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**Evaluation of the PhD thesis of Mag. Jaroslav Šoun  
“Taxonomy of selected groups of the genus *Caloplaca*”**

Lichens are one of the most typical examples of symbiosis and more than 17.000 species have been estimated to occur worldwide. The lichen genus *Caloplaca*, for which more than 500 species have been recognized, is one of the species-richest genera of lichenized Ascomycota. It includes species characteristic for their bright thalli which show a high morphological diversity. Due to its intricate nomenclature and the systematic position of the species, the genus *Caloplaca* was the interest of several authors in the past and nowadays phylogenetic analyses are increasingly used to resolve the affiliation of its taxa and delimiting subgroups. The recently published molecular studies presented new phylogenetic relationships which could not be completely satisfied by morphological analyses alone. Though, this genus still needs a large-scale study to be safely accommodated in the family Teloschistales. Further, the morphologic and phylogenetic approaches often complement each other, but at the same time encounter also different concepts of species, namely morphologic and phylogenetic species concepts, which have to be coped with.

Mag. Jaroslav Šoun focused his study on circumscribed groups of *Caloplaca* in Europe, the *Caloplaca cerina*- groups, *C. citrina*-groups and additional taxa not included in these two groups, for which he presents taxonomic revisions and phylogenetic analyses. In Europe the genus *Caloplaca* is well-known and many recent studies revealed new species, some of which are part of the present PhD thesis.

The work is divided into several chapters. A first introduction giving a general overview on the taxonomy and systematic of the genus *Caloplaca* is followed by six chapters, each focusing on morphological, taxonomical or phylogenetic analyses for selected taxa. The chapters are already accepted or already published contributions in lichenological journals, compiled between 2008 and 2011, and are presented here in their published form. Each paper includes a detailed taxonomic study. In some cases the analyses of phenetic characters is complemented by phylogenetic analyses of the nuclear rDNA marker ITS.

Mag. Jaroslav Šoun is the sole author of one paper and coauthored the remaining five with different degree of contribution.

The first two papers present a combination of morphological and phylogenetic data for species of the *Caloplaca cerina*-group in Europe. The selected samples were analyzed in their nuclear internal transcribed spacer rDNA locus (ITS), and morphological characters were used to compile determinations keys of fertile and saxicolous species in the group. A detailed description of each species follows.

In the second three papers Mag. Jaroslav Šoun and coauthors principally dealt with a taxonomic revision of selected *Caloplaca* species. The author(s) analyzed a great number of specimens and consulted in details old literature, trying to clarifying the nomenclatural status of the taxa.

In the last paper, Paper VI, the authors combines morphological and molecular data to demonstrate the genetic identity of the two different phenotypes and ecotypes of *Caloplaca phlogina* occurring in North Europe and Black Sea regions.

The present PhD thesis represents a source of profitable contributions for any lichenologist. As it is a collection of already published scientific contribution, the revision of each of them is beyond my scope. Therefore I would like more to focus on the results of these works and on the opinions and questions that these information raise in the scientific lichenological community.

Likewise it happens more and more in the last years, lichenologist have to cope with different species concepts in order to place newly described or resurrected taxa, either at the species lever or at higher ranking in the lichen systematic. *Caloplaca* species are not excluded from this context, which is well demonstrated by Mag. Šoun and coauthors. Phylogenetic species concepts, when applied, are not always corroborated by morphological data and cryptic speciation can be assumed to happen, or morphologically homogeneous species do not present a clear monophyletic relationship, as the case of *Caloplaca cerina*. Alternatively, genetically undistinguishable taxa may differ only for few morphological characters or even represent different chemotypes or ecotypes, as *C. phlogina* and *C. scythica*, previously segregated and now synonymized. How can lichenologist properly resolve this issue? Maybe this question will still remain without answer, as the segregation of taxa depends on the type and the number of characters analyzed, and these vary from lichen group to lichen group. In some cases one genetic locus may not be even satisfying, and comparison of the genetic signal of multiple loci is required to get a proper resolution. The ITS locus is commonly used in studies of closely related lichen species, and was the one presented in the published papers. Thought, it may not always be a useful marker to test monophyly. Which other marker would the author compare in order to find a better resolution and support for the circumscribed groups of the analyzed *Caloplaca* species?

In several cases among lichen specie an unclear genetic pattern is seen and process of incomplete lineage sorting or alleles fixation are speculated. How would it be possible to figure out this in the *Caloplaca cerina/stillicidiorum* group, in which several small "ITS-clades" are recognized, the majority of them correlated to ecological features?

Interestingly, *Caloplaca* species are well ecologically characterized and we hardly find taxa able to colonize different substrata, i.e. bark and rock. Is this a phenomenon which directly and exclusively involves the mycobionts and its propagation mode, or is there any preference for the algal partners

involved? Are the genetic different *C. cerina* associated to different photobionts according their ecology (as demonstrated for other cosmopolitan lecanoralean lichens)?

