Absence of anthraquinone pigments is paraphyletic and a phylogenetically unreliable character in the Teloschistaceae

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Absence of anthraquinone pigments is paraphyletic and a phylogenetically unreliable character in the *Teloschistaceae*

Jan VONDRA´K, Jaroslav ŠOUN, Olga VONDRA´KOVA´, Alan M. FRYDAY, Alexander KHODOSOVTSEV and Evgeny A. DAVYDOV

**Abstract:** It has been suggested that the absence of anthraquinones is not a synapomorphic character, but appears independently in unrelated lineages of *Teloschistaceae*. We analyzed ITS nrDNA regions in species of the genus Caloplaca and present evidence for five such examples: the *Caloplaca cerina* group, *C. obscurella*, the *C. servitiana* group, the *C. xerica* group and the *C. variabilis* group (*Pyrenodesmia*). In some cases, loss of anthraquinones is observed only in individuals within ordinarily pigmented populations, but sometimes the loss covers whole lineages containing one or more species. Both situations are observed in the *C. servitiana* group. Loss of anthraquinones is always followed by the synthesis of 'alternative' pigments (often *Sedifolia*-grey). In the specimens with anthraquinone-containing apothecia studied, these pigments are not visible in apothecial sections after dissolving anthraquinones in K. Fully unpigmented apothecia have not been observed.

The *Caloplaca xerica* group is a newly established, infraspecific grouping of species related to, and similar to, *C. xerica*. The *Caloplaca servitiana* group is also newly established and represents an isolated lineage covering two rather different, but related species. *Caloplaca neotaurica* is described here as a new species with apothecia of two colour variants; orange-red (with anthraquinones) and grey (with *Sedifolia*-grey).

The genus *Huea* represents another taxon lacking anthraquinones within *Teloschistaceae*. The genera *Apatoplaca* and *Cephalophysis*, which lack anthraquinones, are tentatively placed in *Teloschistaceae*, but their phylogenetic identity has not been recognized. *Hueidea* is reported to have no anthraquinones, but its secondary metabolites should be studied further and its possible placement in *Teloschistaceae* assessed.

We suggest that *Caloplaca abbreviata* var. *lecioides* and *C. celata* represent variants of *C. stillicidiorum* lacking anthraquinones.

**Key words:** *Apatoplaca*, *Caloplaca neotaurica*, *Cephalophysis*, *Huea*, *Hueidea*, lichens, phylogeny, taxonomy, UV-radiation

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**Introduction**

In one of the largest lichen families, *Teloschistaceae*, the majority of lichens produce yellow-orange-red anthraquinone pigments in the superficial tissues of their fruiting bodies and/or thallus (e.g., Sochting 1997). They are produced in various *Teloschistaceae* in 1) both thallus and apothecia, 2) in apothecial disc and margin only, 3) in apothecial disc only or 4) are entirely absent. Anthraquinones are known to protect lichens from absorption of UV radiation (Solhaug et al. 2003); their content in *Xanthoria parietina* (L.) Beltr. (*Teloschistaceae*) significantly increases with exposition to solar radiation (Gauslaa & McEvoy 2005).
Some *Teloschistaceae* also have the ability to synthesize green or grey pigments of unknown structure, for example, *Lecidea-green* (Wetmore 1996; Arup *et al.* 2007) or *Sedifolia-grey* (e.g., Wetmore 1996; Meyer & Printzen 2000), which may have the same function, that is, protection against UV radiation (e.g., Hauck *et al.* 2007). These pigments usually occur in parts of the thalli or apothecia where anthraquinones are absent, although both pigments may co-occur in the superficial tissues of the apothecia of some species. These species, for example, *Caloplaca conversa* (Kremp.) Jatta, *C. exsecuta* (Nyl.) Dalla Torre & Sarnth., *C. oleicola* placa conversa (Kremp.) Jatta, *Calopolis* species. These species, for example, superficial tissues of the apothecia of some though both pigments may co-occur in the thcia where anthraquinones are absent, usually occur in parts of the thalli or apo-thecia sections (K+ strongly purple-violet, K→HCl+ yellow, N+ orange, N→K+ purple-violet-blue, N→K→HCl+ yellow; Vondrák *et al.* 2010a).

The absence of anthraquinones is known at various levels; their absence in the thallus has arisen in many lineages throughout *Teloschistaceae*, for example, the *C. cerina* group (Šoun *et al.* 2011). Occasional loss of anthraquinones in the thallus is also documented in individuals or populations of species that usually have a pigmented thallus (Søchting 1973; Vondrák *et al.* 2010b). Aberrant populations lacking anthraquinones completely are suggested in *C. tiroliensis* Zahlbr. (Hansen *et al.* 1987; Søchting 1989) or *C. xerica* Poelt & Vězda (Poelt 1975).

