

## *Caloplaca concreticola* (Teloschistaceae), a new species from anthropogenic substrata in Eastern Europe

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**Abstract:** *Caloplaca concreticola* is described here as new. It is a morphologically well-characterized, sorediate species of the subgenus *Pyrenodesmia*, that comprises a part of the “black-fruited” taxa with an absence of anthraquinones in their apothecia. Currently it is known only from concrete and always occurs close to water (mainly on the walls of water channels). Analysis of nuclear ribosomal ITS sequence data of the new species, together with most European species of the subgenus, supports the recognition of the new species as a monophyletic taxon within the *Pyrenodesmia* clade.

**Key words:** ITS, lichenized fungi, taxonomy, *Teloschistales*, Ukraine

### Introduction

The subgenus *Pyrenodesmia* of the large genus *Caloplaca* is characterized mainly by the absence of anthraquinones in all parts of the thallus. Apothecial discs of its members are usually dark coloured, often containing dark acetone-insoluble pigments (most frequently K+ violet *Sedifolia*-grey). *Pyrenodesmia* contains rather few species and has been studied for monographs in Europe including the Mediterranean and the Near East (Wunder 1974) and in North America (Wetmore 1994); recently some new species from Europe have been described (Khodosovtsev *et al.* 2002; Tretiach *et al.* 2003; Tretiach & Muggia 2006). Molecular analyses using ITS data for this group have been presented by Arup & Grube (1999), Tretiach *et al.* (2003), and Muggia & Grube (2007).

In southern Ukraine, we discovered some populations of a black-fruited, sorediate *Caloplaca*, restricted to concrete water channels. It could not be assigned to any known taxon, and is therefore formally described here as a new species.

### Materials and Methods

#### Morphology

A total of 20 characters were analysed quantitatively for the new species: size of areoles, width of thallus, phenocortex and epinecral layer, size of soredia, size of algal and fungal cells in algal layer, size of apothecia, thalline and true exciple width, size of outer cells in true exciple, hypothecium and hymenium height, size of asci and ascospores, width of spore septa, width of paraphyses tips, and size of conidiomata, conidiogenous cells and conidia. For qualitative traits, characters of each tissue (e.g. paraplectenchymatous vs. prosoplectenchymatous type, occurrence of anastomoses, and presence of thin-walled vs. thick-walled cells), and colour of thallus and apothecia were used. The term *phenocortex* (*sensu* Ryan *et al.* 2002) is used here in a modified sense for the hyaline tissue between the epinecral layer and algal layer, formed by living fungal cells among dead algal cells or gaps left by dead algal cells (Fig. 1D).

Sections for anatomical examination were cut by hand and observed in water. Measurements were made with an accuracy of 0.5 µm (for cells, e.g. conidia and ascospores), 1 µm (for asci and true exciple) and 10 µm (for larger structures: e.g. hymenium and hypothecium height, size of apothecia). All measurements of cells included their walls, not only the lumina. Ascospores were examined after heating for a short time (Steiner &

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

Species/Herbarium Accession No.	Locality	GenBank Accession No.
<i>C. albopustulata</i> CBFS JV5468 (topotype)	Ukraine, Crimea, Alushta (coll. <i>Vondrák</i> 2007)	EU192150
<i>C. concreticola</i> CBFS JV4636 (holotype)	Ukraine, Kakhovskiy magistral channel (coll. <i>Vondrák &amp; Šoun</i> 2006)	EU192151
<i>C. concreticola</i> CBFS JV4637	Slovakia, Rožňava (coll. <i>Vondrák</i> 2006)	EU192152
<i>C. concreticola</i> CBFS JV5297	Ukraine, Crimea, Armiansk (coll. <i>Vondrák &amp; Šoun</i> 2007)	EU192153
<i>C. concreticola</i> CBFS JV5298	Romania, Dobrogea, Constanța (coll. <i>Vondrák &amp; Šoun</i> 2007)	EU192154
<i>C. concreticola</i> CBFS JV5306	Ukraine, Kakhovskiy magistral channel (coll. <i>Khodosovtsev</i> 2006)	EU192155
<i>C. transcaspica</i> CBFS JV5466	Ukraine, Crimea, Feodosia (coll. <i>Vondrák</i> 2007)	EU192156

