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**Taxonomy of selected groups of the genus  
*Caloplaca***

Ph.D. Thesis

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### **Annotation**

The thesis deals with phylogeny, taxonomy and nomenclature of selected groups of the lichen genus *Caloplaca*. Particularly, the *C. cerina* group was closely investigated using molecular methods (ITS sequences), morphology and chemistry, based on material from Europe, and to some extent also from North America and western Asia. This approach resulted in the description of three new species (*C. sterilis*, *C. subalpina*, *C. thracopontica*), and detected an unexpected richness of lineages. Nomenclature, taxonomy, morphology and ecology of *C. aurantia* and *C. flavescens* from the *C. aurantia* group were studied in detail, including selection of the neotype of the former species. Their distribution was reviewed for the territory of the Czech Republic. Poorly known taxon *C. aurantiomurorum* from Algeria was lectotypified and synonymized with *C. aurantia*. Apart from the two groups, *C. phlogina* and *C. scythica*, differing partly in thallus colour and distinctly in distribution, were examined using both molecular (ITS sequences) and phenotypic data and found to be conspecific.

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**Jaroslav Šoun**

### Author's contribution to the papers

- I The author obtained all molecular and morphological data except most data for *Caloplaca sterilis*, performed all analyses except HPLC, collected a part of herbarium material used, and wrote the first draft of the ms. and edited the co-authors' amendments and revisions. Overall author's contribution is c. 90 %.
- II The author performed the molecular analysis, participated in the fieldwork and contributed to writing the paper. Overall author's contribution is c. 30 %.
- III This paper is based on the author's master thesis. The author (JŠ) added some new data and wrote the first draft of the ms. and edited the co-author's amendments and revisions. Overall author's contribution is c. 90 %.
- V The author participated in obtaining the data and writing the paper. Overall author's contribution is c. 40 %.
- VI The author performed the molecular and phylogenetic analyses and prepared them for publication. Overall author's contribution is c. 30 %.

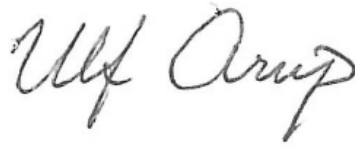
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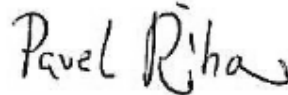
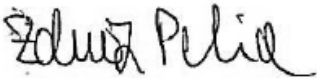
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## Introduction

Taxonomy of lichens – unique symbiotic associations between a filamentous fungus and at least one photosynthetic organism – is passing through a fast progress at this time. New insights from molecular data are considerably changing our understanding of Ascomycota, whose lichen-forming fungi are the indivisible part (e.g. Printzen 2010). Except a few representatives of lichens which belong to Basidiomycota. This thesis is devoted to several groups of one of the most species-rich genera in lichens, the genus *Caloplaca*.

According to the last outline of Ascomycota (Lumbsch & Huhndorf 2007), the genus *Caloplaca* belongs to the family Teloschistaceae, which is along with Letrouitiaceae, Megalosporaceae and Physciaceae included in the order Teloschistales (subclass Lecanoromycetidae, class Lecanoromycetes, subphylum Pezizomycotina). Close alliance of the large family Physciaceae was expected by several older authors, fascinated by many cases of their parallel phenotypic development as evidenced e.g. by the superficial similarity of *Caloplaca carphinea* (Teloschistaceae) and *Dimelaena oreina* (Physciaceae). Physciaceae (incl. Caliciaceae) was firstly assigned into Teloschistales by Miadlikowska et al. (2006) in a separate suborder Physciineae with remaining families sorted in Teloschistineae. For more detailed review of families' classification see Kärnefelt (1989). There is no single phenotypic character shared by all genera in the order but most representatives of the Teloschistaceae and Physciaceae possess polar diblastic ascospores.

A decade before the start of molecular studies on Teloschistales, Kärnefelt (1989) in his throughout revision of the order attempted to implement modern phylogenetic analyses using morphological data. His results were naturally different from following molecular phylogenies (Gaya et al. 2008b), but can serve as an example of limits of morphological taxonomy. Twelve genera were accepted in the family Teloschistaceae in the last outline of ascomycetes (Lumbsch & Huhndorf 2007): *Caloplaca*, *Cephalophysis*, *Fulgensia*, *Huea*, *Ioplaca*, *Josefpoeltia*, *Seirophora*, *Teloschistes*, *Xanthodactylon*, *Xanthomendoza*, *Xanthopeltis* and *Xanthoria*. Some other recently circumscribed xanthorioid genera *Oxneria*, *Rusavskia*, *Xanthoanaptychia* (Kondratyuk & Kärnefelt 2003), distinguished only by phenotypic characters, were not accepted in that treatment. Three new genera *Jackelixia*, *Ovealmbornia*, *Xanthokarrooa* were recently described in a phylogenetic study of xanthorioid lichens by Fedorenko et al. (2009), but they have not been accepted in the recent notes to the ascomycete systematics (Lumbsch & Huhndorf 2009). Detailed review of generic classification history in Teloschistales provided Gaya et al. (2008b).

As in other lichen groups, the taxonomic research in Teloschistaceae progresses from two opposite directions. Several phylogenetic works tried to find borders among the genera within the family (Fedorenko et al. 2009; Gaya et al. 2003, 2008b; Kasalicky et al. 2000; Søchting & Lutzoni 2003; Søchting et al. 2002), while the other group of studies focused at the species delimitation in difficult species aggregates. Nevertheless, none of these studies involved representatives of all accepted genera. The most exhaustive sampling for phylogenetic studies were conducted by Gaya et al. (2008b) using one locus (ITS) and focusing particularly at *Caloplaca*, and by Fedorenko et al. (2009) using two loci (ITS, mtSSU), and focusing on xanthorioid taxa. Polyphyly of all traditionally recognized genera *Caloplaca*, *Fulgensia* and *Xanthoria* was detected in these studies, finding the distinguishing morphological characters to be homoplastic, and implying thus the need for a complete generic reassessment of Teloschistaceae or even Teloschistineae. Although the monophyly of some xanthorioid genera was recently proven by molecular data and some new genera were described (Fedorenko et al. 2009), countless members of the polyphyletic *Caloplaca* still remain to be accommodated, awaiting ultimate large-scale multi-gene study of the suborder and final generic consensus

among lichenologists studying Teloschistineae. The first step to multi-gene study was accomplished by Gaya et al. (2008a), reporting selecting new generation of genes.

*Caloplaca* is far the most species-rich genus within Teloschistineae, and even one of the richest within lichen-forming fungi, comprising an estimated 800 to 1000 species (Arup & Åkeliuss 2009). Species richness of *Caloplaca* is the biggest in non-tropic parts of the world. The genus was never monographed as a whole. However, many taxonomic studies of the genus treating a particular group and/or region have been published, but only some of the recent ones were done using molecular methods (see above). The best explored part is Europe, irrespective of the fact that new species are described from that territory almost every year (Arup 2006b, c; Gaya 2009; Khodosovtsev et al. 2002, 2003; Kondratyuk et al. 2006; Navarro-Rosinés et al. 2000a, b; **paper I**; **paper II**; Roux et al. 2009; Söchting & Stordeur 2001; Söchting et al. 2007; Tretiach & Muggia 2006; van den Boom & Rico 2006; Vondrák & Hrouzek 2006; Vondrák et al. 2008, 2009c). Given this situation in well-explored Europe, it is not hard to imagine what could be found by specialists in other parts of the world. *Caloplaca* in North and Central America is much better known thanks to series of recent works of C. Wetmore and U. Arup, and some other papers (e.g. Arup 1995, Breuss 2001; Nimis et al. 1994; Söchting 2004; Wetmore 1994, 1996, 2001, 2003, 2004a, b, 2007, 2009; Wetmore & Kärnefelt 1998). Polar regions are also quite well explored (e.g. Hansen et al. 1987; Söchting & Olech 1995; Söchting et al. 2008). Asia is still not well investigated, but there are some recent comprehensive treatments (e.g. Khodosovtsev et al. 2004; Poelt & Hinteregger 1993) and on-going research is carried out mainly in India (e.g. Joshi & Upreti 2006, 2007; Joshi et al. 2009). The research on *Caloplaca* in Australia is also in fast progress (Kärnefelt & Kondratyuk 2004; Kondratyuk et al. 2007a, b, 2009a, b, 2010). Africa and South America are still the least investigated continents, although probably host an extensive amount of the *Caloplaca* diversity. Thus the world-wide *Caloplaca* monograph is so far unforeseeable.

Phylogenetic studies using molecular data (ITS or rarely mtSSU sequences) on particular groups of species within the genus *Caloplaca* started to appear at the end of the last century. Molecular data were firstly used by Arup & Grube (1999), who revealed where sterile *Lecanora demissa* really belonged. Muggia et al. (2008), Tretiach et al. (2003) and Vondrák et al. (2008) studied and also newly described some black-fruited species from the section *Pyrenodesmia*. Corrected phylogenetic position of the *C. aurantia* group was presented by Söchting & Arup (2002). Several species from the *C. ferruginea* group were studied by Arup & Åkeliuss (2009) and Arup et al. (2007). Söchting et al. (2007) described new species within the *C. lactea* group exceptionally using mtSSU sequences. The first newly described Asian species supported by DNA data was *C. lenae* by Söchting & Figueras (2007). In the **paper VI** molecular data and phenotype examinations convinced us that two previously distinguished species *C. phlogina* and *C. scythica*, believed to differ in colour of soredia and geographical distribution, are conspecific. The *C. citrina* group (Arup 2006b; Vondrák et al. 2009c) and the *C. holocarpa* group (Arup 2009) are the only ones, which taxonomy were studied by molecular data, at least in restricted regions (Scandinavia and Black Sea region respectively). The *Caloplaca cerina* group has usually been taxonomically used in a broader morphological sense (Clauzade & Roux 1985; Hansen et al. 1987; Wetmore 1996, 1997, 2004b, 2007b), but in the **paper I** and **paper II** is treated for the first time in a narrower sense as a monophyletic group of species around *C. cerina*. The **paper I** presents phylogeny and revised taxonomy of the group in Europe and in some extent also North America and western parts of Asia. Results show unexpected richness of lineages only partly supported by morphology and pointing out on the problem with cryptic or semi-cryptic species (sensu Vondrák et al. 2009c). First data about the phylogeny of the group was published in the **paper II**, where two new species *C. subalpina* and *C. thracopontica* were described. DNA data were several times used to help in determination of peculiar *Caloplaca* species (Arup 2006a; Rosato & Arup 2010; Vondrák et al.

2009a, b, 2010). The standard use of molecular methods in the taxonomy on the species level, with increasing accessibility of them, is anticipated. Nevertheless, another problem has arisen with deeper view into species phylogeny – cryptic or semi-cryptic species and species delimitation in general (see e.g. Crespo & Pérez-Ortega 2009; Del-Prado et al. 2010; Grube & Kroken 2000; **paper I**; Vondrák et al. 2009c).

*Xanthoria* has been also studied by molecular methods. Franc & Kärnefelt (1998), Lindblom & Ekman (2005), Lindblom & Søchting (2008) investigated differences between closely related *Xanthoria* species. Population genetics of *X. elegans* was studied by Dyer & Murtagh (2001) and Murtagh et al. (2002) and that of *X. parietina* by Honegger et al. (2004), Lindblom (2009), Lindblom & Ekman (2006, 2007) at different spatial scales. Widespread and conspicuous *X. parietina* has been used as a model organism in a wide spectrum of studies on developmental anatomy, physiology, element accumulation, chemistry, cultivation, reproduction etc., for examples see Nash (2008). The genome of *X. parietina* will be one of the two going to be sequenced in lichen-forming fungi (Lutzoni & Miadlikowska 2009).

A half of the thesis is devoted to the *Caloplaca aurantia* group, a small but distinct group of lobate, saxicolous species unique within the genus by their lemon-shaped ascospores. The group was studied recently by various authors (e.g. Gaya 2009; Søchting & Arup 2002; Śliwa & Wilk 2008; Wetmore & Kärnefelt 1998) and five species were accepted: *C. aurantia*, *C. aegaea*, *C. flavescens*, *C. fuerteventurae* and *C. thallincola*. Confusing situation with respect to their distribution and distinguishing between *C. aurantia* and *C. flavescens* in the Czech Republic was elucidated in the **paper III**. A neotype of *C. aurantia* was selected and historical circumstances associated with this name were discussed in the **paper IV**. In the **paper V**, the identity of *C. aurantiomurorum* as another potential member of the group was studied. The type exsiccate revealed to be a mixture of different species including *C. aurantia*, *C. saxicola* and *Candelariella senior*. The lectotype of *C. aurantiomurorum* was selected and the name was put into synonymy of *C. aurantia*.

Contents of lichen substances, mainly anthraquinones, in Teloschistaceae and especially in the genus *Caloplaca* have been studied for a long time (e.g. Bohman 1969, Santesson 1970, Søchting 1997, 2001). Formerly TLC and nowadays HPLC or HPTLC have been employed. Their assessment became a standard part of recent taxonomical treatments (e.g. Arup 2006b; **paper I**; **paper II**; **paper VI**; Søchting & Fröberg 2003; Vondrák & Hrouzek 2006).



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# Paper I

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Taxonomy and phylogeny of the *Caloplaca cerina* group in Europe.

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# Taxonomy and phylogeny of the *Caloplaca cerina* group in Europe

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**Abstract:** Using ITS nrDNA sequence data, the *Caloplaca cerina* group (Teloschistaceae) is defined here as a monophyletic, but internally richly branched lineage. The group is also characterized by a combination of morphological and anatomical characters. Its internal lineages are supported by phenotypic characters in addition to ecology and distribution. Within the large *C. cerina* group, we have found at least 20 phylopecies in the temperate zone of the Northern Hemisphere. Two species complexes do not produce any vegetative diaspores; the polyphyletic, corticolous *Caloplaca cerina* s.lat. (six separated cryptic or semi-cryptic species) and the monophyletic *C. stillicidiorum* s.lat. that grows mainly on plant debris, small shrubs and bryophytes and consists of at least four internal lineages. All lineages producing vegetative diaspores (soredia, blastidia, isidia or lobules) are phenotypically characteristic and represent fairly easily distinguishable species; *C. chlorina*, *C. isidiigera*, *C. monacensis*, *C. subalpina*, *C. thracopontica*, *C. turkuensis* and *C. ulmorum*. Only the North American sorediate *C. pinicola* possibly represents an aggregate of species. *Caloplaca sterilis* is described as a new species. A key to the phenotypically distinguishable species is provided here.

Lectotypes are designated here for *C. albolutea*, *Caloplaca cerina* f. *coronulata* and for *C. monacensis*. The Australian *C. hanneshertelii* belongs to this group, but this and other possible species from the Southern Hemisphere are not treated here in detail. Some species traditionally placed into the *C. cerina* group due to their similar morphology are excluded here on the basis of our phenotype examinations and molecular data. *Caloplaca albolutea*, *C. mydalaea* and *C. virescens* are intricate taxa and their identities still remain unclear.

**Key words:** cryptic species, key, lichens, lichen-forming fungi, nrDNA ITS, semi-cryptic species, *Teloschistales*

## Introduction

The *Caloplaca cerina* group is treated here as a monophyletic group of species around *C. cerina* (Hedw.) Th. Fr. It is morphologically characterized by a crustose thallus, which is dark grey to white, devoid of anthraquinones but containing the grey pigment Sedifolia-grey (K+ violet in section). The apothecia can be classified as lecanorine with yellow to orange discs containing non-chlorinated anthraquinones, eventhough there is a strongly reduced true exciple containing anthraquinones in all species. The thalline exciple is distinct, persistent and in shades of grey, devoid of anthraquinones and with a conspicuous cortex in the lower part. The ascospores are *c.* 10–16 µm long, *c.* 6–9 µm wide and have a septum *c.* 4–7 µm thick and the conidia are bacilliform, 3–4 µm long and *c.* 1 µm wide.

The large morphological variation within the group (e.g. in type of vegetative diaspores, disc colour, presence of pruina, size of apothecia) has resulted in many specific and infraspeci-

TABLE 1. List of species that belong to or have formerly been included in the *Caloplaca cerina* group by Clauzade & Roux (1985) and Wetmore (2007). Only the infraspecific names of *C. cerina* with examined type material are listed. Cce – affiliation to the *C. cerina* monophyletic group according our ITS-sequence data; when brackets are used, the placement is based on phenotype characters only. Names representing accepted species within the *C. cerina* monophyletic group are in bold

| Name   | Cce | Note  |
|--|-----|---|
| <i>C. albolutea</i> (Nyl.) Zahlbr.   | (Y) | Intricate name, probably belonging to <i>C. stillicidiorum</i> s.lat.   |
| <i>C. amabilis</i> (Fink) Zahlbr.  | N   | Synonym to <i>C. pelloidella</i>  |
| <i>C. aractina</i> (Fr.) Häyren  | N   | Differs from the <i>C. cerina</i> group by a distinct true exciple (often with blackish outer pigmentation) and c. 4–5 µm long conidia      |
| <i>C. areolata</i> (Zahlbr.) Clauzade  | N   | Differs from the <i>C. cerina</i> group by a distinct true exciple, presence of chlorinated anthraquinones, etc.                            |
| <i>C. asserigena</i> (J. Lahm) H. Olivier                                    | N   | Differs from the <i>C. cerina</i> group by a distinct true exciple and different anthraquinone content                                      |
| <i>C. borealis</i> (Vain.) Poelt   | N   | Differs from the <i>C. cerina</i> group by a distinct true exciple (often with dark outer pigmentation)                                     |
| <i>C. cerina</i> (Hedw.) Th. Fr.   | Y   | Included in <i>C. cerina</i> s.lat.   |
| <b><i>C. cerina</i> s.lat.</b>   | Y   | Polyphyletic taxon including at least six putative cryptic or semi-cryptic species  |
| <i>C. cerina</i> var. <i>chloroleuca</i> (Sm.) Th. Fr.                       | Y   | Included in <i>C. stillicidiorum</i> s. lat.  |
| <i>C. cerina</i> f. <i>coronulata</i> Th. Fr.                                | Y   | Here synonymized with <i>C. turkuensis</i>  |
| <i>C. cerina</i> var. <i>muscorum</i> (A. Massal.) Jatta                     | Y   | Included in <i>C. stillicidiorum</i> s.lat.   |
| <i>C. cerinoides</i> (Anzi) Yatta  | ?   | Intricate name  |
| <b><i>C. chlorina</i> (Flot.) H. Olivier</b>                                 | Y   |   |
| <i>C. chlorina</i> var. <i>cyanopolia</i> (Nyl.) H. Olivier                  | Y   | Here synonymized with <i>C. chlorina</i>  |
| <i>C. chlorina</i> var. <i>paradoxa</i> (Vain.) H. Olivier                   | Y   | Here synonymized with <i>C. chlorina</i>  |
| <i>C. conglomerata</i> (Bagl.) Jatta   | N   | Perhaps synonym to <i>C. pelloidella</i>  |
| <i>C. congregiendens</i> J. Steiner  | N   | Differs from the <i>C. cerina</i> group e.g. by brown thallus   |
| <i>C. haematites</i> (Chaub. ex St.-Amans) Zwackh                            | N   | Differs from the <i>C. cerina</i> group by a distinct true exciple (sometimes with dark grey outer pigmentation) and c. 4–5 µm long conidia |
| <b><i>C. isidiigera</i> Vězda</b>  | Y   |   |
| <i>C. jemtilandica</i> H. Magn.  | Y   | Here synonymized with <i>C. turkuensis</i>  |
| <i>C. jemtilandica</i> var. <i>cerinosora</i> E. S. Hansen, Poelt & Söchting | Y   | Here synonymized with <i>C. turkuensis</i>  |
| <b><i>C. monacensis</i> (Leder.) Lettau</b>                                  | Y   |   |
| <i>C. muscorum</i> (A. Massal.) M. Choisy & Werner                           | Y   | Included in <i>C. stillicidiorum</i> s. lat.  |
| <i>C. mydalaea</i> (Körb.) Zahlbr.   | (Y) | Intricate name, probably belonging to <i>C. stillicidiorum</i> s. lat.  |

TABLE 1. *Continued*

| Name   | Cce | Note   |
|--|-----|--|
| <i>C. pellodella</i> (Nyl.) Hasse                          | N   | Differs from the <i>C. cerina</i> group by a distinct true exciple and a distinctly squamulose thallus           |
| <b><i>C. pinicola</i> H. Magn.</b>                         | Y   |  |
| <i>C. pyracea</i> (Ach.) Th. Fr.                           | N   | Differs from the <i>C. cerina</i> group by a distinct true exciple, belonging to the <i>C. holocarpa</i> group   |
| <i>C. spatatensis</i> Zahlbr.                              | N   | Differs from the <i>C. cerina</i> group by a distinct true exciple, presence of chlorinated anthraquinones, etc. |
| <i>C. squamuloisidiata</i> van den Boom & V. J. Rico       | ?   | Species with unsettled phylogenetic position; phenotypically fits the <i>C. cerina</i> group                     |
| <i>C. stillicidiorum</i> (Vahl) Lyngé                      | Y   | Included in <i>C. stillicidiorum</i> s.lat.  |
| <b><i>C. stillicidiorum</i> s. lat.</b>                    | Y   | Monophyletic taxon with at least four internal phylospesies  |
| <b><i>C. sterilis</i> Šoun, Khodosovtsev &amp; Vondrák</b> | Y   | A new species described here   |
| <b><i>C. subalpina</i> Vondrák, Šoun &amp; Palice</b>      | Y   | Described in a previous paper (Vondrák et al. 2008), not treated here in detail                                  |
| <b><i>C. thracopontica</i> Vondrák &amp; Šoun</b>          | Y   | Described in a previous paper (Vondrák et al. 2008), not treated here in detail                                  |
| <b><i>C. ulmorum</i> (Fink) Fink</b>                       | Y   |  |
| <b><i>C. turkuensis</i> (Vain.) Zahlbr.</b>                | Y   |  |
| <i>C. virescens</i> (Sm.) Coppins                          | (Y) | Intricate name, its identity unclear   |
| <i>C. xerica</i> Poelt & Vězda                             | N   | Differs from the <i>C. cerina</i> group by a distinct true exciple, presence of chlorinated anthraquinones, etc. |

fic names described from all over the world (Table 1). *Caloplaca cerina* itself is an important species to understand as it is the type for the genus *Caloplaca*.

The group name has usually been applied in a broader morphological sense (Clauzade & Roux 1985, Hansen *et al.* 1987, Wetmore 1996, 1997, 2004, 2007a), but our unpublished molecular data and phenotype evaluation show that some species commonly placed in this group belong elsewhere (Table 1) and they are not treated in detail here. The first data about the phylogeny of the group was published by Vondrák *et al.* (2008), who described two new species; *C. subalpina* Vondrák, Šoun & Palice and *C. thracopontica* Vondrák & Šoun. The present study deals with the phylogeny of the entire group in Europe and to some extent also North America and western parts of Asia.

### Material and Methods

Our study is mainly based on material from the temperate zone of Europe, North America and Asia. Samples from ASU, B, BM, C, CBFS, GZU, H, KHER, LD, MIN, PRM, TUR, UPS and the private herbaria of P. v. d. Boom, J. Malíček, Z. Palice, T. Spribille and the first author have been used. Morphological data were taken only from sequenced specimens, listed in Table 2. The specimens were examined with light microscopy. Anatomical features were measured on hand-cut sections or squash pre-

TABLE 2. Voucher specimens and GenBank accession numbers of the ITS sequences used in the phylogenetic analysis. Specimens in bold were already at hand and the rest are newly produced. Sequences marked by an asterisk (\*) have an intron 218 bases long in the 18S region

| Species                   | Specimen   | GenBank accession number |
|---------------------------|--|--------------------------|
| <i>C. aurantia</i>        | <b>Spain, Gaya et al. 13681 (BCC)</b>                            | <b>AY233219</b>          |
| <i>C. flavescens</i>      | <b>Spain, Gaya 368 (BCN)</b>                                     | <b>EU639601</b>          |
| <i>C. cerina</i> 1 'A'    | Greece, <i>Vondrák JV4061</i> (CBFS)                             | HM538546                 |
| <i>C. cerina</i> 2 'A'    | Greece, <i>Vondrák JV3862</i> (CBFS)                             | HM538548                 |
| <i>C. cerina</i> 3 'A'    | Greece, <i>Vondrák JV4035</i> (CBFS)                             | HM538547                 |
| <i>C. cerina</i> 4 'A'    | Greece, <i>Vondrák JV3794</i> (CBFS)                             | HM538542                 |
| <i>C. cerina</i> 5 'A'    | Turkey, <i>Šoun 250</i> (hb. Šoun)                               | HM538543                 |
| <i>C. cerina</i> 6 'A'    | Spain, <i>Vondrák JV7619</i> (CBFS)                              | HM538544                 |
| <i>C. cerina</i> 7 'A'    | Greece, <i>Vondrák JV3824</i> (CBFS)                             | HM538545                 |
| <i>C. sp.*</i>            | Tasmania, <i>Frödén 1246</i> (LD)                                | HM538484                 |
| <i>C. subalpina</i> 1     | <b>Spain, Vondrák JV692 (CBFS)</b>                               | <b>EU365854</b>          |
| <i>C. subalpina</i> 2     | <b>Czech Republic, Palice 6983 (hb. Palice)</b>                  | <b>EU365853</b>          |
| <i>C. subalpina</i> 3     | <b>Ukraine, Vondrák JV6072 (CBFS)</b>                            | <b>EU365855</b>          |
| <i>C. cerina</i> 8 'B'    | Iran, <i>Šochting 11063</i> (C)                                  | HM538539                 |
| <i>C. cerina</i> 9 'B'    | Iran, <i>Šoun 253</i> (hb. Šoun)                                 | HM538541                 |
| <i>C. cerina</i> 10 'B'   | Iran, <i>Šochting 11150</i> (C)                                  | HM538540                 |
| <i>C. cerina</i> 11 'C'   | Iran, <i>Šoun 252</i> (hb. Šoun)                                 | HM538485                 |
| <i>C. cerina</i> 12 'C'   | <b>Sweden, Arup L03347 (LD)</b>                                  | <b>EU365861</b>          |
| <i>C. cerina</i> 13 'C'   | Norway, <i>Arup L02355</i> (LD)                                  | HM538486                 |
| <i>C. turkuensis</i> 1    | Sweden, <i>Arup L04229</i> (LD)                                  | HM538490                 |
| <i>C. turkuensis</i> 2    | Czech Rep., <i>P. &amp; B. v. d. Boom 33926</i> (hb. v. d. Boom) | HM538489                 |
| <i>C. turkuensis</i> 3    | USA, Washington, <i>Spribille 15393</i> (hb. Spribille)          | HM538487                 |
| <i>C. turkuensis</i> 4    | Czech Republic, <i>Malíček &amp; Palice 1908</i> (hb. Malíček)   | HM538491                 |
| <i>C. turkuensis</i> 5    | Italy, <i>Thor 19355</i> (UPS)                                   | HM538488                 |
| <i>C. turkuensis</i> 6    | Czech Republic, <i>Palice et al. s.n.</i> (hb. Palice)           | HM538492                 |
| <i>C. hanneshertelii*</i> | Australia, <i>Kondratyuk 20415</i> (LD)                          | HM538483                 |
| <i>C. isidiigera</i> 1    | Austria, <i>Türk RT32349</i> (GZU)                               | HM538532                 |
| <i>C. isidiigera</i> 2    | USA, Montana, <i>Spribille 12617</i> (hb. Spribille)             | HM538533                 |
| <i>C. isidiigera</i> 3    | <b>Sweden, Arup L04227 (LD)</b>                                  | <b>EU365856</b>          |
| <i>C. isidiigera</i> 4    | <b>Ukraine, Vondrák JV6073 (CBFS)</b>                            | <b>EU365857</b>          |
| <i>C. isidiigera</i> 5    | Slovakia, <i>Šoun 283</i> (hb. Šoun)                             | HM538536                 |
| <i>C. isidiigera</i> 6    | Sweden, <i>Arup L04228</i> (LD)                                  | HM538538                 |
| <i>C. isidiigera</i> 7    | Sweden, <i>Arup L02352</i> (LD)                                  | HM538537                 |
| <i>C. isidiigera</i> 8    | Switzerland, <i>v. d. Boom 17968</i> (hb. v. d. Boom)            | HM538534                 |
| <i>C. isidiigera</i> 9    | <b>Austria, Arup L97333 (LD)</b>                                 | <b>AF353959</b>          |
| <i>C. isidiigera</i> 10   | Austria, <i>Hafellner &amp; Muggia JH68548</i> (GZU)             | HM538535                 |
| <i>C. ulmorum</i> 1*      | USA, South Dakota, <i>Lich. East. N. Am. Exs. 268</i> (GZU)      | HM538509                 |
| <i>C. ulmorum</i> 2*      | USA, Minnesota, <i>Wetmore 90256</i> (GZU)                       | HM538510                 |
| <i>C. ulmorum</i> 3       | USA, Missouri, <i>Wetmore 69226</i> (LD)                         | HM538511                 |
| <i>C. monacensis</i> 1    | Spain, <i>P. &amp; B. v.d. Boom 38821</i> (hb. v. d. Boom)       | HM538493                 |
| <i>C. monacensis</i> 2    | Bulgaria, <i>Vondrák JV3236</i> (CBFS)                           | HM538494                 |
| <i>C. monacensis</i> 3*   | Bulgaria, <i>Vondrák JV762</i> (CBFS)                            | HM538495                 |
| <i>C. monacensis</i> 4*   | Bulgaria, <i>Vondrák JV2244</i> (CBFS)                           | HM538503                 |
| <i>C. monacensis</i> 5*   | Hungary, <i>Vondrák &amp; Khodosovtsev JV6393</i> (CBFS)         | HM538505                 |
| <i>C. monacensis</i> 6*   | Bulgaria, <i>Vondrák JV2127</i> (CBFS)                           | HM538504                 |
| <i>C. monacensis</i> 7*   | Slovakia, <i>Bayerová et al. 8875</i> (hb. Palice)               | HM538506                 |
| <i>C. monacensis</i> 8*   | Slovakia, <i>Bayerová et al. 6150</i> (hb. Palice)               | HM538508                 |
| <i>C. monacensis</i> 9    | Bulgaria, <i>Vondrák JV2026</i> (CBFS)                           | HM538507                 |
| <i>C. monacensis</i> 10*  | Czech Republic, <i>Vondrák 164</i> (hb. Šoun)                    | HM538496                 |

TABLE 2. *Continued*

| Species                               | Specimen   | GenBank accession number |
|---------------------------------------|--|--------------------------|
| <i>C. monacensis</i> 11               | Ukraine, <i>Khodosovtsev 3042</i> (KHER)                           | HM538498                 |
| <i>C. monacensis</i> 12               | Slovakia, <i>Bayerová et al. 5366</i> (hb. Palice)                 | HM538497                 |
| <i>C. monacensis</i> 13*              | Czech Republic, <i>Vondrák JV548</i> (CBFS)                        | HM538499                 |
| <i>C. monacensis</i> 14*              | Slovakia, <i>Palice 85</i> (hb. Palice)                            | HM538500                 |
| <i>C. monacensis</i> 15*              | Slovakia, <i>Bayerová et al. 6154</i> (hb. Palice)                 | HM538501                 |
| <i>C. monacensis</i> 16*              | Czech Republic, <i>Vondrák &amp; Šoun JV2885</i> (CBFS)            | HM538502                 |
| <i>C. aff. thracopontica</i> *        | Greece, <i>Sipman &amp; Raus 54369</i> (B)                         | HM538523                 |
| <i>C. sterilis</i> 1*                 | Romania, <i>Vondrák JV6963</i> (CBFS) – apothecium                 | HM538528                 |
| <i>C. sterilis</i> 2                  | Romania, <i>Vondrák JV6963</i> (CBFS) – soredia                    | HM538531                 |
| <i>C. sterilis</i> 3*                 | Romania, <i>Vondrák JV6955</i> (CBFS)                              | HM538530                 |
| <i>C. sterilis</i> 4*                 | Bulgaria, <i>Vondrák JV6954</i> (CBFS)                             | HM538529                 |
| <i>C. thracopontica</i> 1             | Bulgaria, <i>Vondrák JV3420</i> (CBFS)                             | HM538524                 |
| <b><i>C. thracopontica</i> 2*</b>     | <b>Bulgaria, <i>Vondrák Sel. Exs. Caloplaca 15</i> (CBFS)</b>      | <b>EU365846</b>          |
| <b><i>C. thracopontica</i> 3*</b>     | <b>Bulgaria, <i>Vondrák JV3419</i> (CBFS)</b>                      | <b>EU365847</b>          |
| <i>C. thracopontica</i> 4*            | Turkey, <i>Šoun 301</i> (hb. Šoun)                                 | HM538525                 |
| <i>C. thracopontica</i> 5             | Turkey, <i>Šoun 302</i> (hb. Šoun)                                 | HM538527                 |
| <b><i>C. thracopontica</i> 6*</b>     | <b>Turkey, <i>Vondrák JV5419</i> (CBFS)</b>                        | <b>EU365848</b>          |
| <i>C. thracopontica</i> 7*            | Turkey, <i>Šoun 363</i> (hb. Šoun)                                 | HM538526                 |
| <i>C. chlorina</i> 1                  | Denmark, <i>Søchting 9233</i> (C)                                  | HM538512                 |
| <i>C. chlorina</i> 2                  | USA, Montana, <i>Spribille 11895</i> (hb. Spribille)               | HM538513                 |
| <i>C. chlorina</i> 3                  | Czech Republic, <i>Vondrák JV1292</i> (CBFS)                       | HM538521                 |
| <i>C. chlorina</i> 4                  | Canada, <i>Søchting 7086</i> (C)                                   | HM538522                 |
| <b><i>C. chlorina</i> 5</b>           | <b>Bulgaria, <i>Vondrák JV2055</i> (CBFS)</b>                      | <b>EU365859</b>          |
| <i>C. chlorina</i> 6                  | Bulgaria, <i>Vondrák JV2226</i> (CBFS)                             | HM538514                 |
| <i>C. chlorina</i> 7                  | Czech Republic, <i>Vondrák JV2056</i> (CBFS)                       | HM538517                 |
| <i>C. chlorina</i> 8                  | Czech Republic, <i>Vondrák JV1294</i> (CBFS)                       | HM538516                 |
| <i>C. chlorina</i> 9                  | Czech Republic, <i>Vondrák JV3034</i> (CBFS)                       | HM538515                 |
| <i>C. chlorina</i> 10                 | Belgium, <i>P. &amp; B. v.d. Boom 30644</i> (hb. v. d. Boom)       | HM538518                 |
| <i>C. chlorina</i> 11                 | Czech Republic, <i>Vondrák JV2982</i> (CBFS)                       | HM538520                 |
| <b><i>C. chlorina</i> 12</b>          | <b>Czech Republic, <i>Vondrák JV3120</i> (CBFS)</b>                | <b>EU365858</b>          |
| <i>C. chlorina</i> 13                 | Austria, <i>Vondrák JV1879</i> (CBFS)                              | HM538519                 |
| <i>C. pinicola</i> *                  | USA, North Dakota, <i>Wetmore 80302</i> (GZU)                      | HM538479                 |
| <i>C. aff. pinicola</i> 1             | USA, Montana, <i>Spribille &amp; Arvidson 4731</i> (hb. Spribille) | HM538480                 |
| <i>C. aff. pinicola</i> 2*            | Canada, British Columbia, <i>Spribille 18340</i> (hb. Spribille)   | HM538482                 |
| <i>C. aff. pinicola</i> 3*            | USA, Montana, <i>Spribille 9397</i> (hb. Spribille)                | HM538481                 |
| <i>C. cerina</i> 14 ‘D1’              | Finland, <i>Palice 11906</i> (hb. Palice)                          | HM538476                 |
| <i>C. cerina</i> 15 ‘D1’              | Czech Republic, <i>Vondrák JV2316</i> (CBFS)                       | HM538475                 |
| <b><i>C. cerina</i> 16 ‘D1’</b>       | <b>Sweden, <i>Foucard s.n.</i> (LD)</b>                            | <b>AF353958</b>          |
| <i>C. cerina</i> 17 ‘D2’              | Greece, <i>Vondrák JV3776</i> (CBFS)                               | HM538477                 |
| <i>C. cerina</i> 18 ‘D2’              | Greece, <i>Spribille 16209</i> (hb. Spribille)                     | HM538478                 |
| <i>C. cerina</i> 19 ‘D3’              | Slovakia, <i>Palice 9009</i> (hb. Palice)                          | HM538471                 |
| <i>C. cerina</i> 20 ‘D3’              | Norway, <i>Søchting 10045</i> (C)                                  | HM538472                 |
| <i>C. cerina</i> 21 ‘D3’              | Austria, <i>Hafellner JH70279</i> (GZU)                            | HM538473                 |
| <i>C. cerina</i> 22 ‘D3’              | Spain, <i>Vondrák JV2548</i> (CBFS)                                | HM538474                 |
| <i>C. stillicidiorum</i> 1            | Spain, <i>Vondrák JV6287</i> (CBFS)                                | HM538470                 |
| <i>C. stillicidiorum</i> 2 ‘1’        | Austria, <i>Hafellner JH62279</i> (GZU)                            | HM538468                 |
| <i>C. stillicidiorum</i> 3 ‘1’        | Greece, <i>Vondrák JV4054</i> (CBFS)                               | HM538469                 |
| <i>C. stillicidiorum</i> 4 ‘1’        | Austria, <i>J. &amp; A. Hafellner JH52814</i> (GZU)                | HM538466                 |
| <b><i>C. stillicidiorum</i> 5 ‘1’</b> | <b>France, <i>Gueidan s.n.</i> (BCN)</b>                           | <b>EU639607</b>          |
| <i>C. stillicidiorum</i> 6 ‘1’        | Austria, <i>Hafellner &amp; Muggia JH67075</i> (GZU)               | HM538467                 |
| <i>C. stillicidiorum</i> 7 ‘1’        | Spain, <i>P. &amp; B. v. d. Boom 27071</i> (hb. v. d. Boom)        | HM538461                 |