*Concluding comments*

By reviewing the present PhD work I found it really appreciable that young lichenologist as Mag. Šoun still deal in detail with taxonomy and nomenclatural problems in lichen systematic, as it is supposed to be in lichen systematic, but sometimes forgot, since we are lead to use more direct and modern molecular approaches. Mag. Šoun demonstrates in these contributions his interest and his knowledge in lichen taxonomy and displays his specialization in the genus *Caloplaca*, which will surely prove helpful in the development of his scientific career. Nevertheless, the PhD thesis has in my opinion two weak points: (i) the dissertant is not the first author of all the papers he included in the PhD; (ii) one of the published papers, namely Paper II, was already included in the dissertation of Dr. Vondrák in 2008.

In conclusion, I would like to evaluate with the note “**good**” this PhD thesis

Lucia Muggia Dr. Mag., 28<sup>th</sup> February 2011

Lucia Muggia

## Review of the PhD Thesis submitted by Jaroslav Šoun „Taxonomy of selected groups of the genus *Caloplaca*“

The thesis presents taxonomic research in several groups of the large, paraphyletic and phenotypically heterogeneous cosmopolitan genus *Caloplaca*, published in six articles; the *cerina*, *aurantia* and the *citrina* group were treated.

The dissertation consists of eight main parts. „Introduction“ describes the long history of exploration of the genus and explains the recent progress in phylogeny of the family *Teloschistaceae* and the genus *Caloplaca*, „References“ and six articles published in international peer reviewed journals of high scientific standards. Two of the presented studies bring descriptions of three new species for science (*Caloplaca sterilis*, *C. subalpina* and *C. thracopontica* (Paper II), for four species lectotypes were designated (*C. albolutea*, *C. mydalaea*, *C. virescens* Paper I, *C. aurantia* Paper V) and for *C. aurantia* the neotype has been established (Paper VI).

Used methodology involves several modern biosystematic techniques including the morphometry, HPLC for analysis of lichen substances and sequencing of nuclear ITS region. Several hundreds of newly acquired sequences were uploaded in the GenBank.

The first two papers aimed at solving the complicated taxonomy of the *Caloplaca cerina* group, characteristic by a high level of phenotypic heterogeneity. In Paper II, two new species were described, the subalpine *C. subalpina* and the maritime *C. thracopontica*. Both species differ clearly from each other in both ecology and distribution. In Paper I, more 20 phylopecies within *C. cerina* group could be distinguished using ITS of the nrDNA. Two species complexes do not produce any vegetative diaspores; the polyphyletic, corticolous *Caloplaca cerina* s.lat. consists of six cryptic species and the monophyletic *C. stillicidiorum* s.lat. is well defined by ecological claims.

Polyphyletic *C. cerina* s. lat. was not possible to distinguish because the phenotype-based traditional taxonomy was affected by choosing incorrect diagnostic characters. The study confirms the decisive role of vegetative diaspores between *C. chlorina* and *C. isidiigera*. Lineages producing vegetative diaspores (7 species) are phenotypically characteristic and can easily be determined. Two species complexes without vegetative propagules contains two branches. The second group represents monophyletic *Caloplaca stillicidiorum* s.lat.

Three species were excluded from this group as intricate taxa of unclear identity. Useful result from the practical point of view is a key to 12 fertile species known to belong to the *C. cerina* group.

The third paper presents a revision of two similar lobate species *C. aurantia* and *C. flavescens* of *C. aurantia* group in the Czech Republic. The morphological investigation revealed a new diagnostic character - the thickness of the cortex and confirmed the possibility of segregation of both species according to the presence of hyaline crystals in cortex layer. The author revised all available localities of *C. aurantia* and *C. flavescens* in the Czech Republic and found several new ones for *C. aurantia* in South Moravia, Austria, England, Romania, Spain, Turkey and Ukraine, and for *C. flavescens* in Bohemian Karst, South Moravia, Crimea, England, Hungary, Slovakia and Turkey. In the discussion, the intricate nomenclature of mentioned species was resolved, and the respective entries in our lichen catalogue could be corrected. The neotype of *Caloplaca aurantia* was designated in the fourth article.

The fifth paper is dedicated to the forgotten lobate species *C. aurantiomurorum* from Algeria and Israel. Its basionym, *Placodium aurantiomurorum*, was lectotypified and put into synonymy of *C. aurantia*.

The last article of the dissertation compares molecular data, morphology and geographic distribution of *C. phlogina* and *C. scythica* from the *C. citrina* group. The phenotypically and geographically clearly differentiated lichens are shown to be actually very closely related. In author's opinion *C. scythica* could be moved into the synonymy of *C. phlogina*.