Peveling 1984), but without pretreatment with KOH. Measurements are given as (min.–)  $x \pm SD$  (–max.), where  $x$ =mean value and  $SD$ =standard deviation. Total numbers of measurements ( $n$ ) are given in parentheses. At least five measurements were taken from all available samples; pycnidia and well-developed asci and ascospores were not always observed. The material studied and collected by the authors is deposited in CBFS and KHER.

### Chemistry

Acetone-soluble compounds in the samples CBFS JV4637 and the holotype were investigated using HPLC-MS analysis. Acetone-insoluble pigments were examined and named according to Meyer & Printzen (2000). Crystalline pruina on thallus and apothecia were investigated by polarized light before and after washing with 5% acetic acid (*Giordani et al.* 2003).

### Phylogenetic analysis

A fairly large number of nuclear ribosomal ITS sequences of the subgenus *Pyrenodesmia* is available in GenBank (Fig. 2). In addition to these, five new ITS sequences of the new species were produced together with one each of *C. albopustulata* and *C. transcaspica* (Table 1; highlighted in Fig. 2); extraction, amplification, purification and sequencing follow Søchting & Figueras (2007). 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; 530 positions were retained. Parsimony analysis was conducted with PAUP 4.0b10 (Swofford 2002) with tree bisection reconnection (TBR) branch swapping, sequence addition option set to random (with 10 replicates at each step) and maxtrees unrestricted. Steepest descent option was not in effect and the analysis ran under the MulTrees option. All gaps were treated as missing data. Bootstrap analysis adopted the full heuristic search settings and included 1000 resamplings.

To test the stability of nodes, Bayesian posterior probabilities in the program MrBayes 3.0 (Ronquist and Huelsenbeck 2003) were computed. In accord with the best-fit likelihood settings proposed by hLRT algorithm in MrModeltest 2.2 (Nylander 2004), the analysis was conducted under general time reversible model with some sites assumed to be invariable and the others varying in rates according to the gamma distribution approximated by four categories. The prior probability densities of the substitution rates and the stationary nucleotide frequencies were let as preset: a flat Dirichlet with all values set to 1.0. The Markov chain Monte Carlo run included four parallel chains, three of which were incrementally heated by a temperature of 0.2°C. The MCMC process was in progress for 10 000 000 generations and every 100th generation was sampled. In order to assess stationarity of the MCMC run, two simultaneous independent analyses were conducted and the standard deviation of split frequencies was monitored; finally we decided to discard the first 10% of samples as burn-in.

## The Species

### *Caloplaca concreticola* Vondrák & Khodosovtsev sp. nov.

Apotheciis nigro-brunneis (subg. *Pyrenodesmia*), pigmentis anthraquinoneis in thallo et apotheciis nullis. Thallus areolatus, areolae soraliis marginalibus; soredia (17–)  $31 \pm 7$  (–53)  $\mu\text{m}$  diametro. Ascospores (13.0–)  $15.7 \pm 2.0$  (–20.5)  $\mu\text{m}$  longae, (5.0–)  $7.1 \pm 1.0$  (–9.0)  $\mu\text{m}$  latae; conidia late ellipsoidea vel subglobosa (1.5–)  $2.1 \pm 0.4$  (–2.5)  $\mu\text{m}$  longa (1.0–)  $1.5 \pm 0.2$  (–2.0)  $\mu\text{m}$  lata.

