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

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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*

Introduction

The concept of the *Caloplaca cerina* group 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 ± 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do not, however, belong to the *C. cerina* clade, for example *C. squamuloisidiata* van den Boom & V. J. Rico (J. Šoun, unpublished data).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 ($<0{\cdot}5$ µm) vs. thick-walled ($>0{\cdot}5$ 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 & Søchting, 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.) Häyrén. **Bulgaria:** *Black Sea coast*: Tsarevo, 2004, *J. Vondrák* 2248 (CBFS).—**Czech Republic:** *Central Bohemia*: Křivoklát, 2003, *J. Vondrák* 1163 (CBFS).—**Ukraine:** *Crimean Peninsula*: Karadag, 2007, *J. Vondrák* 5948 (CBFS).

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

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

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

C. isidiigera. **Austria:** *Eastern Alps*: Seckauer Alpen Mts, 2007, *J. Vondrák* 6081 (CBFS).—**Ukraine:** *Eastern Carpathians*: Svidovets Mts, 2007, *J. Vondrák* 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. Vondrák, 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, *J. Vondrák* 2114 (CBFS).—**Morocco:** *Anti-Atlas Mts*: Tafraoute, 2003, *J. Vondrák* 1429 (CBFS).

C. percrocata (Arnold) J. Steiner **Italy:** *Southern Alps*: Castelnuovo, 1902, *J. Baumgartner* (holotype of *C. cerina* var. *areolata*, W); Trento, 2006, *Š. Hulová* 4634 (CBFS).—**Ukraine:** *Eastern Carpathian*: Svidovets Mts, 2007, *J. Vondrák* 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, *J. Vondrák* 2177 (CBFS).—**Czech Republic:** *Central Bohemia*: Točník, 2003, *J. Vondrák* 1124 (CBFS).—**Iran:** *East Azerbaijan*: Nir, 2007, *J. Vondrák* 5607 (CBFS).—**Romania:** *Munţii Zărandului Mts*: Şoimoş, 2005, *J. Vondrák* 3647 (CBFS).—**Ukraine:** *Mykolaivska oblast*: Pervomaisk, 2006, *J. Vondrák* 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.
C. cerina LD L03347	Sweden, Lycksele Lappmark, Rönä (*Arup* 2003)	EU365861
C. chlorina CBFS JV2055	Bulgaria, Rhodope Mountains, Haskovo (*Vondrák* 2004)	EU365859
C. chlorina CBFS JV3120	Czech Republic, Czech-Moravian Highland, Kamenice nad Lipou (*Vondrák* 2005)	EU365858
C. isidiigera CBFS JV6073	Ukraine, Zakarpatska oblast region, Svidovets Mts (*Vondrák* 2007)	EU365857
C. isidiigera LD L04227	Sweden, Lule Lappmark, Padjelanta national park (*Arup* 2004)	EU365856
C. stillicidiorum CBFS, Sel. Exs. *Caloplaca*, 12	Bulgaria, Rhodope Mountains, Asenovgrad (*Vondrák* 2004)	EU365860
C. subalpina CBFS JV6072 (holotype)	Ukraine, Zakarpatska oblast region, Svidovets Mts (*Vondrák* 2007)	EU365855
C. subalpina CBFS JV692	Spain, Pyrenees, Jaca (*Vondrák* 2002)	EU365854
C. subalpina Hb. Palice 6983	Czech Republic, Jeseníky Mts, Velký kotel corrie (*Palice* 2001)	EU365853
C. thracopontica CBFS JV3419	Bulgaria, Coast of Black Sea, Sozopol (*Vondrák* 2005)	EU365847
C. thracopontica CBFS JV5419 (holotype)	Turkey, Coast of Black Sea, Sinop (*Vondrák* 2007)	EU365848
C. thracopontica CBFS JV5621	Turkey, Coast of Black Sea, Trabzon (*Vondrák* 2007)	EU365852
C. thracopontica CBFS JV5623	Turkey, Coast of Black Sea, Sinop (*Vondrák* 2007)	EU365851
C. thracopontica CBFS JV6065	Turkey, Coast of Black Sea, Giresun (*Vondrák* 2007)	EU365849
C. thracopontica CBFS, Sel. Exs. *Caloplaca*, 15 (sub *C.* aff. *chlorina*)	Bulgaria, Coast of Black Sea, Tsarevo (*Vondrák* 2004)	EU365846
C. thracopontica Hb. Šoun 302	Turkey, Coast of Black Sea, Sarp (*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_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).