TABLE 2. *Continued*

| Species                                | Specimen   | GenBank accession number |
|--|--|--------------------------|
| <i>C. stillicidiorum</i> 8 '1'         | Hungary, Šoun 61 (hb. Šoun)  | HM538462                 |
| <i>C. stillicidiorum</i> 9 '1'         | Czech Republic, Halda & Palice 11720 (hb. Palice)                  | HM538463                 |
| <b><i>C. stillicidiorum</i> 10 '1'</b> | <b>Bulgaria, Vondrák Sel. Exs. Caloplaca 12 (CBFS)</b>             | <b>EU365860</b>          |
| <b><i>C. stillicidiorum</i> 11 '1'</b> | <b>Ukraine, Smerychyns'ka 0401(4)3 (KW)</b>                        | <b>EU681284</b>          |
| <i>C. stillicidiorum</i> 12 '1'        | Hungary, Vondrák & Šoun JV4382 (CBFS)                              | HM538465                 |
| <i>C. stillicidiorum</i> 13 '1'        | Bulgaria, Vondrák JV1992 (CBFS)                                    | HM538464                 |
| <b><i>C. stillicidiorum</i> 14 '2'</b> | <b>Russia, Kondratyuk 20709 (KW)</b>                               | <b>EU681283</b>          |
| <i>C. stillicidiorum</i> 15* '2'       | Austria, Hafellner JH42134 (GZU)                                   | HM538569                 |
| <i>C. stillicidiorum</i> 16* '2'       | Austria, Vondrák JV3821 (CBFS)                                     | HM538570                 |
| <i>C. stillicidiorum</i> 17 '2'        | Austria, Kocourková 900101 (PRM)                                   | HM538573                 |
| <i>C. stillicidiorum</i> 18 '2'        | Austria, Hafellner & Muggia JH67073 (GZU)                          | HM538572                 |
| <i>C. stillicidiorum</i> 19* '2'       | Sweden, Arup L04172 (LD)   | HM538571                 |
| <i>C. stillicidiorum</i> 20 '3'        | Alaska, Søchting 7440 (C)  | HM538568                 |
| <i>C. stillicidiorum</i> 21 '3'        | Svalbard (Norway), Košnar 667 (hb. Šoun)                           | HM538575                 |
| <i>C. stillicidiorum</i> 22 '3'        | Greenland (Denmark), Hansen 7525 (C)                               | HM538574                 |
| <i>C. stillicidiorum</i> 23 '3'        | Greenland (Denmark), Hansen 04074 (C)                              | HM538550                 |
| <i>C. stillicidiorum</i> 24 '4'        | USA, Montana, Spribille 14195 (hb. Spribille)                      | HM538556                 |
| <i>C. stillicidiorum</i> 25 '4'        | Canada, British Columbia, Spribille & Goward 15291 (hb. Spribille) | HM538557                 |
| <i>C. stillicidiorum</i> 26 '3'        | Norway, Košnar 669 (hb. Šoun)                                      | HM538576                 |
| <i>C. stillicidiorum</i> 27 '3'        | Norway, Košnar 668 (hb. Šoun)                                      | HM538577                 |
| <i>C. stillicidiorum</i> 28 '4'        | Czech Republic, Vondrák JV6330 (CBFS)                              | HM538551                 |
| <i>C. stillicidiorum</i> 29 '4'        | Czech Republic, Vondrák & Šoun JV2911 (CBFS)                       | HM538553                 |
| <i>C. stillicidiorum</i> 30 '4'        | Czech Republic, Vondrák & Šoun JV2910 (CBFS)                       | HM538552                 |
| <i>C. stillicidiorum</i> 31 '4'        | Ukraine, Khodosovtsev 2937 (KHER)                                  | HM538554                 |
| <i>C. stillicidiorum</i> 32 '4'        | Kazakhstan, Khodosovtsev & Vondrák JV7644 (CBFS)                   | HM538549                 |
| <i>C. stillicidiorum</i> 33 '4'        | Austria, Vondrák JV2974 (CBFS)                                     | HM538555                 |
| <i>C. stillicidiorum</i> 34 '5'        | Romania, Vondrák JV3617 (CBFS)                                     | HM538559                 |
| <i>C. stillicidiorum</i> 35 '5'        | Ukraine, Khodosovtsev 2938 (KHER)                                  | HM538558                 |
| <i>C. stillicidiorum</i> 36 '6'        | Czech Republic, Vondrák JV4824 (CBFS)                              | HM538456                 |
| <i>C. stillicidiorum</i> 37 '6'        | Czech Republic, Kocourková & Šafránek 908963 (PRM)                 | HM538460                 |
| <i>C. stillicidiorum</i> 38 '6'        | Ukraine, Nadyeina JV7620 (CBFS)                                    | HM538459                 |
| <i>C. stillicidiorum</i> 39 '6'        | Hungary, Vondrák JV4867 (CBFS)                                     | HM538458                 |
| <i>C. stillicidiorum</i> 40 '6'        | Hungary, Vondrák & Šoun JV4388 (CBFS)                              | HM538457                 |
| <i>C. stillicidiorum</i> 41 '5'        | Spain, Vondrák JV2612 (CBFS)                                       | HM538560                 |
| <i>C. stillicidiorum</i> 42 '5'        | Finland, Palice 11679 (hb. Palice)                                 | HM538561                 |
| <i>C. stillicidiorum</i> 43 '5'        | Turkey, Šoun 246 (h. Šoun)   | HM538562                 |
| <i>C. stillicidiorum</i> 44 '5'        | Spain, Søchting 9685 (C)   | HM538564                 |
| <i>C. stillicidiorum</i> 45 '5'        | Uzbekistan, Mishutin 2940 (KHER)                                   | HM538563                 |
| <i>C. stillicidiorum</i> 46 '5'        | USA, Montana, Spribille & Goward 10676 (hb. Spribille)             | HM538565                 |
| <i>C. stillicidiorum</i> 47 '5'        | Spain, Vondrák JV2690 (CBFS)                                       | HM538566                 |
| <i>C. stillicidiorum</i> 48 '5'        | Turkey, Šoun 247 (hb. Šoun)  | HM538567                 |

parations mounted in water. Morphological characters were measured on dry material using a dissecting microscope ( $\times 45$ ). Measurements are given as (min.–)  $x \pm SD$  (–max.), where  $x$  = mean value,  $SD$  = standard deviation and min./max. = extremes. The total number of measurements ( $n$ ) is given in brackets. Only the ascospores with well-developed septa (loculi connected by a thin and distinct cytoplasmic channel) were measured.



## Molecular markers, DNA extraction, PCR amplification and sequencing

Initially, we tried to use two loci, mtSSU and nrITS, for molecular analysis. However, the former proved to be too conserved for our study, thus we continued only with the ITS gene, which has suitable variability. Direct PCR was used for PCR-amplification of the ITS regions including the 5.8S gene of the nuclear rDNA following Arup (2006). Primers for amplification were ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990). PCR cycling parameters follow Ekman (2001). Products were cleaned using JETquick PCR purification Spin Kit (Genomed). Both complementary strands were sequenced either by Macrogen Inc., Korea or by Laboratory of Genomics, Biology Centre of the Academy of Sciences of the Czech Republic.

## Sequence alignment and phylogenetic analyses

One hundred twenty two newly obtained ITS sequences were included in the phylogenetic analysis along with seventeen sequences of the *Caloplaca cerina* group available from GenBank (Table 2). Sequences of *Caloplaca aurantia* (AY233219) and *C. flavescens* (EU639601) from the GenBank were selected as an outgroup. Sequences were aligned using MAFFT 6 (on-line version in the Q-INS-i mode; see Katoh *et al.* 2002) and manually cut to eliminate the unalignable ends, the intron and ambiguously aligned regions of ITS1 and ITS2. The final alignment included 596 positions. Bayesian phylogenetic analysis was carried out using the program MrBayes 3.1.1 (Ronquist & Huelsenbeck 2003). The General Time Reversible substitution model (Rodriguez *et al.* 1990) with estimation of invariant sites and assuming a gamma distribution with four categories (GTR+I+G) was used for likelihood calculations. The optimal nucleotide substitution model was found with the program MrModeltest v2.3 (Nylander 2004) using the Akaike Information Criterion and the hierarchical likelihood ratio test (Posada & Crandall 1998). The MCMC analysis was run for ten million generations, performed in two runs, each with four chains starting from a random tree and using the default temperature of 0.2. Every 100th tree was sampled, and the first 30% of the trees were discarded as burn-in.

## Chemistry

Lichen substances in the apothecia were extracted in 150 µl of acetone at room temperature. The extract was subjected to high-performance liquid chromatographic analysis (HPLC). Reverse phase column (C18, 5 µm, Lichrocart 250-4) was eluted with MeOH/30%MeOH+1% $H_3PO_4$  for 77 min and the absorbance at 270 nm was recorded (for details see Søchting 1997). The compounds were determined on the basis of their retention times and absorption spectra. Acetone insoluble pigments were examined according to Meyer & Printzen (2000).

## Results

### Phylogeny

The final alignment of 596 nucleotides had 134 variable positions. The following species had an insertion 218 bases long in the 18S region: *C. stillicidiorum* (only ecotype 2), *C. pinicola*, *C. aff. pinicola*, *C. monacensis* (except v. d. Boom 38821 and CBFS JV3236), *C. ulmorum*, *C. thracopontica*, *C. aff. thracopontica*, *C. sterilis*, *C. hanneshertelii* and *C. sp.* (Frödén 1246, LD). The insertion was not included in the analysis.

The phylogenetic tree, based on the Bayesian inference analysis is presented in Fig. 1 along with the posterior probabilities for the nodes. The species *C. isidiigera*, *C. sterilis*, *C. subalpina*, *C. turkuensis* and *C. ulmorum* form well supported clades (PP > 0.95) in the cladogram. The species *C. chlorina*, *C. monacensis* and *C. thracopontica* are not so well supported. *Caloplaca cerina* s.lat. is a polyphyletic taxon with six lineages, possibly cryptic or semi-cryptic species. *Caloplaca stillicidiorum* s.lat. is a monophyletic taxon (PP = 0.92) forming the crown group of the tree. This clade is internally diverged into three major lineages (ecotype 1, ecotype 2, ecotype 3–4, ecotype 5–6), possibly representing several semi-cryptic species. The only available sequence of *C. pinicola* has a sister relationship to three similar sequences of North

TABLE 3. Anthraquinone contents (% concentrations) of selected species of the *Caloplaca cerina* group

|  | emodin | fallacinal | parietin | parietinic acid | teloschistin | unknown |
|--|--------|------------|----------|-----------------|--------------|---------|
| <i>C. cerina</i> A (CBFS JV2548)             | 4      | 4          | 89       | 1               | 2            | 0       |
| <i>C. cerina</i> A (CBFS JV3776)             | 1      | 4          | 91       | 3               | 1            | 0       |
| <i>C. cerina</i> A (hb. Palice 11906)        | 4      | 4          | 90       | 1               | 0            | 1       |
| <i>C. cerina</i> B (LD L02355)               | 5      | 3          | 91       | 1               | 0            | 0       |
| <i>C. cerina</i> C (hb. Šoun 253)            | 2      | 5          | 86       | 2               | 3            | 2       |
| <i>C. cerina</i> D (hb. Šoun 250)            | 2      | 3          | 92       | 1               | 1            | 1       |
| <i>C. chlorina</i> (CBFS JV1879)             | 4      | 9          | 85       | 0               | 2            | 0       |
| <i>C. isidiigera</i> (hb. v.d. Boom 10756)   | 3      | 4          | 91       | 1               | 1            | 0       |
| <i>C. monacensis</i> (CBFS JV548)            | 1      | 3          | 95       | 1               | 0            | 0       |
| <i>C. pinicola</i> (GZU 80302)               | 4      | 3          | 92       | 1               | 0            | 0       |
| <i>C. aff. pinicola</i> (hb. Spribille 4731) | 2      | 2          | 94       | 1               | 1            | 0       |
| <i>C. stillicidiorum</i> 2 (hb. Šoun 667)    | 2      | 5          | 91       | 2               | 0            | 0       |
| <i>C. stillicidiorum</i> 6 (hb. Šoun 61)     | 2      | 4          | 83       | 1               | 10           | 0       |
| <i>C. turkuensis</i> (CBFS JV6868)           | 5      | 4          | 89       | 2               | 0            | 0       |
| <i>C. ulmorum</i> (GZU 90256)                | 2      | 3          | 93       | 1               | 1            | 0       |

American sorediate *C. cerina*-like samples which probably represent an unknown species (see the note under *C. pinicola*). The only sequence of *C. aff. thracopontica* from the Mediterranean region is close to the *C. thracopontica* clade but possibly not conspecific with it. One available sequence of the Australian *C. hanneshertelii* and one sequence of an undescribed species from Tasmania confirmed their affinities to the *C. cerina* group.

## Chemistry

The anthraquinone content of all analysed species from the *C. cerina* group is uniform (Tab. 3) and corresponds with chemosyndrome A (Søchting 1997). Sedifolia-grey, a pigment insoluble in acetone, is present in the cortex of the thallus, the thalline margin and in the vegetative diaspores, except soredia of *C. sterilis*, of all studied species from the group (C+, K+, N+ pinkish violet/violet/sordid violet in section).

## Key to fertile species known to belong to the *Caloplaca cerina* group. Sterile specimens can be confused with various species outside the group or even outside of the genus *Caloplaca*

- 1 Ascospore septum relatively thin, 1.5–4.0 µm; thallus dark gray; soredia often present; North America ..... **C. pinicola**
- Ascospore septum wider, 4.0–7.0 µm..... 2
- 2 (1) Thallus without any vegetative diaspores ..... 3
- Thallus with vegetative diaspores (lobules, isidia, granules, soredia/blastidia)..... 4
- 3 (2) On trees and shrubs..... **C. cerina s.lat.**
- On mosses, plant debris, wood, bark of exposed roots, dwarf-bushes and rarely on rocks ..... **C. stillicidiorum s.lat.**
- 4 (2) Thallus with lobules, isidia or granules (usually over 50 µm in diam.)..... 5
- Thallus with soredia/blastidia (usually up to 50 µm in diam.) ..... 9
- 5 (4) Thallus surface with pustules and lobules, 100–400 µm wide or rarely without any vegetative diaspores; saxicolous, maritime..... **C. thracopontica**
- Thallus with isidia or granules..... 6

- 6 (5) Thallus with isidia; apothecia absent or present ..... 7  
 Thallus with granules on margins of areoles or completely granulose; granules (53–) 96 ± 27 (–163) µm in diam.; usually richly fertile ..... 8
- 7 (6) Thallus areolate covered by small globose to shortly vertically elongated isidia, (25–) 66 ± 20 (–140) µm wide ..... **C. isidiigera**  
 Thallus with branched coralloid isidia and erect lobules .....  
 ..... *C. squamuloisidiata* (species with unsettled phylogenetic position)
- 8 (6) Thallus areolate with marginal granules, rarely predominantly granular; corticolous; North America ..... **C. ulmorum**  
 Thallus nearly to completely granulose; corticolous, rarely saxicolous; Europe, Asia ...  
 ..... **C. monacensis**
- 9 (4) Soralia crater-like, Australia ..... **C. hanneshertelii**  
 Soralia different ..... 10
- 10 (9) Areoles at thallus margin forming squamules, (0.16–) 0.58 ± 0.32 (–2.04) mm wide; thallus, at least in marginal parts, white-pruinose ..... **C. subalpina**  
 Thallus without marginal squamules ..... 11
- 11 (10) Thallus with white-pruinose delicate areoles, (0.05–) 0.16 ± 66 (–0.30) mm wide, soralia (sordid) grey-green to pale green soralia ..... **C. sterilis**  
 Thallus without pruina; soralia darker, usually with grey or blue tinge ..... 12
- 12 (11) Areoles usually relatively large, up to 1.2 mm wide; soredia/blastidia (15–) 33 ± 7 (–53) µm in diam.; apothecia common, apothecial margin not sorediate; saxicolous or on nutrient-rich tree bases ..... **C. chlorina**  
 Thallus with very small areoles up to 0.2 mm wide; areoles usually very soon entirely sorediate; soredia smaller (15–) 22 ± 3 (–30) µm in diam.; apothecial margin often sorediate, at least in part, however some specimens completely without sorediate margin; corticolous, lignicolous ..... **C. turkuensis**  
 (morphologically resembles another phylogenetically distant undescribed *Caloplaca* species, differing in soralia and apothecia with an endophloeic origin, not developing from areoles, the true exciple usually more apparent and the thalline margin not so stout)

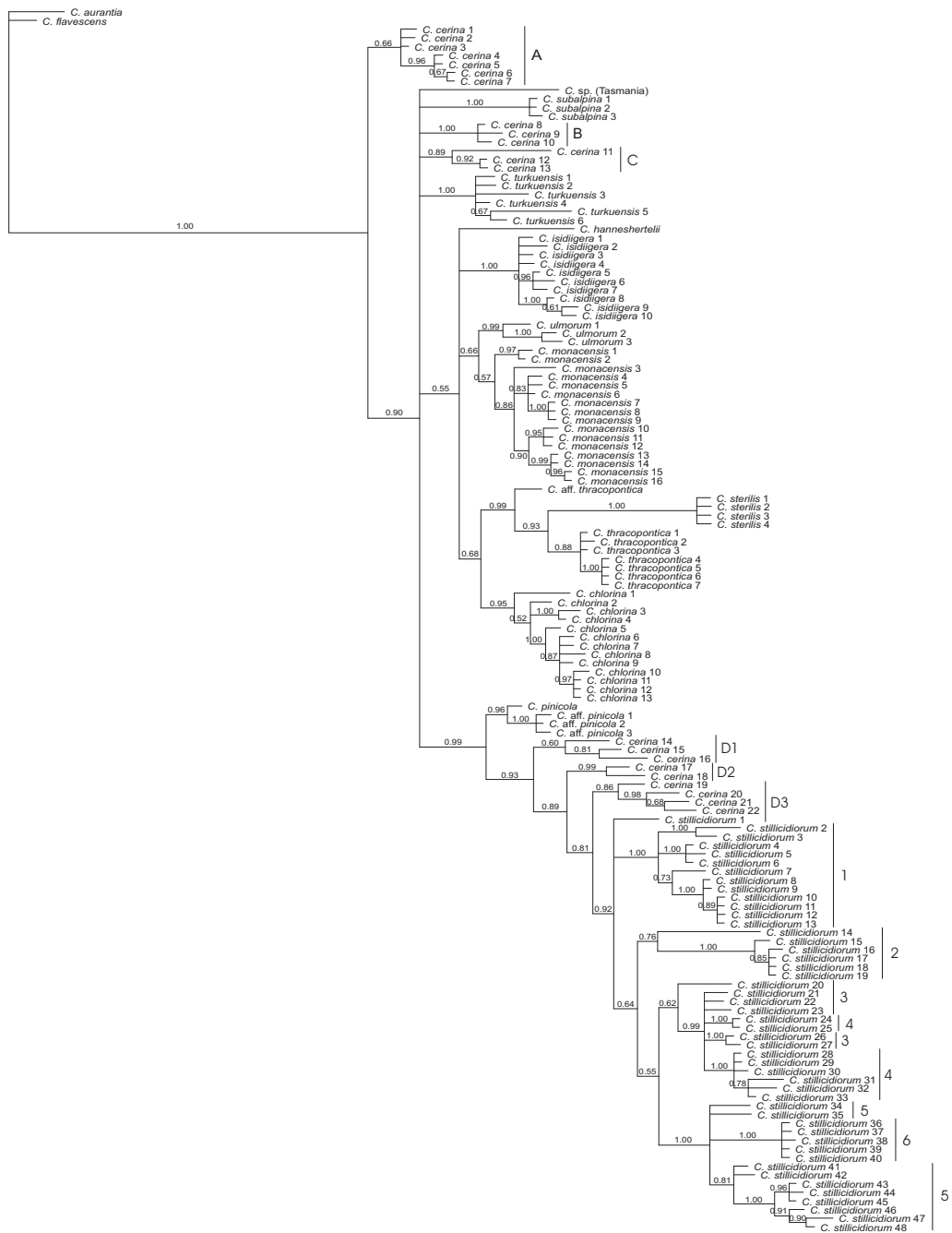
## The Species

### **Caloplaca cerina s. lat.**

Including *Caloplaca cerina* (Hedw.) Th. Fr. s. str., *C. gilva* (Hoffm.) Zahlbr., *C. cerina* var. *azaleae* (Wainio) Zahlbr., *C. cerina* var. *circumponens* (Nyl.) Boist., *C. cerina* var. *subfuscescens* (Nyl.) Blombg., *C. cerina* f. *chryasapis* (Ach.) Th. Fr., *C. cerina* f. *corni* Brtzm.

This is a polyphyletic taxon splitted into six different clades scattered within the cladogram, comprising corticolous specimens lacking vegetative diaspores. Three of the clades, D1, D2 and D3, are very closely related and seems to have similar distribution and ecology and they are here treated together (group D). We keep all the lineages unnamed, without nomenclatural status, because we failed to find sufficient morphological characters to separate them. Short characteristics of each of the molecularly based groups are presented here:

**A:** On bark of *Cupressus*, *Olea*, *Pistacia*, and *Platanus* in the Mediterranean region (Greece, Spain, Turkey). Thallus like in clade B but tends to be darker; apothecia yellow to orange-yellow, strongly pruinose on disc and inner part of margin, to 1.1 mm in diam.; spores (12–) 13.8 ± 1.1 (–16) × (5–) 6.4 ± 0.7 (–8) µm; septum (5–) 5.9 ± 0.7 (–7) µm wide (*n*=60).



**B:** On bark of deciduous trees in northern Iran. Thallus crustose, thick, pale grey to beige, sometimes whitish; apothecia orange-yellow to deep orange, disc sometimes slightly pruinose when young, to 0.9 mm in diam.; spores (14–)  $14.8 \pm 0.8$  (–17)  $\times$  (7–)  $8.3 \pm 1.1$  (–11)  $\mu\text{m}$ ; septum (4–)  $5 \pm 0.5$  (–6)  $\mu\text{m}$  wide ( $n=20$ ).

**C:** On bark of *Salix* in Scandinavia (Norway, Sweden) and on *Fraxinus* in Hyrcanian forest in Iran. Thallus and apothecia similar to the type D but smaller; apothecia orange-yellow, not pruinose, to 0.8 mm in diam.; spores (10–)  $12.6 \pm 2$  (–17)  $\times$  (5–)  $6.8 \pm 1$  (–9)  $\mu\text{m}$ ; septum (3–)  $4.1 \pm 1$  (–6)  $\mu\text{m}$  wide ( $n=30$ ).

**D1, D2 and D3:** On bark of *Acer*, *Juglans*, *Populus*, *Prunus*, *Sambucus* throughout Europe (Austria, Czech Republic, Finland, Greece, Norway, Slovakia, Spain). Thallus crustose, pale to dark grey, sometimes warted, thick or thin; apothecia orange-yellow to orange, rarely pruinose, to 1.6 mm in diam.; spores (11–)  $14.6 \pm 1.7$  (–20)  $\times$  (6–)  $7.9 \pm 0.9$  (–10)  $\mu\text{m}$ ; septum (5–)  $6.7 \pm 1.2$  (–10)  $\mu\text{m}$  wide ( $n=90$ ).

The holotype of *C. cerina* (Hedw.) Th. Fr. is the illustration in Hedwig (1789: 62, tab. 21, f. B). Wetmore (2007a) designated an epitype (MIN!) and several isoepitypes (ASU, COLO, MIN, US) using the exsiccate *Lichenotheca Fenn. 116* (Finland, Ostrobothnia Borealis: Simo, insula Kumurainen, leg. Räsänen). The type illustration is difficult to assign to any of the clades given above. The epitype probably belongs to clade C because of its relatively small apothecia (to 1 mm diam.) and small spores (11–13  $\times$  7–8  $\mu\text{m}$ , septum 5–5.6  $\mu\text{m}$ ). However, a placement in the other Scandinavian clades, D1 or D3, is also possible.

### **Caloplaca chlorina (Flot.) H. Olivier**

*Mém. Soc. natl. Sci. nat. et math. Cherbourg* **37**: 122 (1909).—*Zeora cerina*  $\alpha^*$  [var.] *chlorina* Flot., *Jahresber. Schlesisch. Gesellsch. für vaterl. Kultur* **27**: 126 (1849); type: *Calloposma cerinum*  $\alpha^{**}$  *chlorinum*, *Körper Lichenes Selecti Germanici Exsiccati* no. 128 (1857), ad saxa granitica circa “Drachenburg” prope Hirschbergam Silesiae, *Körper* (O—neotype designated by Wetmore (1997), L, M!, MIN, S!—isoneotypes).

*Caloplaca chlorina* var. *cyanopolia* (Nyl.) H. Olivier, *Mém. Soc. natl. Sci. nat. math. Cherbourg* **37**: 123 (1909).—*Lecanora cerina* f. *cyanopolia* Nyl., *Notiser ur Sällsk. pro Fauna et Flora Fennica Förhandlingar* **8**: 128 (1866); type: Russia: Republic of Karelia, Sortavala, 1863, *Kullhem* (H-NYL 29770!—lectotype designated by Kärnefelt (1990).

*Caloplaca chlorina* var. *paradoxa* (Vain.) H. Olivier, *Mém. Soc. natl. Sci. nat. math. Cherbourg* **37**: 123 (1909).—*Lecanora cerina* var. *cyanopolia* \*[f.] *paradoxa* Vain., *Meddel. Societas pro Fauna et Flora Fennica* **3**: 55 (1878); type: Karelia australis, Wiipuri, Monrepos [Russia, Leningrad Oblast, Vyborg], 1875, *Lang* (TUR-V 07552!).

(Fig. 2A)

*Thallus* areolate, up to several cm wide, non-pruinose, up to 0.27 (–0.53) mm thick, always sorediate/blastidiate; sometimes also with tiny isidiate outgrows especially at margins of older areoles; *areoles* flat to convex, up to 1.2 mm wide; colour variable, sordid white, pale grey, brownish grey, dark grey to almost black, usually with bluish tinge; *soralia* and *blastidia* pale to dark grey, with bluish tinge, rarely greenish grey, arising from margins of areoles, sometimes spreading over the whole areole; *soredia/blastidia* (15–)  $33 \pm 7$  (–53)  $\mu\text{m}$  diam. ( $n=100$ ); soredia sometimes forming consoredia; *prothallus* sometimes present, thin and black; *cortex* up to 10 (–30)  $\mu\text{m}$  thick, paraplectenchymatous.

*Apothecia* lecanorine, abundant to absent, up to 1.5 mm diam., sessile, non-pruinose; *disc* flat to slightly convex, pale to dark orange; *true exciple* indistinct, very thin, up to 15  $\mu\text{m}$  thick, prosoplectenchymatous; *thalline exciple* of thallus colour, raised above disc when young, some-

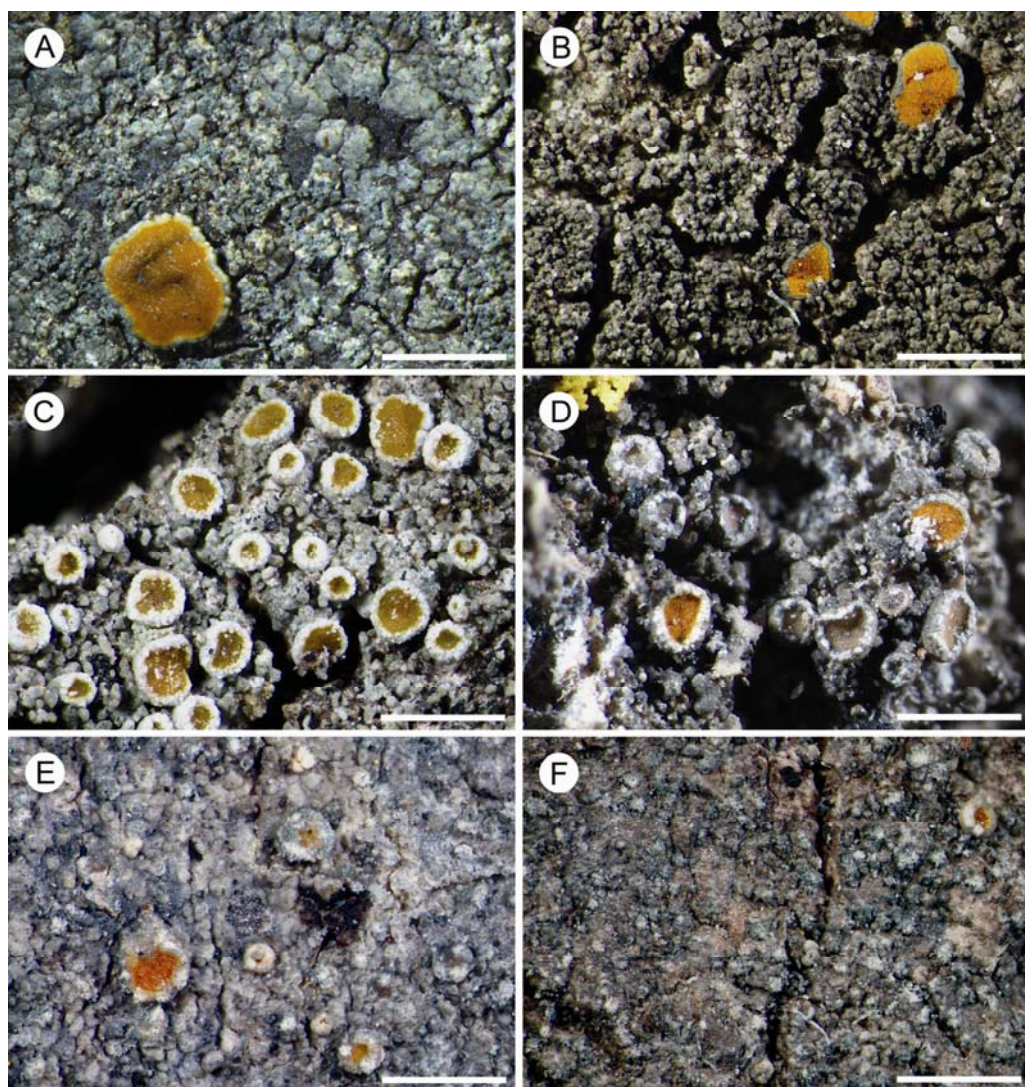


FIG. 2. Species of *Caloplaca*. A, *C. chlorina* (CBFS JV2055); B, *C. isidiigera* (Šoun 283); C, *C. monacensis* (v. d. Boom 38821); D, *C. monacensis*, Arnold Lich. Monacenses exs. 1896 no. 422, note apothecial discs without normal yellow-orange color (BP—*isotype*); E & F, *C. turkuensis* (CBFS JV2921). Scales: A–F = 1 mm.

what reduced in old apothecia, 60–90  $\mu\text{m}$  thick; *epihymenium* orange, with inspersed granules; *hymenium* 50–85  $\mu\text{m}$  thick, hyaline; *hypothecium* (55–) 80–110  $\mu\text{m}$  thick, hyaline; *paraphyses* simple to branched, 2  $\mu\text{m}$  broad with upper cells wider, 3–5  $\mu\text{m}$  thick; *asci* 8-spored, 35–59  $\times$  10–18  $\mu\text{m}$  ( $n=50$ ); *spores* polarilocular, ellipsoid, (10.0–) 12.4  $\pm$  0.9 (–15.0)  $\times$  (5.0–) 6.6  $\pm$  0.6 (–8.0)  $\mu\text{m}$ ; septum (4.0–) 4.7  $\pm$  0.5 (–6.0)  $\mu\text{m}$  wide ( $n=100$ ); ratio of septum width/spore length (0.3–) 0.4  $\pm$  0.04 (–0.5).

*Pycnidia* rare to scattered, immersed, distinct by elevated darker ostiole. *Conidia* bacilliform, 3–4  $\times$  1  $\mu\text{m}$ .

*Habitat and distribution.* This species grows from lowlands to mountains on basic or nutrient enriched siliceous (e.g. gneiss, slate, granite) and limestone rocks both in natural and anthropogenic habitats, especially on old stone walls, pebbles in railroads, bricks, roofing-tiles and mortar. It is occasionally found on bark of broad-leaved trees (e.g. *Acer*, *Fraxinus*, *Juglans*, *Ulmus*) at trunk bases. It seems to prefer humid or shaded sites and substrates near the ground. Probably it is quite frequent throughout Europe but often overlooked or confused with similar taxa. There are confirmed records from Austria, Belgium, Bulgaria, Czech Republic, Denmark, Germany, Ireland, Netherlands, Norway, Portugal, Romania, Russia, Slovakia, Sweden, Ukraine and United Kingdom. The world distribution (see Fletcher & Laundon 2009) needs revision but three records from North America are confirmed.