Species (or individuals) of *Teloschistaceae* with a complete loss of yellow-orange-red pigments, sometimes grouped under *Pyrenodesmia* (*Caloplaca variabilis* group), have been studied since the 19th century but attempts to treat them systematically began with Magnusson (1950), followed by Wunder (1974) in the Old World and Wetmore (1994) in North America. More recently, several works have dealt with this group using single locus (ITS) molecular data (Arup & Grube 1999; Tretiach *et al.* 2003; Tretiach & Muggia 2006; Muggia *et al.* 2008; Vondrák *et al.* 2008; Xahidin *et al.* 2010).

Although the absence of anthraquinones in *Teloschistaceae* has often been treated as an important grouping character, no studies to date have tested whether or not this is a reliable character for classification, or whether species with such characters represent a monophyletic group. The purpose of the present study is to test this hypothesis using a broad sampling of species lacking anthraquinones, which would traditionally be treated as closely related (e.g., Muggia *et al.* 2008). Specifically, we will test whether or not these species can be recovered as a well-supported monophyletic group.

**Materials and Methods**

Lichen samples were collected by the authors from various localities in the Northern Hemisphere. Detailed herbarium data for specimens used in our phylogenetic studies are given in Table 1. Data for paratypes of *C. neotaurica* are abbreviated; full information for samples from CBFS is available at http://botanika.bf.jcu.cz/ lichenology/data.php.

**Morphological examinations**

A detailed morphological description is given only for the new species. Measurements are accurate to 0.25 μm for cells (e.g., conidia and ascospores), 1 μm for width of asci, or 10 μm for larger structures (e.g., hymenium, width of exciple). All measurements of cells (ascospores, conidia, asci, paraphyses) include their walls. Paraphyses tips were observed after treatment with c. 10% KOH. Only those ascospores with well-developed septa were measured; in these ascospores, loculi were connected with a thin cytoplasmatic channel, never disconnected. Measurements with more than ten repeats (n ≥ 10) are given as (min.–) x ± SD (–max.), where x = mean value and SD = standard deviation. Morphological terminology follows Smith *et al.* (2009).

**Chemistry**

The acetone extracts from apothecia were subjected to high-performance liquid chromatography (HPLC) analysis. Reverse phase column (C18, 5 μm, Lichrocart 250-4) was eluted with MeOH / 30%MeOH+ 1%H3PO4 for 77 min and the absorbances at 270 nm were recorded (for details see Søchting 1997). The compounds were determined on the basis of their retention times and absorption spectra. In the newly described species, five specimens were examined (CBFS JV2048, 5925, 6023, 7094, 7105).
<table>
<thead>
<tr>
<th>Species</th>
<th>Specimen</th>
<th>Group</th>
<th>GenBank accession number</th>
</tr>
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<td>Caloplaca albolutescens</td>
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<td>C. xerica group</td>
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<td>C. aractina</td>
<td><strong>Sweden</strong>: Bohuslän; Askum par., Barlindarna, 2006, U. Sechting 10579 (C)</td>
<td>C. haematties group</td>
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<td>C. atroflava</td>
<td><strong>Czech Republic</strong>: North Moravia: Šumperk, Raškov, on serpentine, 2007, Palec 11741 (PRA)</td>
<td>C. xerica group</td>
<td>JN641764</td>
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<td>C. erythrocarpa</td>
<td><strong>Bulgaria</strong>: Strandza Mts; Malko Tarnovo, on limestone, 2005, J. Vondrakov (CBFS JV3208)</td>
<td>C. xerica group</td>
<td>JN641767</td>
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<tr>
<td>C. fuscoatroides</td>
<td><strong>Bulgaria</strong>: Black Sea coast; Burgas, Sozopol, 2007, J. Vondrakov (CBFS JV6468)</td>
<td>C. xerica group</td>
<td>JN641768</td>
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<tr>
<td>C. haematties</td>
<td><strong>Russia</strong>: Caucasus: “Dagestanskiy zapovednik” National park, 2009, A. Gabibova (CBFS)</td>
<td>C. haematties group</td>
<td>JN641769</td>
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<td><strong>Great Britain</strong>: Wales: seashore rocks, U. Arup (LD, material lost)</td>
<td>C. xerica group</td>
<td>JN641770</td>
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<tr>
<td>C. neotaurica</td>
<td><strong>Greece</strong>: Peloponnese: 2010, on serpentineite, J. Vondrakov &amp; O. Vondrakova (CBFS JV8322)</td>
<td>C. xerica group</td>
<td>JN641771</td>
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<td>C. neotaurica</td>
<td><strong>Ukraine</strong>: Crimean Peninsula: Alushta, 2007, on siliceous stone, J. Vondrakov (CBFS JV6023)</td>
<td>C. xerica group</td>
<td>JN641772</td>
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<td>C. neotaurica</td>
<td><strong>Ukraine</strong>: Crimean Peninsula: Karadag, 2007, on siliceous stone, J. Vondrakov (CBFS JV6229, isotype)</td>
<td>C. xerica group</td>
<td>JN641773</td>
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<td><strong>Ukraine</strong>: Crimean Peninsula: Yalta, on siliceous stone, 2009, J. Vondrakov &amp; A. Khodosov (CBFS JV7121)</td>
<td>C. xerica group</td>
<td>JN641774</td>
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<td>C. neotaurica, grey</td>
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<td>C. xerica group</td>
<td>JN641775</td>
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<td>C. neotaurica, grey (1)</td>
<td><strong>Greece</strong>: Peloponnese: Argolis Peninsula, on serpentineite, 2010, J. Vondrakov &amp; O. Vondrakova (CBFS JV8322)</td>
<td>C. xerica group</td>
<td>JN641776</td>
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<td>C. neotaurica, grey (2)</td>
<td><strong>Greece</strong>: Peloponnese: Argolis Peninsula, on serpentineite, 2010, J. Vondrakov &amp; O. Vondrakova (CBFS JV8322)</td>
<td>C. xerica group</td>
<td>JN641777</td>
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<tr>
<td>C. servitiana</td>
<td><strong>Greece</strong>: Pindos Mts; Profitis Ilias, on bark of Quercus coccifera, 2005, T. Spribille 16225 (CBFS JV6974)</td>
<td>C. servitii group</td>
<td>JN641778</td>
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<td>C. soralicera</td>
<td><strong>Ukraine</strong>: Khmelnitsk’ region; Kamenets-Podolsky, on limestone, 2006, J. Vondrakov (CBFS JV4594)</td>
<td>C. xerica group</td>
<td>JN641780</td>
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<td>C. aff. soralicera</td>
<td><strong>Czech Republic</strong>: Prachatice, on gneiss, 2009, J. Vondrakov &amp; O. Vondrakova (CBFS JV8325)</td>
<td>C. xerica group</td>
<td>JN641781</td>
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</table>
Apothecial pigments were also examined by reactions in sections with Cl (C), KOH (K), HCl and HNO₃ (N) according to Meyer & Printzen (2000).

