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To whom it concerns

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Here is my review of the PhD thesis of Mgr. Jan Vondrak "The lichen genus *Caloplaca* (*Teloschistales*) and its lichenicolous fungi: contributions to their taxonomy, nomenclature and biodiversity".

Yours sincerely,

Dr. H. J. M. Sipman

It was a pleasure for me to read this thesis. It is a kind of a result that every taxonomist would have loved to produce, a profound revision of a clearly delimited group by investigating all relevant aspects carefully. In normal life taxonomists have to deal with preparing lectures, doing courses, receiving guests, herbarium management, specimen identification, etc. In between they come across some taxonomical problem and as far as time permits they try to tackle this, saving as much as possible on time and materials, and aware that the job is in fact incompletely done. The present work appears the result of ample attention to a well-argued set of research steps, each requiring its own special technique which is carefully applied, and the results are well evaluated and presented in accordance with the newest standards, and under consultation of relevant specialists. At the basis stands a step, which is very basic but rarely done because it is so time-consuming and requires much experience: the author went in the field to the habitats of his study group, and collected representative material of all available populations; thus making himself independent of the usual, often biased and haphazard herbarium collections. These samples were analysed systematically and scrupulously, using the most modern set of taxonomically relevant characters, including the highly specialized pigment chemistry of the epithecium and DNA analyses. The evaluation of the obtained data was certainly not easy because it concerns closely related taxa with only subtle differences. This view of the work may be a bit idealised because the candidate no doubt was also involved in many other activities and the supposed optimal working conditions are mainly a suggestion resulting from a very careful planning of the work. Certainly the thesis shows that the candidate has mastered the discipline very well and was able to select and develop a promising research project and to pursue it to good results. These in my opinion are the core criteria for qualification for a PhD degree and that is what the candidate deserves well.

Questions:

1. One part of your thesis deals with the *Caloplaca citrina* group. This is not an official taxon complying with the rules for botanical nomenclature. Still the group is familiar to me from my identifications with the lichen flora of Clauzade and Roux, but apparently in a different circumscription. Can you explain why you deviate from earlier definitions and follow this circumscription of the group and why you do not treat it as official taxon?
2. Since the remarkable results of Helmut Mayrhofer, Mireilla Giralt and their colleagues with the use of spore ontogeny for classification in the genus *Rinodina* I have become a fan of the spore ontogeny character complex, and have made interesting observations in Graphidales. According to the latest phylogenetic results of DNA analyses, *Rinodina* is rather closely related to Teloschistaceae and *Caloplaca*. Did you pay attention to the value of this character complex in *Caloplaca* and what is your experience? Could there be an ontogenetic link between the two spore types of *Caloplaca calcitrapa*?
3. After the delimitation of the species, the phylogenetic relations between the species are the second most important goal of systematics. Its knowledge is important because all supraspecific categories, like genera, sections, should be in accordance with the phylogeny, and also because closest relatives are likely to have the most characters in common,

which is valuable information to know when a species has come to use for something. Consequently cladograms are a great thing to look at and to discuss. Before DNA analyses hypotheses about phylogeny were too speculative, and now all taxonomists rightly are busy to extract DNA and calculate cladograms, and questions about your cladogram cannot be lacking today. To be honest, I am not actively working in the DNA lab myself, do not be surprised when my questions seem somewhat simple. First one: why do you use *C. holocarpa* as outgroup. It looks very similar to the other species and could be a good candidate for the group; in that case your cladogram would be distorted.

4. Second cladogram question: How certain are you that *C. calcitrapa* has to be included in this group, it looks so much like *C. inconnexa* and *C. polycarpa*. Moreover in the cladogram published by Arup et al. 2006 it seems to have a different position, as sister of *C. arcis* instead of *C. marina* agg. while *C. marina* agg. is sister to *C. flavocitrina*.

5. It is in my opinion very fortunate that you went in the field yourself to sample the representatives of your study group. Non-specialist collectors tend to restrict themselves to the more conspicuous species and to popular localities where many collect the same species, and the holdings of the herbaria reflect this biased sampling. However, with specialist collecting there is a risk to collect selectively what one thinks that are different species. When a modern lichenologists encounters sorediate specimens, some with pronounced round soralia, others with confluent soralia, he tends to think that these might well be two separate species and will collect a specimen with clearly separate rounded soralia and one with confluent soralia. After visiting several localities his collections may become strong evidence for the existence of two species. What have you done to avoid biased sampling?