*Remarks.* The name *C. chlorina* has often been used in a broad sense for saxicolous and corticolous specimens of the *C. cerina* group with any vegetative diaspores. Recently Wetmore (1997) selected a neotype for *C. chlorina* and stated that the isidiolate *C. isidiigera* is a synonym. However, according to our results, *C. chlorina* s. str. is always characterised by the presence of soredia/blastidia. Only some morphotypes of *C. chlorina*, usually overgrown and by grazing affected thalli, possessing various isidia-like secondary outgrowths and consoredia can resemble *C. isidiigera*. Sterile specimens can be confused with some unrelated species, e.g. *C. soralifera* Vondrák & Hrouzek.

*Selected specimens examined.* **Austria:** Lower Austria: Wolfsthal, castle ruins Pottenburg, Vondrák JV1879 (CBFS). Carinthia: Gailtaler Alpen, Hermagor, Weibriach, van den Boom 16234 (hb. v. d. Boom). Burgenland: Bernsteiner Gebirge, Bernstein, Hafellner & Maurer JH31242 (GZU).—**Belgium:** Luxembourg: Martelange, Witry, van den Boom & van den Boom 30644 (hb. v. d. Boom).—**Bulgaria:** Haskovo: Eastern Rodopi Mts, Malko Gradishte, Vondrák JV2226 (CBFS); Madzharovo, Vondrák JV2055 (CBFS).—**Canada:** British Columbia: Wells Gray Educ. and Res. Centre, Søchting 7086 (C). Newfoundland, Waghorne (BM).—**Czech Republic:** Central Bohemian Reg.: Rakovník, castle ruins Týřov, Vondrák JV2982, JV2764 (CBFS). South Bohemian Reg.: Písek, castle Zvíkov, Vondrák JV3034, (CBFS). Zlín Reg.: Kroměřř, castle Buchlov, Vondrák JV1294 (CBFS). Liberec Reg.: Jizerské hory Mts, Jizerka, Kocourková & Kocourek 907659 (PRM). Karlovy Vary Reg.: Karlovy Vary, Andělská Hora, Vondrák JV1103 (CBFS). Plzeň Reg.: Konstantinovy Lázně, castle ruins Krasíkov, Vondrák JV2294 (CBFS). Vysočina Reg.: Nové Město na Moravě, Kuták 762037 (PRM). Moravian-Silesian Reg.: Hrubý Jeseník Mts, Mt Vysoká hole, Vondrák JV1932 (CBFS). South Moravian Reg.: Kyjov, Milotice, Vondrák JV1373 (CBFS).—**Denmark:** Bornholm: Hammershus, Larsen & Vondrák JV4714 (CBFS).—**Germany:** Baden-Württemberg: Heidelberg (M).—**Ireland:** Connemara: Lough Feagh and Lough Muck, Larbalestier 23 (BM).—**Netherlands:** North Holland: Naarden-Vesting, van den Boom 15417 (hb. v. d. Boom). North Brabant: Werkendam, Woudrichem, van den Boom 12545 (hb. v. d. Boom).—**Norway:** Aust-Agder, Grimstad, Tønsberg 13551 (BM).—**Portugal:** Trás-os-Montes: Bragança, Zeive, van den Boom 19357 (hb. v. d. Boom).—**Romania:** Arad: Arad, Nädlac, Vondrák JV2222 (CBFS).—**Slovakia:** Trenčín Reg.: Považský Inovec Mts, Tematín, Halda & Palice 10186 (hb. Palice). Bratislava Reg.: Bratislava, Mt Devínská kobyła, Vondrák JV1822 (CBFS).—**Sweden:** Värmland: Frykerud, Arup L03566 (LD). Skåne: Maltesholm, Hessikahuset, Arup L08033 (LD).—**Ukraine:** Crimea: Karadag Mts, Mt Svyataya, Vondrák 290 (hb. Šoun).—**United Kingdom:** Scotland: Dumfries, Drumlanrig Castle, James (BM); Banff, Mountblairy, James (BM); Mid Ebudes, Ballyhaugh, James (BM). Wales: Monmouthshire, Abergavenny, James (BM).—**USA:** Montana: Lincoln Co., W of Libby, Spribille 11895 (hb. Spribille).  
*Exsiccata examined:* **Finland:** Finland Proper Reg.: Kakskerta, Lich. Fenn. Exs. no. 234 [as *Placodium gilvum* var. *chlorina*].—**Russia:** Karelia, Kurkiyeki, Lich. Fenn. Exs. no. 85 [as *Placodium gilvum* var. *chlorina*] (BM).—**Sweden:** Närke, Mellösa, Arnold: Lich. Exs. no. 1550a [as *Calloplisma cerinum* f. *chlorinum*] (BM).

### **Caloplaca hanneshertelii S. Y. Kondr. & Kärnefelt**

*Bibliotheca Lichenologica* **88**: 262 (2004); type: Australia, Victoria, NW of Swan Hill, 22 km W of Nyah West, on shrubs along the road, c. 50 m alt., 35°10'61"S, 143°09'14"E, growing together with *Xanthoria filsonii*, 15 Jan. 1999, Kärnefelt 992101 (LD—holotype).

This Australian corticolous species is characteristic by crater-like soralia with dark-bluish soredia erupting from pustule-like outgrowths at whitish grey areoles. Apothecia are small, 0.2–0.5 mm diam. *Caloplaca ahtii* Søchting, *C. obscurella* (J. Lahm) Th. Fr. and *C. ulcerosa* Coppins & P. James have similar type of soralia but differ in apothecia. For detailed description of this species see Kärnefelt & Kondratyuk (2004).

### **Caloplaca isidiigera Vězda**

*Folia Geobot. Phytotax. Bohemoslov.* **13**: 417 (1978); type: *Caloplaca isidiigera*, Vězda *Lich. Sel. Exs.* no. 1494, Czechoslovakia, Eu-Carpaticum, Tatra Minor, in monte Velký bok, ca. 1550 m s. m., ad saxa calcarea, Sept. 1974, Vězda (PRA-V—holotype, PRM!, BM!—isotypes).

(Fig. 2B)

*Thallus* areolate, up to several cm wide, non-pruinose, up to 0.4 (–0.8) mm thick, isidiate; *areoles* convex when very young, later flat, up to 2.1 mm wide; colour varies from sordid pale grey, dark gray to black, usually with bluish tinge; lower part of very thick areoles often stained purple-black (in section); *isidia* initially arising mainly from margins of areoles, but soon covering whole surface of areoles, globose to shortly elongated or flattened, concolorous with the areoles; (25–) 66 ± 20 (–140) µm diam. (*n*=82); *prothallus* usually present, thin and black; *cortex* up to 20 µm thick, paraplectenchymatous.

*Apothecia* lecanorine, abundant to absent, up to 1.3 mm diam., sessile, non-pruinose; *disc* flat to slightly convex, pale to dark orange; *true exciple* indistinct, very thin, up to 10 µm thick, prosoplectenchymatous; *thalline exciple* of thallus colour, raised above disc when young, somewhat reduced in old apothecia, sometimes crenulate to isidiate, 65–110 µm thick; *epihymenium* orange, with inspersed granules; *hymenium* 55–85 µm thick, hyaline; *hypothecium* 60–110 µm thick, hyaline; *paraphyses* simple to branched, 2 µm broad with upper cells wider, 3–5 µm thick; *asci* 8-spored, 38–55 × 9–15 µm (*n*=24); *spores* polarilocular, ellipsoid, (10.0–) 12.4 ± 1.4 (–15.0) × (5.0–) 6.5 ± 0.9 (–8.0) µm; septum (3.0–) 4.6 ± 0.7 (–6.0) µm wide (*n*=48); ratio of septum width/spore length (0.3–) 0.4 ± 0.05 (–0.5).

*Pycnidia* rarely seen, immersed. *Conidia* bacilliform, 3 × 1 µm.

*Habitat and distribution.* The species seems to grow only in subalpine and alpine habitats on natural limestone, dolomite or calciferous slate and base-rich sandstone rocks. Confirmed records are from the Alps (Austria, Germany, Italy and Switzerland), Carpathians (Slovakia, Ukraine) and Scandinavia (Sweden). One record is confirmed from North America.

*Remarks.* This species is characterized by the presence of isidia and the type specimens are clearly isidiate. However, Wetmore (1996) considered these structures seen in the isotypes as lobules and van den Boom & Rico (2006) regarded them as granules or microsquamules. This has caused many misinterpretations and the name has for example been applied on the corticolous *C. monacensis*. Sterile specimens might be confused with unrelated *C. xerica* Poelt & Vězda, but it differs in growing on base-rich siliceous rocks in xerothermic habitats.

*Nomenclatural note.* *Caloplaca isidiigera* was synonymized by Wetmore (1997) with *C. chlorina*, and not accepted as separate species afterwards. This study clearly shows that both taxa are separate species (for more details see the note under *C. chlorina*).

*Selected specimens examined.* **Austria:** Styria: Ennstaler Alpen, Reichenstein-Massiv, *Hafellner, Muggia & Hafellner* JH68548 (GZU); Schladminger Tauern, Ursprungalm, *Türk* RT32349 (GZU); Gurktaler Alpen, Turrach, *Hafellner* JH61513 (GZU); Steirisches Randgebirge, Grazer Bergland, Mixnitz,



*Hafellner* JH64492 (GZU); Nördliche Kalkalpen, Hochschwab-Gruppe, Trenchtling, *Hafellner & Hafellner* JH62455 (GZU); Müritzsteiger Alpen, Schneealpe, *Mayrhofer & Bilovitz* 17752 (GZU); Wölzer Tauern, Lachtal, *Mayrhofer & Sterner* 13973 (GZU); Stubalpe, Wölkerkogel, *Wilfling & Unger* AW2315 (GZU); Niedere Tauern, Gumpeneck, *Hafellner & Wilfling* AW1728 (GZU); Eisenerzer Alpen, Reiting-Massiv, *Hafellner & Hafellner* JH43577 (GZU). *Carinthia*: Karnische Alpen, Schloßhüttensattel, *Poelt* IN52-84 (GZU). *Tyrol*: N. P. Hohe Tauern, Kals, *Hafellner* JH47074 (GZU).—**Germany**: *Bavaria*: Berchtesgaden N. P., *Türk & Wunder* (M).—**Italy**: *Trentino-Alto Adige*: Merano, NP Stilfser Joch, *van den Boom* 10756 (hb. v. d. Boom).—**Slovakia**: *Žilina Reg.*: Nízke Tatry Mts, Mt Veľký bok, *Šoun* 283 (hb. Šoun).—**Sweden**: *Lule Lappmark*: Padjelanta national park, Allak, *Arup* L04227 (LD). *Torne Lappmark*: Torneträsk, Bessisjohka, *Arup* L04228 (LD). *Pite Lappmark*: Arjeplog par., Mt Skärrim, *Arup* L03570 (LD). *Härjedalen*: NE of Ramundberget, Mt Stor-Mittåkläppen, *Arup* L02352 (LD).—**Switzerland**: *Berner Oberland*: Brienz, Chüemad, *van den Boom* 17968 (hb. v. d. Boom). *Valais*: Val de Moiry, Fêta d'Aouët de Chateaupré, *van den Boom* 10108 (hb. v. d. Boom).—**Ukraine**: *Zakarpatska oblast*: Svidovets Mts, *Vondrák* JV6073 (CBFS).—**U.S.A.**: *Montana*: Lincoln Co., Whitefish Range, Mt Marston, *Spribille* 12617 (hb. Spribille).

*Exsiccata examined*: **Ukraine**: *Zakarpatska oblast*: Svidovets Mts, *Vondrák*: *Sel. Exs. of Caloplaca* no. 42 (CBFS).

### **Caloplaca monacensis (Leder.) Lettau**

*Hedwigia* **52**: 240 (1912).—*Pyrenodesmia monacensis* Leder., *Bericht. Bayr. Botan. Gesellsch.* **4**: 26 (1896); type: *Pyrenodesmia monacensis*, *Arnold Lich. Monacenses Exs. 1896* no. 422, an alten Strassenpappeln nicht weit vom Warthof bei Giesing, München, März 1896, *Lederer* (M [M-0023624])!—lectotype designated here, BP!, BM!, M [M-0053460]!—isolectotypes).

(Figs 2C & D)

*Thallus* predominantly to entirely granular, scarcely with distinct areoles, especially around apothecia, scattered to continuous, thin to thick, non-pruinose, (sordid) pale to dark grey; *granules* (53–)  $96 \pm 27$  (–163)  $\mu\text{m}$  diam. ( $n=99$ ). Sometimes very minutely tomentose by inconspicuous hairs  $4\text{--}10 \times 2\text{--}3 \mu\text{m}$  formed by projecting hyphae (visible in section), particularly on granules; *prothallus* not seen.

*Apothecia* lecanorine, abundant to rare, up to 1.2 mm diam., sessile, distinct, sometimes pruinose; *disc* flat to slightly convex, yellow-orange to orange, sometimes pruinose, especially when young; *true exciple* indistinct, very thin, up to 20  $\mu\text{m}$  thick, prosoplectenchymatous; *thalline exciple* of thallus colour and often whitish pruinose, raised above disc when young, somewhat reduced in old apothecia, 55–110  $\mu\text{m}$  thick; *epihymenium* orange, with interspersed granules; *hymenium* (65–) 85–102  $\mu\text{m}$  thick, hyaline; *hypothecium* (45–) 75–90  $\mu\text{m}$  thick, hyaline; *paraphyses* simple to branched, 2  $\mu\text{m}$  broad with upper cells wider, 4–6  $\mu\text{m}$  diam.; *asci* 8-spored, 40–67  $\times$  10–22  $\mu\text{m}$  ( $n=48$ ); *spores* polarilocular, ellipsoid, (11.0–)  $13.4 \pm 1.3$  (–16.0)  $\times$  (5.0–)  $6.5 \pm 0.7$  (–8.0)  $\mu\text{m}$ , septum (3.0–)  $4.9 \pm 0.7$  (–7.0)  $\mu\text{m}$  wide ( $n=100$ ), ratio of septum width/spore length (0.2–)  $0.4 \pm 0.05$  (–0.5).

*Pycnidia* rare to scattered, immersed, distinct by elevated darker ostiole. *Conidia* bacilliform, 3–4  $\times$  1  $\mu\text{m}$ .

*Habitat and distribution*. This species grows on bark of wayside trees, solitary trees in pastures and in other types of cultural landscapes and in open deciduous or mixed forests. It has been recorded on a variety of broad-leaved trees like *Acer*, *Fagus*, *Fraxinus*, *Juglans*, *Ostrya*, *Pistacia*, *Populus*, *Pyrus*, *Quercus*, *Salix*, *Tilia*, *Ulmus*, but it is also rarely found on bark of shrubs and rotten wooden fences. Once it was found growing on shaded cherty limestone in Bükk Mts, Hungary (*Vondrák et al.* 2009b). Probably it is widely distributed from the lowlands to medium altitudes in the mountains throughout temperate and Mediterranean Europe and

western Asia, but its collections has been mistaken for *C. cerina*. We confirmed it from Austria, Bulgaria, Czech Republic, Germany, Greece, Hungary, Italy, Russia, Slovakia, Slovenia, Spain, Sweden, Turkey, Ukraine and the United Kingdom.

*Remarks.* *Caloplaca monacensis* is characterized by its granular thallus and usually pruinose apothecia. The North American *C. ulmorum*, a closely related taxon, is very similar, but it usually has a more developed areolate thallus with granules at the thallus margins.

*Nomenclatural note.* The name *C. monacensis* was never widely used and just adopted by Lettau (1912), Migula (1929), Zahlbruckner (1931) and Poelt (1969) without any new records. The reason is that the apothecia in the exsiccate (syntype) are mostly brownish and only rarely with normal yellow-orange color (see Fig. 2D). Therefore it was originally described in the genus *Pyrenodesmia*. In all other aspects the specimens in the exsiccate are morphologically completely consistent with recent samples of the species.

*Selected specimens examined.* **Austria:** *Styria:* Schladminger Tauern, Schladming, *Kocourková* 900419 (PRM). *Lower Austria:* Mitterbach am Erlaufsee, Neuhaus, *Poelt* IN10-84 (GZU). *Carinthia:* Koralpe, Oberauerling, *Maurer* 1131 (GZU); Görtschitztal, Lölling Graben, *Hafellner* JH14855 (GZU). *Burgenland:* Mittelburgenland, Bernstein, *Maurer* 356 (GZU). Salzburg, *Kondratyuk* 2936 (KHER).—**Bulgaria:** *Kardzhali:* Eastern Rodopi Mts, Ptichar, *Vondrák* JV2026 (CBFS). *Burgas:* Strandzha Mts, Gramatikovo, *Vondrák* JV3236 (CBFS).—**Czech Republic:** *South Moravian Reg.:* Pavlovské vrchy hills, Soutěska, *Vondrák* 164 (hb. Šoun). *Vysočina Reg.:* Nové Město na Moravě, *Kučák* 697020 (PRM). *Central Bohemian Reg.:* BR Křivoklátsko, Týřov, *Palice* 9151 (hb. Palice).—**Greece:** *Crete:* Orosira Dikti Mts, Ano Viannos, *Vondrák* JV3768 (CBFS).—**Hungary:** *Borsod-Abaúj-Zemplén:* Bükk Mts, Látó-kövek, *Hafellner* & *Khodosovtsev* JV6393 (CBFS).—**Italy:** *Trentino-Alto Adige:* Val Venosta, Glorenza, *Hafellner* 61270 (GZU).—**Russia:** *Orenburg:* Buzuluk distr., Nat. park "Buzulukskiy bor", Zapovednoye, *Vondrák* JV7336 (CBFS).—**Slovakia:** *Banská Bystrica Reg.:* Muránska planina Mts, Mt Šiance, *Bayerová, Halda & Palice* 5366, 8875 (hb. Palice).—**Slovenia:** *Cerknica:* Rakek, *Prügger, Suppan, Mayrhofer & Batič* SN059.8/1 (GZU).—**Spain:** *Extremadura:* SW of Cáceres, *van den Boom & van den Boom* 38821 (hb. v. d. Boom).—**Sweden:** *Uppland:* Norrsunda par., Rosenberg, *Nordin* 2081 (UPS).—**Turkey:** *Eastern Anatolia Reg.:* Lake Van, Yassica, *Vondrák* JV6514 (CBFS).—**Ukraine:** *Crimea:* Czernigiv, *Khodosovtsev* 3042 (KHER).—**United Kingdom:** *England:* Colchester, *Crombie* 380 (BM).

*Exsiccata examined:* **Austria:** *Lower Austria,* Rosenau, Mt Sonntagberg, *Krypt. Exs.* no. 252 [as *C. cerina* var. *ehrharti*] (PRM, BM).—**Bulgaria:** *Haskovo,* Rhodope Mts, Rabovo, *Vondrák: Sel. Exs. Caloplaca* no. 11 [as *C. virescens*] (CBFS).—**Czech Republic:** *Central Bohemian Reg.,* Benešov u Prahy, Hodčice, Věžda: *Lich. Sel. Exs.* no. 2469 [as *C. cerina* var. *cyanolepra*] (BM).—**Germany:** *Baden-Württemberg,* Constance, Rabenhorst: *Lich. Eur.* no. 697 [as *Placodium cerinum* var. *ehrharti*] (BM).—**Italy:** *Veneto,* Vicenza, Trevisan: *Lichenotheca veneta* no. 183 [as *C. cerina* var. *ehrharti*] (BM).—**Slovakia:** *Žilina Reg.,* Nízke Tatry Mts, *Lich. Slovak. Exs.* no. 227 [as *C. cerina*] (BM).

### ***Caloplaca pinicola* H. Magn.**

*Bot. Not.* **1953**(2): 188 (1953); type: USA, Arizona, [Coconino Co.] Grand Canyon National Park, Coconino Plateau, Aug. 1926, *E. & G. Du Rietz* (S!)—holotype, UPS!—isotype selected by Wetmore 2004 as superfluous lectotype).

*Taxonomical note:* Detailed descriptions are given in Wetmore (2004, 2007a, b). According to specimens of *C. pinicola* seen from ASU, GZU and MIN, the species seems fairly variable in thallus morphology, especially in number and characteristics of the soralia. The uniting character is a relatively thin spore septum (3.0–4.0 µm and never up to 5.5 µm). Unfortunately, the type specimen has a poorly developed thallus. The species needs further study.

There is one closely related species, also from North America, here provisionally called *C. aff. pinicola* (Fig. 1), but it differs in having larger ascospores with thicker septa.

*Nomenclatural note:* The holotype of *C. pinicola* was identified by Magnusson himself in S; on the label is written in Magnusson's handwriting "*Caloplaca pinicola* H. Magn. n. sp." and "Typus!". Wetmore's lectotypification (Wetmore 2004) is superfluous.

*Selected specimens examined.* **USA:** *Arizona:* Coconino Co., Grand Canyon Nat. Park, *Nash* 10513 (GZU); Gila Co., Flowing Springs, *Nash* 39318 (MIN); Pima Co., Saguaro Nat. Mon., *Wetmore* 54893 (MIN). *Colorado:* San Juan Co., Mesa Verde Nat. Park, *Nash* 18141 (ASU); Rio Grande Co., South Fork, *Wetmore* 17214 (MIN). *New Mexico:* Catron Co., San Francisco Mts, *Nash* 22562 (ASU); San Juan Co., *Marsh & Rankert* 419, 448 (ASU); Union Co., Capulin Mt. Nat. Mon., *Wetmore* 17433 (MIN). *North Dakota:* McKenzie Co., T. Roosevelt Nat. Park, Warford City, *Wetmore* 80071 (MIN); Billings Co., T. Roosevelt Nat. Park, Medora, *Wetmore* 45047 (MIN), 80302 (GZU).

### ***Caloplaca sterilis* Šoun, Khodosovtsev, Vondrák sp. nov.**

Thallus areolatus albido-pruinose, areolae soraliis marginalibus; soredia viridi-grisea, pigmentis anthraquinoneis et Sedifolia-grisea in thallo et soraliis nullis; apothecia lecanorina (*Caloplaca cerinae*-typus).

Typus: Ukraine, AR Cimea, Chornomorskiy district, village Olenevka, cape Tarchankut, coast of Black Sea, on twigs of *Artemisia lerchiana*, 45°20'61.7"N, 32°30'47.6"E, alt. 15 m, 4 v 2010, A. Khodosovtsev (KHER—holotypus; KHER, CBFS —isotypi).

(Figs 3A & B)

*Thallus* of scattered areoles/squamules to thin, inconspicuous, grey-green, often white pruinose, sorediate; *areoles* or *squamules* (50–)  $157 \pm 66$  (–300)  $\mu\text{m}$  wide ( $n=22$ ), slightly convex, (50–)  $85 \pm 22$  (–130)  $\mu\text{m}$  thick ( $n=20$ ); *cortex* alveolate (sensu Vondrák *et al.* 2009a) 8–12  $\mu\text{m}$  thick, loosely paraplectenchymatous with crystals dissolving in N; *algal layer* thick, reaching lower thallus surface; *medulla* not always conspicuous, formed by loose prosoplectenchymatous tissue; *photobiont* trebouxoid; *soralia* developed on the areole margins or rarely punctiform on thallus surface, greenish or greenish-grey, (80–)  $145 \pm 44$  (–250)  $\mu\text{m}$  in size ( $n=20$ ); sterile thalli sometimes forming continuous greenish sorediate crusts; *soredia* (15–)  $24 \pm 6$  (–35)  $\mu\text{m}$  diam. ( $n=30$ ), sometimes forming *consoredia* (50–)  $70 \pm 15$  (–100) ( $n=20$ ), K–; *prothallus* not seen.

*Apothecia* lecanorine, very rare, (0.2–)  $0.3 \pm 0.1$  (–0.6) mm diam. ( $n=29$ ), constricted at the base; *disc* flat to slightly convex, yellow-orange to orange, sometimes pruinose, especially when young, K+ purple; *true exciple* indistinct or very rarely visible on section as 2–3 rows of radiate hyphae; *thalline exciple* whitish pruinose, sometimes sorediate, raised above disc, (50–)  $87 \pm 42$  (–200)  $\mu\text{m}$  thick ( $n=20$ ); *cortex* of thalline exciple well-developed, (12–)  $23 \pm 9$  (–40)  $\mu\text{m}$  thick ( $n=21$ ), paraplectenchymatous; cells (2.5–)  $3.1 \pm 0.5$  (–4.3)  $\mu\text{m}$  thick ( $n=20$ ); *epihymenium* orange, granular interspersed; *hymenium* 65–75  $\mu\text{m}$  tall, hyaline; *hypothecium* 50–75  $\mu\text{m}$  thick, hyaline; *paraphyses* simple to branched, 2.0–2.5  $\mu\text{m}$  wide with upper cells widened to (2.8–)  $3.4 \pm 0.4$  (4.3)  $\mu\text{m}$  ( $n=20$ ); *asci* 8-spored, (33–)  $40.5 \pm 3.2$  (–46)  $\times$  (10–)  $13 \pm 2.4$  (–20)  $\mu\text{m}$  ( $n=20$ ); *ascospores* polarilocular, wide ellipsoid, rarely subglobose, (7.5–)  $10.4 \pm 2.0$  (–16.0)  $\times$  (7.5–)  $7.9 \pm 1.0$  (–11.3)  $\mu\text{m}$  ( $n=32$ ), septum (2.3–)  $4.7 \pm 1.1$  (–7.0)  $\mu\text{m}$  wide ( $n=32$ ), ratio of septum width/spore length (0.25–)  $0.5 \pm 0.1$  (–0.5).

*Pycnidia* immersed, inconspicuous. *Conidia* bacilliform,  $c. 3 \times 1 \mu\text{m}$ .

*Etymology.* The name reflects the often sterile state of this species.

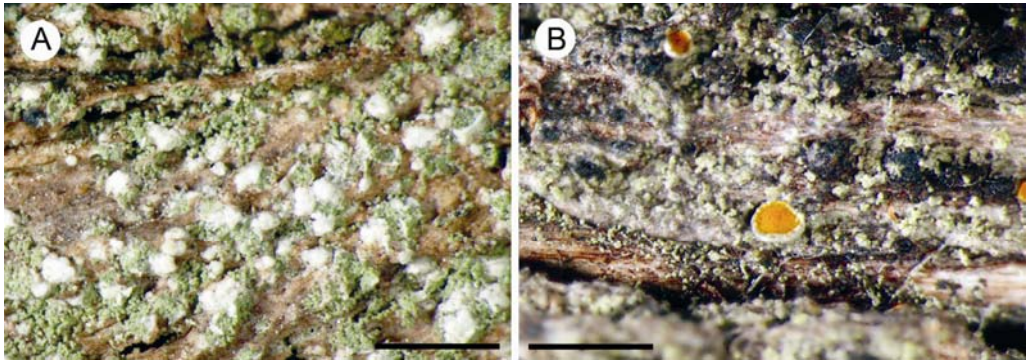


FIG. 3. *Caloplaca sterilis*. A, specimen with white pruinose areoles from steppe (CBFS JV6962); B, fertile specimen without pruina from coastal sand dunes (CBFS JV7421). Scales: A & B = 1 mm.

*Habitat and distribution.* *Caloplaca sterilis* grows on basal parts of woody plant stems (*Artemisia*, *Halocnemum*, *Thymus*), plant debris and dead wood in steppe and sand dune habitats in the Black Sea region. It is an inconspicuous but common species in steppe landscapes and in coastal habitats on sand dunes in SE Europe. Associated species are *Caloplaca raesaenii* Bredkina (= *C. thuringiaca* Søchting & Stordeur), *C. phlogina* (Ach.) Flagey, *C. skii* ad int., *Lecania cyrtella* (Ach.) Th. Fr., *Lecanora hagenii* (Ach.) Ach., *Physcia adscendens* (Fr.) H. Olivier, *Rinodina pityrea* Ropin & H. Mayrhofer, and *Xanthoria parietina* (L.) Beltr. Hitherto known from Bulgaria, Romania, SW Russia and Ukraine.

Two old specimens from old palings from Cherry Hinton near Cambridge (U.K.) deposited in BM (Larbalestier Exs. no. 167, sub *Lecanora cerina* var. *dispersa* Larbal., 9/1879; Ibid., sub *Lecanora cerina* var. *cyanolepra*), seems to be identical with this species. However, molecular data is needed to confirm the identity of British material.

*Remarks.* The species is characterized by commonly white-pruinose minute squamules / areoles with contrasting pale greyish-green to greenish soredia in punctiform, marginal or diffuse soralia. However, our specimens from coastal sand dunes lack pruina and their thalli do not contrast with soralia. The colour of soredia is unique within the *C. cerina* group as they do not contain Sedifolia-gray. The species is easily overlooked and hardly identifiable when completely sorediate and sterile.

*Specimens examined.* **Bulgaria:** *Burgas Prov.:* Burgas, Sozopol, sand dunes near seashore c. 5.5 km S of town, on stems of *Artemisia*, 9 iv 2007, *Vondrák* JV6954, JV7421, JV7624 (CBFS).—**Romania:** *Tulcea Co.:* Babadag, limestone outcrops 250 m SE of Enisala castle ruin, on old dead wood in steppe, 3 iv 2007, *Vondrák* JV6955 (CBFS); coast c. 5.5 km E of Sinoie, on stems of steppe shrubs, 4 iv 2007 *Vondrák* in JV6616 (CBFS); Razim Lake, Popina Island, on dead twig of shrub, 2 iv 2007, *Vondrák* JV6963 (CBFS).—**Russia:** *Krasnodar krai:* Taman Peninsula, steppe near road E of Primorskiy, on shrub twig, 19 v 2007, *Vondrák* & *Šoun* JV7386 (CBFS).—**Ukraine:** *Kherson Reg.:* Belozerskiy district, vill. Sofievka, Sofievska balka, on *Artemisia* twigs, 14 ii 2009, *Gavrilenko* & *Khodosovtsev* JV6962 (CBFS), *Vondrák* JV7100 (CBFS, KHER); vill. Kirove, steppe slope, 11 m alt., N46°53'92.8" E32°50'54.9", 24 iv 2010, *Khodosovtsev* (KHER); Beryslavskiy district, village Novokairy, Kamenska balka valley, steppe slope with limestone outcrops, on twigs of *Thymus dimorphus*, alt. 20 m, 1 iv 2009, *Khodosovtsev* (KHER); Golopristskiy district, Black sea reserve, Tendriv's'ka kosa island, sand littoral dunes, on plant debris of *Artemisia*, 30 iv 2009, *Khodosovtsev* (KHER); Gornostaiivskiy district, vill. Kairy, Kairska balka, steppe slopes, on plant debris, 21 iii 2010, *Khodosovtsev* (KHER); Verhnerogacheskiy district, vill. Ushkalka, cliff to Kahovskoye artificial sea, on plant debris, 27 iii 2010, *Khodosovtsev* (KHER); Novotroitskiy district, 4 km S of vill. Drushelyubovka, cape Kutara, on coast of Sivash like, N46°08'64.0"

E34°02'76.5", 25 iv 2010, *Khodosovtsev* (KHER). *AR Crimea*: Leninskiy district, Arabatskaya strelka peninsula, Valok, sand littoral dunes, on *Thymus*, 10 viii 2009, *Khodosovtsev* (KHER); Krasnoperekopsk, Nadezdino, at small shallow gulf S of village, on stems of *Halocnemum strobilaceum*, 8 vi 2006, *Vondrák* in JV5164 (CBFS); Pervomaiskiy district, upland Sary-Bash, 5 km SW from vill. Vypasne, near road H05, 71 m alt., N45°35'37.9" E33°51'49.8", 28 iv 2010, *Khodosovtsev* (KHER).

### ***Caloplaca stillicidiorum* s. lat.**

Including *Caloplaca stillicidiorum* (Vahl) Lyngé, *C. cerina* var. *chloroleuca* (Sm.) Th. Fr., *C. cerina* var. *muscorum* (A. Massal.) Jatta, *C. cerina* (Hedw.) Th. Fr. p.p., *C. muscorum* (A. Massal.) M. Choisy & Werner

This taxon comprises *Caloplaca cerina*-like lichens growing on or near the ground on various substrata like bryophytes, plant debris, wood, bark of exposed roots and chamaephytes, rarely rocks. Such specimens form a monophyletic group in the cladogram. The molecular topology shows basically four monophyletic groups. These groups may be only partly interpreted on the basis of their morphology and ecology. Six provisional 'ecotypes', more or less reflecting the phylogeny (Fig. 1), are distinguished here but they are not always separable by morphology. Short characteristics of the ecotypes based on sequenced material are presented here.

1. On bryophytes and plant debris, in uplands and mountains on limestone in Austria, Bulgaria, Czech Republic, France, Greece, Hungary, Spain and Ukraine; apothecia to 1.1 mm in diam.; apothecial margin strongly white pruinose, disc sordid yellow-orange, sordid yellow-green to sordid grey-brown, pruinose or rarely not; thallus consisting of small white pruinose areoles; spores (12–)  $16.0 \pm 1.8$  (–19)  $\times$  (6–)  $8.7 \pm 1.0$  (–11)  $\mu\text{m}$ , septum (5–)  $7.1 \pm 1.1$  (–9)  $\mu\text{m}$  wide ( $n=59$ ). The type of *Lichen chloroleucus* Sm. (BM!—holotype) and the epitype of *Lichen stillicidiorum* Vahl (FH!—isoeptype) selected by Wetmore (2007a) probably belong to this ecotype. However, the holotype illustration of *Lichen stillicidiorum* in Vahl (1792: 6, tab. 1063, f. 2) may belong elsewhere.

2. On plant debris (e.g. *Saxifraga*) and moss, in alpine calcareous habitats in Austria, Russia and Sweden; apothecia to 1.2 mm in diam.; apothecial margin dark grey, white pruinose; disc (sordid) yellow to orange, pruinose or not; thallus invisible; spores (13–)  $15.2 \pm 1.4$  (–19)  $\times$  (6–)  $7.7 \pm 0.8$  (–10)  $\mu\text{m}$ , septum (5–)  $6.3 \pm 0.9$  (–8)  $\mu\text{m}$  wide ( $n=43$ ).

3. On plant debris and moss, in arctic areas in Alaska, Greenland, Norway and Svalbard; apothecia to 1.3 mm in diam.; apothecial margin dark grey, white pruinose or not, disc yellow-orange to orange, not pruinose; thallus invisible; spores (12–)  $14.7 \pm 1.2$  (–17)  $\times$  (7–)  $8.1 \pm 0.7$  (–9)  $\mu\text{m}$ , septum (5–)  $5.7 \pm 0.7$  (–7)  $\mu\text{m}$  wide ( $n=39$ ).

4. On plant debris, exposed wood of roots, and rock in calcareous xerothermous habitats in Canada, Czech Republic, Kazakhstan, Ukraine and USA and on wooden fences in mountains in Austria; apothecia to 1.5 mm in diam.; apothecial margin pale to dark grey, white pruinose or not, disc yellow-orange to orange, pruinose or not; thallus invisible or formed by white pruinose tiny areoles; spores (12–)  $14.4 \pm 0.9$  (–16)  $\times$  (5–)  $7.7 \pm 0.6$  (–9)  $\mu\text{m}$ , septum (4–)  $4.9 \pm 0.5$  (–6)  $\mu\text{m}$  wide ( $n=58$ ).