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 (Katoh *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+Γ (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 exteriore bene evoluto e cellulis crassae tunicatis. Soredia parva, (18–) 30 ± 8 (–54) µm, 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, *J. 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) µm 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) µm diam. (*n*=40); consoredia rare and small. *Epinecral layer* up to *c.* 15 µm high. *Cortex* conspicuous, (5–) 17 ± 11 (–53) µm 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 µm thick-walled, large, isodiametric cells, (4·0–) 6·5 ± 1·0 (–8·5) µm diam. Cortex in lower part of thalline exciple distinctly thickened, up to 70 µm. *Algal layer* (30–) 65 ± 20 (–110) µm high (*n*=15), formed of algal cells (6·0–) 11·0 ± 4·0 (–21·0) µm diam. (*n*=32) and mostly isodiametric fungal cells, (3·5–) 5·5 ± 1·5 (–9·0) µm diam. (*n*=15), with walls up to 1 µm thick. *Medulla* not always conspicuous, formed by loose prosoplectenchymatous tissue, of thin-walled, 2–4 µm 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) µm thick (*n*=18). *True exciple* indistinct, very thin, up to 40 µm thick, prosoplectenchymatous, formed of thin-walled, *c.* 2–4 µm thick, cells; prosoplectenchymatous tissue usually continuous with the lowermost part of the hypothecium. *Hypothecium* hyaline, very variable in height, (30–) 90 ± 40 (–160) µm high (*n*=15), formed by a mixture of isodiametric and elongated hyphal cells. *Hymenium* hyaline, (60–) 69 ± 7 (–80) µm high (*n*=15). *Paraphyses* of thin-walled, *c.* 1·5 – 2 µm thick cells; somewhat

