, but there is no way to ascertain this from the paper; and the type isn't cited so how can we be certain the authors are applying the name correctly?)

6) The extensive geographical range of several species of Teloschistaceae
Comments: I like this paper quite a lot because it deals with attempting to resolve the distributions of species and does so in a rather thoughtful and elegant way.

6a) Where are the datasets for this study deposited? Forgive me if I missed it.

7) Three new species of crustose Teloschistaceae in Siberia
Comments: This is another nice taxonomic study much like the others included in the dissertation. It combined molecular and morphological approaches and is a great example of how taxonomy should be done in a group like this.

7a) Where are the datasets for this study deposited? Forgive me if I missed it.

Sincerely,

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