Typus: Ukraine, Khersonska oblast: Chaplinskiy district, c. 4.5 km W of village Zaozerne, Kakhovskiy kanal water channel, alt. c. 30 m, 46°35'44.37"N, 033°53'13.21"E, on dry surface of concrete water channel, c. 1 m above water level, 7 June 2006, *Ĵ.*

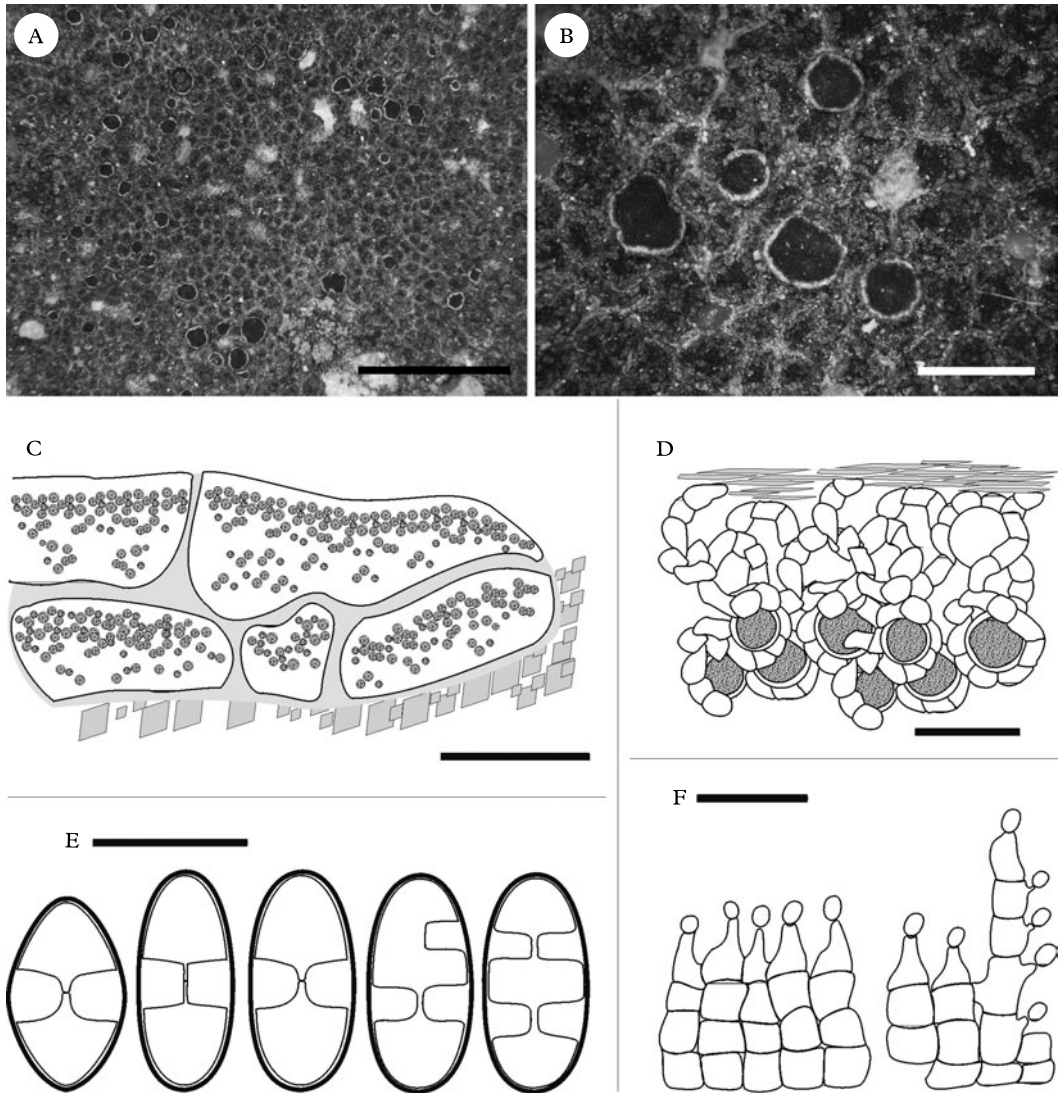


FIG. 1. *Caloplaca concreticola*. A, thallus with apothecia (isotypus); B, detail of apothecia and areoles with marginal soralia (isotypus); C, section of a thallus composed of old but living areoles overgrown by younger areoles. (CBFS JV5301); D, section of phenocortex formed of loose tissue with gaps left by dead algal cells (CBFS JV5298); E, ascospores, right ascospores deformed, 3-locular (CBFS JV5306); F, conidiophores with conidia (left—CBFS JV5301, right—CBFS JV5304). In Figs C and D, shapes of cells, substratum crystals and structure of epinecral layer are schematic, not realistic. Scales: A=5 mm, B=1 mm, C=100  $\mu$ m, D, E, F=10  $\mu$ m.

Vondrák & J. Šoun (CBFS JV4636—holotypus; *Selected exsiccates of Caloplaca*, fasc. 2: <http://botanika.bf.jcu.cz/lichenology/index.php?pg=7>—isotypi).

(Fig. 1)

*Thallus* crustose, areolate, forming patches up to 1.5 cm diam., whitish-grey (by pruina)

to grey, greenish when wet, (50–)  $170 \pm 50$  (–270)  $\mu$ m high ( $n=28$ ). *Areoles* angular to minutely squamiform, (0.26–)  $0.56 \pm 0.21$  (–0.99) mm diam. ( $n=31$ ), flat or with raised margins, sorediate (Fig. 1A); old areoles sometimes overgrown by young thalli (Fig. 1C). *Soralia* marginal, erupting from

lower surfaces of areoles, dark-grey to green-grey, darker than surfaces of areoles (containing Sedifolia-grey), old areoles sometimes completely sorediate, often confluent and forming a dark grey sorediate crust. *Soredia* (17–)  $31 \pm 7$  (–53)  $\mu\text{m}$  diam. ( $n=46$ ). True *cortex* not developed or rarely present, thin, up to 10  $\mu\text{m}$ , formed of 1–2 rows of paraplectenchymatous, thin-walled cells. *Phenocortex* (see Materials and Methods) usually present, *c.