5. On acrocarpous mosses (esp. *Grimmia*), on basic siliceous and conglomerate rocks in Finland, Romania, Spain, Turkey, Ukraine, USA and Uzbekistan; apothecia to 1.6 mm in diam.; apothecial margin pale to dark grey, white pruinose; disc yellow-orange to dull orange, pruinose or not; thallus thin to thick crustose or formed by well developed small lobules; spores (11–)  $14.8 \pm 1.6$  (–19)  $\times$  (6–)  $8.7 \pm 1.3$  (–11)  $\mu\text{m}$ , septum (4–)  $5.2 \pm 0.6$  (–7)  $\mu\text{m}$  wide ( $n=69$ ).

6. On bark of chamaephytes (esp. *Thymus*) and exposed roots, in steppe-like habitats on limestone in the Czech Republic, Hungary and Ukraine; apothecia to 0.9 mm in diam.; apothecial margin pale to medium grey, white pruinose or not; disc yellow to yellow-orange,

pruinose or not; thallus invisible or formed by small areoles; spores (10–)  $12.8 \pm 1.2$  (–16)  $\times$  (6–)  $7.1 \pm 0.7$  (–8)  $\mu\text{m}$ , septum (4–)  $4.8 \pm 0.4$  (–5)  $\mu\text{m}$  wide ( $n=39$ ).

Burgaz *et al.* (1999) dealt with samples of *C. cerina* on *Dryas octopetala* from the Pyrenees with extremely wide septa, 7–9  $\mu\text{m}$ , more than half of the length of the ascospore. Such wide septa have not been observed in the studied material and future attention should be given to those samples.

### **Caloplaca subalpina Vondrák, Šoun & Palice**

*Lichenologist* **40**: 378 (2008); type: Ukraine, Eastern Carpathians, Svidovets Mts, glacial cirque in NE slope below Mt Bliznitsa, alt. c. 1500 m, 48°14'21"N, 24°14'E, on lime-rich schist outcrop, beneath overhang, in subalpine belt, 29 June 2007, *Vondrák* CBFS JV6072 (CBFS!—holotype; GZU, L—iso-types).

This saxicolous species grows on well-lit rocks beneath overhangs and is known from several localities throughout the mountains in Europe. Apothecia are rarely developed on the white-pruinose areolate thallus which merges into squamules at margins. The soralia are dark grey, arising from margins of areoles. For a detailed description see *Vondrák et al.* (2008).

### **Caloplaca thracopontica Vondrák & Šoun**

*Lichenologist* **40**: 381 (2008); type: Turkey, Black Sea coast, Sinop, coastal rocks on NE coast of peninsula, alt. c. 100 m, 42°01'57.81"N, 35°11'34.42"E, on coastal volcanic rock, 21 April 2007, *Vondrák* CBFS JV5419 (CBFS!—holotype; GZU, hb. M. Seaward—iso-types).

This is a maritime saxicolous species that grows on hard siliceous outcrops in the Black Sea region, where it is locally common. It is characteristic by the presence of lobules and pustules on the thallus. On the Mediterranean coasts there is a similar, but possibly different species, hitherto not well known (see Results). For a detailed description see *Vondrák et al.* (2008).

### **Caloplaca turkuensis (Vain.) Zahlbr.**

*Cat. Lich. Univ.* **8**: 589 (1931) [as '*turkuensis*'].—*Placodium turkuense* Vain., *Ann. Soc. zool.-bot. Fenn. Vanamo* **9**: 320 (1929); type: Ab. Turku, Vartiovuori, 2 Oct. 1927, *Vainio* 35118 (TUR-V 35374!—holotype).

*Caloplaca jemtlandica* H. Magn., *Bot. Notiser* **1945**: 304 (1945); type: Sweden, Jämtland, Undersåker, 30 June 1912 *Malme* (S!—holotype, UPS!—isotype).

*Caloplaca jemtlandica* var. *cerinosora* E.S. Hansen, Poelt & Søchting, *Meddelelser om Grønland, Bioscience* **25**: 33 (1987); type: Grønland, Disko, Lyngmark, untere Hänge des Lyngmarksfjeld N Godhavn, an *Salix*, 27 July 1983, *Poelt & Ullrich* (GZU!—holotype).

*Caloplaca cerina* f. *coronulata* Th. Fr., *Lichenogr. Scandinavica* **I**: 175 (1871).—*Lecanora cerina* f. *coronulata* Nyl., *Notiser ur Sällsk. pro Fauna et Flora Fennica Förhandlingar* **8**: 128 (1866); type: Lapponia orientalis. Knäsä [Russia, Murmansk Oblast, Knyazhaya Guba], 1861, *Fellman* (H!—lectotype designated here).

(Figs 2E & F)

*Thallus* with very small areoles, sorediate, up to several cm wide, non-pruinose, up to 0.13 (–0.3) mm thick; *areoles* sordid white to pale (sordid) grey, initially convex but later flat, up to 0.2 mm wide, usually very soon entirely sorediate. *Soralia* pale to dark grey or greenish grey, with bluish tinge, whitish or greenish inside (seen when external soredia are detached), arising from upper surface or margins of areoles, scattered to confluent, slightly convex; *soredia* (15–)

22 ± 3 (–30) µm diam. (*n*=95); *prothallus* sometimes present, very thin, grey to black; *cortex* up to 10 (–20) µm thick, paraplectenchymatous.

*Apothecia* lecanorine, abundant to absent, up to 0.8 mm diam., sessile; *disc* flat to slightly convex, yellow-orange, rarely pruinose; *true exciple* indistinct, up to 15 µm thick, prosoplectenchymatous; *thalline exciple* grey to white, rarely pruinose, raised above disc when young, somewhat reduced in old apothecia, 50–85 µm thick, sometimes becoming sorediate; *epihymenium* orange, with interspersed granules; *hymenium* 55–100 µm thick, hyaline; *hypothecium* 55–110 µm thick, hyaline, sometimes oil-inspersed; *paraphyses* simple to branched, 2 µm broad with upper cells wider, 3–5 µm diam.; *asci* 8-spored, 38–54 × 10–17 µm (*n*=19); *spores* polarilocular, ellipsoid, (10.0–) 12.1 ± 1.1 (–15.0) × (6.0–) 6.9 ± 0.8 (–9.0) µm, septum (3.0–) 4.3 ± 0.7 (–6.0) µm wide (*n*=58), ratio of septum width/spore length (0.2–) 0.4 ± 0.05 (–0.5).

*Pycnidia* sometimes present, immersed, distinct by elevated darker ostiole. *Conidia* bacilliform, 3–4 × 1 µm.

*Habitat and distribution.* This species grows on bark of broad-leaved trees (*Acer*, *Alnus*, *Betula*, *Fraxinus*, *Malus*, *Populus*, *Sorbus*, *Ulmus*) and rarely of conifers (*Picea*). Also found on wood of spruce stumps and wooden fences. It occurs from lowlands to mountains in deciduous and mixed forests or on wayside trees. The specimen from Greenland (*C. jemtlandica* var. *cerinosora*) grew on a twig of *Salix glauca*. Probably, this species is widespread throughout Europe and North America, but it has not previously been recognized. Hitherto recorded from Austria, Czech Republic, Finland, Greenland, Norway, Russia, Slovakia, Sweden, United Kingdom and USA.

*Remarks.* This species is characterized by the often sorediate apothecial margins, slightly convex, punctiform to confluent blue-grey soralia, minute or often absent areoles. However, specimens without sorediate apothecial margins and with persistent corticated areoles with delimited soralia are common and can be confused with small thalli of *C. chlorina*, but the latter usually has larger soredia. Sterile specimens can be confused with *Rinodina colobina* (Ach.) Th. Fr. or *Rinodina pityrea* Ropin & H. Mayrhofer.

During the phylogenetic analyses a taxon similar to *C. turkuensis* was discovered, but several ITS sequences showed that it belonged outside of the *C. cerina* group (not included in the cladogram in Fig. 1). These specimens from Sweden are morphologically distinguished from *C. turkuensis* by soralia and apothecia with an endophloeic origin, not developing from areoles, the true exciple is usually more apparent and the thalline margin is not so stout. This probably unknown species needs further study. The types of *C. turkuensis* and the here synonymized *C. jemtlandica* incl. var. *cerinosora* and *C. cerina* f. *coronulata* are mostly old and were not molecularly analyzed. However, morphologically they seem to correspond with our material molecularly confirmed to belong to the *C. cerina* group.

*Nomenclatural note.* Specimens of this species have been commonly misidentified as various species, i.e., *C. virescens*, *C. chlorina* or *C. cerina*. However, the names *Caloplaca jemtlandica* (including var. *cerinosora*) and *Caloplaca turkuensis* have been applied to different forms of the same species, but the latter name is the oldest and has therefore priority over the younger ones.

*Caloplaca turkuensis* has probably only been used for the type collection, which has fairly well developed, thick, sorediate thallus and abundant apothecia, with completely sorediate margins; it is congruent in phenotype with specimens used for getting molecular data.

*Caloplaca jemtlandica* is also a little known taxon. Surprisingly, the type specimens are sorediate, contrary to the description in the original paper, although both thallus and apothecial

margin are by far not so soresiate as in the type of *C. turkuensis*. The specimen labeled as isotype in S is not conspecific with holotype in S and isotype in UPS, but at the moment it is not possible to tell where it belongs.

*Caloplaca jemlandica* var. *cerinosora* is another poorly known taxon, described and hitherto known only from one twig of *Salix glauca* from Greenland. The type specimen of *C. jemlandica* var. *cerinosora* is a poorly developed specimen of *C. turkuensis*.

*Caloplaca cerina* f. *coronulata* is a form with distinctly soresiate thallus and apothecial margin clearly conspecific with *C. turkuensis*.

*Selected specimens examined.* **Austria:** Vorarlberg: Verwall-Gruppe, Nenzigast-Tal, Poelt IN85-86 (GZU). Styria: Schladming, Aich, Vondrák JV7251 (CBFS).—**Czech Republic:** Central Bohemian Reg.: Příbram, Lešetice, Malíček et al. 1258 (hb. Malíček). Plzeň Reg.: Šumava Mts, Modrava, Mt Medvěď, Liška JV2921 (CBFS). South Bohemian Reg.: Šumava Mts, České Žleby, Palice, Dětinský, Horáková & Liška (hb. Palice). Moravian-Silesian Reg.: Beskydy Mts, Staré Hamry, Salajka, Malíček & Palice 1908 (hb. Malíček).—**Finland:** Uusimaa: Karjalohja, Maila, Laurila (H). Tavastia Proper: Tammela, Mustiala, Räsänen (H).—**Norway:** Oslo, Tønsberg 9926 (BM). Buskerud: Sigdal, Prestfoss, Tønsberg 13312 (BM). Oppland: Vang, Lykkja, Søchting (BM).—**Russia:** Orenburg: Buzuluk distr., Nat. park "Buzulukskiy bor", Zapovednoye, Vondrák JV7429 (CBFS).—**Slovakia:** Prešov Reg.: E Carpathians, N.P. Nízke Poloniny, Mt Pľaša, Palice & Šárová 9302 (PRA).—**Sweden:** Torne Lappmark: Jukkasjärvi par., Torneträsk, Arup L04229 (LD). Gotland: Atlingbo par., Lillo Atlings, Sundin & Thor 57 (UPS).—**United Kingdom:** Scotland: Elgin, Ballindalloch Castle, James (BM).—**USA:** Washington: Pend Oreille Co., Selkirk Mts, Lower Granite Falls, Spribille 15393 (hb. Spribille).

### **Caloplaca ulmorum (Fink) Fink**

*Lichen Fl. U. S.:* 358 (1935).—*Placodium cerinum* var. *ulmorum* Fink, *Contr. U. S. Nat. Herb.* **14:** 215 (1910); type: USA, Kansas, Rooks County, on *Juniperus virginiana*, 7 Dec. 1893, E. Bartholomew (MICH 00062622!)—lectotype designated by Wetmore 2007a, MICH 00067693!—isolectotype).

*Thallus* areolate to predominantly granular, up to 0.16 (–0.3) mm thick; *areoles* flat to slightly convex, scattered to continuous, non-pruinose, beige, beige-grey to pale greenish grey, up to 0.8 mm wide; *granules* (50–) 107 ± 32 (–200) µm diam. (*n*=90); *cortex* up to 25 (–40) µm thick, paraplectenchymatous, sometimes very minutely tomentose by inconspicuous hairs 4–10 × 2–3 µm formed by projecting hyphae (visible in section), particularly on granules; *prothallus* not seen.

*Apothecia* lecanorine, usually abundant, up to 1.3 mm diam., sessile, distinct, usually pruinose; *disc* flat to slightly convex, yellow to yellow-orange, usually pruinose; *true exciple* indistinct, very thin, up to 10 µm thick, prosoplectenchymatous; *thalline exciple* of thallus colour, usually white pruinose, raised above disc when young, somewhat reduced in old apothecia, 75–120 µm thick; *epihymenium* orange, with interspersed granules; *hymenium* 80–100 µm thick, hyaline; *hypothecium* 50–140 µm thick, hyaline; *paraphyses* simple to branched, 2 µm broad with upper cells wider, 3–6 µm thick; *asci* 8-spored, 42–70 × 10–18 µm (*n*=45); *spores* polarilocular, ellipsoid, (11.0–) 13.6 ± 1.4 (–18.0) × (6.0–) 6.9 ± 0.8 (–9.0) µm; septum (4.0–) 5.6 ± 0.9 (–8.0) µm wide (*n*=90); ratio of septum width/spore length (0.3–) 0.4 ± 0.05 (–0.5).

*Pycnidia* rare to abundant, immersed, distinct by elevated darker ostiole. *Conidia* bacilliform, 3–4 × 1 µm.

*Habitat and distribution.* *Caloplaca ulmorum* is an exclusively North American species usually growing on bark of broad-leaved trees, mainly *Ulmus* but also on e.g. *Acer* and *Quercus*. Nevertheless, the type collection is from bark of a conifer, *Juniperus virginiana*. The complete distribution is not known.



*Remarks.* The species is characterized by a usually well developed areolate thallus with marginal granules. Poorly developed thalli consisting of only granules are morphologically hard to separate from *C. monacensis*. A good image is in Wetmore (2007a: 805).

*Nomenclatural note.* Wetmore (2007a) synonymized this name with *C. cerina* but we show that it represents a separate species.

*Selected specimens examined.* **USA:** *Minnesota:* Hennepin Co., Minneapolis, Wayzata Big Woods, Wetmore 90256 (GZU), *ibid.*, Wolsfeld Woods, Trana 9973 (GZU); Olmsted Co., Oronoco, Wetmore 24185 (GZU). *Missouri:* Newton Co., Joplin, George Washington Carver Nat. Mon., Wetmore 69226 (LD). *South Dakota:* McCook Co., Lake Vermillion St. Rec. Area, Wetmore 93154 (MIN). *Wisconsin:* Bayfield Co., Rainbow Lake Wilderness, Bellevue Lake, Wetmore 70829 (GZU).

*Exsiccata examined.* **Canada:** *Ontario:* Ontario Co., Beaverton, *Lich. Can. Exs.* nr. 157 (GZU).—**USA:** *South Dakota,* Grant Co., Marvin, Blue Cloud Benedictine Abbey, *Lich. E. N. Am. Exs.* no. 265 [as *Caloplaca cerina*] (GZU). *Wisconsin,* Iowa Co., Barneveld, *Lich. Wiscon. Exs.* no. 107 (GZU).

### Intricate names

*Caloplaca virescens* (Sm.) Coppins seems to be morphologically and ecologically close to *C. monacensis*, but differs in the well developed, areolate, rarely fertile thallus with a thick layer of small granules (45–70 µm in diam.). Unfortunately, no fresh material was available for sequencing. Four very old specimens, including the part of the holotype (see Laundon 1992), and only one more recent specimen (1973) have been found in BM. This species needs further study.

The little used name *C. albolutea* (Nyl.) Zahlbr. (Type: *Supra muscos pulvinatos* in *Lapponia orientali eam in insulis Maris Albi, legit G. Selin, H-NYL!*—lectotype designated here) and perhaps *C. mydalaea* (Körb.) Zahlbr. (Type: UPS—“fragment. specimin. original.”!) probably belong to *C. stillicidiorum* s. lat. but both type specimens are too exiguous.

### Discussion

Our results show again, what has already been proved many times; the phenotype-based traditional taxonomy was affected by choosing incorrect diagnostic characters or by the incorrect interpretation of the characters. For example, in the British lichen flora, Fletcher & Laundon (2009) list three species of the *Caloplaca cerina* group: a sorediate "*C. virescens*", an isidiate "*C. chlorina*" and "*C. cerina*" without vegetative diaspores. This is a very practical simplification, but the situation is much more complex. As we have shown, sorediate and isidiate species and species without vegetative diaspores occur repeatedly within the phylogenetic tree, and extremely similar species are often not closely related. Based on our data, we can not say which thallus characters are plesiomorphic and which are derived but convergent evolution is evident here in phenotypes even with or without vegetative diaspores. Similar patterns were observed in the phylogeny of the *C. citrina* group (Arup 2006, Vondrák *et al.* 2009a) and the *C. ferruginea* group (Arup & Åkelius 2009).

Our molecular analysis corroborates the sometimes underestimated role of the precise type of vegetative diaspores as a crucial phenotypic character in this complex. For instance, presence of soredia (or blastidia) vs. isidia clearly separate the formerly often unrecognized *C. chlorina* and *C. isidiigera*. Undoubtedly, other important phenotype characters are substrate specificity and geographical distribution. This is shown in a provisional taxonomy of *C. cerina* s. lat. and *C. stillicidiorum* s. lat., where vegetative diaspores are absent, and only little data on ecology and distribution are available. Both groups are formed of several phylogenetic lineages,

polyphyletic in *C. cerina* s. lat. and monophyletic in *C. stillicidiorum* s. lat., which may be called cryptic species or semi-cryptic species, if they have at least some phenotypic tendencies (Vondrák *et al.* 2009a).

Grube & Kroken (2000) recommended that a phylogenetic species should be congruent with at least one phenotypic character when a single locus such as ITS is used. Crespo & Pérez-Ortega (2009) suggested for recognition of cryptic species that molecular analysis should be based on more than one independent genetic region, in order to detect recombination events among lineages. We are so far not able to fulfill these conditions in the two species complexes. Several loci, more extensive sampling to avoid present sampling bias and/or more advanced phenotype appraisal should be used to separate some putative cryptic species within these tentative aggregate names.

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## Letter of acceptance

17-Oct-2010

Dear Mr. Šoun:

It is a pleasure to accept your manuscript entitled "Taxonomy and phylogeny of the Caloplaca cerina group in Europe" in its current form for publication in The Lichenologist.

Thank you for your fine contribution. We look forward to your continued contributions to the Journal.

Yours sincerely

Peter Crittenden

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# Paper II

Vondrák, J., Šoun, J., Hrouzek, P., Říha, P., Kubásek, J., Palice, Z. & Söchting, U.

(2008)

*Caloplaca subalpina* and *C. thracopontica*, two new saxicolous species from the *Caloplaca cerina* group (*Teloschistales*).

*Lichenologist* 40: 375–386.



## *Caloplaca subalpina* and *C. thracopontica*, two new saxicolous species from the *Caloplaca cerina* group (*Teloschistales*)

Jan VONDRÁK, Jaroslav ŠOUN, Pavel HROUZEK, Pavel ŘÍHA, Jiří KUBÁSEK,  
Zdeněk PALICE and Ulrik SØCHTING

**Abstract:** *Caloplaca subalpina* Vondrák, Šoun & Palice and *C. thracopontica* Vondrák & Šoun are described here as new to science. The former is a sorediate, often sterile, saxicolous species inhabiting subalpine base-rich overhanging rocks in European mountains; the latter grows on maritime cliffs of the Black Sea and is conspicuous by the lobules and pustules which are usually present on its thallus and by its apothecia which are typically large and abundant. The placing of the two species in the *C. cerina* group was confirmed by molecular studies using nrDNA ITS sequences. The chemosyndromes of both new species correspond to chemosyndrome A, which is in accordance with their position in the *C. cerina* group. A key to the saxicolous species of the *C. cerina* group is provided.

**Key words:** Black Sea, Europe, lichenized fungi, nrDNA ITS, *Teloschistaceae*

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### Introduction

The concept of the *Caloplaca cerina* group has varied with different authors. For example, Clauzade & Roux (1985) and more recently Wetmore (2007) have interpreted it in a broad sense to include species with zeorine apothecia, which are not related

to *C. cerina* (Ehrh. ex Hedw.) Th. Fr. We consider the *C. cerina* group in its strict sense as a monophyletic group that is morphologically characterized by lecanorine apothecia with strongly reduced, superficially  $\pm$  invisible true exciple. The thallus is not placodioid and does not contain anthraquinones. The apothecial characters show little variability in this group, but thallus morphology, such as vegetative diaspores (lobules, pustules, isidia, consoredia, and soredia), is highly variable; thus the diagnostic characters of particular species are mainly concerned with thallus structures. Some species with the morphology of the *C. cerina* group do not, however, belong to the *C. cerina* clade, for example *C. squamuloisidiata* van den Boom & V. J. Rico (J. Šoun, unpublished data).

We present here data which are part of a major project on the taxonomy of the *Caloplaca cerina* group in Europe. Not many species have been described in the *Caloplaca cerina* group, and the majority are from Europe, but species are to be found on a wide range of substrata (bark, soil, mosses, rock and plant debris).

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This paper concerns two new saxicolous species that grow specifically beneath base-rich overhanging rocks in the subalpine mountain belt in Europe and maritime cliffs of the Black Sea, respectively. Both new species are shown here in two well-supported clades in a brief phylogenetic tree of the *Caloplaca cerina* group. A key to the saxicolous species of this group is also provided.

## Materials and Methods

### Morphology

A total of 19 characters were measured in the new species: size of areoles, height of thallus, cortex, and algal layer, size of vegetative diaspores, size of cortical cells and of algal and fungal cells in the algal layer, size of apothecia, thalline and true exciple width, hypothecium and hymenium height, size of asci and ascospores, width of equatorial wall thickenings in ascospores (referred to as 'septa' in the text), width of paraphyses tips, and size of conidiomata, conidiogenous cells and conidia. Qualitative characters such as type of each tissue, for example paraplectenchymatous vs. prosoplectenchymatous, occurrence of anastomoses and presence of thin-walled (<0.5 µm) vs. thick-walled (>0.5 µm) cells, and colour of the thallus and apothecia were also studied.

Sections for morphological examination were cut by hand and mounted in water. Accuracies of 0.5 µm (for cells, e.g. conidia and ascospores), 1 µm (asci size and cortex height) and 10 µm (larger structures, e.g. hymenium and hypothecium height) were achieved; all measurements of cells include their walls as well as lumina. Paraphyses tips and thallus characters were observed after pretreatment with KOH. Ascospores with well-developed septum (loculi only connected with thin cytoplasmatic channel, but not disconnected) were measured. Measurements are given as (min.–)  $x \pm SD$  (–max.), where  $x$  = mean value,  $SD$  = standard deviation and min./max. = extremes. Total numbers of measurements ( $n$ ) are given in parentheses. Morphological data were taken from all specimens available; in *C. subalpina*, apothecial characters were investigated in only two populations with well-developed ascocarps. For both species, at least 15 measurements of each character were determined, except for pycnidial size in *Caloplaca subalpina*, where  $n=6$ .

Nomenclature generally follows Nimis & Martellos (2003) and Santesson *et al.* (2004), but Hansen *et al.* (1987) for *Caloplaca jemtlandica* var. *cerinosora* E. S. Hansen, Poelt & Söchting, and van den Boom & Rico (2006) for *C. squamuloisidiata* van den Boom & V. J. Rico. Names with single quotation marks are incorrect or unclear. For instance, corticolous samples commonly named *Caloplaca isidiigera* Vězda or *C. chlorina* (Flot.) Sandst. belong to a different species (J. Šoun,

unpublished data), thus both names are in inverted commas, when used for corticolous material.

*Material used for comparison.* *Caloplaca aractina* (Fr.) Håyrén. **Bulgaria:** Black Sea coast: Tsarevo, 2004, *ř. Vondřák* 2248 (CBFS).—**Czech Republic:** Central Bohemia: Křivoklát, 2003, *ř. Vondřák* 1163 (CBFS).—**Ukraine:** Crimean Peninsula: Karadag, 2007, *ř. Vondřák* 5948 (CBFS).

*C. chlorina.* **Bulgaria:** Rhodopes: Madzharovo, 2004, *ř. Vondřák* (CBFS JV2055).—**Czech Republic:** South Bohemia: Milevsko, 2004, J. Vondřák (CBFS JV2056).

*C. conversa* (Kremp.) Jatta. **Iran:** East Azerbaijan: Khalkhal, 2007, *ř. Vondřák* 5566 (CBFS).—**Ukraine:** Crimean Peninsula: Alushta, 2007, *ř. Vondřák* 6007 (CBFS).

*C. furax* Egea & Llimona. **Spain:** Sierra del Relumbrar: 1978, *ř. Egea & X. Llimona* (isotype, Murc. Lichenotheca 3039, GZU).

*C. isidiigera.* **Austria:** Eastern Alps: Seckauer Alpen Mts, 2007, *ř. Vondřák* 6081 (CBFS).—**Ukraine:** Eastern Carpathians: Svidovets Mts, 2007, *ř. Vondřák* 6073 (CBFS).—**Slovakia:** Low Tatras: Mt Vel'ký Bok, 1974, *A. Vězda* (isotype, *Vězda Lich. sel. exs.* no. 1494, PRM). Note: *C. isidiigera* is a morphologically well-characterized species forming a monophyletic group (J. Šoun & J. Vondřák, unpublished data), which should not be reduced to synonymy under *C. chlorina*, as done by Wetmore (1997).

*C. pellodella* (Nyl.) Hasse. **Bulgaria:** Rhodopes: Madzharovo, 2004, *ř. Vondřák* 2114 (CBFS).—**Morocco:** Anti-Atlas Mts: Tafraoute, 2003, *ř. Vondřák* 1429 (CBFS).

*C. percrocata* (Arnold) J. Steiner **Italy:** Southern Alps: Castelnuovo, 1902, *ř. Baumgartner* (holotype of *C. cerina* var. *areolata*, W); Trento, 2006, Š. Hulová 4634 (CBFS).—**Ukraine:** Eastern Carpathian: Svidovets Mts, 2007, *ř. Vondřák* 6082 (CBFS).

*C. squamuloisidiata.* **Spain:** Extremadura: Sierra de las Villuercas, 2001, P. & B. v. d. Boom 27264 (paratype, hb. v. d. Boom).

*C. xerica* Poelt & Vězda. **Bulgaria:** Rhodopes: Lyubimets, 2004, *ř. Vondřák* 2177 (CBFS).—**Czech Republic:** Central Bohemia: Točník, 2003, *ř. Vondřák* 1124 (CBFS).—**Iran:** East Azerbaijan: Nir, 2007, *ř. Vondřák* 5607 (CBFS).—**Romania:** Munții Zărandului Mts: Șoimoș, 2005, *ř. Vondřák* 3647 (CBFS).—**Ukraine:** Mykolaivska oblast: Pervomaisk, 2006, *ř. Vondřák* 5650 (CBFS).

*A new morphological term.* The term *algonecral medulla* is established here for the hyaline, paraplectenchymatous tissue below the algal layer, formed by thin-walled fungal cells among dead algal cells or gaps created after the death of algal cells (Fig. 2A). The true medulla is a loose prosoplectenchymatous tissue situated below this layer. The algonecral medulla is present in both new species, mainly in places where the thallus height is above-average. Its presence in other species of the *C. cerina* group will be discussed in a forthcoming paper.



TABLE 1. Sample data and GenBank numbers of the new ITS sequences used in the phylogenetic analysis

| Species/Herbarium Accession No.   | Locality (collector)   | GenBank Accession No. |
|---|--|-----------------------|
| <i>C. cerina</i> LD L03347  | Sweden, Lycksele Lappmark, Rönä<br>(Arup 2003)                                   | EU365861              |
| <i>C. chlorina</i> CBFS JV2055  | Bulgaria, Rhodope Mountains,<br>Haskovo (Vondrák 2004)                           | EU365859              |
| <i>C. chlorina</i> CBFS JV3120  | Czech Republic, Czech-Moravian<br>Highland, Kamenice nad Lipou<br>(Vondrák 2005) | EU365858              |
| <i>C. isidiigera</i> CBFS JV6073  | Ukraine, Zakarpatska oblast region,<br>Svidovets Mts (Vondrák 2007)              | EU365857              |
| <i>C. isidiigera</i> LD L04227  | Sweden, Lule Lappmark,<br>Padjelanta national park (Arup<br>2004)                | EU365856              |
| <i>C. stillicidiorum</i> CBFS,<br>Sel. Exs. <i>Caloplaca</i> , 12                               | Bulgaria, Rhodope Mountains,<br>Asenovgrad (Vondrák 2004)                        | EU365860              |
| <i>C. subalpina</i> CBFS JV6072 (holotype)  | Ukraine, Zakarpatska oblast region,<br>Svidovets Mts (Vondrák 2007)              | EU365855              |
| <i>C. subalpina</i> CBFS JV692  | Spain, Pyrenees, Jaca (Vondrák<br>2002)  | EU365854              |
| <i>C. subalpina</i> Hb. Palice 6983   | Czech Republic, Jeseníky Mts,<br>Velký kotel corrie (Palice 2001)                | EU365853              |
| <i>C. thracopontica</i> CBFS JV3419   | Bulgaria, Coast of Black Sea,<br>Sozopol (Vondrák 2005)                          | EU365847              |
| <i>C. thracopontica</i> CBFS JV5419 (holotype)  | Turkey, Coast of Black Sea, Sinop<br>(Vondrák 2007)                              | EU365848              |
| <i>C. thracopontica</i> CBFS JV5621   | Turkey, Coast of Black Sea,<br>Trabzon (Vondrák 2007)                            | EU365852              |
| <i>C. thracopontica</i> CBFS JV5623   | Turkey, Coast of Black Sea, Sinop<br>(Vondrák 2007)                              | EU365851              |
| <i>C. thracopontica</i> CBFS JV6065   | Turkey, Coast of Black Sea,<br>Giresun (Vondrák 2007)                            | EU365849              |
| <i>C. thracopontica</i> CBFS,<br>Sel. Exs. <i>Caloplaca</i> , 15 (sub <i>C. aff. chlorina</i> ) | Bulgaria, Coast of Black Sea,<br>Tsarevo (Vondrák 2004)                          | EU365846              |
| <i>C. thracopontica</i> Hb. Šoun 302  | Turkey, Coast of Black Sea, Sarp<br>(Šoun 2007)                                  | EU365850              |

### Chemistry

Lichen substances in apothecia were extracted in 150 µl of acetone at room temperature. The extract was subjected to high-performance liquid chromatographic analysis. Reverse phase column (C18, 5 µm, Lichrocart 250-4) was eluted with MeOH/30%MeOH+1%H<sub>3</sub>PO<sub>4</sub> for 77 min and the absorbance at 270 nm was recorded (for details see Sochting 1997). The compounds were determined on the basis of their retention times and absorption spectra. Acetone-insoluble pigments were examined according to Meyer & Printzen (2000).

### DNA extraction, amplification and sequencing

Direct PCR was used for PCR-amplification of the ITS regions including the 5.8S gene of the nuclear rDNA following Arup (2006). Primers for amplification were ITS1F (Gardes & Bruns 1993) and ITS4 (White

et al. 1990). PCR cycling parameters follow Ekman (2001). Products were cleaned using JETquick PCR purification Spin Kit (Genomed). Both complementary strands were sequenced with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) using the primers mentioned above, and run on an ABI 3130xl Genetic Analyzer.

### Phylogenetic analyses

Newly obtained ITS sequences were included in the phylogenetic analyses of the ingroup (Table 1) and *C. crenularia* along with *C. demissa* (AF353965 and AF353961 downloaded from the GenBank database) were used as the outgroup. On-line version of MAFFT 6 in the Q-INS-i mode (Kato et al. 2002) was employed to align the sequences.

Maximum parsimony analysis was conducted using PAUP\*4.0b10. Gaps were treated as missing data and

all characters were equally weighted. A heuristic search was performed with 100 random-addition-sequences (RAS), using tree bisection-reconnection (TBR) branch-swapping. The steepest descent option was not in effect and the analysis ran under the MulTrees option; no restriction was applied to the maximum number of trees in memory using the MaxTrees option. Non-parametric bootstrap analysis encompassed 1000 resamplings and kept the same settings as the parsimony heuristic search.

An additional analysis aimed to test the credibility of nodes was conducted in MrBayes 3.0 (Ronquist and Huelsenbeck 2003), set in accordance with the best-fit model suggested by MrModeltest 2.2 (Nylander 2004) to GTR+ $\Gamma$  (gamma approximated by four categories). A flat Dirichlet prior distribution with all values set to 1.0 was used to model the prior probability densities of the substitution rates as well as the stationary nucleotide frequencies. In order to assess the stability of the MCMC process, we monitored the standard deviation of split frequencies of two simultaneous independent runs, each including four parallel chains (one 'cold' and three incrementally heated by a temperature of 0.2). Each parallel run proceeded 5 000 000 generations and 75 000 trees were selected from both runs after sampling every 100th count and excluding the first 25 000 trees (burn-in) in order to avoid trees that might have been sampled prior to convergence of the Markov chains. A majority-rule consensus tree was obtained by pooling the selected trees; Bayesian posterior probabilities for its nodes are shown in Fig 4.

### The Species

#### *Caloplaca subalpina* Vondrák, Šoun & Palice sp. nov.

Lichen areolatus cum margine thalli convexis, sublobatis, sorediatis, cum cortice exteriori bene evoluto e cellulis crassae tunicatis. Soredia parva, (18–) 30 ± 8 (–54)  $\mu\text{m}$ , in consoredias non aggregata. Apothecia rariora cum margine apotheciorum lecanoraceanum.

Typus: Ukraine, Eastern Carpathians, Svidovets Mts, glacial cirque in NE slope below Mt Bliznitsa, alt. c. 1500 m, 48° 14' 21" N, 24° 14' E, on lime-rich schist outcrop, beneath overhang, in subalpine belt, 29 June 2007, *ř. Vondrák* 6072 (CBFS—holotypus; GZU, L— isotypi).