**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 follow Ekman (2001).

**Phylogenetic analyses**

Sequences were aligned using MAFFT 6 (on-line version in the Q-INS-i mode; see Katoh et al. 2002) and manually trimmed to eliminate the unaligned ends and ambiguously aligned regions of ITS1 and ITS2. Bayesian phylogenetic analyses were carried out using the program MrBayes 3.1.1 (Ronquist & Huelsenbeck 2003). The optimal nucleotide substitution models were found using the program MrModeltest v2.3 (Nylander 2004). The MCMC analyses were performed in two runs, each with four chains starting from a random tree and using the default temperature of 0°C1°C. Every 100th tree was sampled and standard deviation of splits between runs less than 0.01 as a convergence criterion was used to assess burn-in. Models of nucleotide substitution, numbers of generations and discarded generations, numbers of sequences and lengths of each alignment are given in Table 2. Posterior probabilities are shown in all depicted phylogenetic trees (Figs 1–6). Tree terminals of samples

<table>
<thead>
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<th>Species</th>
<th>Specimen</th>
<th>Group</th>
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<tr>
<td>Caloplaca sp.</td>
<td><strong>Russia</strong>: Southern Ural Mts: Orenburg, on limestone, 2009, J. Vondrák (CBFS JV8182)</td>
<td>Pyrenodesmia 2</td>
<td>JN641784</td>
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<td>Caloplaca sp.</td>
<td><strong>Russia</strong>: Southern Ural Mts: Orenburg, on conglomerate, 2009, J. Vondrák (CBFS JV8179)</td>
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<td><strong>Russia</strong>: Southern Ural Mts: Orenburg, on conglomerate, 2009, J. Vondrák (CBFS JV8326)</td>
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<td>JN641782</td>
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<td>Caloplaca sp.</td>
<td><strong>Kazakhstan</strong>: Mangistau region: Beyney, on limestone, 2009, J. Vondrák &amp; A. Khodosovtsev (CBFS JV7635)</td>
<td>Pyrenodesmia 2</td>
<td>JN641785</td>
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<tr>
<td>Caloplaca sp.</td>
<td><strong>Ukraine</strong>: Doneck upland: Luhansk, Rozkishne, on marl, 2007, O. Nadyeina (hb. O. Nadyeina)</td>
<td>Pyrenodesmia 2</td>
<td>JN641786</td>
</tr>
<tr>
<td>Caloplaca sp., grey</td>
<td><strong>China</strong>: Xinjiang: Qinghe county, Mongolian Altai, west slope of Mt. Kara-Balchigtau, alt. 2400 m, on plant debris, 2007, E. A. Davydov 6876b (CBFS JV8413)</td>
<td>C. servitii group</td>
<td>JN641787</td>
</tr>
<tr>
<td>Caloplaca sp., yellow</td>
<td><strong>China</strong>: Xinjiang: Qinghe county, Mongolian Altai, west slope of mt. Kara-Balchigtau, alt. 2400 m, on plant debris, 2007, E. A. Davydov 6876a (CBFS JV8414)</td>
<td>C. servitii group</td>
<td>JN641788</td>
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<tr>
<td>C. stillicidiorum, grey</td>
<td><strong>USA</strong>: Alaska: Atqasuk, on bone, 2001, A. Fryday 8158 (MSC 57393)</td>
<td>C. cerina group</td>
<td>JN641789</td>
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<td>C. teicholyta</td>
<td><strong>Romania</strong>: Dobrogea: Tulcea, on limestone, 2007, J. Vondrák (CBFS JV5381)</td>
<td>C. xerica group</td>
<td>JN641790</td>
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<td>C. teicholyta</td>
<td><strong>Ukraine</strong>: Crimean Peninsula: Bakhchisaray, on limestone, 2006, J. Vondrák (CBFS JV5675)</td>
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<td>C. xerica group</td>
<td>JN641793</td>
</tr>
</tbody>
</table>
without anthraquinones are in green (absence on population level) or yellow (absence on species level).