* 20–40  $\mu\text{m}$  high (Fig. 1D). *Epinecral layer* *c.* 5–10  $\mu\text{m}$  thick, crystalline *pruina* white, sometimes strongly developed, up to 25  $\mu\text{m}$  thick. *Algal layer* formed of cells (6.0–)  $14.0 \pm 4.5$  (–23.0)  $\mu\text{m}$  diam. ( $n=43$ ), surrounded with thin-walled, isodiametric fungal cells, (2.0–)  $4.9 \pm 1.6$  (–8.5)  $\mu\text{m}$  diam. ( $n=34$ ), or elongated cells *c.* 3–5  $\mu\text{m}$  thick. *Medulla* inconspicuous,  $\pm$  prosoplectenchymatous, loose, formed by hyphal strands of rather elongated cells, *c.* 2.5–5  $\mu\text{m}$  thick, growing among substratum particles.

*Apothecia* first urceolate, later flat, (0.26–)  $0.54 \pm 0.18$  (–1.1) mm diam. ( $n=43$ ), with brown to black disc (darker apothecia contain more Sedifolia-grey in epihymenium) and whitish margin (Fig. 1B). Apothecial margin zeorine, sometimes seemingly lecanorine, but both true and thalline exciples always present. *True exciple* (35–)  $64 \pm 18$  (–100)  $\mu\text{m}$  thick ( $n=28$ ), formed of thin-walled, elongated cells, (2.0–)  $4.0 \pm 0.9$  (–5.0)  $\mu\text{m}$  wide ( $n=21$ ), that may be almost isodiametric in uppermost part. Upper part of true exciple grey-brown (Sedifolia-grey). *Thalline exciple* (70–)  $103 \pm 37$  (–210)  $\mu\text{m}$  thick ( $n=29$ ), without cortex, sometimes crenulate when old. *Hypothecium* (40–)  $119 \pm 48$  (–230)  $\mu\text{m}$  high in central part ( $n=23$ ), hyaline,  $\pm$  paraplectenchymatous, of cells (3.0–)  $6.25 \pm 2.5$  (–12.0)  $\mu\text{m}$  diam. ( $n=16$ ). *Hymenium* (70–)  $92 \pm 10$  (–110)  $\mu\text{m}$  high ( $n=27$ ), with *epihymenium*  $\pm$  slightly greyish, usually covered by crystalline *pruina*. *Paraphyses* *c.* 1.5–3.5  $\mu\text{m}$  wide, but terminal and subterminal cells usually inflated, (2.5–)  $4.0 \pm 0.9$  (–5.5)  $\mu\text{m}$  thick ( $n=33$ ). *Asci* of *Teloschistes*-type, clavate to cylindrical, 8-spored, (47–)  $59 \pm 6$  (–71)  $\times$  (13–)  $17 \pm 3$  (–24)  $\mu\text{m}$  ( $n=31$ ). *Ascospores*