(Figs 1A–C; 2A, B & D)

*Thallus* (Fig. 1B, C) areolate, but areoles merge into squamules at thallus margins, sorediate, of various shades of grey or rarely dark green, usually white pruinose in spots or over most of thallus surface, up to several cm in diam. *Areoles* flat (mainly in central part of tightly closed areoles) to convex,

(60–) 164 ± 74 (–450)  $\mu\text{m}$  high ( $n=37$ ) and (0.16–) 0.58 ± 0.32 (–2.04) mm wide ( $n=52$ ). *Areoles* close to thallus margin usually larger and more discrete. Grey to black *prothallus* sometimes visible around marginal areoles. *Soralia* dark grey, arising from margins of areoles, sometimes spreading over whole areole. *Soredia* strongly K+ violet in section, (18–) 30 ± 8 (–54)  $\mu\text{m}$  diam. ( $n=40$ ); consoredia rare and small. *Epinecral layer* up to c. 15  $\mu\text{m}$  high. *Cortex* conspicuous, (5–) 17 ± 11 (–53)  $\mu\text{m}$  high ( $n=54$ ), hyaline in lower part, sordid-grey (K+ violet in section) in upper part, formed of tight paraplectenchymatous tissue of 0.5–1.5  $\mu\text{m}$  thick-walled, large, isodiametric cells, (4.0–) 6.5 ± 1.0 (–8.5)  $\mu\text{m}$  diam. *Cortex* in lower part of thalline exciple distinctly thickened, up to 70  $\mu\text{m}$ . *Algal layer* (30–) 65 ± 20 (–110)  $\mu\text{m}$  high ( $n=15$ ), formed of algal cells (6.0–) 11.0 ± 4.0 (–21.0)  $\mu\text{m}$  diam. ( $n=32$ ) and mostly isodiametric fungal cells, (3.5–) 5.5 ± 1.5 (–9.0)  $\mu\text{m}$  diam. ( $n=15$ ), with walls up to 1  $\mu\text{m}$  thick. *Medulla* not always conspicuous, formed by loose prosoplectenchymatous tissue, of thin-walled, 2–4  $\mu\text{m}$  thick hyphae. *Algonecral medulla* (Fig. 2A) derived from decaying algal layer present in thick thalli.

*Apothecia* lecanorine (Fig. 1A), medium-sized, (0.26–) 0.48 ± 0.11 (–0.70) mm diam. ( $n=33$ ), found in three of four populations, but usually not abundant, almost always white-pruinose but growing mainly on non-pruinose parts of thallus, discs orange or pale orange to yellow when pruinose. *Thalline exciple* same colour as thallus, raised above discs when young, lowered in old apothecia, (80–) 100 ± 16 (–140)  $\mu\text{m}$  thick ( $n=18$ ). *True exciple* indistinct, very thin, up to 40  $\mu\text{m}$  thick, prosoplectenchymatous, formed of thin-walled, c. 2–4  $\mu\text{m}$  thick, cells; prosoplectenchymatous tissue usually continuous with the lowermost part of the hypothecium. *Hypothecium* hyaline, very variable in height, (30–) 90 ± 40 (–160)  $\mu\text{m}$  high ( $n=15$ ), formed by a mixture of isodiametric and elongated hyphal cells. *Hymenium* hyaline, (60–) 69 ± 7 (–80)  $\mu\text{m}$  high ( $n=15$ ). *Paraphyses* of thin-walled, c. 1.5–2  $\mu\text{m}$  thick cells; somewhat

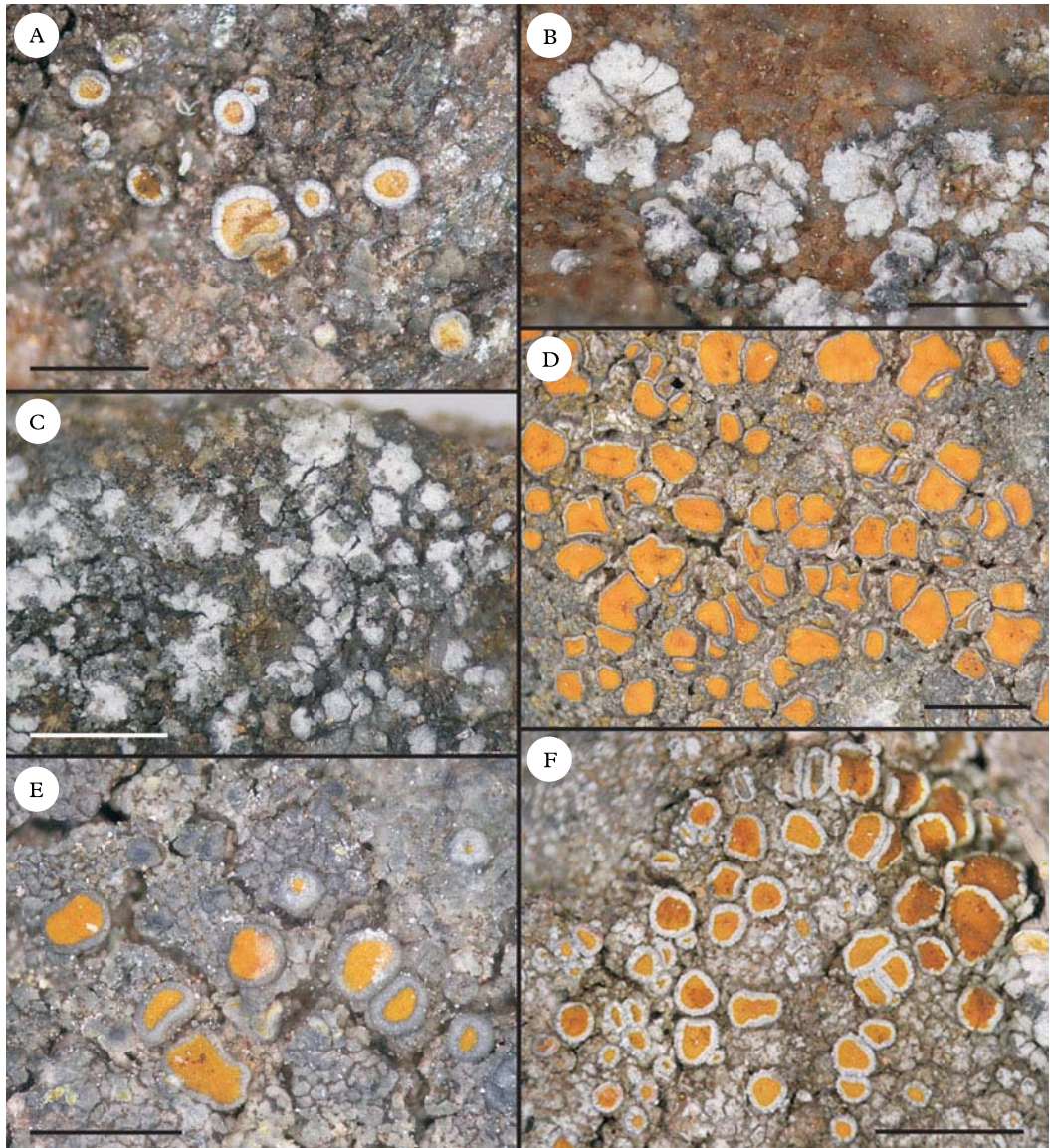


FIG. 1. A–C, *Caloplaca subalpina*. A, apothecia (CBFS JV6071); B, sublobate marginal parts of thallus (CBFS JV6071); C, thallus with soralia (isotype). D–F, *Caloplaca thracopontica*. D, thallus with abundant apothecia (CBFS JV3419); E, detail of a thallus with pustules and apothecia (CBFS JV6066); F, non-typical specimen with crystalline pruina on thallus and apothecia (CBFS JV5421). Scales: A–C, E=1 mm, D, F=2 mm.

branched and anastomosed; upper 1–2 (–3) cells swollen; terminal cells  $(2.5\text{--}3.5 \pm 0.5\text{--}5.0) \mu\text{m}$  wide ( $n=17$ ). *Epihymenium* orange from granules of anthraquinones dissolving in K; crystalline pruina insoluble in K often present. *Asci* 8-spored,  $(41\text{--}49 \pm 6$

$(\text{--}61) \times (10\text{--}12 \pm 1.5\text{--}17) \mu\text{m}$  ( $n=19$ ). *Ascospores* (Fig. 2B) polarilocular, ellipsoid,  $(9.0\text{--}11.5 \pm 1.5\text{--}15.0) \times (4.5\text{--}6.0 \pm 1.0\text{--}7.0) \mu\text{m}$  ( $n=21$ ), length/breadth ratio *c.* 1.9, *ascospore septa*  $(3.0\text{--}4.0 \pm 0.75\text{--}5.5) \mu\text{m}$  thick ( $n=21$ ), septa/spore length

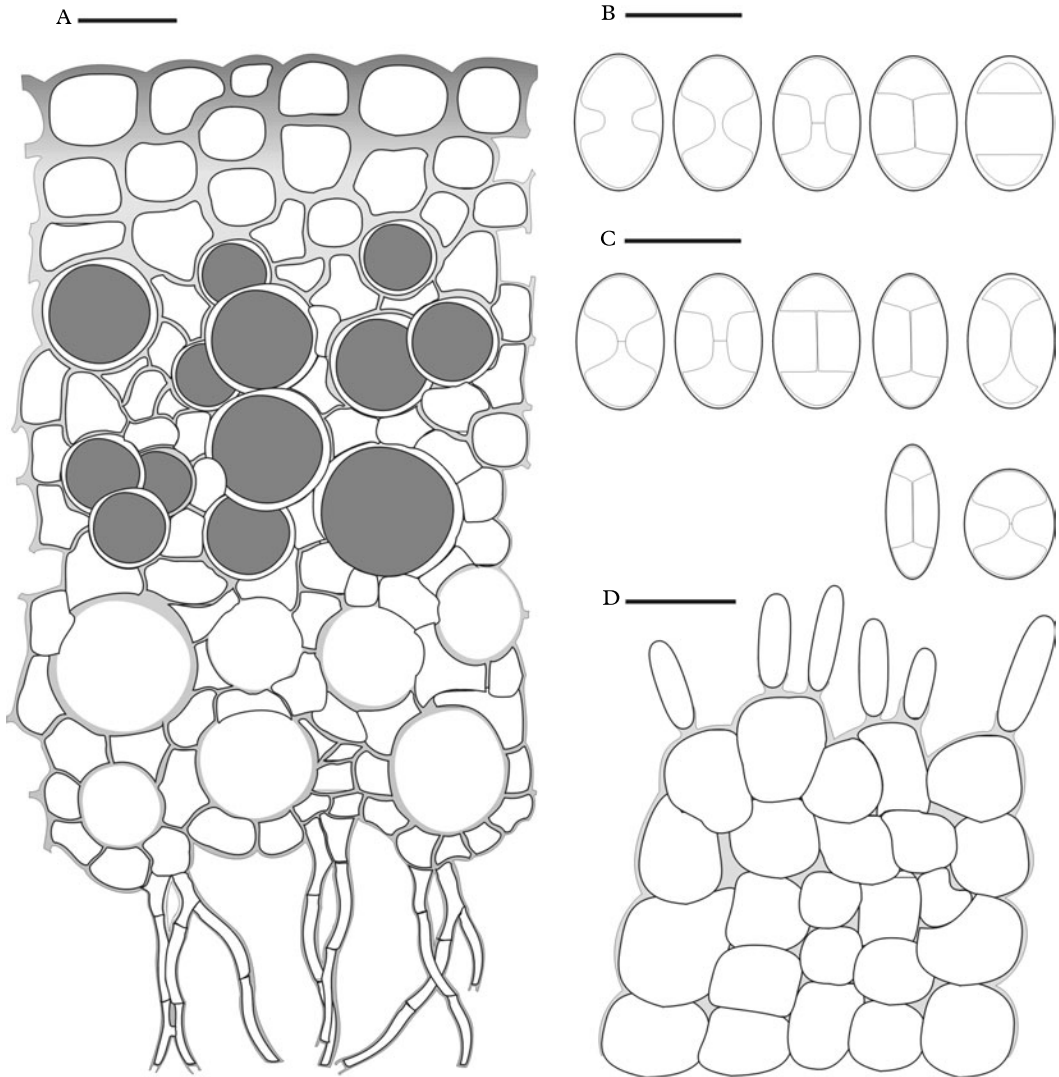


FIG. 2. A, B, D, *Caloplaca subalpina*. A, vertical section through a thallus with thick-walled cortical cells and with pseudomedulla in lower part (CBFS JV692); B, development of ascospores; D, conidiophores with attached conidia (holotype). C, *Caloplaca thracopontica*, ascospore variability, lower spores non-typical, with extreme shapes. Scales: A–C=10  $\mu$ m, D=5  $\mu$ m.

ratio *c.* 0.35, ascospore wall thin, but thicker in old spores (up to *c.* 0.5  $\mu$ m).

*Conidiomata* pycnidia, with centrum *c.* 50–90  $\mu$ m wide ( $n=6$ ). *Conidiophores* tightly packed together forming paraplectenchymatous tissue (Fig. 2D) or rarely solitary. *Conidiogenous cells* smaller than cortical cells, thin-walled, isodiametric, (2.5–) 4.0  $\pm$  1.0 (–5.5)  $\mu$ m diam. ( $n=16$ ). *Conidia* mostly

acrogenous, bacilliform, (2.0–) 3.5  $\pm$  0.75 (–5.0)  $\times$  (0.5–) 1.0  $\pm$  0.25 (–1.5)  $\mu$ m ( $n=17$ ).

*Chemistry.* Anthraquinones are only present in apothecial discs. Parietin was found to be the dominant anthraquinone (mean=91% of total anthraquinone content). Low proportions of teloschistin,

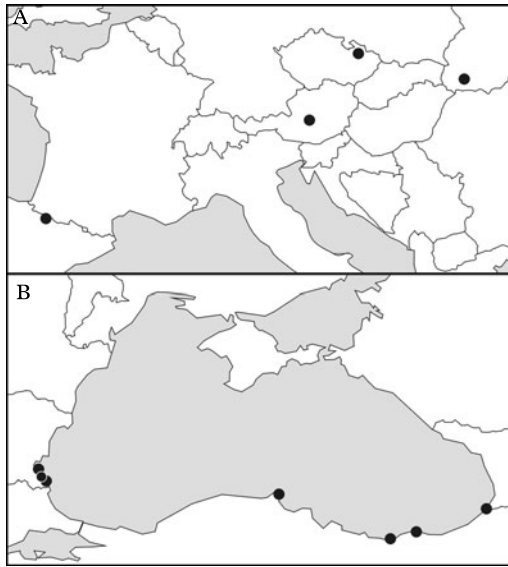


FIG. 3. Distribution of *Caloplaca subalpina* (A) and *Caloplaca thracopontica* (B).

fallacinal, parietinic acid and emodin were also recorded. This anthraquinone content corresponds with chemosyndrome A (Søchting 1997). Sedifolia-grey, a pigment insoluble in acetone, is present in the thallus cortex and soralia (C+, K+, N+ pink/violet/sordid violet in section).

**Etymology.** All known localities are situated on intermediate rocks in the subalpine vegetation belt.

**Ecology and distribution.** The species is known from base-rich schist and conglomerate outcrops in glacial cirques and similar localities in the subalpine vegetation belt (alt. 1190–1800 m). It prefers vertical, sheltered, but well-lit rocks beneath overhangs; only a few lichen species are usually associated, for example *Caloplaca arenaria*, *C. obliterans*, *C. saxicola* s. l., and *Physcia dubia*. The known distribution in the Alps, Carpathians, Pyrenees and Sudetes is shown in Fig. 3A.

**Remarks.** Only a few sorediate species from the *Caloplaca cerina* group are known. Corticolous species producing soredia,

identified as '*C. chlorina*', '*C. virescens*' and *C. jemlandica* var. *cerinosora* differ in the character of their soralia, which originate from blastidia or are less delimited, often forming a sorediate crust on the whole surface of the areoles. The Australian corticolous species *C. hanneshertelii* S. Y. Kondr. & Kärnefelt differs, among other characters, in having a K – cortex and soralia erupting from pustules on the thallus surface (Kärnefelt & Kondratyuk 2004). The North American corticolous species *C. pinicola* H. Magn. differs in, for example, its thinner, 70–85 µm thick thallus and thinner ascospore septa, c. 2.0–3.5 µm (Wetmore 2004). Differences from the predominantly saxicolous *C. chlorina* are shown in the key below.

**Additional specimens examined.** **Austria:** *Seckauer Alpen:* Knittelfeld, Seckau, at chalet Ober Boden Alm below Mt Hämmer Kogel, alt. c. 1630 m, 47°21'16"N, 14°46'34"E, on base-rich overhung schist outcrop in subalpine belt, 2007, *ř. Vondrák* 6071 (CBFS, BM).—**Czech Republic:** *North Moravia:* Hrubý Jeseník Mts, glacial cirque "Velká kotlina", outcrop named "Beckeho skála", alt. 1190 m, 50°03'22"N, 17°14'20.5"E, on dry overhung SE-exposed phyllitic schist rock, 2001, *Z. Palice* 6983 (hb. Palice, B, BM).—**Spain:** *The Pyrenees:* Jaca, Candanchu, valley of Rio de Canal Roya, alt. 1800 m, 42°47'30"N, 0°28'W, on base-rich, N-exposed conglomerate, under overhang, 2002, *ř. Vondrák* 692 (CBFS).

### ***Caloplaca thracopontica* Vondrák & Šoun sp. nov.**

Thallus crassus, (90–) 184 ± 65 (–350) µm, pustulae seu lobulae ad thallo 100–400 µm crassae, soralia nulla. Cortex bene evoluto cellulis crassis composito. Apothecia magna et copiosa.

Typus: Turkey, Black Sea coast, Sinop, coastal rocks on NE coast of peninsula, alt. c. 100 m, 42°01'57.81"N, 35°11'34.42"E, on coastal volcanic rock, 21 April 2007 5419 (CBFS—holotypus; GZU, hb. M. Seaward—isotypi).

(Figs 1D–F; 2C)

**Thallus** (Fig. 1D & E) grey to dark grey, rarely dark green (with whitish spots from crystalline pruina in *Vondrák* 5421), conspicuous, several cm diam., areolate and occasionally minutely sublobate in thallus margins; thallus surface usually covered by pustules or lobules (Fig. 1E), c. 100–400 µm

wide and up to 150  $\mu\text{m}$  high. *Areoles* (90–)  $184 \pm 65$  (–350)  $\mu\text{m}$  high ( $n=40$ ) and (0.29–)  $1.32 \pm 0.75$  (–3.40) mm wide ( $n=38$ ). *Prothallus* conspicuous, glossy lead-grey, rarely with whitish outer margin. *Epinecral layer* usually distinct, up to 30  $\mu\text{m}$  high. *Cortex* conspicuous, (5–)  $23 \pm 14$  (–75)  $\mu\text{m}$  high ( $n=54$ ), hyaline in lower part, sordid-grey (K+ violet in section) in upper part, formed of tight paraplectenchymatous tissue of 0.5–2  $\mu\text{m}$  thick-walled, large, isodiametric cells, (5.0–)  $7.5 \pm 1.5$  (–11.0)  $\mu\text{m}$  diam. ( $n=22$ ). *Cortex* in lower part of thal-line exciple distinctly thickened, up to 90  $\mu\text{m}$ . *Algal layer* (40–)  $81 \pm 37$  (–210)  $\mu\text{m}$  high ( $n=20$ ), formed of algal cells (6.5–)  $12.5 \pm 3.0$  (–18.0)  $\mu\text{m}$  diam. ( $n=22$ ) and mostly isodiametric fungal cells with thin-walls (up to 0.5  $\mu\text{m}$ ). *Medulla* not always conspicuous, formed by loose prosoplectenchymatous tissue, of thin-walled, 2–4  $\mu\text{m}$  thick hyphae. *Algonecral medulla* derived from decaying algal layer is present in thick thalli, mainly below pustules.

*Apothecia* (Fig. 1D, F) lecanorine, often abundant, large, (0.22–)  $0.71 \pm 0.28$  (–1.52) mm diam. ( $n=46$ ), with orange to dark red, flat discs. *Thal-line exciple* same colour as thallus, raised above discs when young, somewhat reduced in old apothecia, (50–)  $100 \pm 24$  (–170)  $\mu\text{m}$  thick ( $n=35$ ). *True exciple* indistinct, very thin, up to 25  $\mu\text{m}$  thick, prosoplectenchymatous, formed of thin-walled cells, up to 6  $\mu\text{m}$  thick in uppermost part, *c.* 2–4  $\mu\text{m}$  thick in lower part; prosoplectenchymatous tissue usually extending to the lowermost part of hypothecium. *Hypothecium* hyaline, very variable in height, (40–)  $116 \pm 37$  (–180)  $\mu\text{m}$  high ( $n=34$ ), formed by a mixture of isodiametric and elongated hyphal cells. *Hymenium* hyaline, (60–)  $81 \pm 11$  (–110)  $\mu\text{m}$  high ( $n=34$ ). *Epihymenium* orange from granules of anthraquinones, these dissolving in K; crystalline pruina insoluble in K rarely present (e.g. *Vondrák* 5421). *Paraphyses* of thin-walled, *c.* 1.5–2.5  $\mu\text{m}$  thick cells; branched (in upper one-third) and somewhat anastomosed; upper 1–4 (–7) cells swollen; terminal cells (2.5–)  $5.0 \pm 1.0$  (–6.5)  $\mu\text{m}$  wide ( $n=52$ ). *Asci* 8-spored,

(39–)  $51 \pm 6$  (–64)  $\times$  (8–)  $13 \pm 3$  (–21)  $\mu\text{m}$  ( $n=37$ ). *Ascospores* (Fig. 2C) polarilocular, ellipsoid (rarely narrowly ellipsoid), (10.0–)  $12.5 \pm 1.5$  (–15.5)  $\times$  (3.0–)  $6.0 \pm 1.0$  (–10.0)  $\mu\text{m}$  ( $n=62$ ), length/breadth ratio *c.* 2.1; *wall* thin, but thicker in old spores (up to *c.* 0.5  $\mu\text{m}$ ); *septa* (2.5–)  $5.0 \pm 1.0$  (–7.0)  $\mu\text{m}$  thick ( $n=62$ ), *septa/spore length ratio c.* 0.4.

*Conidiomata* pycnidia, with centrum (80–)  $132 \pm 27$  (–180)  $\mu\text{m}$  wide ( $n=22$ ). *Conidiphores* tightly packed forming paraplectenchymatous tissue or  $\pm$  solitary. *Conidiogenous cells* smaller than cortical cells, thin-walled, isodiametric, (3.5–)  $5.0 \pm 1.0$  (–7.5)  $\mu\text{m}$  diam. ( $n=24$ ) or elongated, up to *c.* 7  $\mu\text{m}$  long. *Conidia* acro- or pleurogenous, bacilliform, (2.5–)  $3.5 \pm 1.0$  (–5.5)  $\times$  (1.0–)  $1.25 \pm 0.25$  (–1.5)  $\mu\text{m}$  ( $n=44$ ). Detached conidia sometimes form a conglutinated mass on thallus surface around ostioles (blackish dots, translucent when wet, when observed under the stereomicroscope).

*Chemistry.* Similar to the previous species, the anthraquinone composition of *C. thracopontica* is consistent with chemosyndrome A, with parietin as the principal component (94%) and teloschistin, fallacinal, parietinic acid and emodin in lower concentrations. Anthraquinones are absent from the thallus. Sedifolia-grey, pigment insoluble in acetone, is present in thallus cortex (C+, K+, N+ pink/violet/sordid violet in section).

*Etymology.* *Thracia* and *Pontus* are the Latin names for the areas around the Black Sea, where the new species was collected.

*Ecology and distribution.* *Caloplaca thracopontica* is a maritime species, mainly inhabiting the supralittoral zone of coastal cliffs at 14–180 m alt. at Sinop, Turkey (an extremely exposed shore), and at 3–10 m alt. at Sinemorets, Bulgaria (a sheltered shore). It occurs on exposed, hard siliceous outcrops associated, for example, with *Caloplaca aractina*, *C. aff. crenularia*, *C. fuscoatroides*, *C. maritima*, *C. aff. thallicola*, *Candelariella plumbea*, *Catillaria chalybeia*, *Rinodina genarii*, and *Xanthoria calcicola*. It is distributed on the Black Sea coast (Fig. 3B) in South

Bulgaria (several localities between Burgas and Rezovo) and in NE Turkey (very abundant in localities between Sinop and the Georgian border). According to our field-work, its absence from the Romanian, North Bulgarian, Georgian, and Russian coast of the Black Sea is probably caused by the scarcity of suitable substrata, but surprisingly, it was not found on numerous hard siliceous rocks in NW Turkey and the well-surveyed Crimean Peninsula.

*Remarks.* The species is clearly characterized by its wide and tall areoles usually covered by pustules or small lobules. Corticolous specimens of *C. cerina* s. l. differ in their thin thallus, devoid of vegetative diaspores; corticolous specimens named '*C. chlorina*', '*C. isidiigera*' and '*C. virescens*' possess soredia or blastidia, but not pustules or lobules, as vegetative diaspores. Some terricolous or muscicolous *C. stillicidiorum* s. l. produce pustule-like structures, but their thallus is clearly different, being significantly less conspicuous. For differences from the saxicolous species see the key below.

*Additional specimens examined.* **Bulgaria:** Black Sea coast: Burgas, Sozopol, siliceous cliffs at seashore c. 4 km S of town, 42°22'58.86"N, 27°42'43.81"E, on siliceous coastal rock, 2007, *f. Vondrák* 6066 (CBFS); *ibid.*: coastal rocks near camp Veselie, 42°22'46.2"N, 27°43'19"E, on siliceous rock in upper supralittoral zone in alt. c. 15–25 m, 2005, *f. Vondrák* 3419, 3420 (CBFS); Burgas, Tsarevo, Sinemorets, coastal rocks c. 2 km SE of village, alt. 3–10 m, 42°00'30"N, 28°00'E, on coastal rocks in mesic-supralittoral zone, 2004, *f. Vondrák (Sel. Exs. Caloplaca*, 15, sub *Caloplaca* aff. *chlorina*).—**Turkey:** Black Sea coast: Giresun, 40°58'15.75"N, 38°38'15.95"E, on siliceous coastal rock, 2007, *f. Vondrák* 6065 (CBFS); Sinop, coastal rocks on E coast of peninsula, alt. 180 m, 42°01'12.86"N, 35°12'19.56"E, on siliceous coastal rock, 2007, *f. Vondrák* 5623 (CBFS); *ibid.*: alt. c. 100 m, 42°01'13"N, 35°12'20"E, 2007, *f. Vondrák* 6067 (CBFS); Sarp (Turkish-Georgian border), coastal rocks 1.3 km SW of village, alt. c. 10 m, 41°30'34.44"N, 41°32'14.80"E, on siliceous coastal rock, 2007,

*f. Šoun* 302, *f. Vondrák* 6107 (CBFS); Trabzon, coastal rocks in village Akçakale, 41°04'56.69"N, 39°30'08.72"E, on siliceous coastal rock, 24 Apr. 2007, *f. Vondrák* 5621 (CBFS).

### Phylogeny

The dataset of 18 aligned ITS sequences included 842 positions, with 154 variable positions 68 of which were parsimony informative. The parsimony analysis yielded six equally parsimonious trees with the length of 207 steps, all belonging to the same island (hit 100 times). The consistency index (CI) of the trees was 0.859, with a retention index (RI) of 0.717. The bootstrap tree showed 9 supported internodes (BS>50%), 3 of which give evidence of interspecific relationships, 4 confirm conspecificity of multiple isolates (in the case of *C. chlorina*, *C. isidiigera*, *C. subalpina* and *C. thracopontica*) and 2 message the intra-specific relationships among isolates. The Bayesian inference revealed only 8 supported internodes, one of which represents additional resolution to the bootstrap tree (grouping of *C. cerina* with *C. subalpina*, further in text). *Caloplaca subalpina* and *C. thracopontica* form two well-supported clades among the analyzed sequences (Fig. 4), with bootstrap support 100% in the former and 97% in the latter species; the monophyly of the *C. subalpina* sequences is moreover supported by the Bayesian posterior probabilities equal to 1.00. Bayesian inference statistically proves the grouping of *C. subalpina* with *C. cerina* (PP=0.94), in spite of the bootstrap support (45%, not shown in Fig. 4), which is quite low for this clade. The analyses reveal that *C. chlorina* is the sister taxon to *C. thracopontica*, but further work is necessary as bootstrap support values (68%) show low confidence and this clade was not revealed by the Bayesian analysis.

### Key to saxicolous species of the *Caloplaca cerina* group

The key is confined to those species of the *Caloplaca cerina* group characterized by lecanorine, anthraquinone pigmented apothecia with strongly reduced true exciple, never with placodioid thalli and without anthraquinones in the thallus and thalline margin. It deals with the species occurring in Europe, but we know of no saxicolous species of the *C. cerina*

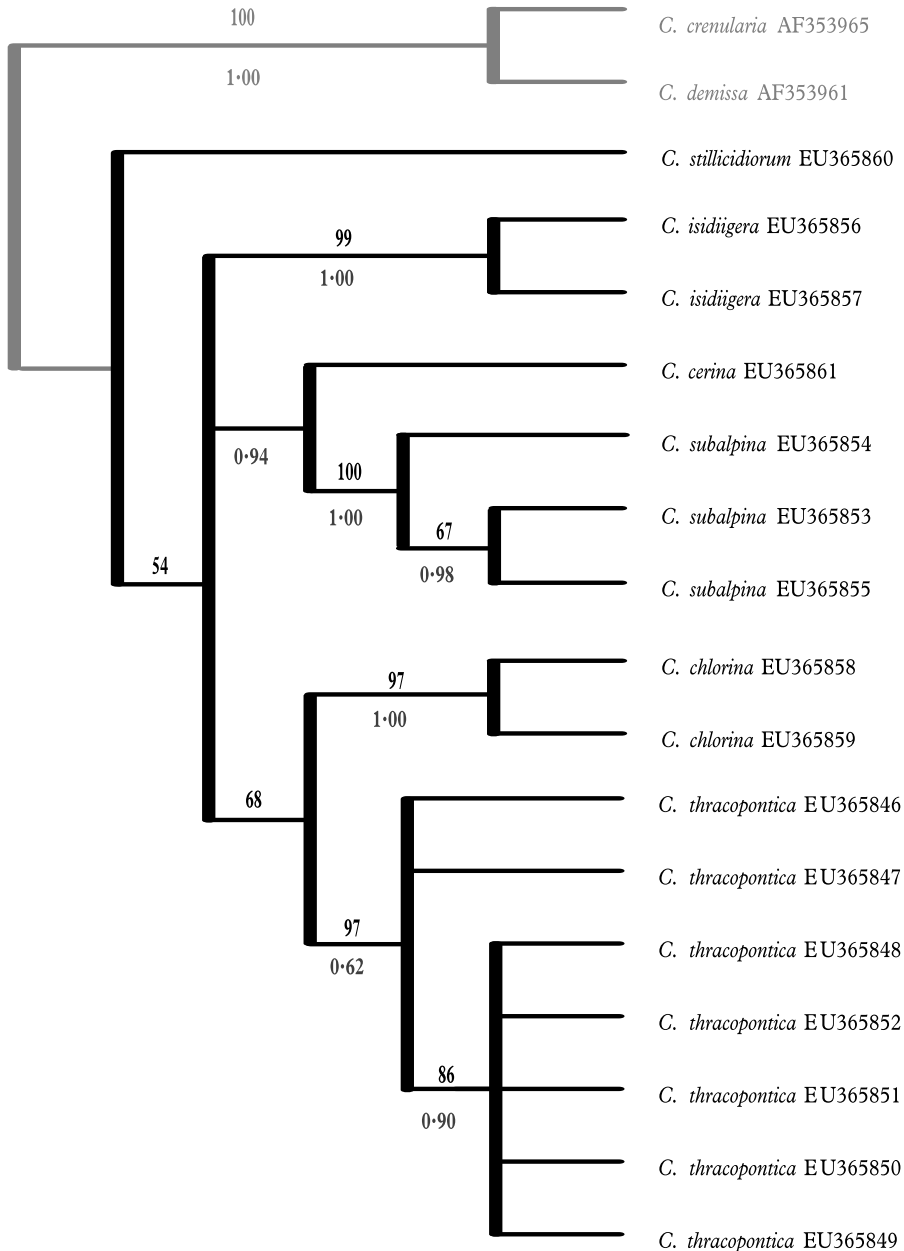


FIG. 4. Phylogenetic relationships of newly acquired ITS sequences of the *Caloplaca cerina* group, rooted by the *C. crenularia* and *C. demissa* (outgroup shown in grey, names of taxa accompanied by their GenBank accession numbers). Topology respects the bootstrap consensus tree for 1000 replicates computed using parsimony heuristic search under the TBR algorithm with tree bisection reconnections and random sequence addition. Numbers above branches stand for bootstrap values for clades present in 500 or more bootstrap replicates, lighter numbers below branches denote posterior probabilities for the following node calculated in MrBayes (37 500 trees were sampled among 5 000 000 generations using the GTR+ $\Gamma$  model). Branches having neither bootstrap support above 50% nor Bayesian posterior probability above 0.90 are presented as collapsed.





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# Paper III

Šoun, J. & Vondrák, J.

(2008)

*Caloplaca aurantia* and *Caloplaca flavescens* (*Teloschistaceae*, lichen-forming fungi) in the Czech Republic; with notes to their taxonomy and nomenclature.

*Czech Mycology* 60: 275–291.



***Caloplaca aurantia* and *Caloplaca flavescens*  
(*Teloschistaceae*, lichen-forming fungi) in the Czech Republic;  
with notes to their taxonomy and nomenclature**

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Šoun J. and Vondrák J. (2008): *Caloplaca aurantia* and *Caloplaca flavescens* (*Teloschistaceae*, lichen-forming fungi) in the Czech Republic; with notes to their taxonomy and nomenclature. – Czech Mycol. 60(2): 275–291.

A revision of *Caloplaca aurantia* and *C. flavescens* in the Czech Republic is provided. Both species are confirmed from the territory; their distribution is reviewed and their ecological demands commented upon. Morphological investigations have confirmed the structure of the cortex and shape and colour of the lobes as the best diagnostic characters of both species; the thickness of the cortex has been found to be another useful character. Nomenclatural confusions regarding both species are discussed and clarified.

**Key words:** Czech Republic, distribution, morphology, nomenclature, *Teloschistales*.

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Jsou presentovány výsledky revize druhů *Caloplaca aurantia* a *C. flavescens* v České republice. Je potvrzen výskyt obou druhů na tomto území. Zrevidováno a opraveno je jejich rozšíření a okomentovány jsou jejich ekologické nároky. Studie morfologických znaků potvrdila, že nejlepšími diagnostickými znaky mezi těmito druhy jsou struktura kůry a tvar a barva laloků. Jako nový užitečný znak byla nalezena výška kůry. Diskutovány a objasněny jsou nomenklatorické nejasnosti týkající se těchto druhů.

## INTRODUCTION

The *Caloplaca aurantia* group, characterised by the unique, lemon-shaped ascospores and conspicuous, lobate thalli, currently represents a small group of six saxicolous species: *C. aegaea* Sipman, *C. aurantia* (Pers.) Hellb., *C. flavescens* (Huds.) J.R. Laundon, *C. fuerteventurae* van den Boom & Etayo, *C. scrobiculata* H. Magn. and *C. thallincola* (Wedd.) Du Rietz. *C. aurantia* and *C. flavescens* grow on both inland and maritime calcareous rocks throughout the mild-temperate to subtropical zone of the Old World (e. g. Nordin 1972, Wasser and Nevo 2005).

*C. thallincola* is a silicolous maritime species in North and West Europe (e. g. Clauzade and Roux 1985, Laundon 1992, Nordin 1972). The recently described *C. aegaea* is known from maritime siliceous rocks in the Mediterranean (Sipman and Raus 2002) and *C. fuerteventuræ* from inland volcanic rocks in Fuerteventura, Canary Islands (van den Boom and Etayo 2006). *C. scrobiculata* is a calcicolous species of the southern Eurasiatic mountains (Poelt and Hinteregger 1993).