Results and Discussion

We generated 29 new sequences and used them in six different alignments. Five alignments were for specifically studied groups and one for a broad context. We found that the character of anthraquinone absence is polyphyletic, occurring in five different clades; the *Caloplaca cerina* group, *C. obscurella*, the *C. servitiana* group, the *C. xerica* group and the *C. variabilis* group (*Pyrenodesmia*). In some cases, whole lineages of anthraquinone-containing species contain one or more species that lack these pigments (‘absence on species level’), whereas in others loss of yellow-orange pigments is restricted to individuals or populations of species that normally contain anthraquinones (‘absence on population level’). In the latter case, specimens lacking anthraquinones always have some alternative pigment in their apothecia (usually *Sedifolia*-grey), which is not identified in apothecia with anthraquinones; that is, it is not apparent in the apothecial sections after dissolving the anthraquinones in K. Each case will be discussed specifically.

**Caloplaca cerina group (absence on population level)**

This homogeneous group (*Šoun et al.* 2011) clusters crustose *Teloschistaceae* with a white/grey/black thallus without anthraquinones, and lecanorine apothecia with distinct thalline exciple lacking anthraquinones but with anthraquinone pigmented (yellow/orange/red) discs.

We have observed individuals entirely lacking anthraquinones in the Mediterranean lineage of *Caloplaca cerina* (Hedw.) Th. Fr. s. lat. (group A in *Šoun et al.* 2011) and in the arctic lineage of *Caloplaca stillicidiorum* (Vahl) Lynge (group 3 in *Šoun et al.* 2011). Both examples are morphologically similar to their closest relatives; important similarities are lecanorine exciples, distinct cortex in lower exciple and presence of *Sedifolia*-grey pigment in the thalline exciple. The arctic sample has smaller ascospores (c. 10–15 × 6–8 μm) than the phylogenetically closest observed specimens of *C. stillicidiorum* with orange discs (c. 13–16 × 7–9 μm). Both samples differ from other specimens in the *C. cerina* group by the presence of *Sedifolia*-grey in the apothecial disc (in section: K+ violet, C+ orange-carmine, N+ orange-red-purple); we have not observed this pigment in orange, anthraquinone-containing discs. Both specimens investigated are placed into the named groups (PP = 1·00 in both; Fig. 1A). In the available material, non-anthraquinone phenotypes occur side by side with their counterparts with orange discs (Fig. 1B & C).

The arctic specimen of *C. stillicidiorum* lacking anthraquinones is probably conspecific with *C. celata* Th. Fr., which appears to

<table>
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<th>Tree</th>
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<th>Substitution model</th>
<th>Number of generations</th>
<th>Number of discarded generations</th>
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<td><em>Caloplaca cerina</em> group</td>
<td>25</td>
<td>433</td>
<td>GTR+G</td>
<td>1 × 10^6</td>
<td>297,000</td>
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<tr>
<td><em>Caloplaca obscurella</em></td>
<td>21</td>
<td>528</td>
<td>GTR+I+G</td>
<td>1 × 10^6</td>
<td>259,000</td>
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<tr>
<td><em>Caloplaca servitiana</em></td>
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<td>536</td>
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<tr>
<td><em>Caloplaca xerica</em> group</td>
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<td>477</td>
<td>GTR+G</td>
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<td><em>Pyrenodesmia</em> group</td>
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<td><em>Teloschistaceae</em></td>
<td>33</td>
<td>398</td>
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<td>1,582,000</td>
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</table>
Fig. 1. Caloplaca cerina group. A, Bayesian phylogeny (ITS nrDNA regions) of a part of the group containing lineages of *C. cerina* and *C. stillicidiorum* with individuals lacking anthraquinones (in green); B, two phenotypes of *C. cerina* (CBFS JV8329), grey apothecia without anthraquinones (left) and normally coloured apothecia (right); C, *C. stillicidiorum* (Fryday 8158; MSC) with grey, white pruinose apothecia (right) and with orange, normally coloured apothecia (left) and with *C. cf. tiroliensis* in the upper part. Scales: B & C = 1 mm.
have the same ecology and identical morphology (Magnusson 1950; Poelt 1955; Hansen et al. 1987; Sochting et al. 2008). Caloplaca abbreviata var. lecideoides H. Magn., a lignicolous lichen from the Russian arctic, seems to be another name referring to C. stilllicidorum lacking anthraquinones; the type specimen in S (http://andor.nrm.se/kryptos/kbo/kryptobase/200803/max/32948.jpg) apparently contains a mixed population with orange and grey apothecia.