polarilocular (Fig. 1E, left), (13.0–)  $15.7 \pm 2.0$  (–20.5)  $\times$  (5.0–)  $7.1 \pm 1.0$  (–9.0)  $\mu\text{m}$  in size ( $n=31$ ); length/width ratio *c.* 2.2. Ascospore walls *c.* 0.2–0.5  $\mu\text{m}$  wide; *septa* (2.0–)  $3.2 \pm 0.5$  (–4.0)  $\mu\text{m}$  thick ( $n=31$ ), *c.* 0.2 of spore length. Deformed ascospores with three loculi (Fig. 1E, right) rarely observed (CBFS JV5306).

*Conidiomata* pycnidia, *c.* 80–100  $\mu\text{m}$  diam. ( $n=4$ ); wall around ostium slightly greyish (Sedifolia-grey). *Conidiophores* usually arranged in a tightly-packed tissue (Fig. 1F), variable in length, formed of 2–5 thin-walled  $\pm$  isodiametric cells. *Conidiogenous cells* often obtuse triangular, (3–)  $4.15 \pm 0.6$  (–5)  $\mu\text{m}$  ( $n=20$ ). *Conidia* broadly ellipsoid to subglobose (Fig. 1F), (1.5–)  $2.1 \pm 0.4$  (–2.5)  $\times$  (1.0–)  $1.5 \pm 0.2$  (–2.0)  $\mu\text{m}$  ( $n=28$ ).

*Chemistry.* No acetone-soluble compounds were detected by HPLC in the thallus or apothecia. Sedifolia-grey, an acetone-insoluble pigment, was detected in the soralia, upper part of the true exciple (strong reactions), epihymenium, and the walls of pycnidia (weak reactions). The upper surface of the thallus (incl. phenocortex) and surface of the thalline exciple do not contain Sedifolia grey pigment, except for rare patches with a true cortex (weak reactions). The chemical composition of the crystalline *pruina* is unknown, but some crystals in the epihymenium were observed in polarized light, and most of them dissolved after treatment with 5% acetic acid.

*Etymology.* Named after its specific substratum, concrete.

*Phylogeny.* Based on nuclear ITS data, *Caloplaca concreticola* forms a well-supported monophyletic group (100% bootstrap support) within *Pyrenodesmia* (Fig. 2). Although morphologically highly dissimilar, *Caloplaca badioreagens* (together with the sequence AY313973 assigned to *C. alociza* in GenBank) is shown here to be the most closely related known species, possibly having a paraphyletic position in relation to *C. concreticola*. However, the low bootstrap support