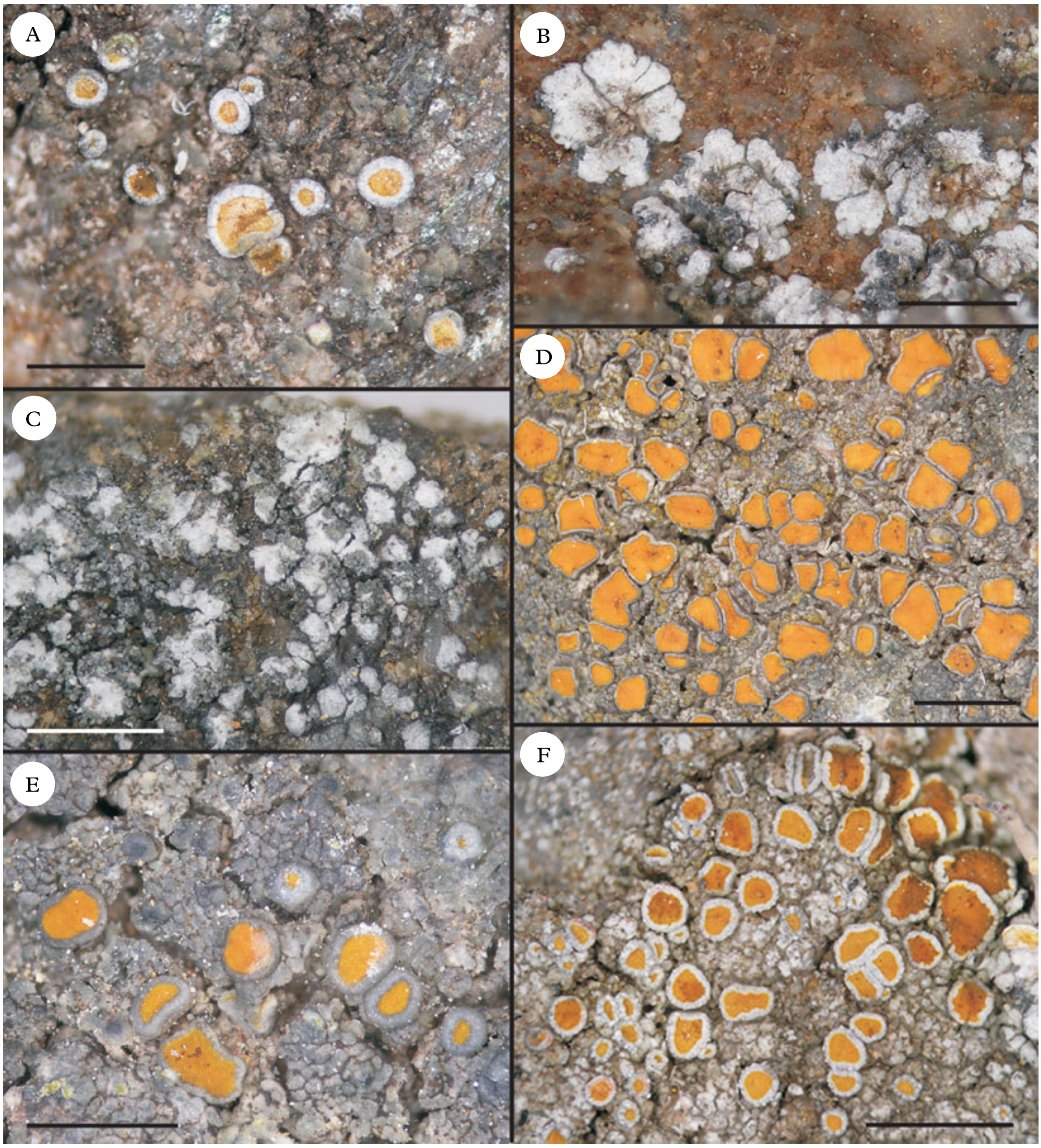

FIG. 1. A–C, *Caloplaca subalpina*. A, apothecia (CBFS JV6071); B, sublobate marginal parts of thallus (CBFS JV6071); C, thallus with soralia (isotype). D–F, *Caloplaca thracopontica*. D, thallus with abundant apothecia (CBFS JV3419); E, detail of a thallus with pustules and apothecia (CBFS JV6066); 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–) 3·5 ± 0·5 (–5·0) µm wide (n=17). *Epihymenium* orange from granules of anthraquinones dissolving in K; crystalline pruina insoluble in K often present. *Asci* 8-spored, (41–) 49 ± 6 (–61) × (10–) 12 ± 1·5 (–17) µm (n=19). *Ascospores* (Fig. 2B) polarilocular, ellipsoid, (9·0–) 11·5 ± 1·5 (–15·0) × (4·5–) 6·0 ± 1·0 (–7·0) µm (n=21), length/breadth ratio *c.* 1·9, *ascospore septa* (3·0–) 4·0 ± 0·75 (–5·5) µm thick (n=21), septa/spore length