The work as a whole represents a significant contribution to our knowledge, primarily in taxonomy, ecology and distribution of treated taxa.

In my opinion, the dissertation brings a great amount of new information about this group of lichens and resolves several serious taxonomic questions. The results are clear and original, they give evidence about huge amount of work and time spent in the laboratory and in the field. I recommend the dissertation to be accepted as a foundation for awarding the PhD. degree to the author.

My comments on the work.

The author has not presented a general outline of the genus *Caloplaca* as a whole and to evaluate the historical role of morphologically significant characters as confronted with the recently obtained molecular data in proposing the subdivision of the genus. It could help better understanding of the dissertation. In some groups, great changes occurred following the last modern studies. Can you explain your idea of subdivision into the major subgenera (*Caloplaca*, *Gasparrinia*, *Gyalolechia*, *Leproplaca*, *Pyrenodesmia*, *Xanthocarpia*) and the largest characteristic groups (*aurantia*, *aurea*, *carphinea*, *citrina*, *dolomiticola*, *ferruginea*, *holocarpa*, *lactea*, *persica*, *saxicola*, *sideritis*, *squamosa*)? Which of them have been confirmed monophyletic to date?

In *C. cerina* and *citrina* groups, the vegetative diaspores and thallus structure seem to be the most relevant for identification. What other phenotypic characters are still important?

From Paper I: Can you tell us about the ecology of *C. squamuloisidiata*?

Can you speculate about the phylogenetic affinities of *C. xerica*, *C. furax* and *C. percrocata*, mentioned in the key in Paper II?

In a work of Garty et al. 1986, it is noted that *Caloplaca aurantia* is the dominant lichen on concrete roof tiles in urban and rural areas in Israel. Do you know it from similar anthropogenic habitats from Central Europe?

Results and comments of Paper VI show that the *C. citrina* group belongs to the most difficult at all. Vondrák et al 2009 describe three types of type development of soralia in *C. citrina* group. Have you checked the development in *C. phlogina*?

In *C. citrina* group, most of morphological characters are shared with other, phylogenetically unrelated species. The position of each species must therefore be confirmed by molecular data. Some superficially similar species of the *lactea* group and of the *squamosa* group do not fit the *citrina* morphology. The former has large, narrow-ellipsoid ascospores with thin septa and the latter has a paraplectenchymatous true exciple. For most collectors in the field, it is hardly possible to use traditional keys for e.g. *C. citrina* group after recent result. What common species from that group we can collect and identify by standard examination without fear?

The dissertation unfortunately lacks concluding chapters like „DISCUSSION“ or „OPTIONS for PROGRESS on CALOPLACA in the FUTURE“. The genus *Caloplaca* is not explored sufficiently – as admitted in Introduction. More than 1000 species names have been published for *Caloplaca* alone. Which other groups in your opinion are necessary to be analysed? What is your view on *Caloplaca* subdivision in future? Would it be necessary to split it in a high number of smaller genera?



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## Report on the dissertation of Jaroslav Šoun "Taxonomy of selected groups of the genus *Caloplaca*"

by Christian Printzen, Senckenberg Forschungsinstitut und Naturmuseum,  
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The thesis presented by Jaroslav Šoun comprises six single publications on the systematics and taxonomy of lichen species from the large genus *Caloplaca*. Two of the manuscripts (I and II) deal with species from the *Caloplaca cerina* group, three with the *C. aurantia* group (III-V), and the sixth one with a species that is closely related to the *C. citrina* group. The six papers do not form a unit in the sense that they all are part of a monographic study about *Caloplaca*. Considering the size of this genus, even a regional monographic treatment is impossible to achieve in the time frame that is usually available for a doctoral thesis. The six papers do not only deal with different species but also with different questions of taxonomic and systematic research. This makes it a bit difficult to evaluate the thesis as a whole. In the following, I will therefore describe and assess the papers one by one, describing them and adding comments and questions (in bold) where appropriate. I will conclude by summarizing my impression of the thesis.

**Paper I** constitutes the most comprehensive part of the thesis and Jaroslav Šoun is first author of this paper. It presents a molecular phylogenetic and taxonomic study on European species of the *C. cerina* group. About 140 specimens were included in the phylogenetic analysis. *C. cerina* appears as highly polyphyletic and *C. stillicidiorum* is a monophyletic assemblage of partly well supported clades. Some specimens that were identified as *C. cerina* are closely related to *C. stillicidiorum*. Two further clades of *C. cerina* are more or less well supported but may also be close relatives of *C. stillicidiorum*. This situation is not really satisfying from a nomenclatural perspective. Ecologists and lichenologists want to know how they shall call the specimens which they collect in the field. Unfortunately, it is exactly the taxonomic treatments of *C. cerina* and *C. stillicidiorum* that are very brief and no lists of specimens examined are given. **What can be done to improve this situation? How can we clarify, whether *C. cerina* and *C. stillicidiorum* are really different species and which of the clades should carry which name?** This is an important question. The example of *C. thuringiaca* which is now regarded as a synonym of *C. raesae-nenii* shows that taxonomists are always eager to describe new taxa even in large and confusing groups, in which the chances are high that older names can be found for almost any deviant morphological form.