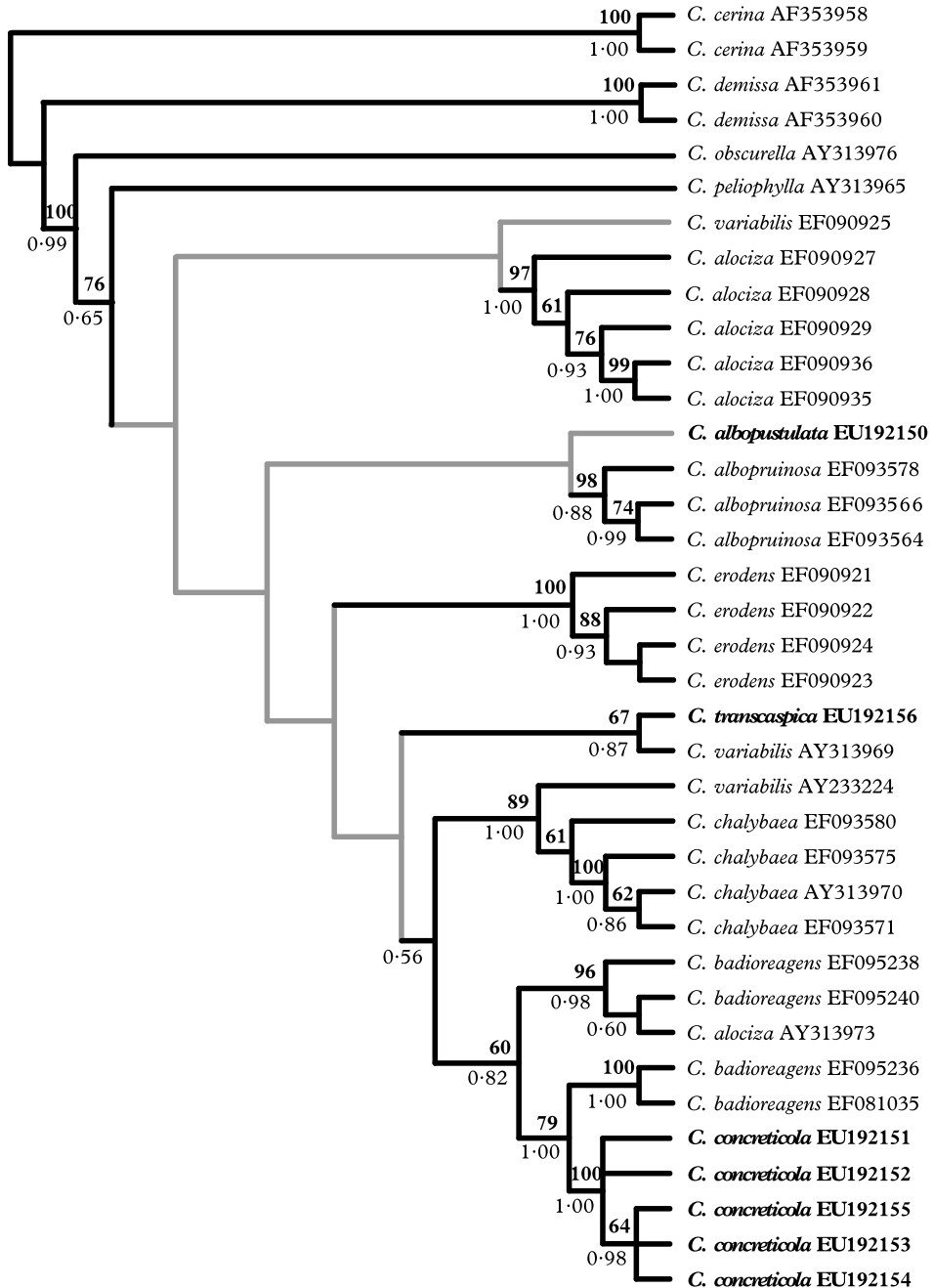


FIG. 2. Phylogeny of *Caloplaca* subgenus *Pyrenodesmia* including the new species *Caloplaca conreticola*. Majority-rule consensus of sixteen most parsimonious cladograms based on  $530 \times 37$ -nucleotide matrix plus bipartitions found during bootstrap analysis; heuristic search option in PAUP was employed using random sequence addition and TBR algorithm (more in text). Numbers in bold are bootstrap portions for clades present in 500 or more of 1000 bootstrap replicates. Light numbers below branches denote posterior probabilities for the following node calculated in MrBayes (90 000 trees were sampled among 10 000 000 generations). The highly ambiguous mid portion of the cladogram with low statistical support is drawn in grey. Bold terminals represent new sequences acquired for this work.