The description of Pyrenodesmia monacensis Leder., currently known as Caloplaca monacensis (Leder.) Lettau, is also based on non-anthraquinone containing specimens (Lederer 1896). Nevertheless, our data have shown that C. monacensis is a well-characterized, widely distributed and usually anthraquinone-containing species from the C. cerina group (Sˇoun et al. 2011).

Caloplaca xerica group (absence on population level)

The ‘Caloplaca xerica group’ represents a monophyletic group (PP = 1·00 in Fig. 2A) of mainly saxicolous species with the following characters: thallus without orange-red pigmentation, devoid of anthraquinones but with Sedifolia-grey in cortical tissues (K+ violet reaction in sections); apothecia orange to red, containing anthraquinones; ascospores never narrowly ellipsoid, length/breadth ratio 1·4–2·6; pycnidia grey-black, devoid of anthraquinones. The C. xerica group is related to Pyrenodesmia and the C. haematites complex (Fig. 3).

This species-rich group contains many unnamed taxa, one of which is Caloplaca neotaurica (Fig. 2B) that is formally described at the end of this paper. Caloplaca neotaurica is unusual within the group because it also has phenotypes with complete loss of anthraquinones, which usually occur along with phenotypes with red apothecia (Fig. 2C). Non-anthraquinone phenotypes are morphologically ± identical to their counterparts with red apothecia, but all samples observed without anthraquinones had apothecia containing Sedifolia-grey (in section K+ violet, C+ orange-carmine, N+ orange-red-purple-black); this pigment is present only in the thallus of the variant with red apothecia. The non-anthraquinone specimen from the Crimean Peninsula (CBFS JV6942) nests safely inside the C. neotaurica monophylum (PP = 1·00), but specimens examined from Greece have aberrant ITS sequences and the Bayesian phylogeny placed them as a sister group to other C. neotaurica samples (Fig. 2A).

Although he did not do so explicitly, Poelt (1975) already recognized the absence of anthraquinones at the population level within this group in Caloplaca xerica; its anthraquinone-lacking populations are named C. xerica var. venostana.

Pyrenodesmia group (absence on species level)

The C. variabilis group (Pyrenodesmia) is a well-known group containing most of the European saxicolous species without anthraquinones but with Sedifolia-grey in the apothecia (in section K+ violet, C+ orange-carmine, N+ orange-red-purple-black). Our data show that Pyrenodesmia is unresolved within the Caloplaca xerica group and the C. haematites group (PP = 0·97 for the whole group; Fig. 3). The internal taxonomy and phylogeny of this group have been reported (Tretiach et al. 2003; Tretiach & Muggia 2006; Muggia et al. 2008; Vondrák et al. 2008), but the group is still only partially known in Europe and North America and it is little known in Asia. For instance, the group is species-rich in Central Asia (J. Vondrák, unpublished data). Based on our set of sequences, two main internal lineages are to be found within Pyrenodesmia; Pyrenodesmia 1 with most of the European taxa (unsupported) and Pyrenodesmia 2 containing mainly unknown taxa from the southern Ural Mountains, the Near East and the steppe-desert zone of the most SE region of Europe (PP = 0·97).

Caloplaca obscurella (absence on species level)

Currently, we know little about the closest phylogenetic relatives of Caloplaca obscurella.
Fig. 2. Caloplaca neotaurica. A, Bayesian phylogeny (ITS nrDNA regions) of the C. xerica group (delimited by the red line) with the monophylum of C. neotaurica (delimited by the blue line); species without anthraquinones in yellow; specimens of C. neotaurica without anthraquinones in green; B, C. neotaurica, isotype (CBFS JV6229); C, C. neotaurica, phenotype with anthraquinones (left) and phenotype with grey apothecia (right) in Peloponnese (CBFS JV8322). Scales: B & C = 1 mm.
(J. Lahm) Th. Fr. (Fig. 4A). It forms a strongly supported clade ($PP = 1.00$), sister to the large monophylum containing the C. haematites group, the C. xerica group and Pyrenodesmia ($PP = 1.00$), but we failed to find closer relatives. It is also unclear whether the C. obscurella clade contains only a single species. We suggest that the North American species C. brunneola Wetmore, C. camptidia (Tuck.) Zahlbr. C. dakotensis Wetmore (Wetmore 1994) and C. lecanoroides Lendemer (Lendemer et al. 2010) might belong here.
Fig. 4. *Caloplaca obscurella*. A, Bayesian phylogeny of ITS nrDNA regions; sister relationship of *C. obscurella* is shown to the clade containing *C. haematites* group, *C. xerica* group and *Pyrenodesmia* (delimited by the red square); species without anthraquinones highlighted in yellow; B, richly fertile specimen from Russia (CBFS JV7641). Scale = 1 mm.
because all these species contain brown pigments in the epihymenium that resemble those in *C. obscurella* (K−, C−, N−, acetone insoluble, not detectable by HPLC).