The monophyly of *C. aegaea*, *C. aurantia*, *C. flavescens* and *C. thallincola* was corroborated molecularly by Søchting and Arup (2002) and Gaya et al. (2008). *C. scrobiculata* is not related to the other species in the group (Gaya et al. 2008), differing in its morphology by the thin ascospore septa. The position of *C. fuerteventuræ* is as yet undetermined.

*C. aurantia* and *C. flavescens* have been treated in many floras and keys (e. g. Clauzade and Roux 1985, Khodosovtsev et al. 2004, Laundon 1992, Poelt 1969, Wasser and Nevo 2005, Wirth 1995) and also specialised *Caloplaca* papers (Alon and Galun 1971; Laundon 1984; Nordin 1972; Poelt 1954; Verseghe 1970, 1971, 1972; Wade 1965; Wetmore and Kärnefelt 1998). However, in comparison with many European countries, the situation regarding these species has remained unclear in the Czech Republic. *C. aurantia* is the only species of the group included in the Catalogue of Lichens of the Czech Republic (Vězda and Liška 1999), but *C. flavescens* has been also recently recorded from the territory. The aim of this paper is to clarify the situation about the distribution and morphological differences between these species in the Czech Republic.

#### MATERIAL AND METHODS

Fourty specimens from the Czech Republic and some reference samples from other countries were used for detailed morphological investigations (specimens in bold in the studied material); these specimens are deposited in private herbaria of the first author, D. Svoboda and Z. Palice and the herbarium CBFS. Seven selected quantitative characters were measured: lobe length (from tip to the closest apothecium), maximum lobe width, cortex thickness, length and width of ascospores, width of ascospore septa, and conidium length. Ascospore length / width ratio and ascospore length / septum width ratio were calculated. The characters were measured with a dissecting microscope and light microscope (magnification 1000×). Sections for examination were cut by hand and all structures were measured with an accuracy of 1 µm in water. Measurements are given as (min.–) X ± SD (–max.), where X = mean value, SD = standard deviation and min./max. = extremes. Ten measurements of each character for 20 specimens of each species were carried out (= 200 measurements), except for lobe length and size of ascospores in *C. flavescens*, where n = 170 and 190, respectively. Qualitative charac-

ters such as colour, shape of lobes, presence of crystalline layer in cortex and surface structure were also recorded. A JEOL 6300 scanning electron microscope was used for a detailed study of the cortex structure.

Distributional data of both species in the Czech Republic were gathered from recent field surveys, herbarium specimens (PRM, PRC, CBFS, and the private herbaria of D. Svoboda, Z. Palice and A. Vězda) and the literature (Ginzberger 1913; Kovář 1908; Kuřák 1911, 1914; Podpěra 1928; Servít 1910, 1911, 1930; Suza 1913, 1914, 1921, 1925, 1933, 1935, 1943a, 1943b, 1947; Vězda 1955; Vězda and Gruna 2000; Svoboda 2007). Distribution maps were drawn using the DMAP mapping software (Morton 2001).

## RESULTS

***Caloplaca aurantia*** (Pers.) Hellb. nomen sed non planta, Bih. Kgl. Svensk. Vetensk.-Akad. Handl. 16(3): 60. 1890.

Bas.: *Lichen aurantius* Pers., Neue Ann. Bot. 5: 14. 1794. Typus: Prope Witgenhausen [Witzenhausen] in Hassia [Hessen] (according to G. Thijssse in litt. absent in Persoon's herbarium in L).

Syn.: *Caloplaca callopisma* (Ach.) Th. Fr.

**Short description.** Thallus matt, light yellow-white to dull orange. Marginal lobes long, mostly flattened for their whole length. Thallus often faintly pruinose on older parts of lobes, rarely strongly pruinose in thallus centre. Cortex paraplectenchymatous, thinner than in *C. flavescens*, without a crystalline layer (Fig. 3). Ascospores lemon-shaped, slightly wider and shorter with less pointed apices than in *C. flavescens*. For quantitative characters see Table 1.

**Variability.** The lobes in *C. aurantia* are usually flattened along their whole length, but rarely slightly convex, particularly when overgrowing thalli of other lichens. The thallus has many colour forms: typically, it has dull orange-coloured margins, often with a few narrow alternating zones of lighter ( $\pm$  pale yellow) and darker ( $\pm$  yellow-orange) strips towards the centre. Individuals with lighter lobe tips and a darker centre of thallus exist, as well as wholly orange-yellow to orange forms. Forms with an almost white pruinose centre and light yellow margins are also known. Dark clusters of cyanobacteria sometimes occur at the surface in the thallus centre.

**Chemistry.** Thallus and apothecia K<sup>+</sup> (purple), chemosyndrome A<sub>3</sub> (Søchting and Lutzoni 2003).

**Ecology.** *C. aurantia* is restricted to natural habitats in limestone areas in the Czech Republic, but outside the territory, it also grows on other calcareous rocks and building materials, including mortar and concrete. It is considered to be a nitrophilous, heliophilous, thermophilous and xerophilous species preferring

slightly inclined surfaces with southern exposition, and occasionally it is found on vertical surfaces with other expositions.

**Distribution in the Czech Republic.** The species only occurs in the warmest regions of South Moravia (Fig. 1) and is absent from the xerothermic limestone area in central Bohemia (Bohemian Karst) as already pointed out by Suza (1935, 1943b, 1947). It is rather abundant in the Pavlovské vrchy Hills where it has been recorded (as *Caloplaca / Gasparrinia callopisma*) since the beginning of the 20<sup>th</sup> century (Kovář 1908; Ginzberger 1913; Podpěra 1928; Suza 1913, 1921, 1925, 1943b). A recent field survey revealed a new locality on soft tertiary limestone at Sedlec, close to Pavlovské vrchy Hills.

Another three populations are situated at the rim of the south-Moravian xerothermic vegetation region; two of them have been confirmed by the recent field survey, but one is extinct. In the Podyjí National Park it grows on the Moravian side of the Dyje River valley on several crystalline limestone outcrops in the vicinity of the Austrian Hardegg (Suza 1933, 1943b, 1947; Vězda and Gruna 2000). The locality on Stránská skála Hill at the periphery of Brno (Suza 1914, 1925, 1943b, 1947) is rather small and the lichen grows there unusually also on N-faced rocks. The most outlying population at Čebínka Hill near Tišnov, already cited by Servít (1910) and Suza (1925, 1943a, 1943b, 1947), is extinct and was probably destroyed by a large limestone quarry on the southern slope of the hill. During the revision of Suza's specimens, it was discovered that he wrongly identified the specimens of *C. aurantia* from the last three localities as *C. aurantia* var. *intermedia* (= *C. flavescens*), except for Servít's sample from Čebínka Hill, which was identified as *C. callopisma* by Suza, but he doubted his determination.

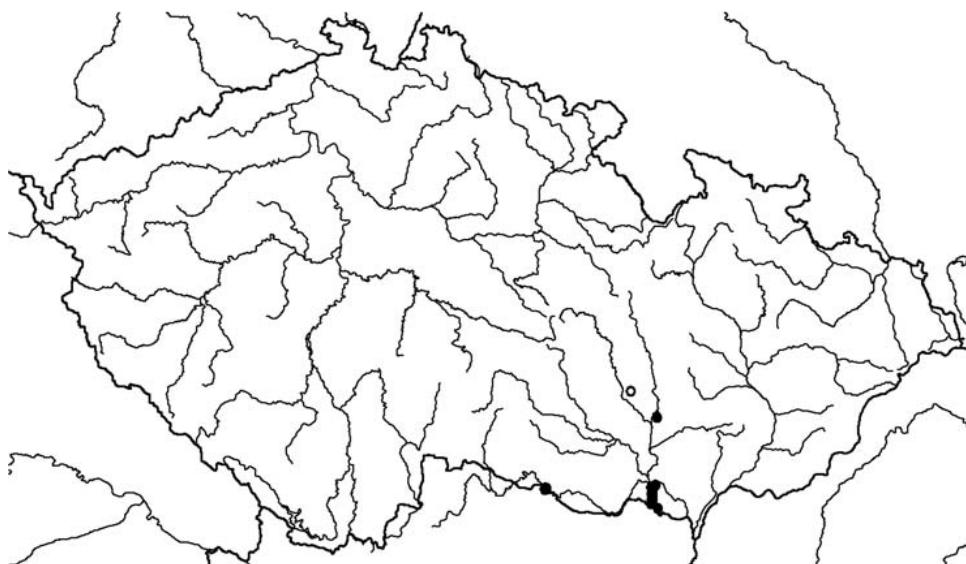
The records of *C. callopisma* from the Bohemian Karst by Servít (1911), Bayer, Hora and Pejška (unpublished specimens in PRM) are erroneous and refer to *C. flavescens*. The record of *C. (G.) callopisma* from Vápenný Podol in the Železné hory Mts. (Kuřák 1911, 1914) is also incorrect since its voucher (PRM 763079, sub *G. callopisma*) represents *C. saxicola* (Hoffm.) Nordin. Vězda (1955) mentioned *C. aurantia* from a paved, artificial bank of the river Bečva in Vsetín. Its occurrence at this locality is rather improbable (we did not confirm this occurrence in 2006), because it is an anthropogenic site in a relatively cool region, far from any natural limestone outcrops. However, the voucher specimen (if it exists) has not been seen.

## Material studied

Material used for detailed morphological investigations is in bold. The sample data are in their original wording on voucher labels or with small modifications.

AUSTRIA: Lower Austria – Kalkfelsen b. Hainburg, 1887, leg. G. Beck (PRC 14, sub *Amphiloma callopisma*).





**Fig. 1.** Distribution of *Caloplaca aurantia* in the Czech Republic; black dots represent recent distribution, open circle marks destroyed historic locality.

**BULGARIA:** The Rhodopes – ad rupes calcareas in monte Czervenata stena supra monasterium Baczkovo, c. 12–1400 m, 1923, leg. J. Suza (PRM 631187, sub *C. callopisma*); Lyubimets, Malko Gradishte, nearby hill „Svieta Marina“ c. 5 km SW of village, alt. 600 m, 41° 44' N, 26° 00' E, small limestone outcrop in forest, 2004, leg. J. Vondrák (CBFS JV2046); *Ibid.*, Kaloyantsi, Dolishte, limestone rocks in valley c. 1 km W of village, alt. 300 m, 41° 38' N, 25° 35' E, 2004, leg. J. Vondrák (CBFS JV2122); ***Ibid.*, Madzharovo, Silen, Byal Kladenets, limestone rocks in valley below village, alt. 350 m, 41° 37' N, 25° 40' E, hard limestone rocks, 2004, leg. J. Vondrák (CBFS JV2086).**

**CROATIA:** West Croatia – ad saxa calcarea in vicinitate urbis Fiume [Rijeka], leg. F. Blechschmidt et J. Schuler (PRC 12, PRM 631203; Kryptogamae Exsiccatae 1256b, sub *C. callopisma*). Dalmatia – Župa Dubrovačka, ad rupes calc. prope Kupari, ca 20 m, 1917, leg. R. Dvořák (PRM 631180, sub *G. aurantia*); Obrovac, 1907, leg. M. Servít (PRM 631200, sub *C. callopisma*); Krunjacija, 480 m, 1907, leg. M. Servít (PRM 631198, sub *C. callopisma*); Ragusa [Dubrovnik], ad saxa calcarea, c. 60 m, 1908, leg. A. Latzel (PRM 761023, sub *C. callopisma*); Ins. Rab, Tiguaro, in rupib. calcareis, c. 300 m, 1932, J. Šmarda (PRM 631201, sub *C. callopisma*).

**CZECH REPUBLIC:** South Moravia – Pavlovské vrchy [Hills], 1908, leg. Dvořák (PRM 631186, sub *G. callopisma*); *Ibid.*, na Turoldě, 1909, leg. J. Suza (PRM 763078, sub *G. callopisma*); Jurské vápence na Turoldu u Mikulova, 1912, leg. J. Suza (PRM 631181, 631184, sub *G. callopisma*); Ad rupes calcareas colli Sv. Hora prope Mikulov, 1912, leg. J. Suza (PRM 631185, sub *C. callopisma*); Pavlovské vrchy [Hills], 1921, leg. A. Hilitzer (PRM 696984, sub *C. callopisma*); Šibeničník prope Mikulov, c. 230 m, 1922, leg. J. Suza (PRM 631191, 631183, sub *C. callopisma*); Pavlovské vrchy [Hills], loco „Soutěska“, calcicola, 1922, leg. J. Suza (PRM 631188, 631189, hb. Vězda, sub *C. callopisma*); Mikulov, limestone rocks under Svatý kopeček Hill, c. 0,2 km E of town, 48° 48' 29" N, 16° 39' 00" E, limestone rock, 2002, leg. J. Vondrák (CBFS JV933); Mikulov, rocks on E slope of Koží hrádek ruin, c. 270 m, 48° 48' 35" N, 16° 38' 11" E, 2002, leg. J. Vondrák (CBFS JV925); **Mikulov, small limestone outcrops at upper edge of former quarry in Turoid Hill, alt. c. 380 m, 48° 48' 58" N, 16° 38' 21" E, 2003, leg. J. Šoun (hb. Šoun 1); Klentnice, small limestone outcrops near Siroťčí hrádek ruin, alt. c. 420 m, 48° 50'**

48° N, 16° 38' 27" E, 2004, leg. J. Šoun (hb. Šoun 5); Mikulov, limestone rocks at SW foothill of Svätý Kopeček Hill, alt. c. 270 m, 48° 48' 21" N, 16° 38' 33" E, 2004, leg. J. Šoun (hb. Šoun 4); Mikulov, limestone rocks at SW foothill of Svätý Kopeček Hill, alt. c. 270 m, 48° 48' 21" N, 16° 38' 33" E, 2004, leg. J. Šoun (hb. Šoun 3); Mikulov, small limestone outcrops on E slope of Kozí hrádek ruin, alt. c. 270 m, 48° 48' 35" N, 16° 38' 11" E, 2004, leg. J. Šoun (hb. Šoun 2); Bavory, small limestone rocks at locality Kočičí kámen, alt. c. 350 m, 48° 49' 50" N, 16° 38' 13" E, 2005, leg. J. Šoun (hb. Šoun 23); Mikulov, limestone rocks on Šibeničnick Hill, alt. c. 330 m, 48° 51' 18" N, 15° 52' 15" E, 2005, leg. J. Šoun (hb. Šoun 24); Pavlov, limestone outcrop on SE slope of Děvín Hill, alt. c. 420 m, 48° 52' 1.12" N, 16° 39' 11.34" E, 2006, leg. J. Šoun (hb. Šoun 134); Horní Věstonice, limestone rock on W slope of Obora Hill, alt. c. 430 m, 48° 51' 51.89" N, 16° 38' 4.35" E, 2006, leg. J. Šoun (hb. Šoun 137); Sedlec, small limestone outcrops on Skalky Hill, alt. c. 250 m, 48° 46' 00" N, 16° 40' 42" E, 2004, leg. J. Šoun (hb. Šoun 6); Brno, na Stránské skále u Líšně, 1911, leg. J. Suza (PRM 580964, sub *C. aurantia* var. *intermedia*); **Ibid.:** limestone outcrop on N slope of Stránská skála Hill, alt. c. 300 m, 49° 11' 28.6" N, 16° 40' 35.74" E, 2006, leg. J. Šoun (hb. Šoun 138); Calcicola in colle Čebínka prope Tišnov, c. 400 m, 1922, leg. J. Suza (PRM 580962, sub *C. aurantia* var. *intermedia*); Znojmo, Hardek [Hardegg], c. 350 m, in rup. phylliticis-calcareis, 1932, leg. J. Suza (PRM 580963, sub *G. aurantia* var. *intermedia*); In valle fl. Dyje pr. Hardegg in rup. phylliticis (calc. includ.), c. 250 m, 1932, leg. J. Suza (PRM 580960, sub *G. aurantia* var. *intermedia*); Čížov, limestone outcrops near road to Hardegg in Dyje valley, alt. c. 350 m, 48° 51' 17.8" N, 15° 52' 09.7" E, 2005, leg. J. Šoun (hb. Šoun 22); Čížov, limestone outcrop below road to Hardegg in Dyje valley, alt. c. 320 m, 48° 51' 17.59" N, 15° 52' 16.97" E, 2006, leg. J. Šoun (hb. Šoun 133); **Ibid., 48° 51' 17.18" N, 15° 52' 15.93" E, 2006, leg. J. Šoun (hb. Šoun 132).**

GERMANY: Baden-Württemberg – Germania (Württemberg): ad saxa dolomitica ad Eglosheim prope Ludwigsburg, leg. X. Rieber (PRC 15, 24, PRM 761022; Kryptogamae Exsiccatae 1256, sub *C. callopisma*). Hessen – An Kalkfelsen beim Dorfe Wendershausen unweit Witzzenhausen an der Werra (ubi Persoon plantam legit), 1883, leg. Dannenberg (PRM 631175, sub *Physcia aurantia*).

ITALY: Sicily – Cavagrande Nature Reserve, limestone rock, 2002, J. Vondrák (CBFS JV421).

ROMANIA: Dobruja – Tîrgusor, in valle fl. Casimcea prope pagum Casian, c. 50 m., 1974, leg. A. Vězda (hb. Vězda, sub *C. aurantia* var. *aurantia*); Tulcea, Popina Island in Lake Razim, E coast, alt. 0–1 m, 44° 58' 03.16" N, 28° 58' 57.29" E, limestone rock, 2007, leg. J. Šoun (hb. Šoun 171).

SLOVAKIA: Little Carpathians – Malé Karpaty: ad rupes calcareas in decl. merid. collis Devínska Kobyla supra pag. Devín, alt. 300 m s.m., 1965, leg. L. Opold, I. Pišút et B. Wagner (PRC 28; Lichenes Slovakiae Exsiccati. 197, sub *C. c.* var. *heppiana*); Smolenice, Holý vrch pr. pag. Nádaš [Trstín], in rupibus dolomit., c. 230–300 m, 1937, leg. J. Suza (PRM 580958, 580961, sub *C. aurantia* var. *intermedia*); In rupibus calcareis pr. ruinam Dobrá Voda, c. 280–300 m, 1937, leg. J. Suza (PRM 580959, sub *C. aurantia* var. *intermedia*); Čachtické kopce, in colle Veľký Plešivec, in rup. calcar., c. 470–480 m, 1933, leg. J. Suza (PRM 631163, sub *C. aurantia*). Považský Inovec – Piešťany, in colle Holý kopec pr. p. Hubína, in rup. calcareis, c. 200 m, 1930, leg. J. Suza (PRM 631195, sub *G. callopisma*); In rupe dolomitica pr. ruinam Tematín, c. 400 m, 1926, leg. J. Suza (PRM 631197, sub *C. callopisma*); Nové Mesto nad Váhom, Lúka, rocks on S slope below ruin of Tematín castle, alt. c. 530 m, limestone rock, 2006, leg. J. Šoun (hb. Šoun 95). Strážov Mts. – Uhrovské Podhradie, ad pedem occid. m. Rokoš, c. 300 m, in rup. calcareis, 1930, J. Suza (PRM 631194, sub *G. callopisma*). Tribeč – Nitra, in colle calcaris Kalvária, c. 200 m, 1928, leg. J. Suza (PRM 631190, sub *C. callopisma*); Zobor pr. Nitra, in rup. calcareis „Pleška“, c. 400 m, 1934, leg. J. Suza (PRM 631202, sub *C. callopisma*); Zobor, pr. Nitra, apud „Svätý Svorad“ in rup. calcar., c. 350–400 m, 1934, leg. J. Suza (PRM 631199, sub *C. callopisma*); Nitra, calcareous rocks on W slope of Mt Zobor, alt. 400 m, 2004, leg. J. Vondrák (CBFS JV1820, sub *C. flavescens*); **Ibid., 2004, leg. J. Vondrák (CBFS JV6838).** Slovak Karst – Košice, apud ruinam arcis Turňa n. Bodvou ad saxa calcarea, c. 300 m, 1927, leg. J. Suza (PRM 631182, sub *C. callopisma*).

SPAIN: Andalusia – Sierra de Cazorla, Cazorla, on limestone rock c. 2–3 km N of Cazorla village, 950 m, 1998, leg. Z. Palice (hb. Palice s.n.).

**Tab. 1.** Quantitative characters of *Caloplaca aurantia* and *C. flavescens* investigated in this study compared with the literature.

| Characters                          | Reference                  | <i>C. flavescens</i>        | <i>C. aurantia</i>          |
|-------------------------------------|----------------------------|-----------------------------|-----------------------------|
| lobe length (mm)                    | Wade 1965                  | 2–5                         | 5–10                        |
|                                     | Nordin 1972                | 1.5–3                       | 2–3                         |
|                                     | Wetmore & Kärnefelt 1998   | 2–3                         | –                           |
|                                     | Khodosovtsev et al. 2004   | 1.5–3.5                     | –                           |
|                                     | this study                 | (1.55–) 3.05 ± 0.85 (–5.60) | (0.20–) 3.61 ± 1.00 (–6.50) |
| max. lobe width (mm)                | Wade 1965                  | 0.6–0.75(1)                 | –                           |
|                                     | Clauzade & Roux 1985       | 0.5–1                       | 1.5–3                       |
|                                     | Wirth 1995                 | 1(1.5)                      | 1.5–3                       |
|                                     | Wetmore & Kärnefelt 1998   | 0.5                         | –                           |
|                                     | Khodosovtsev et al. 2004   | 0.3–1                       | ±1                          |
|                                     | this study                 | (0.20–) 0.73 ± 0.20 (–1.30) | (0.40–) 0.94 ± 0.26 (–1.83) |
| cortex thickness (µm)               | this study                 | (10–) 37.43 ± 11.65 (–75)   | (0–) 17.87 ± 9.86 (–45)     |
| ascospore length (µm)               | Wade 1965                  | 8–15                        | 10–16                       |
|                                     | Poelt 1969                 | 8–15                        | 10–16                       |
|                                     | Verseghy 1971              | –                           | 8–15                        |
|                                     | Alon & Galun 1971          | –                           | 10–12                       |
|                                     | Nordin 1972                | 11–15                       | –                           |
|                                     | Clauzade & Roux 1985       | 8–16                        | 8–16                        |
|                                     | Laundon 1992               | 12–15                       | 10–13                       |
|                                     | Wirth 1995                 | 8–15                        | 10–16                       |
|                                     | Khodosovtsev et al. 2004   | (10)12–14(16)               | 9.7–13                      |
|                                     | Wasser & Nevo 2005         | 9–15                        | 10–13                       |
|                                     | this study                 | (9–) 11.76 ± 1.35 (–15)     | (9–) 11.08 ± 1.19 (–15)     |
| ascospore width (µm)                | Wade 1965                  | 5–10                        | 7–12                        |
|                                     | Poelt 1969                 | 5–10                        | 5–8                         |
|                                     | Verseghy 1971              | –                           | 6–10                        |
|                                     | Alon & Galun 1971          | –                           | 3–10                        |
|                                     | Nordin 1972                | 6–9                         | –                           |
|                                     | Clauzade & Roux 1985       | 5–13                        | 5–13                        |
|                                     | Laundon 1992               | 8–10                        | 8–10                        |
|                                     | Wirth 1995                 | 5–10                        | 7–12                        |
|                                     | Khodosovtsev et al. 2004   | (7)8–10(12)                 | 7–13                        |
|                                     | Wasser & Nevo 2005         | 6–10                        | 7–10                        |
|                                     | this study                 | (6–) 8.62 ± 0.80 (–11)      | (6–) 9.25 ± 0.95 (–12)      |
| septum thickness (µm)               | Khodosovtsev et al. 2004   | 1.5–2(2.4)–5                | 2.5–3.5                     |
|                                     | Wasser & Nevo 2005         | to 5                        | to 5                        |
|                                     | this study                 | (4–) 4.68 ± 0.55 (–8)       | (3–) 4.83 ± 0.59 (–6)       |
| conidium length (µm)                | Wade 1965                  | 3.6                         | 3.6                         |
|                                     | Khodosovtsev et al. 2004   | 3–6                         | –                           |
|                                     | this study                 | (4–) 5.04 ± 0.34 (–6)       | (4–) 5.02 ± 0.33 (–6)       |
| ascospore length / width            | this study                 | (1.09–) 1.38 ± 0.2 (–2.33)  | (0.82–) 1.21 ± 0.14 (–1.67) |
| ascospore length / septum thickness | Wasser & Nevo 2005         | –                           | 6–3(2)                      |
| this study                          | (1.25–) 2.54 ± 0.32 (–3.5) | (1.67–) 2.32 ± 0.32 (–3.75) |                             |

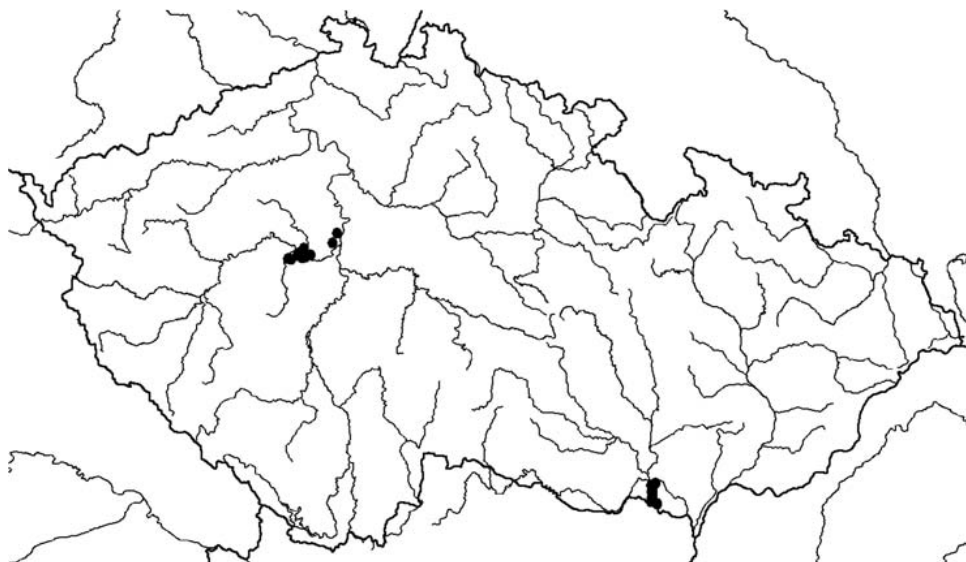


Fig. 2. Distribution of *Caloplaca flavescens* in the Czech Republic.

TURKEY: *Black Sea Coast* – İnebolu, coastal limestone rocks E of Abana, alt. c. 6 m, 41° 58' 52.90" N, 34° 02' 47.26" E, 2007, leg. J. Šoun (hb. Šoun 169). *Sea of Marmara Coast* – Gallipoli Peninsula, coastal limestone cliffs 1 km NE of Abide monument, alt. 1 m, 40° 03' 12.27" N, 26° 13' 41.24" E, 2007, leg. J. Šoun (hb. Šoun 170).

UKRAINE: Crimean Peninsula – Ad rupes calcareas frequentissima prope Sebastopolin, in peninsula Taurica, 1900, leg. A. Elenkin (PRM 631193; Lichenes Florae Rossiae I. 1901, 26, sub *Placodium aurantium*); Bakhchysarai, limestone cliffs above town, alt. c. 300 m, 44° 45' 04.68" N, 33° 53' 06.88" E, 2006, leg. J. Šoun (hb. Šoun 105); Feodosiya, limestone rocks on hill SWW of village Koktebel, alt. c. 300 m, 44° 57' 19.57" N, 35° 12' 39.26" E, 2006, leg. J. Šoun and J. Vondrák (hb. Šoun 106); Kerch Peninsula, Opukskiy zapovednik, coastal limestone cliffs, alt. c. 20 m, 45° 01' 57.18" N, 36° 12' 23.30" E, leg. J. Šoun and J. Vondrák (hb. Šoun 160); Yalta, Nikita, zapovednik "Mys-Martian", limestone rocks in forest near sea, alt. c. 20 m, 44° 30' 26.33" N, 34° 14' 50.83" E, 2007, leg. J. Šoun (hb. Šoun 167, 168).

UNITED KINGDOM: England – Bristol, calcareous rocks in Avon Gorge, next to Clifton Suspension Bridge, 2006, leg. J. Vondrák (hb. Šoun 44).

***Caloplaca flavescens*** (Huds.) Laundon, Lichenologist 16: 53. 1984.

Bas.: *Lichen flavescens* Huds., Fl. Anglica: 445. 1762. Typus: Hist. Musc.: 136, tab. 18, fig. 18A (BM, lectotypus, not seen).

Syn.: *Caloplaca heppiana* (Müll. Arg.) Zahlbr., *Caloplaca aurantia* var. *intermedia* Zahlbr., *Caloplaca aurantia* f. *centrifuga* (A. Massal.) Zahlbr.

**Short description.** Thallus smooth, light yellow to orange. Marginal lobes mostly convex for their whole length. Thallus sometimes pruinose in the centre, rarely slightly pruinose over whole surface. The cortex is loosely paraplecten-

chymatous, with a continuous layer of hyaline crystals, while in *C. aurantia* it is thinner and crystals are absent (Fig. 3). Ascospores lemon-shaped, slightly narrower and longer with more pointed apices than in *C. aurantia*. For quantitative characters see Table 1.

**Variability.** The marginal lobes are usually convex, but in some cases they are almost flattened at their tips. The thallus surface is usually single-coloured, never zonate, orange, orange-yellow to pale yellow; sometimes the tips of lobes can be lighter. Central parts of the thallus may be white due to absence of anthraquinone pigments, or the centre dies off with age. Furthermore, the apothecia may be rare and the thallus is covered by small, flat lobules or pustules in the centre of old specimens.

**Chemistry.** Thallus and apothecia K<sup>+</sup> (purple), chemosyndrome A (Søchting 1997).

**Ecology.** The species possesses almost the same ecology as *C. aurantia*, but there are some differences: it is more frequent not only on pure limestone but also on other calcareous or alkaline rocks and artificial substrata such as concrete, mortar, roofing tiles and monuments, and very rarely on the dusty bark of trees near quarries (Laundon 1992). The occurrence in the Czech Republic is, however, restricted to natural habitats, similarly to *C. aurantia*. There is an evident tendency to grow on more shaded, less xerothermic habitats than *C. aurantia*, but occasionally both species occur side-by-side.

**Distribution in the Czech Republic.** It is restricted to two larger xerothermic limestone areas (Fig. 2): the Pavlovské vrchy Hills (with one new, related locality on soft limestone at Sedlec) in South Moravia (Kovář 1908, Podpěra 1928, Suza 1925, all as *C. / G. aurantia*) and the Bohemian Karst in central Bohemia (Servít 1911 as *C. callopisma*, Servít 1930 as *C. aurantia*, *C. heppiana* and *C. aurantia* f. *centrifuga*, Suza 1943b as *C. aurantia* var. *intermedia*, Svoboda 2007). In both areas *C. flavescens* is rather common in suitable habitats.

Suza's statements regarding occurrences of *C. flavescens* (as *C. aurantia* var. *intermedia*) in Podyjí (Suza 1933, 1943b, 1947), Stránská skála Hill at the periphery of Brno (Suza 1914, 1925, 1943b, 1947) and Čebínka Hill near Tišnov (Suza 1925, 1943a, 1943b, 1947) are based on incorrect identifications and refer to *C. aurantia*.

## Material studied

Material used for detailed morphological investigations is in bold. The sample data are in their original wording on voucher labels or with small modifications.

**AUSTRIA:** Upper Austria – [Laakirchen] an Conglomeratfelsen beim Traunfall, 1887, leg. A. Zahlbruckner (PRC 23, sub *Physcia Heppianum*).

**BULGARIA:** West Bulgaria – Dragoman, Petrov Karst, ad rupes calcareas, c. 900–1100 m, 1923, leg. J. Suza (PRM 631168, sub *C. aurantia*).

CROATIA: West Croatia – ad saxa calcarea in vicinitate urbis Fiume [Rijeka], leg. F. Blechschmidt et J. Schuler (PRC 12; Kryptogamae Exsiccatae 1256b, sub *C. callopisma*); Ibid.: leg. F. Blechschmidt et J. Schuler (PRC 29, 31, PRM 580952; Kryptogamae Exsiccatae 2077, sub *C. aurantia* var. *dalmatica*). Dalmatia – Župa Dubrovačka, ad rupes calc. prope Kupari, c. 20 m, 1917, leg. R. Dvořák (PRM 580955, sub *C. aurantia* var. *dalmatica*); Pola [Pula], 1907, leg. J. Steiner (PRM 580944, sub *C. heppiana*); Mezi Albanese a Bibinjí, 1907, leg. M. Servít (PRC 33, sub *C. aurantia*); Na pobřeží mezi Razancí [Ražanac] a Vinjeraci, 1907, leg. M. Servít (PRC 30, sub *C. aurantia*); Albanese, 1907, leg. M. Servít (PRC 32, sub *C. aurantia*).