Jan Vondrak is to be commended for the excellent body of information he has assembled and interpreted on a taxonomically difficult lichen genus. The quality of this work is borne out by the fact that it has been published in internationally recognised and leading journals which have been peer reviewed by several expert lichenologists. Furthermore, I can vouch for the quality of many of his publications as I was involved in their preparation, albeit my revisions were usually of a minor nature and mostly concerned with correcting the English language. Many of his published papers are based on a sound knowledge of the lichen flora of the Czech Republic derived from first-hand experience in the field, from a detailed examination of herbarium material from a wide range of sources and from extensive literature searches. He has provided a firm foundation on which to build not only our knowledge of a key group of Czech Republic lichens but also of these lichens in Europe. Indeed, his work is of international significance. He has thoughtfully developed his research programme in a logical manner as testified by the sequence of his publications. As senior researcher and author, he has profitably joined forces with some leading lichenologists, and the scope and effectiveness of these cooperative projects is excellently reflected in their results and publications. On the basis of his published output and the research work involved in their production, I have no hesitation in recommending Jan Vondrak for a doctorate.

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24.5.2008

Questions

1. Since I have no *curriculum vitae*, I would find it useful to know the academic background to this project, and more particularly why you chose *Caloplaca* as your topic for a PhD.
2. Which earlier researchers and their publications influenced you – and why?
3. How many taxa do you recognise in the *Caloplaca holocarpa* group – and how satisfied are you with this interpretation?
4. What are the biogeographical differences between *Caloplaca albolutescens* and *C. teicholyta*?
5. Which aspect of your work gave you the greatest pleasure, and which published components are you most proud of – and why?
6. What are your future plans in terms of your *Caloplaca* studies – will you, for example, extend your studies beyond Europe?



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To whom it may concern

Review of the PhD thesis

The lichen genus *Caloplaca* (Teloschistales) and its lichenicolous fungi: contributions to their taxonomy, nomenclature and biodiversity

by Jan Vondrák, University of South Bohemia, Faculty of Science, České Budějovice, Czech Republic

Introduction

The lichen genus *Caloplaca* encompasses species with mostly strikingly bright-orange/yellow thalli, thus well visible and recognizable for amateurs and students of lichenology. On expert level, however, we deal with one of the largest lichen genera, with ca 1000 species. They vary greatly in their morphological characters as well as asexual reproduction modes (soralia/blastidia, isidia, schizidia, fragmentation). Jan Vondrak identified a gap in the knowledge of sorediate *Caloplaca* species geographically delimited by the coastal area of the Black Sea. These lichens, with a special emphasis on *Caloplaca citrina* group, but touching also *C. cerina* group and *Pyrenodesmia* group, are a subject of his PhD. thesis. Great advantage to carry out the work on in this respect "terra incognita" of Black sea region must have been Ulf Arup's treatment of *Caloplaca citrina* group in the Nordic countries published at the beginning of 2006. Jan Vondrak successfully got a grip over the theme since the beginning. To get hold of the working material he carried out own fieldwork in Bulgaria, Romania, Turkey, Crimea (Ukraine), Georgia and Russia, to get additional material also in home Czech Republic, Slovakia, Spain and Austria. To get the data on original descriptions he had at his disposal type material from relevant European collections of cryptogams. He studied also other herbarium material kept in these collections. Apart from this he built a network of cooperating experts – a Lichenological Research Group. This, in combination with almost all the aspects of taxonomic work, resulted in he presented book of PhD thesis, which are challenging to be reviewed. I am happy to have an opportunity to read the yet fresh story of the *Caloplaca* as it was observed, perceived and understood by Jan Vondrák.

Assessment

- Presentation and clarity

The outputs of the studies are presented as a book collecting five aspects of the studied topic – taxonomy, nomenclature, biodiversity, hosting lichenicolous fungi and Exsiccata. It is a combination of a manuscript of the main project (*Caloplaca citrina* group in the Black Sea region), reprints of already published articles or manuscripts of either submitted or accepted papers. The stream of information is logical, starting with the most important taxonomical part, through the "technical" aspects of nomenclature, practical aspects of biodiversity and additional elements bringing information on lichenicolous fungi new for science and enabling tool for the other "lichen aficionados" – Exsiccate collection. The text is telling the story clearly, the style is accurate, without unnecessary duplications or repetitions. The reference lists are accurate.

- Integration and coherence

There is logical and rational link between the chapters of the thesis, as already commented above. Some readers, however, might miss a "classical" review of the literature summarizing the up-to-date knowledge in the four topics of the thesis (taxonomy, nomenclature, biodiversity and hosting lichenicolous fungi).

- Contribution to knowledge

The thesis is built upon 8 articles and 1 exsiccate contribution. Out of this number, 1 article was already published in top cryptogamological peer-reviewed journal (Mycotaxon), 2 were accepted in top cryptogamological peer-reviewed journal (Lichenologist), 2 were submitted in top peer-reviewed journal (Lichenologist, Taxon), 3 were published in international peer-reviewed journals (Preslia, Mycologica Balcanica and Graphis scripta). The schedae of the exsiccate were published in Fritschiana, Graz, AT.