**Caloplaca servitiana group (absence on both species and population level)**

We revealed two species in the *Caloplaca servitiana* group (Fig. 5A); 1) *C. servitiana* Szatala s. str. (absence on species level; Fig. 5B) and 2) an unnamed species from China (absence on population level; Fig. 5C & D). This group is the only one with the lack of anthraquinones found in the ‘Lineage 1’ of Teloschistaceae (*sensu* Gaya *et al.* 2008; Fig. 6). It is a rather isolated group with no known closely related lineages.

1) A brief description of *C. servitiana* is available in Vondrák *et al.* (2010a). It is known only from several sites in the eastern Mediterranean, where it grows on trees with various other *Caloplaca* species. Our comparison of this phenotype with co-occurring

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**Fig. 5. Caloplaca servitiana group.** A, Bayesian phylogeny of ITS nrDNA regions containing *C. servitiana* with absence on species level (in yellow) and a species from China with absence on population level (in green); B, *C. servitiana* (Spribille 13705); C, species from China (*Davydov* 6876; CBFS) with yellow and grey apothecia growing side by side; D, species from China, the phenotype with grey apothecia (*Davydov* 6876b; CBFS) together with *C. stillicidiorum* (orange apothecia with white margins). Scales: B–D = 1 mm.
Caloplaca species showed that it had a strong similarity with *C. aegatica* Giralt et al., which challenged us to test if *C. aegatica* and *C. servitiana* are a single species. Our results show that *C. aegatica* is closely related to *C. cerinella* (Nyl.) Flagey, whereas *C. servitiana* is not closely related to any known European species.

2) The unnamed specimen from the Altai Mountains (north-west China) is characterized by an inconspicuous, simplified thallus and biatorine to zeorine apothecia with a
distinct, c. 70–100 μm wide, proper exciple. The thalline exciple is usually reduced and restricted to the lower part of the apothecial margin. The ascospores, c. 14–18 × 7–9 μm, have a characteristic ‘obtuse rhomboid’ shape. In the specimen observed, the apothecia have two colour variants (Fig. 5C & D). The first is yellow, contains anthraquinones and lacks Sedifolia-grey, whereas the second is grey, devoid of anthraquinones, and contains Sedifolia-grey (in section K+ violet, C+ red, N+ red) in the epihymenium and superficial cells of the excipulum.

Other Teloschistaceae without anthraquinones

Among the genera of Teloschistaceae, the entire loss of anthraquinones is known only in the paraphyletic genus Caloplaca (Fig. 6). Species or populations with entire loss of anthraquinones are not known within foliose and fruticose genera of Teloschistaceae, although in some specimens of, for example, Seirophora lacunosa (Rupr.) Frödén and some Teloschistes species, whole thalli are grey with anthraquinones restricted to the apothecial discs and the walls of pycnidia (e.g., Sochting & Frödén 2002).

Our study has been carried out only on specimens from arctic-temperate-(subtropical) zones of the Northern Hemisphere, but species without anthraquinones are known from the tropics and the Southern Hemisphere: for example, Caloplaca filsonii Hafellner et al. (Australia; Kondratyuk et al. 2007), C. gambiensis Apret (Gambia; Apret 2001), C. jatolensis Y. Joshi & Upreti (India; Joshi et al. 2008), C. lewis-smithii (Antarctica; Sochting & Øvstedal 1998), Caloplaca yammeraensis S. Y. Kondr. et al. (Australia; Kondratyuk et al. 2009) and some tropical species with tri- to tetralocular ascospores (Hafellner & Poelt 1979). It is probable that these species belong to diverse groups that are unrelated to the lineages shown in this study.

Additional lineages with species or individuals that do not produce anthraquinones will also certainly be found in the Northern Hemisphere. We have examined the pigments contained in herbarium specimens of some little-known arctic species, Caloplaca atrocyaneascens (Th. Fr.) H. Olivier, C. conciliascens (Nyl.) H. Olivier and C. diphyes (Nyl.) H. Olivier. These species have blackish apothecia without anthraquinones, but contain different pigments (blue-green–olive) with various reactions with K, N and C in sections, but the identity of these pigments is unknown. The phylogenetic positions of these taxa are also not yet known. Other rare and little-known European species with black apothecia that contain unknown pigments are C. concilians (Nyl.) H. Olivier and C. conciliascens (Nyl.) Zahlbr. In addition, Caloplaca sorocarpa (Vain.) Zahlbr. has brown apothecia but is very rarely fertile and it is not known which pigments are present in its apothecia.