CZECH REPUBLIC: Central Bohemia – [Bohemian Karst], nad Kačákem (PRM 696988, sub *C. callopisma*); Ibid., Kačák, leg. E. Bayer (PRM 696992, sub *C. callopisma*); Císařská rokle u Berouna, leg. J. Podpěra (PRC 18, sub *G. aurantia* var. *areolata*); Sv. Prokop, 1884, leg. P. Hora (PRM 696980, 696986, sub *C. callopisma*); Z okolí Karlova Týna a Srbska, 1887, leg. E. Bayer (PRM 696996, 696994, sub *C. callopisma*); Butovice? (Daleje) za sv. Prokopem, 1891, leg. E. Bayer? (PRM 696999, sub *C. callopisma*); Diabasové skály u Butovic u Prahy, 1893, leg. E. Bayer (PRM 696997, sub *C. callopisma*); Vápenné skály za Butovicemi ku Prokopu, 1893, leg. E. Bayer (PRM 696995, sub *C. callopisma*); Radotín, 1902, leg. J. Podpěra (PRC 27, sub *G. aurantia*); Radotín. Skála nad mlýnem, 1903, leg. E. Bayer (PRM 696991, sub *C. callopisma*); Severní skála hradu Karlštejna, 1906, leg. M. Servít (PRC 19, sub *G. aurantia*); Diabasy v Prokopském údolí, 1919, leg. A. Hilitzer (PRM 696900, sub *C. aurantia*); Sv. Prokop, již. stráž, vápence, skalka v lese, 1920, leg. A. Hilitzer (PRM 696902, sub *C. aurantia*); In valle St. Procopii prope Pragam supra Daleje in summis rupibus calcareis, 1920, leg. E. Bayer (PRM 696993, sub *C. callopisma*); Daleje, 1921, leg. E. Bayer (PRM 696983, sub *C. callopisma*); U Srbska nad Kačákem na vápenných skalách, 1922, leg. A. Pejška et E. Bayer (PRM 696989, 696990, 696982, 696998, sub *C. callopisma*); Císařská rokle u Berouna. 1923, leg. J. Podpěra. (PRC 22, sub *C. aurantia*); Ad rupes calcareas prope „Daleje“ in valle Scti Procopii ad urbem Praga. 1924, leg. A. Pejška (PRM 696987, 697001, sub *C. callopisma*); Radotín, 1925, leg. A. Hilitzer (PRM 696901, sub *C. aurantia*); Praha, Prokopské údolí, in rup. calcar., c. 230 m, 1933, leg. J. Suza (PRM 631162, sub *C. aurantia*); In valle St. Prokop, c. 250 m, in rupibus calcareis, 1938, leg. J. Suza (PRM 631157, sub *C. aurantia*); In valle Prokopské údolí, in clivo cumuli Špičák dicto, ad rupam calcaream, alt. 270 m s.m., 1994, leg. J. Horáková (PRM 886362, sub *C. aurantia*); Prague, Prokopské údolí, Nová ves, in colle Hradiště, ad saxam calcaream, alt. 310 m, 1999, leg. D. Svoboda (hb. Svoboda: Lichenes Bohemiae 396); Beroun, in clivo saxae-stepposo in montis Hřebenec apud Svatý Jan pod Skalou (contre Skála), ad saxam calcaream, alt. 320 m, 2002, leg. D. Svoboda (hb. Svoboda: Lichenes Bohemiae s.n., sub *C. aurantia*); Beroun, secundum viam ferream (km 36,4) prope Tetín in valle fluminis Berounka, in fissura rupis calcareis, alt. 240 m, 2002, leg. D. Svoboda (hb. Svoboda: Lichenes Bohemiae s.n., sub *C. aurantia*); Beroun, in valle rivuli Berounka prope Tomáškův lom prope Srbsko pagum, in valle angusta parva cum rivulo, saxo calcareo in declivibus faucis, alt. 230 m, 2002, leg. D. Svoboda (hb. Svoboda: Lichenes Bohemiae 539); **Beroun, Srbsko, near Barrandova jeskyně cave in valley of Berounka River, alt. 230 m, 49° 56' N, 14° 07' E, 2002, leg. D. Svoboda (hb. Svoboda, Lichenes Bohemiae 389); Beroun, Srbsko, near Tomáškův lom quarry in valley of Berounka River, alt. 240 m, 49° 55' 43" N, 14° 08' 50" E, 2002, leg. D. Svoboda (hb. Svoboda, Lichenes Bohemiae 597, sub *Caloplaca aurantia*); Praha, Nová Ves, Prokopské údolí valley, limestone outcrop on S slope at Hradiště plateau hill, alt. c. 290 m, 50° 2' 20.02" N, 14° 21' 21.16" E, 2006, leg. J. Šoun (hb. Šoun 163); Tetín, limestone rocky slopes in Koda nature reserve, 1 km SW of village, alt. c. 400 m, 49° 56' 11.3" N, 14° 05' 12.8" E, 2006, leg. J. Šoun (hb. Šoun 99); Srbsko, Císařská rokle gorge, alt. c. 370 m, 49° 55' 33.9" N, 14° 07' 38.6" E, 2006, leg. J. Šoun (hb. Šoun 98); Praha, Hlubočepy, Prokopské údolí valley, S-exposed limestone rock 100 m SE of Dalejský Mlýn, alt. c. 260 m, 50° 2' 28.55" N, 14° 22' 32.21" E, 2006, leg. J. Šoun (hb. Šoun 162).** South Moravia – [Pavlovské vrchy Hills], Kesselberg montium, calcicola, 450 m, 1921, leg. J. Suza (PRM 631161, sub *C. aurantia*); Ibid., loco „Soutěska“, ad rupes calcareas, c. 380 m, 1922, leg. J. Suza (PRM 580954, sub *C. aurantia*); Ibid., Tabulová, c. 450 m, ad saxa calcarea, 1962, leg. A. Vězda (hb. Vězda, sub *C. heppiana*); **Horní Věstonice, locality „Soutěska“, limestone outcrops, alt. c. 350 m, 48° 51' 55" N, 16° 38' 29"**

**E, 2003, leg. J. Šoun (hb. Šoun 8, 9, 10, 11); Klentnice, limestone rocks on W slope of Tabulová Hill, alt. c. 420 m, 48° 50' 30" N, 16° 38' 12" E, 2004, leg. J. Šoun (hb. Šoun 12, 13, 14, 15, 16); Klentnice, small limestone outcrops near Sirotní hrádek ruin, alt. c. 420 m, 48° 48' 35" N, 16° 38' 11" E, 2004, leg. J. Šoun (hb. Šoun 17, 18, 19); Mikulov, small limestone outcrops on E slope of Kozí hrádek ruin, alt. c. 270 m, 48° 48' 35" N, 16° 38' 11" E, 2004, leg. J. Šoun (hb. Šoun 20); Klentnice, small limestone outcrops in forest on S slope of Tabulová Hill, alt. c. 370 m, 48° 50' N, 16° 38' E, 2005, leg. J. Šoun (hb. Šoun 29); Pavlov, small limestone outcrops on SE slope below Děvičky ruin, alt. c. 420 m, 48° 52' 33" N, 16° 39' 42" E, 2005, leg. J. Šoun (hb. Šoun 28); Mikulov, Kočičí skála Hill, limestone outcrops, alt. c. 360 m, 48° 49' 34" N, 16° 38' 30" E, 2005, leg. J. Šoun (hb. Šoun 7); Bavorý, small limestone rocks at locality Kočičí kámen, alt. c. 350 m, 48° 49' 50" N, 16° 38' 13" E, 2005, leg. J. Šoun (hb. Šoun 26); Mikulov, limestone rocks on Šibeničník Hill, alt. c. 250 m, 48° 47' 21" N, 16° 37' 48" E, limestone rock, 2005, leg. J. Šoun (hb. Šoun 25, 27); Horní Věstonice, limestone rock on W slope of Obora Hill, alt. c. 420 m, 48° 51' 50.34" N, 16° 38' 1.5" E, 2006, leg. J. Šoun (hb. Šoun 136); Pavlov, limestone outcrop on ridge of Děvín Hill, alt. c. 450 m, 48° 52' 26.35" N, 16° 39' 27.34" E, 2006, leg. J. Šoun (hb. Šoun 135); Sedlec, small limestone outcrops on Skalky Hill, alt. c. 250 m, 48° 46' 00" N, 16° 40' 42" E, 2004, leg. J. Šoun (hb. Šoun 21).**

FRANCE: Rhône-Alpes – Salève, leg. J. Müller Arg. (PRM 580941, 580943, sub *Amphiloma Heppianum*).

GERMANY: Schleswig-Holstein – Krs. Flensburg, an der Kirche in Översee, 1932, C.F.E. Erichsen (PRM 631177, sub *C. aurantia*).

HUNGARY: Bükk Mts. – Repáshuta, Három-kő rock on main edge, 3 km NEE of village, alt. c. 900 m, limestone rock, 2006, leg. J. Šoun (hb. Šoun 57).

ROMANIA: Dobruja – Tulcea, Jurilovca, rocky cliff at Dolosman Cape, 0–20 m, 44° 45' 31.70" N, 28° 56' 27.09" E, calcific claystone, 2007, leg. J. Šoun (hb. Šoun 179); Tulcea, Popina Island in Razim Lake, limestone rock on E coast, 0–1 m, 44° 58' 03.16" N, 28° 58' 57.29" E, 2007, leg. J. Šoun (hb. Šoun 180).

RUSSIA: Black Sea Coast – Tuapse, coastal calciferous sandstone rocks NW of town, alt. 3 m, 44° 06' 42.35" N, 39° 01' 58.02" E, 2007, leg. J. Šoun (hb. Šoun 172).

SLOVAKIA: Little Carpathians – Smolenice, in rupibus calcareis Ostrý Kameň – Burian, c. 450–550 m, 1937, leg. J. Suza (PRM 631171, sub *C. aurantia*). Považský Inovec – Ad ruinam arcis Tematín supra rupes dolomiticis, c. 4–600 m, 1926, leg. J. Suza (PRM 580956, sub *C. aurantia* v. *dalmatica*); Nové Mesto nad Váhom, Lúka, limestone rocks on south slope below ruin of Tematín castle, alt. c. 530 m, 2006, leg. J. Šoun (hb. Šoun 96). White Carpathians – Vršatec, in rup. calcar. Červený Kameň, c. 400–500 m, 1930, leg. J. Suza (PRM 631170, sub *G. aurantia*). Strážov Mts. – Uhrovské Podhradie, in valle dolina Rokoše, c. 500 m, in rup. calcareis, 1930, leg. J. Suza (PRM 631179, sub *C. aurantia*); Uhrovské Podhradie, ad ped. occ. m. Rokoš, c. 300 m, 1930, leg. J. Suza (PRM 631169, sub *G. aurantia*); Beluša, in rup. dolomiticis intre pag. Slatiny et Mojtín, c. 400 m, 1930, leg. J. Suza (PRM 580948, sub *C. aurantia* var. *centrifuga*); Beluša, Slatiny – Mojtín, in rup. dolomit., c. 400 m, 1930, leg. J. Suza (PRM 631176, sub *C. aurantia*); Beluša, Malenica, 800–900 m, dolomit., 1930, leg. J. Suza (PRM 631159, sub *G. aurantia*); calcicola in valle Manínska soutěska prope Záskanie, com. Trenčín, c. 350 m, 1922, leg. J. Suza (PRM 580953, sub *C. aurantia* v. *dalmatica*); Ad rupes arenosas (calcar.) inter Jablonové et Súľov, com. Trenčín, c. 350–500 m, 1922, leg. J. Suza (PRM 631167, sub *C. aurantia*); Žilina, in rupibus calcareis prope ruinam supra pag. Súľov, c. 600 m, 1926, leg. J. Suza (PRM 631174, sub *C. aurantia*, etiam cum f. *centrifuga*). Lesser Fatra – Kláštor pod Znievom, Zniev Hill, c. 980 m, vertical side of limestone rock, 2003, leg. J. Vondrák (CBFS JV1273). Greater Fatra – Plešovica, pr. p. Turč. Blatnica, c. 600 m, in rup. calcareis, 1930, leg. J. Suza (PRM 631165, sub *G. aurantia*); Ostré brdo, c. 1200 m, ad latus in rup. calcareis, 1930, leg. J. Suza (PRM 580950, sub *G. aurantia* var. *centrifuga*). Chočské vrchy Mts. – In valle Prosiecka dolina, c. 800 m, 1930, leg. J. Suza (631178, sub *G. aurantia*). Muráň Plateau – In rupibus calcareis “Cigánka” supra pag. Muráň, c. 950 m, 1929, leg. J. Suza (PRM 631153, 631155, sub *C. aurantia*); Mt. Kľak – S-slope, vertical S-facing rocks near red tourist footpath not far from crossings „Nižná Kľaková“, limestone rock, alt. c. 1250 m, 1999, leg. A. Guttová, J. Halda and Z. Palice (hb. Palice: Lichenes Slovakiae 2058).

Belianske Tatras – In valle rivi Biela prope Tatranská Kotlina, c. 800 m, 1925, leg. J. Suza (PRM 631172, sub *C. aurantia*). Slovak Paradise – „Havrania skala“ pr. pag. Stratená, c. 1050–1100 m, in rupibus calcareis, 1937, leg. J. Suza (PRM 580946, sub *C. aurantia* var. *centrifuga*); Hrabušice nature reserve „Prielom Hornádu“ – upper part, 48° 57' 30" N, 20° 24' 20" E, limestone rock, alt. c. 540 m, 1998, leg. Š. Bayerová, J. Halda and Z. Palice (hb. Palice: Lichenes Slovakiae 913). Volovské vrchy Hills – Rožňava, pr. p. Krásnohorské Podhradie in rup. calcar., c. 330 m, 1933, leg. J. Suza (PRM 631158, sub *G. aurantia*). Slovak Karst – Košice, in valle Zádielska dolina ad rupes calcareas, c. 250 m, 1927, leg. J. Suza (PRM 631173, sub *C. aurantia*). Spiš – Spišské Vlachy, Dreveník, c. 600 m, in rupibus calcareis, 1930, leg. J. Suza (PRM 631154, sub *C. aurantia* v. *centrifuga*). Branisko – Rudník-Rajtopiky, in rupibus dolomit., c. 950–1000 m, 1938, leg. J. Suza (PRM 631156, sub *G. aurantia*). Vihorlat Mts. – Humenné: in monte Krivošianka 500 m, 1930, leg. J. Nádvorník (PRM 760997, sub *C. aurantia* var. *dalmatica*).

SWEDEN: Gotland – Hoburgen, on sandstone by shore, 1918, leg. H. Magnusson (PRM 580945, sub *C. heppiana*); Ljugarn, 1918, leg. G.O. Malme (PRM 580940, sub *C. heppiana*).

SWITZERLAND: Basel-Country – An Kalkfelsen bei Liestal, Kant. (PRC 26, PRM 580942; Rabenhorst: Lichenes europaei 671, sub *Amphiloma heppianum*).

TURKEY: Black Sea Coast – İnebolu, coastal limestone rocks E of Abana, alt. c. 6 m, 41° 58' 52.90" N, 34° 02' 47.26" E, 2007, leg. J. Šoun (hb. Šoun 173); Cide, coastal sandstone rocks near Denizkonak, alt. 4 m, 41° 56' 54.30" N, 33° 08' 10.63" E, 2007, leg. J. Šoun (hb. Šoun 174); Amasra, claystone coastal rocks near Çakrazboz, alt. 2 m, 41° 46' 55.44" N, 32° 29' 07.96" E, 2007, leg. J. Šoun (hb. Šoun 175); Zonguldak, limestone coastal rocks near Ilksu, alt. 5 m, 41° 24' 36.97" N, 31° 40' 49.10" E, 2007, leg. J. Šoun (hb. Šoun 176). Sea of Marmara Coast – Armutlu, coastal limestone rocks 5.5 km SW of Esenköy, alt. 1–3 m, 40° 35' 06.68" N, 28° 53' 44.72" E, 2007, leg. J. Šoun (hb. Šoun 177); **Bandırma, coastal limestone rocks near Yenice, alt. c. 20 m, 40° 22' 44.78" N, 28° 04' 16.09" E, 2007, leg. J. Šoun (hb. Šoun 178).**

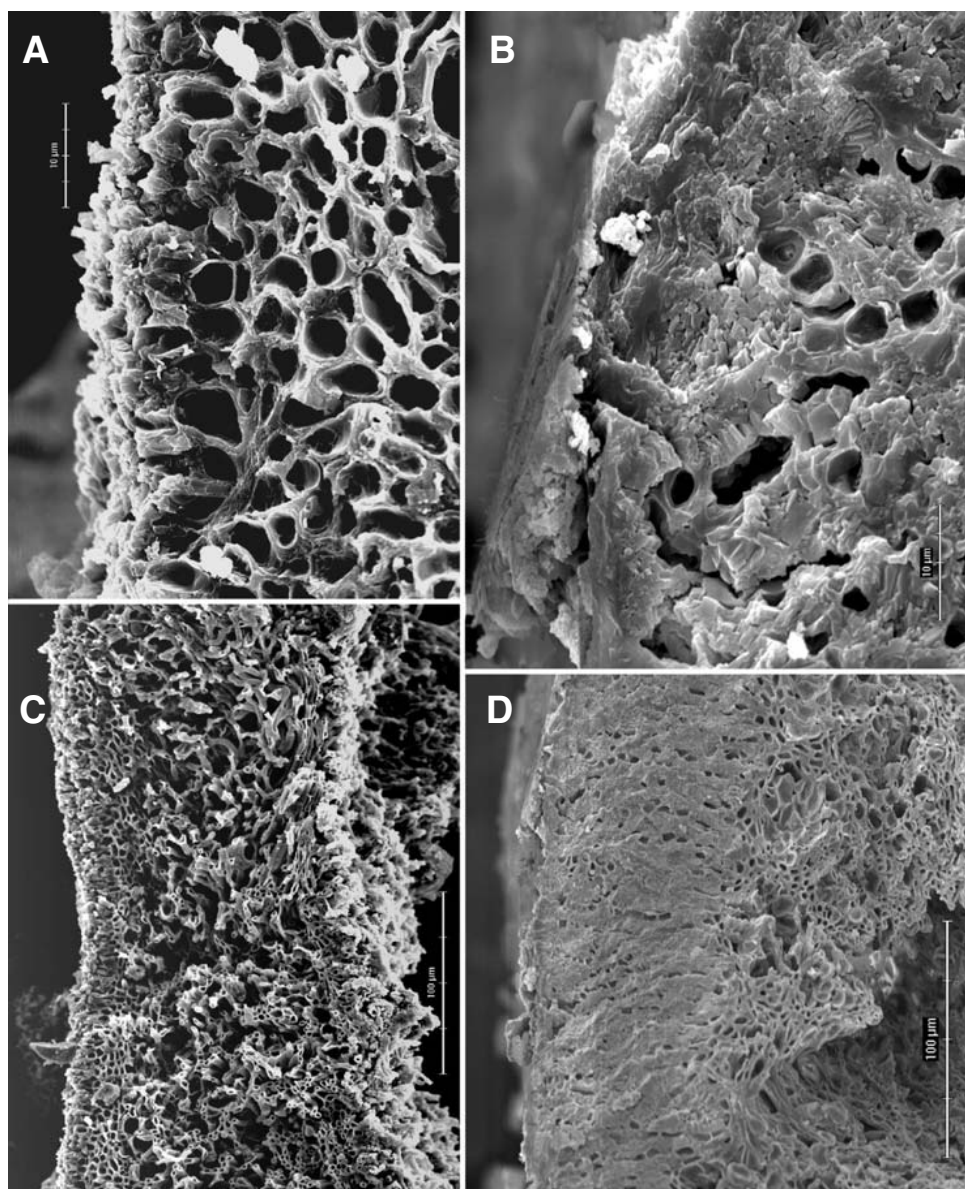
UKRAINE: Crimean Peninsula – Bakhchysarai, limestone cliffs above town, alt. c. 300 m, 44° 45' 04.68" N, 33° 53' 06.88" E, 2006, leg. J. Šoun and J. Vondrák (hb. Šoun 104); Kerch Peninsula, Opukskiy zapovednik, coastal limestone cliffs, alt. c. 20 m, 45° 01' 57.18" N, 36° 12' 23.30" E, 2006, leg. J. Šoun and J. Vondrák (hb. Šoun 159).

UNITED KINGDOM: England – Ruins of Tong Priory, Shropshire, leg. W. Leighton (PRM 631192; Leight. Exs. 113, sub *Parmelia murorum*); **Bristol, calcareous rocks in Avon Gorge next to Clifton Suspension Bridge, 2006, leg. J. Vondrák (hb. Šoun 43).**

## DISCUSSION

Morphology and anatomy. The basic morphology of *C. aurantia* and *C. flavescens* is described in e. g. Alon and Galun (1971), Clauzade and Roux (1985), Khodosovtsev et al. (2004), Nordin (1972), Verseghy (1970, 1971, 1972), Wade (1965), Wasser and Nevo (2005), Wetmore and Kärnefelt (1998) and Wirth (1995), but the diagnostic value of the characters has never been tested. Therefore, this study tries to pin-point diagnostic characters of the treated species. Data from our morphological investigations along with literature data (see Tab. 1 for quantitative characters) show the main differences in shape and colour of lobes and thallus, structure and thickness of cortex, and ascospore shape. However, the intraspecific variability in some characters, such as colour and lobe shapes, has resulted in descriptions of many varieties and forms in both species in the past.





**Fig. 3.** Transverse sections of marginal lobes (C, D) with detailed views of cortical layer structure (A, B) of *Caloplaca aurantia* (A, C) and *C. flavescens* (B, D) from a scanning electron microscope.

The presence vs. absence of a crystalline layer in the cortex seems to be the most reliable character distinguishing *C. aurantia* and *C. flavescens*, because colouring and thallus morphology of some untypical specimens can be confusing.

The presence of crystals as a crucial character is missing from some publications (Alon and Galun 1971; Laundon 1984, 1992; Nordin 1972; Wade 1965; Wasser and Nevo 2005; Wetmore and Kärnefelt 1998), but noted in others (Clauzade and Roux 1985; Khodosovtsev et al. 2004; Poelt 1954, 1969; Verseghy 1971, 1972; Wirth 1995). The thickness of the cortex is another useful diagnostic feature as described here for the first time.

As Verseghy (1971) already pointed out, ascospores in *C. flavescens* are on average slightly narrower and longer with more pointed apices than in *C. aurantia*, but since the variations in ascospore shape considerably overlap in both species (Tab. 1), this is not a reliable character.

**Nomenclature.** The nomenclature of *C. aurantia* and *C. flavescens* has undergone an intricate and somewhat confusing development since the 18th century. This matter will be treated in detail in another publication and only the circumstances concerning the Czech Republic are mentioned here. In the Czech literature (cf. Vězda and Liška 1999, Servít 1930) various names have been used for these species. *C. aurantia* has been named by its younger synonym *Caloplaca / Gasparrinia callopisma*. *C. flavescens* has been named as *C. heppiana*, *C. aurantia* var. *intermedia* and *C. aurantia* f. *centrifuga*. The last name was only used by Servít (1930: 45) and *C. heppiana* by Servít (1930: 8); these names are not included in the Catalogue of Lichens of the Czech Republic (Vězda and Liška 1999). The name *C. aurantia* var. *intermedia* was used by Suza for Bohemian populations of *C. flavescens*, but also incorrectly for some morphotypes of *C. aurantia* from Moravia.

Herbarium specimens of *C. flavescens* collected in the first half of the 20th century by some Czech authors (Kovář, Podpěra, Servít, Suza) were incorrectly named *C. aurantia* (Pers.) Hellb., *C. aurantia* (Pers.) J. Steiner and *Gasparrinia aurantia*. This confusion was caused by an incorrect concept of the name *C. (G.) aurantia* as an older and favoured synonym for *C. heppiana* or *C. sympagea* (= *C. flavescens*) established by some mainly central-European authors from the 19th to the middle of the 20th century (e. g. Steiner 1896, 1919; Sydow 1887; Zahlbruckner 1931).

These nomenclatural difficulties and confusions probably resulted in the omission of *C. flavescens* in Vězda and Liška (1999) and incorrect allocations of some references to *C. aurantia*. The name *C. flavescens* was published for the first time from the Czech Republic by Svoboda (2007).

**Distribution.** *C. aurantia* occurs in subtropical and temperate zones of the Old World, but recently, it was surprisingly reported from Argentina (Calvelo and Liberatore 2002). Its occurrence in North America mentioned by some authors (Esslinger and Egan 1995, Laundon 1992, Nimis 1993) was denied by Wetmore and Kärnefelt (1998). *C. flavescens* shows a similar distribution pattern to *C. aurantia*, but extends to higher altitudes and higher latitudes (up to south-

ern Scandinavia). In the Czech Republic, Moravian occurrences of both species probably represent extensions of their continuous ranges in the Eastern Alps, Carpathians and Pannonia. Somewhat striking is the absence of *C. flavescens* at the three localities of *C. aurantia* at the rim of south-Moravian xerothermic region. Both species are missing in the Moravian Karst where apparently favourable niches exist. The populations of *C. flavescens* in the Bohemian Karst, Central Bohemia, may be related either to its distribution in Germany or Moravia. However, *C. aurantia* is absent there, although it occurs in higher latitudes, e. g. in Germany and Poland (Fałtynowicz 2003, Scholz 2000).

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# Paper IV

**Šoun, J.**

(2010)

Typification and taxonomy of *Caloplaca aurantia*.

*Mycotaxon* 111: 331–336.





**Typification and taxonomy of *Caloplaca aurantia***

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**Abstract** — The sample from the Arnold's Lichenes Exsiccati no 989: *Physcia aurantia*, deposited in M, is designated to serve as neotype of *Caloplaca aurantia*. The sample appears to be a topotype of *C. aurantia*. An overview is presented of the complicated history of the application and misuse of the name. Old literature references to *C. aurantia* should be confirmed by herbarium material, since the species was often confused with *C. flavescens*.

**Key words** — central Europe, lichenized fungi, lichens, *Teloschistales*

**Introduction**

*Caloplaca aurantia* belongs to a small group of lobate, saxicolous species unique within the large genus *Caloplaca* (*Teloschistales*, lichenized *Ascomycota*) on account of their lemon-shaped ascospores. The group also includes *C. aegaea* Sipman, *C. flavescens* (Huds.) J.R. Laundon, *C. fuerteventurae* van den Boom & Etayo and *C. thallincola* (Wedd.) Du Rietz. Despite several recent taxonomic treatments including these species (Nordin 1972, Wetmore & Kärnefelt 1998, Gaya 2009), *C. aurantia* remains the only taxon that has not been typified. Moreover, although the name *C. aurantia* is used unambiguously in recent literature, I have noticed that its intricate evolution since the 18<sup>th</sup> century sometimes causes confusion even today (Šoun & Vondrák 2008). Here I select a type and discuss the historical circumstances associated with this name.

**Typification**

*Caloplaca aurantia* (Pers.) J. Steiner, Sitzungsber. Kaiserl. Akad. Wiss. Wien, Math.-Naturwiss. Cl., Abt. 1, 105: 438, 1896

≡ *Lichen aurantius* Pers., Ann. Bot. (Usteri) 11: 14, 1794

Type – An Kalkfelsen beim Dorfe Wendershausen unweit Witzenhausen an der Werra [Germany, Hesse], 1883, Dannenberg [Arnold's Lichenes Exsiccati no 989 sub *Physcia aurantia*, M (M-0127045)! – **neotype designated here**; PRM! – isoneotype].

- = *Physcia aurantia* (Pers.) Arnold, Flora 67: 246, 1884
- = *Caloplaca callopisma* [unranked] *aurantia* (Pers.) Hellb., Bih. K. Svenska Vet.-Akad. Handl., Afd. 3, 16(1): 60, 1890 [as "*Caloplaca callopisma* \* *C. aurantia*"]
- = *Amphiloma aurantium* (Pers.) Müll. Arg., Hedwigia 31: 153, 1892
- = *Lichen sympageus* Ach., Lichenogr. Suec. Prodr.: 105, 1798
- = *Lecanora callopisma* Ach., Lichenogr. Univ.: 437, 1810
  - Type – in muris et saxis Galliae [France], Dufour (H-Ach #1163A!), lectotype selected by Wetmore 1998).
- = *Calopisma vulgaris* De Not., Giorn. Botan. Ital., anno 2, tomo 2: 199, 1847

*Caloplaca aurantia* was described as *Lichen aurantius* by Persoon (1794). However, there is no specimen named *Lichen aurantius* present in Persoon's herbarium in L (Nordin 1972, G. Thijsse in litt. 2005). Despite the absence of a type specimen, however, the protologue (FIG. 1) is sufficiently detailed to lead one to a proper identification of *C. aurantia* according to current concepts. Any confusion with the potentially most similar species, *C. flavescens*, is avoided by the reference to flat, non-convex lobes. Arnold's Lichenes Exsiccati no 989 of *Physcia aurantia* was collected in 1883 by Dannenberg, most likely at the type locality. The exsiccate was distributed according to Grummann (1974) to C, DUKE, FH, FR, GOET, H, HAL, HSI, K, L, M, NY, O, PC, S, UPS, US, and W;

**II.** *Lichen aurantius, saxatilis, crusta foliacea tartarea: foliis inbricatis expansis obscure aurantiis, scutellis parvis concoloribus.*

Prov. elegans hæcce species ad rupes calcarias. (Prope Witgenhaussen in Haffia.)

Desc. *Ambitus* suborbicularis latitudine 3 — 4 unc., rupibus arcte adhærens foliis expansis adpressis, fibi invicem approximatis, planis finuatis, apice subcrenatis, colore obscure vitellinis. *Substantia* tartarea, fragili, hinc de rupibus se evellendum non finit.

Obs. Foliis nec convexis, nec pulposis, nec inter se distantibus, etiam magnitudine a *Lich. murorum* Hoffm. & *miniato* ejusd. differt. Sic quoque foliis latioribus & imprimis colore a *Lich. circinnato, versicolore & murali* discrepat.

FIG. 1. The original description of *Lichen aurantius* (Persoon 1794: 14).

**989.** — *Physcia aurantia* Pers. in Ust. Ann. 11, 1794 p. 14; 1795 p. 36.  
*L. sympagea* Ach. prodr. 1798 p. 105, univ. p. 437.  
 An Kalkfelsen beim Dorfe Wendershausen unweit Witzenhausen an  
 der Werra (ubi Persoon plantam legit). 1883. **Dannenberg.**

FIG. 2. The label of Arnold's Lichenes Exsiccati no 989 of *Physcia aurantia* (M).

I have also found one in PRM. On the label (FIG. 2) it is claimed that the locality [the village of Wendershausen near Witzenhausen on the Werra] corresponds with Persoon's protologue [at Witzenhausen in Hassia], so this exsiccate specimen is regarded as a topotype. The locality is situated in the northern part of current German state of Hesse, and in Persoon's protologue Witzenhausen is very probably incorrectly spelled Witzenhausen. Arnold's exsiccate agrees well with Persoon's description — especially in its flat, deep orange lobes — while at the same time corresponding to the modern concept of *C. aurantia*; among other characters, the absence of a crystalline layer in the cortex distinguishes it from *C. flavescens*. For some reason, however, Arnold issued this collection as *Physcia aurantia*, a name that he usually misapplied to *C. flavescens*, instead of *Physcia callopisma* (Ach.) A. Massal., the name that he normally used for the true *C. aurantia* (Arnold 1884). I select here this exsiccate in M as the neotype of *C. aurantia*.

### Development of taxonomy

The name *Lichen aurantius* introduced by Persoon (1794) was soon synonymized by Acharius (1798: 105) with a new name, *Lichen sympageus*. Acharius, however, had never seen Persoon's *L. aurantius*, as he himself noted; he likely just excerpted Persoon's original description and also the short note in a further Persoon publication (Persoon 1795). Fries (1871) later also pointed out that Acharius had never seen *L. sympageus* and that the species is absent from Acharius's herbarium. Although by citing an older synonym in the protologue Acharius made the name *L. sympageus* superfluous and illegitimate, it was adopted by some authors. Later, Acharius (1803) incorrectly reduced both *L. sympageus* and *L. aurantius* to synonymy with *Parmelia elegans* (Link) Ach. [= *Xanthoria elegans* (Link) Th. Fr.].

In 1810, Acharius adopted yet another concept. On the basis of specimens from different sites in Europe, he described *Lecanora callopisma* (= *C. aurantia*) with *Lichen sympageus* treated as its variety (*Lecanora callopisma*  $\beta$  *sympagea*) that differed only in its more strongly orange thallus color (Acharius 1810). During the 19<sup>th</sup> century various authors treated *L. callopisma* also under other genera, reflecting the evolution in taxonomy: *Aglaopisma vulgaris* (De Not.) De Not., *Amphiloma callopisma* (Ach.) Körb., *Callopisma vulgaris*, *Gasparrinia*

*callopisma* (Ach.) P. Syd., *Parmelia callopisma* (Ach.) Hepp, *Physcia callopisma*, *Placodium callopismum* (Ach.) Mérat, *Teloschistes callopismus* (Ach.) Trevis., and *Xanthoria callopisma* (Ach.) Th. Fr. Fries (1871) combined *Lecanora callopisma* into the currently accepted genus *Caloplaca*, however he included under this name also lichens from southern Scandinavia known today as *C. flavescens*; nevertheless some authors followed suit (e.g. Hellbom 1890).

Arnold (1881) stated that, in his opinion, *Lichen aurantius* is the oldest name for *Amphiloma heppianum* Müll. Arg. (= *C. flavescens*) described by Müller (1862). He probably based his opinion on specimens of *C. flavescens* that he saw in Meyer's herbarium determined as *L. callopisma* var. *aurantia* or *L. callopisma* var. *sympagea*. Subsequently Arnold (1884) incorrectly replaced the name *Physcia heppiana* (Müll. Arg.) Arnold with *Physcia aurantia* and this was probably the starting point for the misapplication of the epithet *aurantia* for next c. 70 years. Disagreeing with his concept, some authors (e.g. Hue 1886, Crombie 1894, Nylander 1896, Monguillon 1899) used Acharius's epithet *sympagea* for *Physcia heppiana*. From the end of 19<sup>th</sup> to the beginning of 20<sup>th</sup> century, three epithets (*aurantium*, *heppianum* and *sympageum*) were in use simultaneously for *C. flavescens* and two for *C. aurantia* (*aurantium* and *callopismum*; e.g., Flagey 1886, Sydow 1887, Hue 1896 and Nylander 1896).

As Nordin (1972) has noted, Hellbom's combination of *Caloplaca callopisma* \* *aurantia* (Hellbom 1890: 60) does not refer to the correct material but is misapplied to *C. flavescens*. Steiner's later combination of *Caloplaca aurantia* (Steiner 1896: 438) probably refers to the correct lichen. The combination *C. aurantia* has been attributed to both Hellbom and Steiner, but as Laundon (1984) has indicated, only Steiner's combination is at species rank; the Hellbom combination is at an undesignated infraspecific rank, as indicated by the typography and explicitly stated in discussion (Hellbom 1890: 60–62).

Müller's combination of *Amphiloma aurantium* is worth mentioning because it was the first to correctly synonymize *L. aurantius* with *L. callopisma* (Müller 1892). This concept was subsequently adopted by some, but not all, lichenologists (e.g. Hue 1896, Rieber 1901, Migula 1929) in the first half of 20<sup>th</sup> century. Unfortunately Zahlbruckner (1931) also treated *C. aurantia* incorrectly as *C. heppiana* (Müll. Arg.) Zahlbr. (= *C. flavescens*).

After Poelt (1954) the species and names *C. aurantia* and *C. flavescens* (until Laundon 1984 as *C. heppiana*) have been used correctly in general, although some authors initially used these names for two different varieties within *C. aurantia* (Poelt 1954, 1969, Wade 1956, Alon & Galun 1971). Only a few authors persisted in using Zahlbruckner's concept after 1954 (e.g. Moruzi et al. 1967 and Werner 1956).

The intricate history of the application of the name *C. aurantia* means that all old literature references to this species require confirmation by herbarium

material, because some records obviously represent a different species, *C. flavescens*.

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# Paper V

Vondrák, J. & Šoun, J.

(2006)

An appraisal of the syntype material of *Caloplaca aurantiomurorum*  
(*Teloschistaceae*, lichenized fungi).

*Mycotaxon* 97: 67–71.





**An appraisal of the syntype material of  
*Caloplaca aurantiomurorum*  
(*Teloschistaceae*, lichenized fungi)**

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**Abstract**—Sample no. 54 of Flagey: Lichenes Algeriensis exsiccati represents the syntype of *Placodium aurantiomurorum* (= *Caloplaca aurantiomurorum*). However, the samples of this exsiccatum distributed to FH, H, M, PC and UPS contain different lichen species. The lectotype of *P. aurantiomurorum* is selected here (sample in UPS) and this name is treated as a synonym of *Caloplaca aurantia*. In this exsiccatum, *Candelariella senior* has been identified (in H, FH, and PC), which is reported here as a new species to Algeria. The known distribution of *Can. senior* is described.

**Key words**—lichens, nomenclature, typification

## Introduction

*Placodium aurantiomurorum* was described by Flagey (1891: 112) from Algeria “Rochers humides de Sidi-Mecid et seulement là”) in the exsiccatum “Flagey: Lichenes Algeriensis exsiccati (no 54)”. This exsiccate collection was distributed to the herbaria FH (nos 1-200), H, M, PC, and UPS (Grummann 1974: 277). The specimen in the herbarium of the University of Helsinki (H) was investigated, it being the only representative of *Caloplaca aurantiomurorum* (Flagey) Zahlbr. in the section. Surprisingly, it clearly belonged to the genus *Candelariella*, which we later determined as *Can. senior* Poelt. Subsequently, we investigated more samples of this exsiccatum (FH, M, PC, UPS) and found that individual exsiccates represent different species of *Caloplaca* (= *Cal.*) and *Candelariella* (= *Can.*).