**Paper II** can be regarded as a precursor to paper I, in which two new species from the *C. cerina* group are described and their phylogenetic position verified by molecular data. This and the following papers only deal with one or two species at the same time. The descriptions of the two new species are very detailed and it is good that the authors indicate on how many observations their measurements are based. But in this case it allows us to observe some apparent discrepancies. In *C. subalpina* 33 apothecia were measured, but only 17 paraphyses, 19 asci but only 21 ascospores,

although the size variability seems to be high. Similar discrepancies are observed in the description of *C. thracopontica* where 46 apothecia but only 52 paraphyses, 37 asci and 62 ascospores were measured.

In **paper III** the distinction of *C. aurantia* and *C. flavescens*, two morphologically similar species, is revised and their occurrence and distribution in the Czech Republic discussed. I have very little to criticise in this paper. It is usual in taxonomic studies that not all collections are investigated in the same detail. Once a species concept has emerged and taxonomically important characters have been identified, usually only those characters are studied. It is a good idea to highlight those specimens and "Material studied" that were investigated in detail. I also liked the comparison between quantitative observations made by the authors with those found in the literature. But here another obvious discrepancy is visible concerning the septum thickness. There are considerable differences between observations in the literature (*C. flavescens*: 1.5-5  $\mu\text{m}$ , *C. aurantia*: 2.5-3.5  $\mu\text{m}$ ) and those made by the authors (*C. flavescens*: 4-8  $\mu\text{m}$ , *C. aurantia*: 3-6  $\mu\text{m}$ ). In paper I it is mentioned that "only the ascospores with well-developed septa ... were measured." Perhaps this is a source of confusion. **I would be happy to learn from the candidate why septum thickness is such a difficult character. More specifically, Steiner & Peveling (1984) recommended a procedure to ensure that spores in fresh and old herbarium material are comparable, but this is not mentioned under material and methods.**

**Papers IV and V** deal with the typification of two names for the widespread lichen *C. aurantia*. Both papers are very thorough nomenclatural treatments in which I have almost nothing to criticise. The only point that I would like to raise is the typification of *Caloplaca aurantiomurorum* in paper V. On p. 86 the authors write: "In the protologue, Flagey (1891: 112) described one locality but did not designate the holotype. His main herbarium is located in PC and ... the sample placed there should be regarded as the holotype and the others as isotypes." **I would like to discuss how this can be justified in light of Arts. 9.1 and 9.9 of the ICBN.**

Finally, **paper VI** investigates the taxonomic status of two morphological varieties of *Caloplaca phlogina*. Only a few minor points can be made, of which I would like to discuss the following. On p. 98 the authors mention that "... the species [= *C. phlogina*] is ecologically and phenotypically uniform in maritime conditions." **Can anything be said about the genetic diversity? Why would it be interesting to know more about the genetic diversity of the different morphs and how could this help to solve the question of whether pale morphs of *C. phlogina* are the result of long distance dispersal?**

### Summary

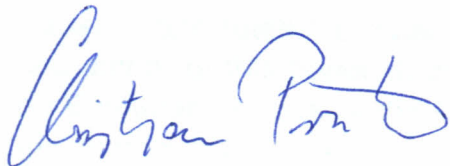
As usual in a compiled work such as this thesis with a differing number of co-authors involved, the taxonomic parts of the paper are of a mixed quality. The nomenclatural papers IV and V are very thorough. Descriptions and discussions of the species are generally very detailed but are missing for some important species. Anatomical measurements are extremely detailed, but in some cases based on relatively few observations. Young scientists today are under a strong pressure to present



good publication lists when they apply for jobs. This also affects taxonomists and in the course the number of monographs decreases and the number of publications on small species groups or even single species increases. The missing comparison of *C. sterilis* with *C. subalpina* and *C. thracopontica* in paper I is an example of the problems that can arise when results are split into several publications. Splitting publications also means that the number of co-authors of a thesis may nowadays be rather high. It becomes then difficult to assess the contribution of the author of the thesis. In this case nine co-authors were involved. Jaroslav Šoun was single author of one paper and main author of two others, among them the most important paper I. Overall the author presents interesting new results that greatly helped in clarifying the taxonomy and systematic relationships within parts of *Caloplaca*. I therefore suggest that – after passing the disputation – the author is awarded a doctoral degree. I would mark the present dissertation as

**Good**

Frankfurt am Main, 18.3.2011



(Dr. Christian Printzen)