TABLE 2. List of *Caloplaca* species in subg. *Pyrenodesmia* producing vegetative diaspores

Species	Type of vegetative diaspores/Key character	Substrata and distribution	References
<i>Caloplaca albopustulata</i> Khodosovtsev & S. Y. Kondr.	Blastidia or schizidia/K+ violet cortex	Calcareous conglomerates in Crimean Peninsula	Khodosovtsev <i>et al.</i> (2002)
<i>C. demissa</i> (Körb.) Arup & Grube	Soredia/thallus rounded, placodioid, apothecia not known	Non-calcareous rocks in Europe and North America	Arup & Grube (1999)
<i>C. diplacioides</i> (Vain.) Zahlbr. (=sorediate form of <i>C. diplacia</i> (Ach.) Riddle)	Soredia/dense granules in apothecial margin, dissolving in KOH; thallus K+ yellow (atranorin)	Non-calcareous, rarely calcareous rocks in North America	Wetmore (1994)
<i>C. erodens</i> Tretiach, Pinna & Grube	Soredia/thallus endolithic, causing specific depressions in substratum	Calcareous rocks in Europe	Tretiach <i>et al.</i> (2003); Hafellner & Muggia (2006); Vondrák <i>et al.</i> (2007)
<i>C. fuscoblastidiata</i> van den Boom & Etayo	Blastidia (isidia)/apothecia similar to <i>C. obscurella</i> , epithecium K-, N-, ascospores 10–14 × 5.5–7 µm	On bark of e.g. <i>Olea</i> and <i>Ceratonia</i> in Portugal and Spain	van den Boom & Etayo (1995); Wetmore (2004)
<i>C. gambiensis</i> Aptroot	Isidia/all parts of apothecia and thallus K-, C-, N-, ascospores 14–15.5 × 5.5–7 µm, septum 5.5–7 µm thick	On savannah trees in tropical Africa and Taiwan	Aptroot (2001); Wetmore (2004)
<i>C. obscurella</i> (J. Lahm ex Körb.) Th. Fr.	Soredia/K- crater-like soralia, ascospores 10–13 × 6–8 µm	Nutrient-rich, basic bark in Europe and North America	Laundon (1992); Søchting (1994); Wetmore (1994)
<i>C. turkuensis</i> (Vain.) Zahlbr.	Soredia/ascospores 9–12 × 5–7 µm	Bark of <i>Ulmus</i> in Finland	Clauzade & Roux (1985)
<i>C. wrightii</i> (Tuck.) Fink (= <i>C. neotropica</i> Wetmore)	Isidia/usual presence of pseudocyphellae, ascosp. with thickened end walls	On bark in tropical and subtropical America	Wetmore (1994, 2004)

(60%) does not exclude the possibility that *C. badioreagens* could be monophyletic. *Caloplaca chalybaea* (together with the sequence AY233224 assigned to *C. variabilis* in GenBank) appears to be the species most closely allied to the *C. badioreagens/C. concreticola* group, although the bootstrap support is below 50% and this grouping is sustained only by the measure of Bayesian posterior probabilities (BPP=0.56).

**Ecology and distribution.** The new species grows on concrete, usually on surfaces of water channels in dry areas; it is not yet known from natural habitats. Most records are from channels with permanent water,

usually in a zone 1–3 m above the water level, but some sterile populations were found in channels with only intermittent water flow. Phytosociologically *C. concreticola* belongs to the pioneer unit *Lecanorion dispersae* described by Laundon (1967). This is a pioneer community on artificial calcareous substrata in urban areas throughout Europe (e.g. Christensen 2004). Accompanying species are *Caloplaca crenulata*, *C. decipiens*, *C. flavocitrina*, *C. saxicola*, *C. teicholyta*, *Candelariella aurella*, *Lecanora albescens*, *L. crenulata*, *L. dispersa*, *Sarcogyne regularis*, *Staurothele frustulenta*, *Verrucaria macrostoma* f. *furfuracea*, *Verrucaria muralis*, and *V. nigrescens* s.l. The new species is

probably widespread on concrete in the semi-arid landscapes of Eastern Europe, but has been overlooked because of its small and often sterile thalli.