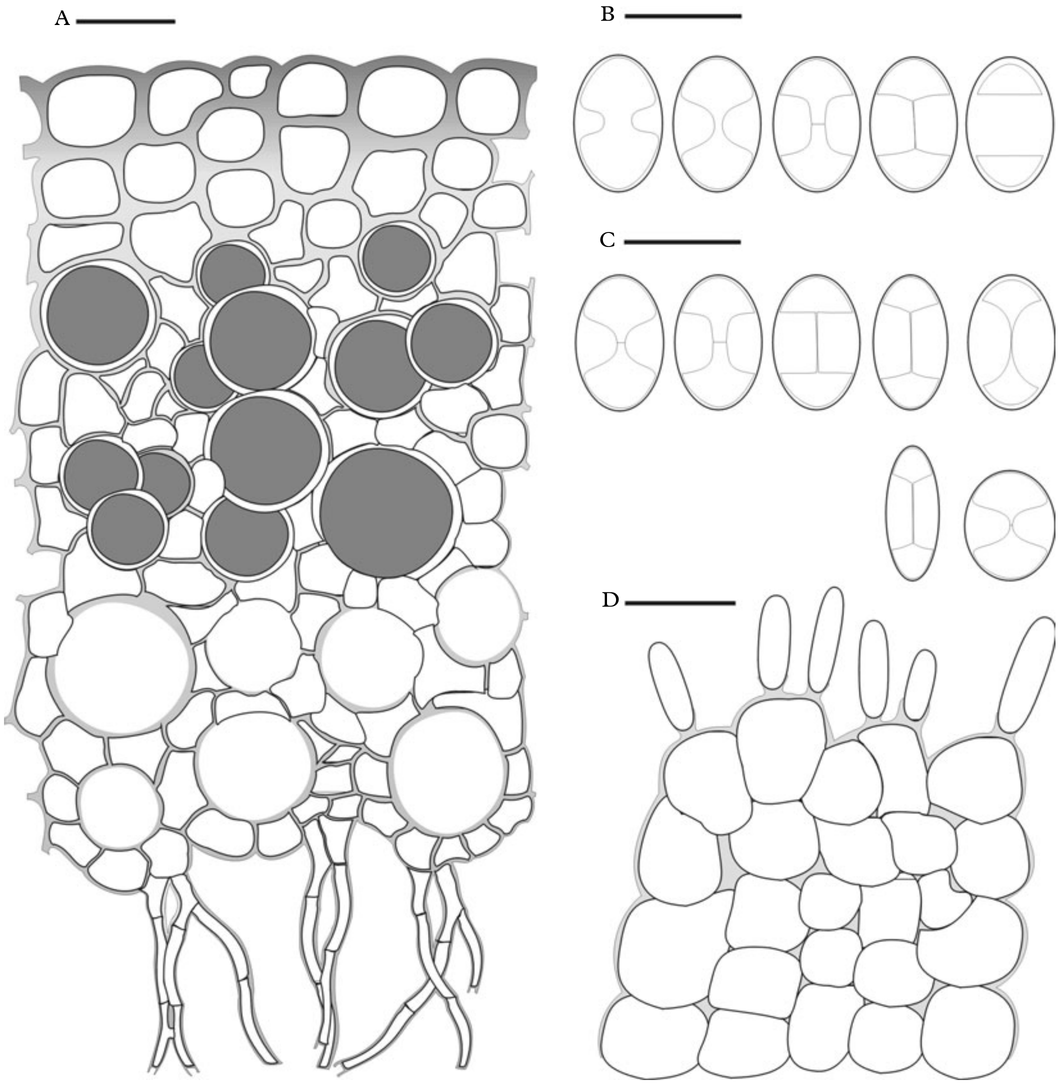

FIG. 2. A, B, D, *Caloplaca subalpina*. A, vertical section through a thallus with thick-walled cortical cells and with pseudomedulla in lower part (CBFS JV692); B, development of ascospores; D, conidiophores with attached conidia (holotype). C, *Caloplaca thracopontica*, ascospore variability, lower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

ratio *c.* 0·35, *ascospore wall* thin, but thicker in old spores (up to *c.* 0·5 μm).