## Materials and Methods

Apart from the investigated exsiccates, reference materials of *Caloplaca aurantia* (Pers.) Hellb., *Cal. flavescens* (Huds.) J.R.Laundon, *Cal. saxicola* (Hoffm.)

Nordin, and *Candelariella senior* from the herbaria CBFS, GZU, M, PRC, and PRM were used. Light microscopy measurements of ascospore characteristics, to an accuracy of 1  $\mu\text{m}$ , were performed on hand-made sections examined in water at a magnification of  $\times 1000$ . These measurements are given as MIN-X ( $\pm$ SD)–MAX, where X = mean value, SD = standard deviation, and MIN and MAX = extremes. Ten measurements (five ascospores in two apothecia) were examined in all samples except that from H, where the numbers of measurements (n) are given in parentheses.

## Results

The material in FH has a yellow-orange, rosette-like thallus with broad and flat lobes. Mature, well-developed ascospores are citriform, 12–14.2 ( $\pm 1.1$ )–16  $\times$  8–9.3 ( $\pm 0.7$ )–10  $\mu\text{m}$ , with septa 3–4.2 ( $\pm 0.6$ )–5  $\mu\text{m}$  wide. This specimen is morphologically identical with typical *Caloplaca aurantia*. *Candelariella senior* (morphologically identical with the material from H) and a small piece of an undetermined *Caloplaca* with a granular thallus are also present in this collection.

The material in H has *Candelariella*-type asci, simple ascospores, and a thallus and apothecia devoid of anthraquinones; therefore it belongs to *Candelariella*, not to *Caloplaca*. This rosette-like lichen was morphologically and anatomically compared with the holotype specimen of *Candelariella senior* (M, 0099854). Both samples are identical in most characters, differences were only observed in the thallus thickness [100–170 ( $\pm 44$ )–250  $\mu\text{m}$  (n=12) in “*Cal. aurantiomurorum*” vs. 120–228 ( $\pm 66$ )–310  $\mu\text{m}$  (n=10) in *Can. senior*] and in the spore width [10–12.5 ( $\pm 1.2$ )–15  $\times$  4–5.3 ( $\pm 0.6$ )–6  $\mu\text{m}$  (n=18) in “*Cal. aurantiomurorum*” vs. 10–12.0 ( $\pm 1.7$ )–16  $\times$  3–4.0 ( $\pm 0.5$ )–5  $\mu\text{m}$  (n=10) in *Can. senior*].

The material in M (M-0100101) has an orange, rosette-like thallus with short broad marginal lobes. Mature ascospores are ellipsoid, never citriform, 10–11.0 ( $\pm 0.5$ )–12  $\times$  6–6.1 ( $\pm 0.3$ )–7  $\mu\text{m}$ , with septa 3–3.4 ( $\pm 0.5$ )–4  $\mu\text{m}$  wide. This specimen is morphologically identical with typical *Caloplaca saxicola*.

The material in PC (PC0107050) is on two pieces of stone (glued on a sheet). The upper one is only covered by a fertile lichen with yellow-orange, rosette-like thallus with broad and flat marginal lobes. Mature, well-developed ascospores are citriform 13–14.1 ( $\pm 1.2$ )–16  $\times$  9–10.4 ( $\pm 1.0$ )–12  $\mu\text{m}$ , with septa 4–4.5 ( $\pm 0.7$ )–6  $\mu\text{m}$  wide. This specimen is morphologically identical with typical *Caloplaca aurantia*. The lichenicolous fungus *Cercidospora caudata* Kernst. occurs in its apothecia. *Cal. aurantia* also prevails on the lower stone, but *Candelariella senior* and an undetermined granulose *Caloplaca* are admixed.

The material in UPS has a yellow-orange, rosette-like thallus with broad and flat lobes. Mature, well-developed ascospores are citriform, 13–14.6 ( $\pm 0.8$ )–16

× 8–9.7 (±0.9)–11 µm, with septa 4–4.9 (±0.7)–6 µm wide. This specimen is morphologically identical with typical *Caloplaca aurantia*. This sample is selected here as the lectotype.

## Discussion

The short Latin diagnosis of *Placodium aurantiomurorum* (Flagey 1891: 112) is translated as follows: “Thallinal lobes flatter than in *P. murorum* (= *Caloplaca saxicola*); spores 16–18 × 8–9 µm, wider than in *P. murorum* and with a shape as in *Physcia aurantia* (= *Cal. flavescens*)”. The extended French description (Flagey 1896: 28), where *Placodium aurantiomurorum* was compared with *P. murorum*, *P. callopismum* (= *Cal. aurantia*) and *P. heppianum* (= *Cal. flavescens*), is translated as follows: “Thallus fairly yellow suede with lobes larger and more flattened than in *P. murorum*, resembling lobes of *P. callopismum*, but with lobes yellow, less reddish. Spores ovoid, ‘placodial’ 16–18 × 8–9 µm, larger than in *P. murorum*, strongly resembling spores of *P. heppianum*, whose thallus is clearly different”.

Based on these descriptions, *Cal. aurantiomurorum* is distinct from *Cal. saxicola* by having a different shape and size of the thallus and a different shape of spores and from *Cal. flavescens* by having a different shape of lobes. However, *Cal. aurantiomurorum* is distinguished from *Cal. aurantia* only by the yellow colour of the thallus. Based on this and the syntype investigation, we decided to place the name *Cal. aurantiomurorum* into the synonymy of *Cal. aurantia*.

In the protologue, Flagey (1891: 112) described one locality but did not designate the holotype. His main herbarium is located in PC and following the usual practice for exsiccates, the sample placed there should be regarded as the holotype and the others as isotypes. In this case, however, due to the heterogeneity of the respective material, we treat all exsiccate samples as syntypes. We have selected the sample in UPS as the lectotype, because it is well-preserved and without any admixture of similar lichen species (cf. the mixture represented by the specimen in PC). The sample in UPS was already revised as *Cal. aurantia* and mentioned in the list of exsiccates of this species by Nordin (1972: 80). This specimen was indicated as an isotype although the typification was not published.

*Cal. aurantiomurorum* has only been reported on calcareous rocks in Sidi-Mecid near Azéba fort and in Djebel Akar Mts in Algeria (Flagey 1896: 28) and in Upper Galilee, Mt Carmel in Israel (Alon & Galun 1971: 287–288). *Cal. aurantiomurorum* was accepted in two lichen checklists of Israel (Galun & Mukhtar 1996: 152, Kondratyuk et al. 1996: 35), until the voucher material from Israel was redetermined as *Cal. flavescens* (Wasser & Nevo 2005: 100) and the name *Cal. aurantiomurorum* was excluded from the Israel lichen flora

(Wasser & Nevo 2005: 321) and erroneously put into the synonymy of *Cal. flavescens* (Wasser & Nevo 2005: 99).

The sample in H and parts of the samples in FH and PC belong to the lobate species of *Candelariella*. In southern Europe, three lobate species are known, *Can. medians*, *Can. rhodax*, and *Can. senior*. While the two former species are clearly different (Poelt & Vězda 1976, 1977), the latter fits well with the respective samples. The differences in the thallus thickness and spore width between the holotype specimen of *Can. senior* and the sample in H can be easily accounted for by intraspecific variation. Having seen more material of *Can. senior* from GZU, we consider them conspecific with the samples of the investigated exsiccatum. Previously, *Can. senior* was only known from the type locality in Spain (Poelt 1958: 440-441), and from Libya (Thor, unpublished data) and Tunisia (cf. Seaward 1996: 123) so far.

Other samples of *Can. senior* seen: **Algeria**. on limestone in "d'Azeba" (GZU, intermixed in sample Flagey: Lich. Alg. Exsic. 93, *Rinodina subconfragosa*). **Libya**. On calcareous stone near Derna (Darnah), Thor, 1982 (GZU). **Tunisia**. Douggha, Poelt, 1968 (GZU); Djebel Goraa Mts, between Thibar and Teboursouk, Poelt, 1968 (GZU).

## Conclusions

Flagey's exsiccatum of *Placodium aurantiomurorum* is composed of heterogeneous material. The samples in FH, PC and UPS are taxonomically indistinguishable from *Cal. aurantia*, and the sample in M is indistinguishable from *Cal. saxicola*. The sample in UPS is selected here as the lectotype of *Placodium aurantiomurorum* and we propose to put the name *Cal. aurantiomurorum* into the synonymy of *Cal. aurantia*. We consider the sample of *Cal. aurantiomurorum* in H and the admixed lichens in samples from FH and PC conspecific with *Candelariella senior*, which is newly reported from Algeria.

In the light of this work, more attention should be given not only to examining distributed material of this particular exsiccatum in other herbaria, but also to appraising the homogeneity of exsiccata in general.

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# Paper VI

Vondrák, J., Šoun, J., Søgaard, M. Z. & Söchting, U. & Arup, U.

(2010)

*Caloplaca phlogina*, a lichen with two faces; an example of infraspecific variability resulting in the description of a redundant species.

*Lichenologist* 42: 685–692.





## ***Caloplaca phlogina*, a lichen with two facies; an example of infraspecific variability resulting in the description of a redundant species**

**Jan VONDRÁK, Jaroslav ŠOUN, Majbrit Zeuthen SØGAARD, Ulrik SØCHTING and Ulf ARUP**

**Abstract:** *Caloplaca phlogina* is shown here to have two kinds of soralia, yellow soralia with anthraquinones versus whitish or white-green soralia lacking pigments. Both kinds are present, growing side by side, in some localities in Scandinavia, but yellow soralia appear to be more common. In contrast, the populations from halophilous shrubs on the Black Sea coast have predominantly white soralia, and they were described as a separate species, *C. scythica*. A single collection from Chile also has white soralia. Molecular data and phenotype examinations convinced us that Scandinavian and Black Sea populations are conspecific. We consider the North European, phenotypically variable population as a source for the Black Sea population which is ecologically and phenotypically more uniform.

**Key words:** *Caloplaca scythica*, founder effect, disjunct distribution, phenotypic variability, species delimitation

### **Introduction**

Lichen species often produce more than one colour morph. Cyanomorphs and chloromorphs in *Lobaria* (e.g. Jørgensen 1998) are an example where even one individual mycelium produces two colour variants. Several examples are also known of non-pigmented phenotypes that occur rarely, but randomly, throughout normally pigmented populations. These include: specimens with unpigmented ascomata in species with normally dark fruit bodies (Gilbert 1996); specimens with unpigmented thalli in for example, *Caloplaca verruculifera* (Vain.) Zahlbr. (Søchting 1973) and *Arctoparmelia centrifuga* (L.) Hale (Santesson 1970); and

the citrine-green morphotypes in various species of *Candelariella* (Gilbert *et al.* 1981). Such variation may be due to occasional mutations blocking specific metabolic pathways. On the other hand, greyish thalli of normally yellow *Xanthoria* species are usually considered to be ecologically induced phenotypic expressions occurring in shaded and wet populations.

Here, we present an example of two sorediate ‘species’ of the genus *Caloplaca* (*Teloschistaceae*) recognized because of the different colours, grey versus yellow, of their thalli.

### ***Caloplaca phlogina* (Ach.) Flagey**

This is a yellow, sorediate morphotype containing anthraquinones (Fig. 1A) that grows on bark (rarely on concrete). It is known from Scandinavia (Arup 2006), Canada (Richardson *et al.* 2009; specimen confirmed by U. Søchting) and Romania (Vondrák *et al.* 2009). Records not confirmed by molecular data are also known from Western and Central Europe (Crespo *et al.* 1980; Sérusiaux *et al.* 1999; Vondrák

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*et al.* 2007; Fletcher & Laundon 2009), and India (Joshi & Upreti 2008).

### ***Caloplaca scythica* Khodosovtsev & Søchting**

This is a whitish, or white-green, sorediate morphotype lacking anthraquinones in the thallus (Fig. 1B) that grows on the maritime shrubs *Halocnemum strobilaceum* and *Limonium suffruticosum*, plant debris and loess soil on the northern coast of the Black Sea (Kondratyuk *et al.* 1998; Vondrák *et al.* 2009a, sub *C. phlogina*). It also occurs on tree bark in Scandinavia, where it often grows side by side with yellow-sorediate morphotypes. Surprisingly, this morphotype was recently collected from a weathered piece of leather at a maritime site in southern Chile (Isla Navarino, Zeuthen Søgaard 84, C). Its ITS sequence was nearly identical to those of *C. scythica* (Fig. 2).

Our aim was to investigate the status of the *Caloplaca* morphotypes using phenotype studies and phylogenetic analysis of the ITS regions of nuclear rDNA.

## **Materials and Methods**

### **Specimens studied**

*Morphotypes with yellow soralia.* **Denmark:** Bornholm: Arnager, on bark, 2006, R. S. Larsen & J. Vondrák (CBFS JV4713). Jutland: Ranum, on bark, T. Jensen (LD 1081648). Sjælland: Haraldsted, 1946, O. Almborn (LD 1081712). Sjælland: Kildebrønne, on wood, 1943, M. Skytte-Christiansen (LD 1081584).—**France:** Seine-et-Marne: Fontainebleau, on bark, 1949, O. Almborn (LD 1094801).—**Germany:** Oldenburg, on wood, 1889, H. Sandstede (LD 1093457).—**Romania:** Black Sea coast: Constanta, Mangalia, on concrete, 2007, J. Vondrák (CBFS JV3437).—**Sweden:** Blekinge, Ringamåla, on bark, 1989, U. Arup & S. Ekman (LD 1025279). Halland: Släp, on bark, 1989, U. Arup & S. Ekman (LD 1025983); Närke, Götlunda, Sickelsjö, on bark, 1952, G. Kjellmert (LD 104335). Skåne: Bollerup, on bark, 1992, L. Lindblom (LD 1068814); Hallands Väderö, 1990, L. Lindblom (LD 1068878); Käseberga, on bark, 1997, P. Frödén (LD 1023711) Vittskövle, on bark, 1943, O. Almborn (LD 1067982).

*Morphotypes with soralia lacking anthraquinones.* **Chile:** Isla Navarino: Puerto Williams, on weathered leather, 2008, M. Z. Søgaard (C).—**Russia:** Black Sea coast: Taman Peninsula, on loess and maritime shrubs, 2007, J. Vondrák (CBFS JV6060, 6061, 6224, 7385, 7500).—**Sweden:** Skåne: on bark, 1942, O. Almborn

(LD 1067918); *ibid.*, on bark, 1964, S.W. Sundell (LD 1068110, anthraquinones present in spots); Kristianstad, on bark, 1935, O. Almborn (LD 1024223, 1068942); Kyrkheddinge, on bark, 2002, D. Göransson (LD 1262223).—**Ukraine:** Crimea: Syvash salt lagoon, on maritime shrubs, 2006 & 2009, J. Vondrák (CBFS JV4648, 4653, 4996, 7209); *ibid.*: 2003, A. Khodosovtsev (KHER 2918). Kherson region: Sadove, Nikolaiivka, on maritime shrubs, 2009, J. Vondrák (CBFS JV7143). Nikolaev region: Kinburnskaya kosa, on maritime shrubs, 2009, J. Vondrák (CBFS JV7132, 7133).

*Samples with both morphotypes:* **Sweden:** Halland: Vallda, on bark, 1932, A. H. Magnusson (Magnusson: *Lich. sel. scand. exs.* 143; LD 1067470). Skåne: Ivotofta, 1942, O. Almborn (LD 1023967, 1067854); Mölleberga, on bark, 1938, O. Almborn (LD 1023903); Kristineberg, on bark, 1995, P. Frödén (LD 1023775); Träne, on bark, 1946, O. Almborn (LD 1024031). Södermanland: Trosa, on maritime wood, 1925, G. O. A. Malme (LD 1015744). Västmanland: Norlund, on wood, 1950, G. Kjellmert (LD 1015552).

### **DNA extraction and amplification**

Direct PCR was used for PCR-amplification of the ITS regions including the 5.8S gene of the nuclear rDNA following Arup (2006). Primers for amplification were ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990). PCR cycling parameters followed Ekman (2001).

### **Phylogenetic analysis**

Five newly obtained ITS sequences (Table 1) were included in the phylogenetic analysis along with ten sequences from GenBank selected to illustrate the phylogenetic position of *Caloplaca scythica* in relation to the *Caloplaca phlogina* clade. Sequences were aligned using MAFFT 6 (on-line version in the Q-INS-i mode; see Katoh *et al.* 2002) and manually cut to eliminate the unaligned ends and ambiguously aligned regions of ITS1 and ITS2; 506 positions were retained. Bayesian phylogenetic analysis was carried out using the program MrBayes 3.1.1 (Ronquist & Huelsenbeck 2003). The optimal nucleotide substitution model (HKY+G) was found using the program MrModeltest v2.3 (Nylander 2004) with the Akaike Information Criterion and the hierarchical likelihood ratio test (Posada & Crandall 1998). The MCMC analysis was run for five million generations, performed in two runs, each with four chains starting from a random tree and using the default temperature of 0.2. Every 100th tree was sampled, and the first 174 000 generations were discarded as burn-in, using standard deviation of splits between runs less than 0.01 as a convergence criterion.

Phenotype investigations were made according to Vondrák *et al.* (2009) and the phenotypic characters selected for the study are listed in Table 2. Anthraquinone content was analyzed in one selected sample by HPLC analysis (for details see Søchting 1997).

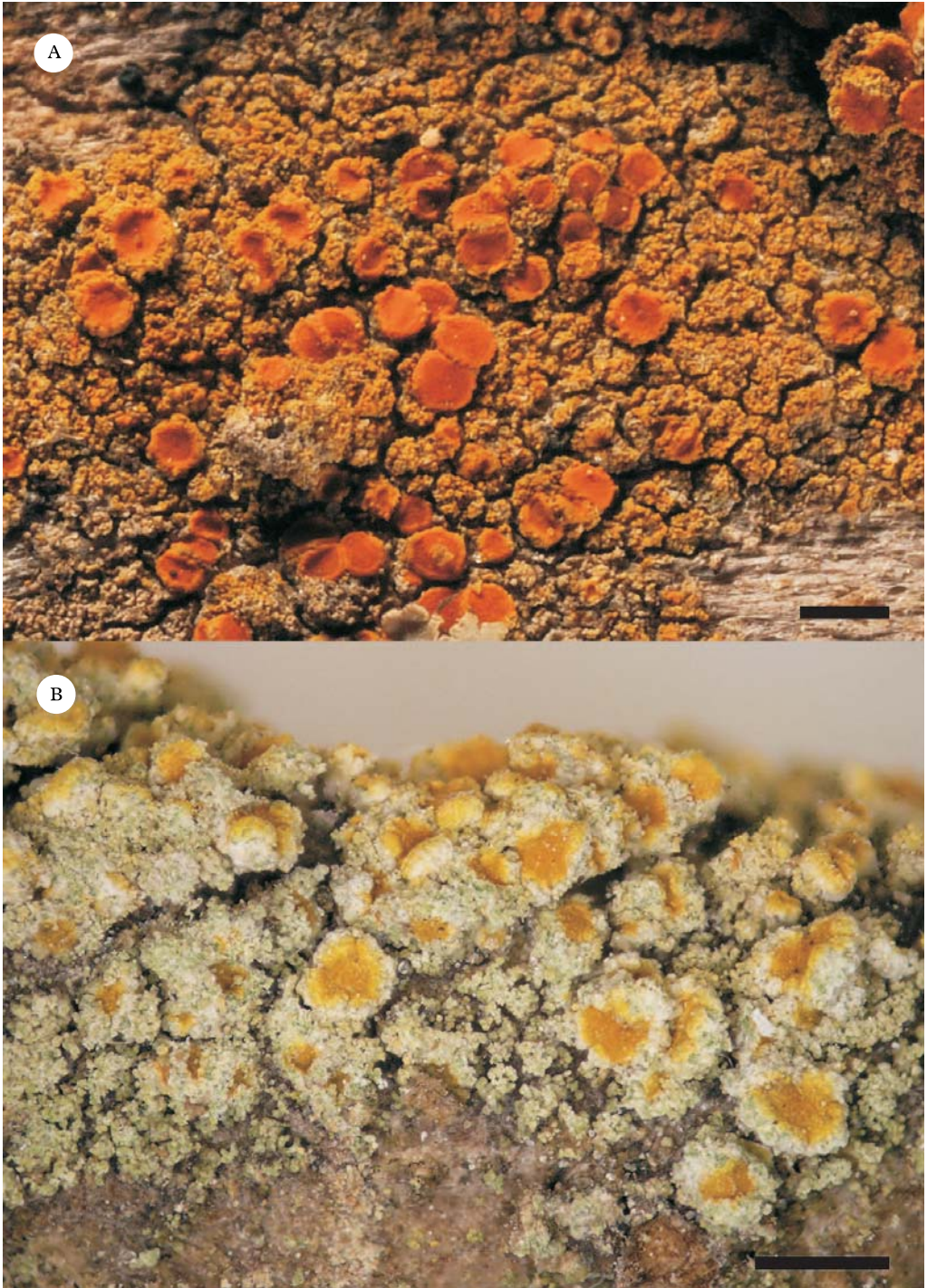


FIG. 1. *Caloplaca phlogina*, thallus and apothecia. A, *C. phlogina* type with anthraquinones in the thallus [Denmark, Nekselø, *Søchting* 11263 (C)]; B, *C. scythica* type (Russia, Black Sea coast, *CBFS JV7059*). Scales: A & B = 1 mm.

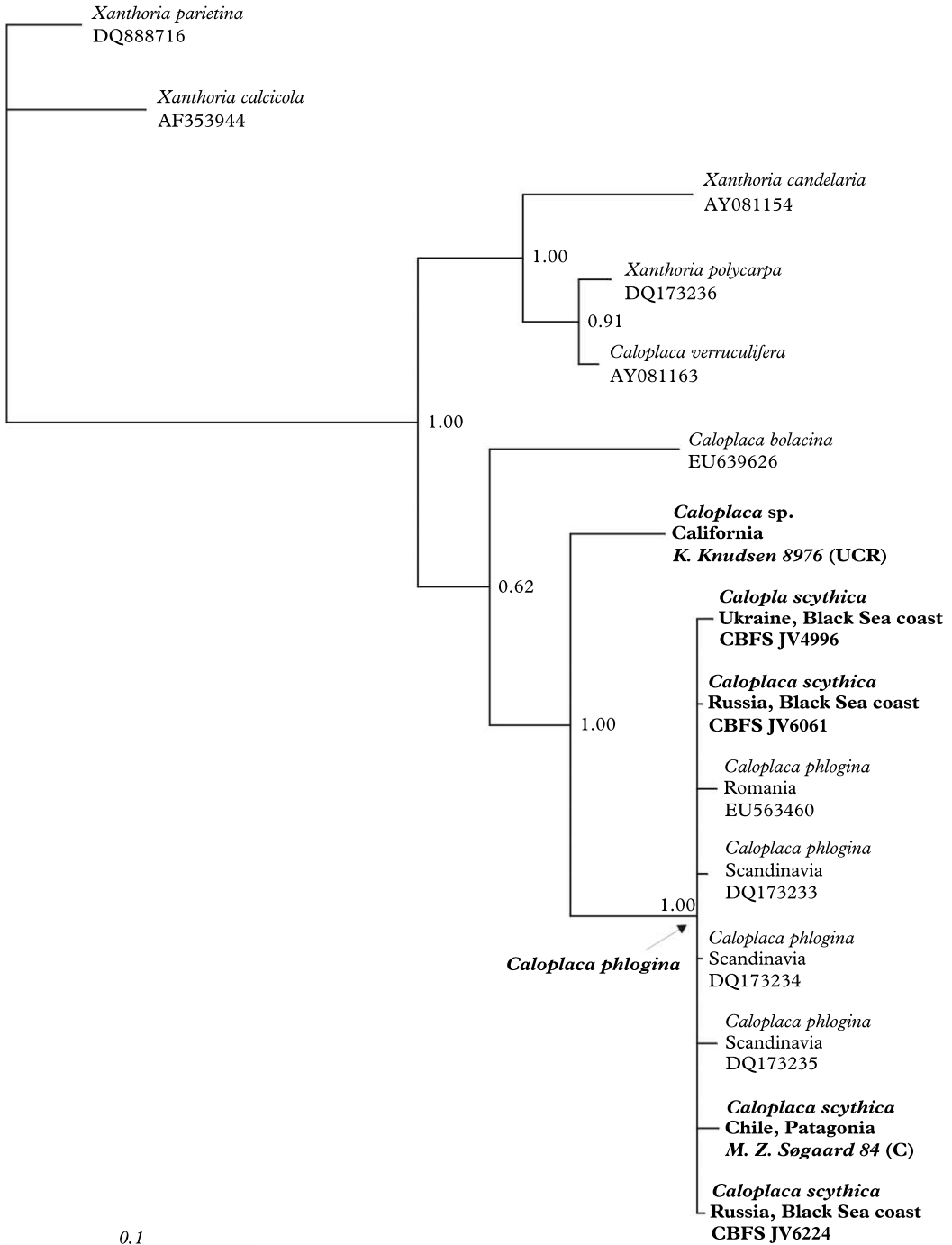


FIG. 2. Bayesian consensus phylogeny of the clade including *Caloplaca phlogina*. Node support values are Bayesian posterior probabilities. Newly obtained ITS sequences are in bold.

TABLE 1. Voucher specimens and GenBank accession numbers of the new ITS sequences used in the phylogenetic analysis.

| Species / Herbarium Accession No.                              | Locality (collector)   | GenBank Accession No. |
|--|--|-----------------------|
| <i>Caloplaca</i> sp. (sub <i>C. holocarpa</i> )<br>UCR; KK8976 | <b>USA</b> , California, Channel Islands ( <i>Kerry Knudsen</i> 2007)                | GU080301              |
| <i>C. scythica</i> C; MZS84                                    | <b>Chile</b> , Isla Navarino, Puerto Williams ( <i>Majbrit Zeuthen Søgaard</i> 2008) | GU080300              |
| <i>C. scythica</i> CBFS JV4996                                 | <b>Ukraine</b> , Syvash salt lagoon ( <i>Vondrák</i> 2006)                           | GU080304              |
| <i>C. scythica</i> CBFS JV6224                                 | <b>Russia</b> , Taman Peninsula ( <i>Vondrák</i> 2007)                               | GU080303              |
| <i>C. scythica</i> CBFS JV6061                                 | <b>Russia</b> , Taman Peninsula ( <i>Vondrák</i> 2007)                               | GU080302              |

## Results

The Bayesian phylogenetic analysis of the ITS nrDNA region revealed that both phenotypic variants together form one well-supported phylogenetic clade (PP = 1.0) without any internal diversification (Fig. 2). Only 10 variable positions (of 506) occurred within this clade. The closest relative we found is a North American species resembling *C. pyracea* (**USA: California: Channel Islands**, on *Eucalyptus* bark, 2007, *K. Knudsen* 8976, UCR, dupl. in CBFS), but which is not related to *C. pyracea* (Ach.) Th. Fr. s. str.

Phenotypic appraisals did not reveal any significant differences between the two variants (Table 2). Both morphotypes are very similar in the 13 characters examined; the colour of their soralia remains the only difference.

However, there are marked differences in the ecological and distributional pattern of the two variants. They grow together on tree bark in southern Scandinavia, but the white-green sorediate morphotype is probably less common there. In contrast, on shrubs in salt marshes on the Black Sea coast, the yellow sorediate morphotype is exceptionally rare, whereas the grey one is very common.

Based on both molecular and phenotypic data, we consider both morphotypes to be conspecific and we propose to reduce *Caloplaca scythica* into synonymy with *C. phlogina*.

## Discussion

The molecular data show *Caloplaca phlogina* to be rather closely related to the morphologically very dissimilar *C. bolacina* (Tuck.) Herre, whereas it is morphologically extremely similar to several species from the unrelated *Caloplaca citrina* group. For example, *Caloplaca citrina* (Hoffm.) Th. Fr. and *C. limonia* Nimis & Poelt are sometimes morphologically indistinguishable from *C. phlogina*. Ecology can help us to determine the species and we can achieve almost 100 % certainty in identification of *C. phlogina* in its 'typical' habitats: bark of broad-leaved trees in southern Sweden or shrubs in salt marshes at the Black Sea coast. Samples from other habitats (e.g., concrete) may be confused with other species and we are sceptical about some records not confirmed by molecular data (e.g. Crespo *et al.* 1980; Vondrák *et al.* 2007).

### Caloplaca phlogina – a species with two different ecologies

In Scandinavia, *C. phlogina* mainly grows inland, has no clear tendency to be maritime (Arup 2006), and grows mainly on tree trunks, not twigs and branches. Its distribution and ecology elsewhere in Europe are not well known, but it is probable that there are other, scattered, non-maritime populations. In contrast, the Black Sea populations are strongly maritime and grow mainly on

TABLE 2. Phenotypic data for both colour variants of Caloplaca phlogina; literature data and our data (bold) are shown.

|   | Morphotype with yellow soralia  |   | Morphotype without anthraquinone-pigmented soralia                             |  |
|---|---|---|--|--|
|   | (Arup 2006)   | (our data)  | (Kondratyuk <i>et al.</i> 1998)  | (our data)   |
| Thallus character                                     | 0.1–0.2(–0.3) mm thick consisting of $\pm$ sorediate areoles  | <b>endophloeodal or endoxic or yellow, forming dispersed granules, small areoles or thin film-like structure</b>                                | areolate to minutely squamiform, sometimes invisible, greenish grey to white   | <b>endophloeodal or grey-white, forming dispersed granules, small areoles or thin film-like structure</b>                                      |
| Character of soralia                                  | convex areoles $\pm$ completely dissolved into soredia  | <b>convex, even punctiform, or confluent, forming sorediate crust</b>   | even punctiform, erumpent from areoles or confluent, forming sorediate crust   | <b>convex, even punctiform, erumpent from areoles or confluent, forming sorediate crust</b>  |
| Size of soredia                                       | (25–)30–50(–60) $\mu$ m diam.   | (20–)36 $\pm$ 10(–70) $\mu$ m in diam. [44]*  | (30–)40–60(–70) $\mu$ m diam.  | (25–)42 $\pm$ 13(–70) $\mu$ m diam. [20]   |
| Size of apothecia                                     | 0.3–0.8(–1.7) mm diam.  | 0.2–1.3 mm diam.  | 0.3–0.7 mm diam.   | 0.2–1.2 mm diam.   |
| Width and character of exciple                        | true exciple 25–50(–60) $\mu$ m thick; thalline exciple 0–75(–125) $\mu$ m thick, uneven to sorediate | <b>apoth. margin c. 50–120 <math>\mu</math>m wide when seen from above, zeorine, with pale yellow, <math>\pm</math> sorediate, outer margin</b> | true exciple c. 130 $\mu$ m thick; thalline exciple 90–130(–150) $\mu$ m thick | <b>apoth. margin c. 70–120 <math>\mu</math>m wide when seen from above, zeorine, with grey-white, <math>\pm</math> sorediate, outer margin</b> |
| Height of hymenium                                    | 55–80 $\mu$ m high  | <b>c. 60–70 <math>\mu</math>m high</b>  | 50–60(–70) $\mu$ m high  | c. 60–70 $\mu$ m high  |
| Size of asci  | 45–58 $\times$ 10–12 $\mu$ m  | <b>c. 40–50 <math>\times</math> 9–12 <math>\mu</math>m</b>  | (40–)42–47(–50) $\times$ (10–)11–13(–15) $\mu$ m                               | c. 45–60 $\times$ 7–17 $\mu$ m   |
| Size of ascospores; width/length ratio                | (9.1–)10.0–13.0(–13.5) $\times$ (4.0–)4.2–6.0(–7.3) $\mu$ m; ratio not calculated                     | (9.0–)10.8 $\pm$ 1.1(–12.75) $\times$ (3.25–)4.7 $\pm$ 0.7 (–6.75) $\mu$ m [21]; ratio (0.35–)0.45 $\pm$ 0.08 (–0.69)                           | 11–13 $\times$ 4.5–6(–7) $\mu$ m; ratio not calculated                         | (9.0–)11.0 $\pm$ 1.4(–14.5) $\times$ (3.5–)5.0 $\pm$ 0.8(–7.0) $\mu$ m in size [21]; ratio (0.36–)0.46 $\pm$ 0.06(–0.58)                       |
| Width of ascospore septum & septum/spore length ratio | (2.5–)2.9–4.0(–4.2) $\mu$ m wide; ratio (0.22–)0.25–0.35(–0.39)                                       | <b>wide [21]; ratio (0.27–)0.36 <math>\pm</math> 0.06(–0.45)</b>  | 3–4(–5) $\mu$ m wide, ratio not counted  | (3.0–)3.9 $\pm$ 0.7(–5.5) $\mu$ m wide [21]; ratio (0.25–)0.36 $\pm$ 0.06(–0.52)   |
| Width of paraphyses tips                              | up to 7 $\mu$ m wide  | <b>up to (4.25–)5.2 <math>\pm</math> 0.6 (–6.5) <math>\mu</math>m wide [19]</b>   | up to 5–6(–7) $\mu$ m wide   | <b>up to (4.5–)5.5 <math>\pm</math> 0.9(–8.0) <math>\mu</math>m wide [24]</b>  |
| Colour of pycnidia & size of conidia                  | conidiomata orange-yellow; conidia c. 2.5–3.0(–3.5) $\times$ 1.2–1.5 $\mu$ m                          | <b>conidiomata yellow around ostiole; conidia c. 3–4 <math>\times</math> 1–1.25 <math>\mu</math>m not analyzed</b>                              | conidiomata not seen   | <b>conidiomata yellowish around ostiole; conidia c. 2.5–4 <math>\times</math> 1–2.5 <math>\mu</math>m</b>                                      |
| Anthraquinone content                                 | apothecia & soralia: parietin (main); teloschistin, fallacinal, parietinic acid and emodin (traces)   | <b>not analyzed</b>   | not analyzed   | <b>apothecia: parietin (main); teloschistin, fallacinal, parietinic acid and emodin (traces)</b>   |

\*no. of measurements

twigs of shrubs in salt marshes. It might be justified to regard the Black Sea population with white soralia as distinct from the Scandinavian one at an infra-specific level, but we prefer to wait for convincing molecular data before making any taxonomic proposals.

### Founder effects in distant populations

The distant maritime populations of *C. phlogina* may have originated from one or a few diaspores. This hypothesis is based mainly on the observations that the species is ecologically and phenotypically uniform in maritime conditions where the yellow-sorediate morphotype is extremely rare and with a low anthraquinone content. We consider the Black Sea populations to be established through distribution from some European population where the species grows on a variety of substrata, in both phenotypic variants and even inland or along sea shores. Even though the distribution of *C. phlogina* in South America is poorly known, we also suspect that the only known Chilean record is a result of young distribution from the source populations in Europe.

The examples listed in the introduction concern phenotypes which appear to have lost their ability to produce anthraquinones or other pigments. This phenomenon, which may be due to a single mutation somewhere in the synthesis pathway, differs from the situation in *C. phlogina*. Here, in the grey thallus phenotype, the anthraquinones are still produced in the apothecia but the synthesis is not expressed in the soralia.

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