Caloplaca atrosanguinea (G. Merr.) I. M. Lamb is a North American corticolous species which has brown (young) or black (old) apothecia. Its apothecial pigment is violet-blue in section and reacts: K+ violet-blue intensifying, K→HCl+ sordid green, Nunchanged, N→K+ weekly purple→discoloured, N→K→HCl– discoloured, Cdiscoloured. These reactions do not correspond to anthraquinones, which are typically K→HCl+ strongly yellow. The HPLC chromatogram shows one major unknown substance with a short retention time (Rt = 13-6 min, which is less than any known anthraquinone in Teloschistaceae) but also a minor substance (Rt = 33-3 min) with the absorption spectrum similar to emodin that we identified as an unknown anthraquinone. Caloplaca atrosanguinea is related to, but not within, the C. cerina group (J. Vondrák, unpublished data).

Caloplaca demissa (Körb.) Arup & Grube also lacks anthraquinones, but it has probably never been found with apothecia. If it is able to produce apothecia, they may contain anthraquinones because its closest related species, C. carpheina (Fr.) Jatta (Gaya et al. 2008), has anthraquinone-containing apothecia.

Another lineage in Teloschistaceae that lacks anthraquinones is represented by the genus Huea C. W. Dodge & G. E. Baker (Dodge & Baker 1938). This genus was erected for a group of crustose Antarctic species of Telo-
schistaceae characterized by lacking anthraquinones, and having black apothecia with a carbonaceous exciple and a bright blue epihymenium. The status of *Huea* is controversial, with some authors accepting it (Øvstedal & Lewis Smith 2001) whereas others include the species in *Caloplaca* (e.g., Poelt & Hafellner 1980). Sochting et al. (2004) cited unpublished preliminary molecular data showing that the two species transferred to *Huea* by Dodge & Baker (1938) formed a distinct clade in *Caloplaca*. Unfortunately, there are problems with the typification of *Huea* because Dodge & Baker (1938) chose as the type species their newly described species *Huea flavia* C.W. Dodge & G.E. Baker. This is usually considered to be a sterile crust of uncertain identity but was shown by Fryday (2011) to be conspecific with *Lecidea capsulata* C.W. Dodge & G.E. Baker. As this species is now included in the synonymy of *Carbonea vorticosa* (Flo¨rke) Hertel, (http://www.indexfungorum.org/Names/NamesRecord.asp?RecordID=107792) this makes *Huea* an earlier name for *Carbonea*. Sochting et al. (2004) proposed a conserved type for *Huea* [*Huea coralligera* (Hue) C.W. Dodge & G.E. Baker] but, because they did not do so as a formal proposal, this has no relevance. The typification of *Huea* is dealt with in detail by Fryday (2011).

*Apatoplaca* Poelt & Hafellner and *Cephaloplysis* H. Kilias are monotypic genera with an absence of anthraquinones affiliated to *Teloschistaceae* mainly because of their *Teloschistaceae*-type asci (Poelt & Hafellner 1980; Kilias 1985); however, their phylogenetic identities are uncertain.

Also of interest is the genus *Hueidea* Kantvilas & P. M. McCarthy (Kantvilas & McCarthy 2003), which was erected for a single Australian species, *H. australiensis* Kantvilas & P. M. McCarthy, with characters intermediate between *Teloschistaceae* and *Fuscideaceae* V. Wirth & Vˇezda. The authors characterized their new genus as lacking thalline chemistry and showing all spot-test reactions negative, and placed it in *Fuscideaceae*. However, inspection of a specimen of *H. australiensis* revealed a weak K+ crimson reaction in the epihymenium, suggesting the presence of anthraquinones and that the genus may be better placed in *Teloschistaceae*.

**Conclusions**

1. Non-anthraquinone lineages or populations represent a minor part of *Teloschistaceae* but they originated repeatedly in various sites in the phylogeny of the family (Fig. 6).

2. We have observed individuals without anthraquinones (absence on population level) in the *Caloplaca cerina* group, the *C. servitiana* group and the *C. xerica* group. Lineages containing non-anthraquinone species (absence on species level) were recognized in *Caloplaca obscurella*, the *C. servitiana* group and *Pyrenodesmia*.

3. Loss of anthraquinones is always followed by synthesis of alternative pigments (often Sedifolia-grey); fully unpigmented apothecia have not been observed by us. Anthraquinones and the alternative pigments may perform the same function, perhaps protection of ascospores against absorption of the UV-B radiation.

**Taxonomy**

*Caloplaca neotaurica* Vondrák, Khodosovtsev, Arup & Sochting sp. nov.

MycoBank No: MB563136

*Caloplaca fuscoatroidis* similis sed thallo ambitu squamulis nullis, tenui, atrogriseo vel nigrescenti, epruinoso vel raro pruinoso.

Typus: Ukraine, Crimean Peninsula, Sudak, Karadag Mt, Mt Svyataya, alt. 320 m, 44°56′03″-27″N, 35°13′06″-17″E, on volcanic rock, 24 May 2007, J. Vondrák (CBFS JV5925—holotypus; CBFS JV5960, 6022, 6229 & C—isotypi). ITS sequence of the isotypus: JN641773.