Conidiomata pycnidia, with centrum *c.* 50–90 μm 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 ± 1·0 (–5·5) μm diam. (n=16). *Conidia* mostly acrogenous, bacilliform, (2·0–) 3·5 ± 0·75 (–5·0) × (0·5–) 1·0 ± 0·25 (–1·5) μm (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,

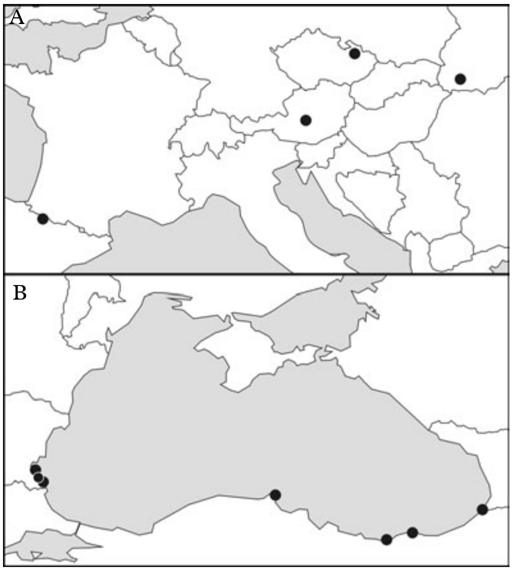

FIG. 3. Distribution of *Caloplaca subalpina* (A) and *Caloplaca thracopontica* (B).

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. jemtlandica* 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, *J. 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, *J. 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 e 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 µm high. *Areoles* (90–) 184 ± 65 (–350) µm high (n=40) and (0·29–) 1·32 ± 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 µm high. *Cortex* conspicuous, (5–) 23 ± 14 (–75) µm 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 µm thick-walled, large, isodiametric cells, (5·0–) 7·5 ± 1·5 (–11·0) µm diam. (n=22). Cortex in lower part of thalline exciple distinctly thickened, up to 90 µm. *Algal layer* (40–) 81 ± 37 (–210) µm high (n=20), formed of algal cells (6·5–) 12·5 ± 3·0 (–18·0) µm diam. (n=22) and mostly isodiametric fungal cells with thin-walls (up to 0·5 µm). *Medulla* not always conspicuous, formed by loose prosoplectenchymatous tissue, of thin-walled, 2–4 µm 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 ± 0·28 (–1·52) mm diam. (n=46), with orange to dark red, flat discs. *Thalline exciple* same colour as thallus, raised above discs when young, somewhat reduced in old apothecia, (50–) 100 ± 24 (–170) µm thick (n=35). *True exciple* indistinct, very thin, up to 25 µm thick, prosoplectenchymatous, formed of thin-walled cells, up to 6 µm thick in uppermost part, *c.* 2–4 µm thick in lower part; prosoplectenchymatous tissue usually extending to the lowermost part of hypothecium. *Hypothecium* hyaline, very variable in height, (40–) 116 ± 37 (–180) µm high (n=34), formed by a mixture of isodiametric and elongated hyphal cells. *Hymenium* hyaline, (60–) 81 ± 11 (–110) µm 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 µm thick cells; branched (in upper one-third) and somewhat anastomosed; upper 1–4 (–7) cells swollen; terminal cells (2·5–) 5·0 ± 1·0 (–6·5) µm wide (n=52). *Asci* 8-spored, (39–) 51 ± 6 (–64) × (8–) 13 ± 3 (–21) µm (n=37). *Ascospores* (Fig. 2C) polarilocular, ellipsoid (rarely narrowly ellipsoid), (10·0–) 12·5 ± 1·5 (–15·5) × (3·0–) 6·0 ± 1·0 (–10·0) µm (n=62), length/breadth ratio *c.* 2·1; *wall* thin, but thicker in old spores (up to *c.* 0·5 µm); *septa* (2·5–) 5·0 ± 1·0 (–7·0) µm thick (n=62), septa/spore length ratio *c.* 0·4.