Jan in cooperation with other colleagues introduces 9 *Caloplaca* species new for sciences. He lectotypifies 5 species (*Placodium aurantiomurorum* and four *Acharius* species), identified holotypes for four *Acharius* species. He also brings additional knowledge (synonymy of particular names, identification of the species *Candelariella senior* and first report for Algeria). More than 80 ITS sequences were supplied to the GenBank. Two lichenicolous *Opegrapha* species were described new for science. Important findings to the diversity of the genus in the Czech Republic and the countries of the Black Sea region was done (six species new for CZ, lots of information on the occurrence of 19 *Caloplaca* species in CZ was gathered, annotated list of *Caloplaca* species occurring in Bulgaria – 50 species listed, 17 reported first time from the country). Very interesting and currently not frequent action is to prepare exsiccate collection, which Jan did. Moreover he studied *Caloplaca* also as a host for other organismi – lichenicolous fungi and described two new lichenicolous *Opegrapha* species for science and reported on a potentially distinct species related to *Opegrapha rupestris*. This brief enumeration of the outputs is an evidence of a high standard of the thesis.

- Originality and creativity

The PhD thesis show Jan Vondrak's great level of independent working, which is in countries with a few number of lichenologists a crucial precondition for success. He created a strategy to deal with the taxonomy of the group. He acquired skills in molecular techniques during the Synthesis supported stay in Denmark, incorporated due study on chemistry of the species (HPLC-MS) and cooperated with the international experts in the topic.

- Review of relevant literature

As is already mentioned in the section "Integration and coherence", a "classical" review of literature is missing in the book. However, each separate article (either manuscript or already published) comprises introduction with an overview of the status quo of the studied problem.

- Statement of the research problem

Research problems are clearly stated in in each separated article and the need why to address them is worthwhile was accurately justified.

- Methods of enquiry adopted

Sense of planning is clearly visible in the work. This includes a reasoned consideration of the techniques that the chosen methods require. As already mentioned in the section Originality and creativity, all relevant methods were thoroughly applied in the study: classical anatomy and morphology, chemistry (HPLC-MS), genetic markers (ITS sequences). Jan Vondrák clearly demonstrated why each particular analysis was conducted, how it was done, and what the analysis tells about the data.

Summary

The results of the thesis were either already published in a peer-reviewed journals or underwent the review procedure, hence underwent a review procedure and major questionable issues were already put in place, or in case of submitted manuscripts they are undergoing it. I would like to ask Jan Vondrák the following questions:

- Most of current works on genetic relationship of the species within different Caloplaca groups has been inferred from nrDNA ITS (e.g. Arup 2006 – Caloplaca citrina group, Caloplaca havaasii, Muggia et al. 2008 - Pyrenodesmia). Do you think it is appropriate to use only this gene to resolve the relationships between the species/cryptic species of Caloplaca citrina group? Is it sufficient?
- Available morphological characters do not enable to frame the newly described species and already known species of the Caloplaca citrina group in a morphological species concept, the species are considered cryptic. Also above the species level the phylogenetic analysis revealed 3 large groups within the Caloplaca citrina clade, which are internally diverse (p. 10). How important is this finding? Are there any characters which might serve as “key” characters to these assemblages?
- The characters other than genetical - morphology, anatomy, substrate preferences, microclimatic conditions, “preferable” zone on the coast, alternatively accompanying species, are very variable within the studied clade, or they are shared with other, phylogenetically unrelated species (e.g. C. dolomiticola group). At the end a set of vegetative characters (p. 11) was found useful. Were these characters, the data set, submitted to any multivariate analysis to get an overall picture of the situation prior to the conclusions about their importance?
- The specimens assigned to Caloplaca confuse from Georgia, Russia, Turkey and Ukraine (p. 18) were not confirmed by molecular data. Do they represent any other entity?
- One of the results of the study “Typification of names of selected taxa described by Acharius and now placed in Caloplaca” (p. 69) is that “Lecidea caesiorufa var. festiva and Lichen craspedius are illegitimate names and both are automatically typified by the type of Lichen arenarius.” This is stated in Abstract. What is the type of Lichen arenarius Pers (basionymum of Caloplaca arenaria)? Which article of the International Code of Botanical Nomenclature justifies this operation?
- Regarding the report on Caloplaca flavocitrina in Italy on p. 20 – <http://dbiodbs.univ.trieste.it/italic/italic02a> indicates its potential distribution in Trentino Alto Adige, with a general comment that the species needs a thorough revision. Have you seen any specimens from that area?
- Tab. 2. p. 36 gives an overview of antrachinone contents in selected species in the Caloplaca citrine clade. The numbers refer to the percentage proportion?

Conclusion

The PhD thesis of Jan Vondrák are of high standard and meeting all the criteria which are set. The success is undoubtedly due to a strategy to create a working group and thus proceed in the research. After successful defence of the PhD thesis I highly recommend that Jan Vondrák is given the PhD degree.

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