(Fig. 2)

**Thallus** thin, less than 150 (rarely 250) μm high, grey to brown-black, rarely white pruinose, indistinctly areolate; areoles (150–) 322 ± 112(−600) μm wide (n = 20). *Prothalus* ± present, dark grey. Marginal squamules and vegetative diaspores absent. Cortex absent; *alveolate cortex* (sensu Vondrák et al. 2009) present in spots (<20 μm thick).
Apothecia <0.7 mm diam., biatorine, orange to red (grey in variants without anthraquinones), K+ purple, C+ purple, I- (disc I+ blue due to reaction with asci), P- (but red pigment dissolves in acetic solution of P), UV-; no visible spot reactions in grey apothecia. Exciple usually paler than the disc. Proper exciple c. 100–130 μm thick prospolctenchymatous. Thalline exciple c. 60–120 μm thick. Hymenium c. 90–110 μm high. Paraphyses ± branched and anastomosed, with tips widened to (3.3–)4.4±1.5(–6.0) μm (n = 10). Asci clavate, c. 55–75 × 18–26 μm. Ascospores (13.0–)14.7±1.4(–17.3) × (7.0–) 8.9±1.0(–11.3) μm (n = 25); ratio of ascospore length/breadth (1.4–)1.7±0.2(–2.0); (n = 25); ascospore septa (3.5–) 5.7±0.9(–8.0) μm wide (n = 25); ascospore septa/spore length (0.27–)0.39±0.07(–0.54); (n = 25).

Pycnidia common, usually observable as darker spots in thallus (higher concentration of Sedifolia-grey around ostiole). Conidia ellipsoid c. 2.5–3.5 × 1.0–1.5 μm.

Chemistry. In apothecia, the main anthraquinone is 7–Cl-emodin; additional anthraquinones: 7–Cl-emodinal, 7–Cl-emodic acid, 7–Cl-citreorosein, ±citreorosein, ±emodinal, ±emodin (chemosyndrome C2, sensu Søchting 2001). Sedifolia-grey in thallus and in grey variants of apothecia (K+ violet, C+ orange-red, N+ purple in section).

Etymology. The species is very common in the Crimean Peninsula, which was known as ‘Taurica’ by the Greeks and Romans. The name Caloplaca taurica Mereschk. nomen nudum [=C. inconexa (Nyl.) Zahlbr.] already exists and, although this is not validly published and so does not prevent the use of that name for our species, we have decided not to use it in order to avoid confusion.

Diagnostic characters. Thallus dark grey to brown-black (rarely white pruinose), thin, usually up to 150 μm thick; without marginal squamules and vegetative diaspores. Apothecia small (up to 0.7 mm), biatorine, orange-red, C+ purple (except in grey apothecial variants). Ascospores c. 14–17 × 7.5–10.5 μm, with septa c. 4.0–7.5 μm wide. Pycnidia grey, conidia ellipsoid. Chemosyndrome C2 (details above).

Variability. Apart from two variants of apothecia (with/without anthraquinones), the species varies strongly in the content of Sedifolia-grey in the thallus. While northern specimens from Great Britain have a pale grey thallus with an indistinct amount of the grey pigment, specimens from the Mediterranean regions have a darker thallus, with an observable Sedifolia-grey content in sections.

Similar species. Caloplaca atroflava (Turner) Mong. differs in its paler and C– apothecia (absence of chlorinated anthraquinones). Caloplaca crenularia (With.) J. R. Laundon and related species have red pycnidial walls (with anthraquinones) and chemosyndrome C3 or C4 (with constant content of parietin and fragilin). Caloplaca fuscoatraoides J. Steiner differs in its thick thallus (100–350 μm high), usual presence of marginal squamules (up to 2 mm diam.) and chemosyndrome C5 (with stable content of fragilin and absence of citreorosein and emodinal), while Caloplaca xerica Poelt & Vězda has a blastidiate-isidiate-lobulate thallus, ±zeorine apothecia and chemosyndrome C5 (as in C. fuscoatraoides).

Chemosyndrome C2 (absence of parietin and fragilin and traces of citreorosein and emodinal) has been observed only in Caloplaca ligustica B. de Lésd. (Søchting 2001), which is probably unrelated to the new species having narrow ascospores with thin septa (Bouly de Lésdain 1936) similar to C. subpallida s. lat.

Phylogeny. Most sequences of the new species examined form a well-supported monophylum (PP = 1.00), but two sequences (not identical) of one Greek specimen with grey apothecia are somewhat different, and their relationship to the core clade is unsupported. Based on phenotype similarities, we decided to place the Greek specimen (containing also individuals with red apothecia) into Caloplaca neotaurica. The nearest related species may be a morphologically different C. xerica, but the relationship is unsupported.
Ecology and distribution. The species occurs on siliceous cliffs, outcrops or stones not far from the sea coast and is only rarely observed further inland (only in the Peloponnese and in the Rhodopes, Bulgaria). It is distributed in the Mediterranean and the Black Sea coast (especially in the Crimean Peninsula) and along the Atlantic coast of Europe (confirmed from Great Britain). Phenotypes with grey apothecia are known only from the Crimean Peninsula, Cyprus and Greece.


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