Conidiomata pycnidia, with centrum (80–) 132 ± 27 (–180) µm wide (n=22). *Conidiophores* tightly packed forming paraplectenchymatous tissue or ± solitary. *Conidiogenous cells* smaller than cortical cells, thin-walled, isodiametric, (3·5–) 5·0 ± 1·0 (–7·5) µm diam. (n=24) or elongated, up to *c.* 7 µm long. *Conidia* acro- or pleurogenous, bacilliform, (2·5–) 3·5 ± 1·0 (–5·5) × (1·0–) 1·25 ± 0·25 (–1·5) µm (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. *thallincola*, *Candelariella plumbea*, *Catillaria chalybeia*, *Rinodina gennarii*, 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 fieldwork, 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, *J. 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, *J. 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, *J. 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, *J. 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, *J. Vondrák* 5623 (CBFS); *ibid.*: alt. *c.* 100 m, 42°01′13″N, 35°12′20″E, 2007, *J. 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, *J. Šoun* 302, *J. 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, *J. 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 intraspecific 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*

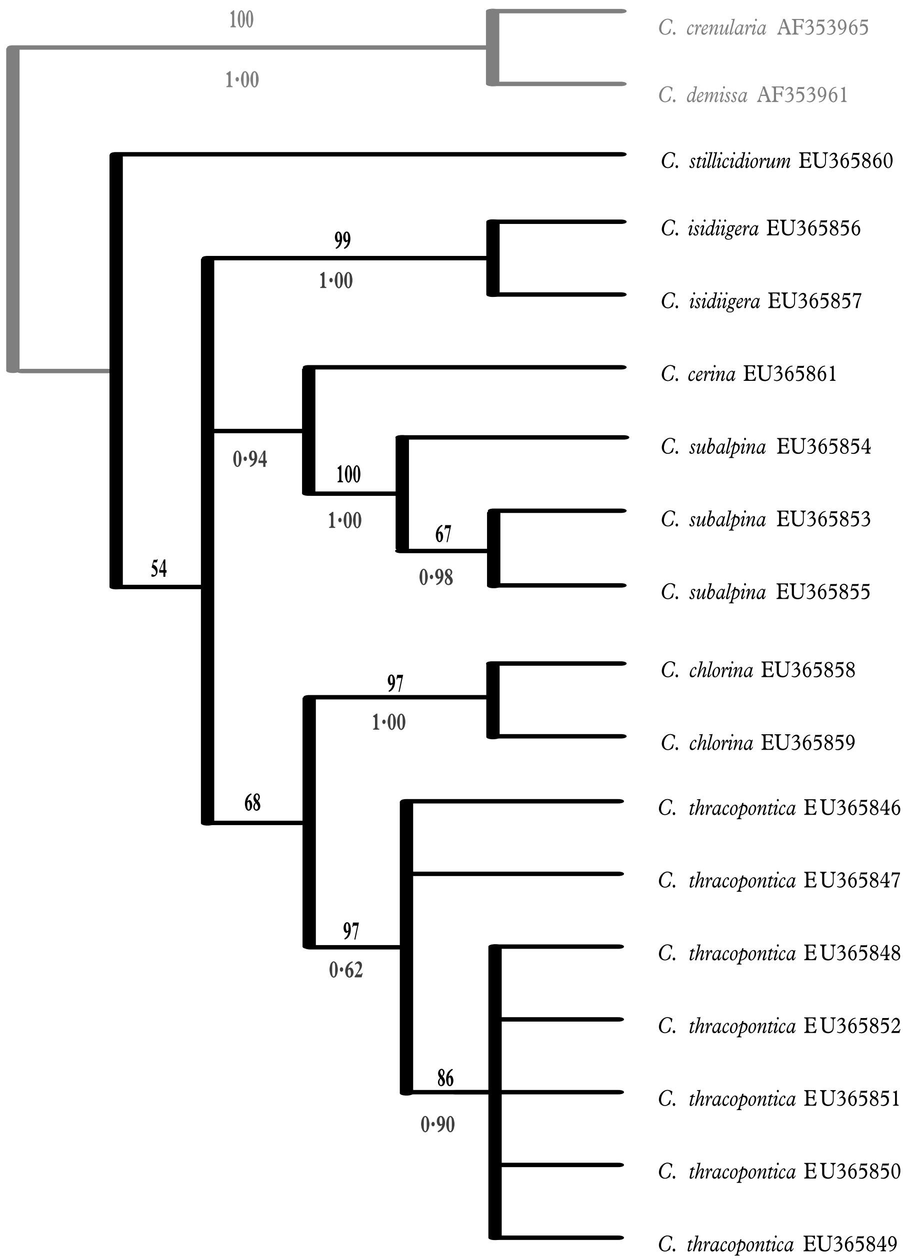

FIG. 4. Phylogenetic relationships of newly acquired ITS sequences of the *Caloplaca cerina* group, rooted by the *C. crenularia* and *C. demissa* (outgroup shown in grey, names of taxa accompanied by their GenBank accession numbers). Topology respects the bootstrap consensus tree for 1000 replicates 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+Γ model). Branches having neither bootstrap support above 50% nor Bayesian posterior probability above 0·90 are presented as collapsed.

group that have been described from outside Europe, at least not from North America (Wetmore 2007). Only fertile lichens can be identified by the key.

1 Apothecia zeorine, with distinct anthraquinone-containing true exciple; however, old apothecia may have a lecanorine appearance, with strongly magnified thalline margin . . . . e.g. **C. furax**, **C. percrocata**, **C. xerica** (not in the *Caloplaca cerina* group)