*Remarks.* The new species is easily distinguished by the combination of marginally sorediate grey areoles and brown to black apothecia lacking anthraquinone. Only a few known species of the subgenus *Pyrenodesmia* produce vegetative diaspores: *Caloplaca albobustulata*, *C. demissa*, *C. diplaciooides* (sorediate morphotype of *C. diplacia*), *C. erodens*, *C. fuscoblastidiata*, *C. gambiensis*, *C. obscurella*, *C. turkuensis*, and *C. wrightii*. These morphologically very different species are listed in Table 2.

When sterile, *C. concreticola* is very similar to *C. soralifera*; the two species being almost indistinguishable in the field, but the former differs in the K<sup>-</sup> reaction of the phenocortex, instead of K<sup>+</sup> violet (Sedifolia-grey) of the true cortex in *C. soralifera* (Vondrák & Hrouzek 2006). *Caloplaca soralifera* also has a similar ecology and may occur with *C. concreticola*. The sterile crust (K<sup>+</sup> violet) of *Rinodina pityrea* on concrete can be distinguished by its blue-green, entirely blastidiate thallus.

*Additional specimens examined.* **Romania:** Dobrogea: Constanța, water channel SW of Basarabi, alt. 25 m, 44°09'48.87"N, 28°23'25.21"E, on dry surface of concrete water channel, c. 4 m above water level, with *C. teicholyta*, 6 iv 2007, *Ĵ. Vondrák & Ĵ. Šoun* (CBFS JV5298).—**Slovakia:** Slovenský kras karst: Rožňava, Turňa nad Bodvou, on road to Host'ovce, c. 2 km SW of town, alt. 170 m, 48°35'13"N, 20°51'23"E, on concrete on bridge over brook, 5 viii 2006, *Ĵ. Vondrák* (CBFS JV4637).—**Ukraine:** Crimean Peninsula: Krasnoperekopskiy district, Armiansk, 6.5 km NW of town, at North-Crimean channel, alt. 10 m, 46°08'57.36"N, 33°37'40.25"E, on dry surface of concrete water channel, 1–2 m above water level, 29 v 2007, *Ĵ. Vondrák & Ĵ. Šoun* (CBFS JV5297); Dzhankovskiy district, North-Crimean channel near road bridge N of Kondratovo, alt. c. 20 m, 45°42'11.54"N, 34°26'27.00"E, on concrete of channel wall, 23 v 2007, *Ĵ. Vondrák & Ĵ. Šoun* (CBFS JV5301); Kirovskiy district, North-Crimean channel near road bridge S of Kirovske, alt. c. 20 m, 45°10'55.34"N, 35°13'21.89"E, on concrete of channel wall, 23 v 2007, *Ĵ. Vondrák & Ĵ. Šoun* (CBFS JV5302, dupl. GZU, UPS). *Khersonska oblast:* Chaplinskiy district, 10 km W of village Askania-Nova, R-2 channel, on concrete of channel wall, 15 xi 2006,

*A. Khodosovtsev* (KHER, dupl. CBFS JV5305); 4.5 km W of village Zaozerne, Kakhovskiy kanal water channel, on concrete of channel wall, 15 xi 2006, *A. Khodosovtsev* (KHER, dupl. CBFS JV5304, 5306, topotypi). *Mykolajivska oblast:* Ochakovskiy district, Radsad, at stadium in town, bank of river Pivdenniy Bug, on concrete, 23 xii 2006, *A. Khodosovtsev* (KHER).

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