Apothecia lecanorine or zeorine, but without distinct anthraquinone-containing true exciple 2

2(1) Apothecia zeorine, with brown to black true exciple (dark excipular ring between disc and thalline exciple) devoid of anthraquinones, but with strong concentration of Sedifolia-grey (K+ deeply violet in section) . . . . e.g. **C. aractina** p.p., **C. conversa**, **C. pellodella** (not in the *Caloplaca cerina* group)

Apothecia lecanorine, with strongly reduced true exciple 3

3(2) Thallus without vegetative diaspores; rare morphotypes without pustules and lobules . . . . **C. thracopontica**

Thallus with soredia, consoredia, isidia, pustules or lobules as vegetative diaspores 4

4(3) Isidia, pustules or lobules on thallus surface 5

Soredia or consoredia produced 7

5(4) Abundant branched coralloid isidia or branched erect thin lobules; sedifolia-grey (K+ violet in section) restricted to cortex at pycnidia and apothecial primordia . . . . **C. squamuloisidiata**

Isidia, when present, not branched and not distinctly coralloid; cortex distinctly pigmented by sedifolia-grey (K+ violet in section) 6

6(5) Thallus surface with small globose to shortly vertically elongated isidia, (37–) 62 ± 17 (–97) µm wide (n=30) . . . . **C. isidiigera**

Thallus surface with pustules and lobules, 100–400 µm wide . . . . **C. thracopontica**

7(4) Thallus usually non-pruinose; soredia (18–) 38 ± 11 (–67) µm diam. (n=40), often united to form consoredia; large consoredia superficially resemble isidia, but microscopically, they are formed of soredia-like units; cortex well-developed only in lower part of thalline exciple; in thallus surface, cortex formed only by 1–2 rows of cells (up to 10 µm high); apothecia common, non-pruinose . . . . **C. chlorina**

Thallus, at least in marginal parts, white-pruinose; soredia usually simple, rarely in consoredia, (18–) 30 ± 8 (–54) µm diam.; cortex well-developed, (5–) 17 ± 11 (–53) µm high; apothecia rare, usually white-pruinose . . . . . **C. subalpina**

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