

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

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

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

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

### **Introduction**

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

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

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

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

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

J. Vondrák (corresponding author) and J. Šoun: Department of Botany, Faculty of Science, University of South Bohemia, Branišovská 31, CZ-370 05, České Budějovice, Czech Republic. Email: j.vondrak@seznam.cz

M. Z. Søgaard and U. Søchting: Section on Ecology and Evolution, Department of Biology, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen Ø, Denmark.

U. Arup: Botanical Museum, Lund University, Östra Vallgatan 18, SE-223 61 Lund, Sweden.

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

### *Caloplaca scythica* Khodosovtsev & Søchting

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

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

## Materials and Methods

### Specimens studied

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

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

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

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

### DNA extraction and amplification

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

### Phylogenetic analysis

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

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

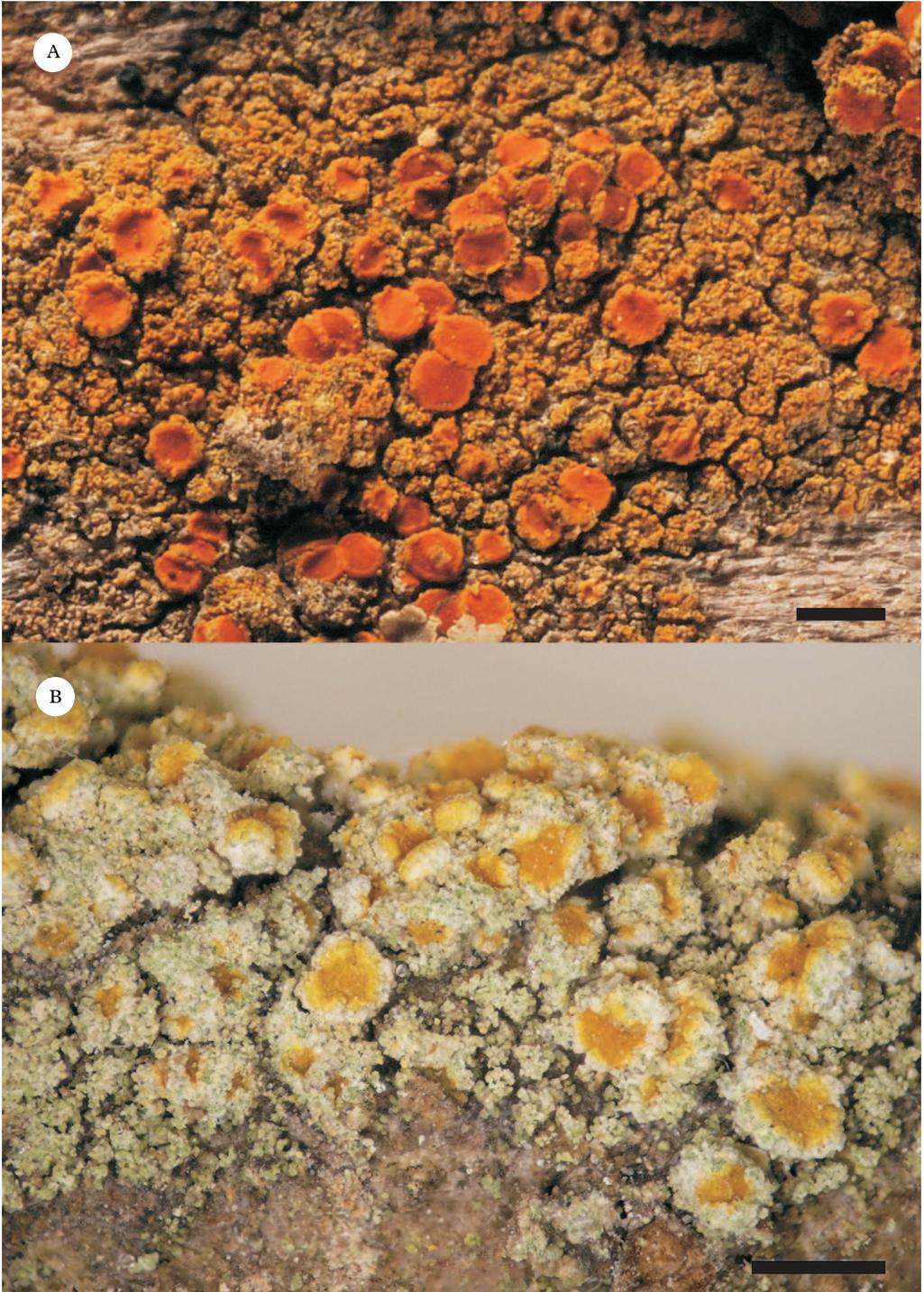


FIG. 1. *Caloplaca phlogina*, thallus and apothecia. A, *C. phlogina* type with anthraquinones in the thallus [Denmark, Nekselø, *Søchting* 11263 (C)]; B, *C. scythica* type (Russia, Black Sea coast, *CBFS* *ŹV7059*). Scales: A & B = 1 mm.

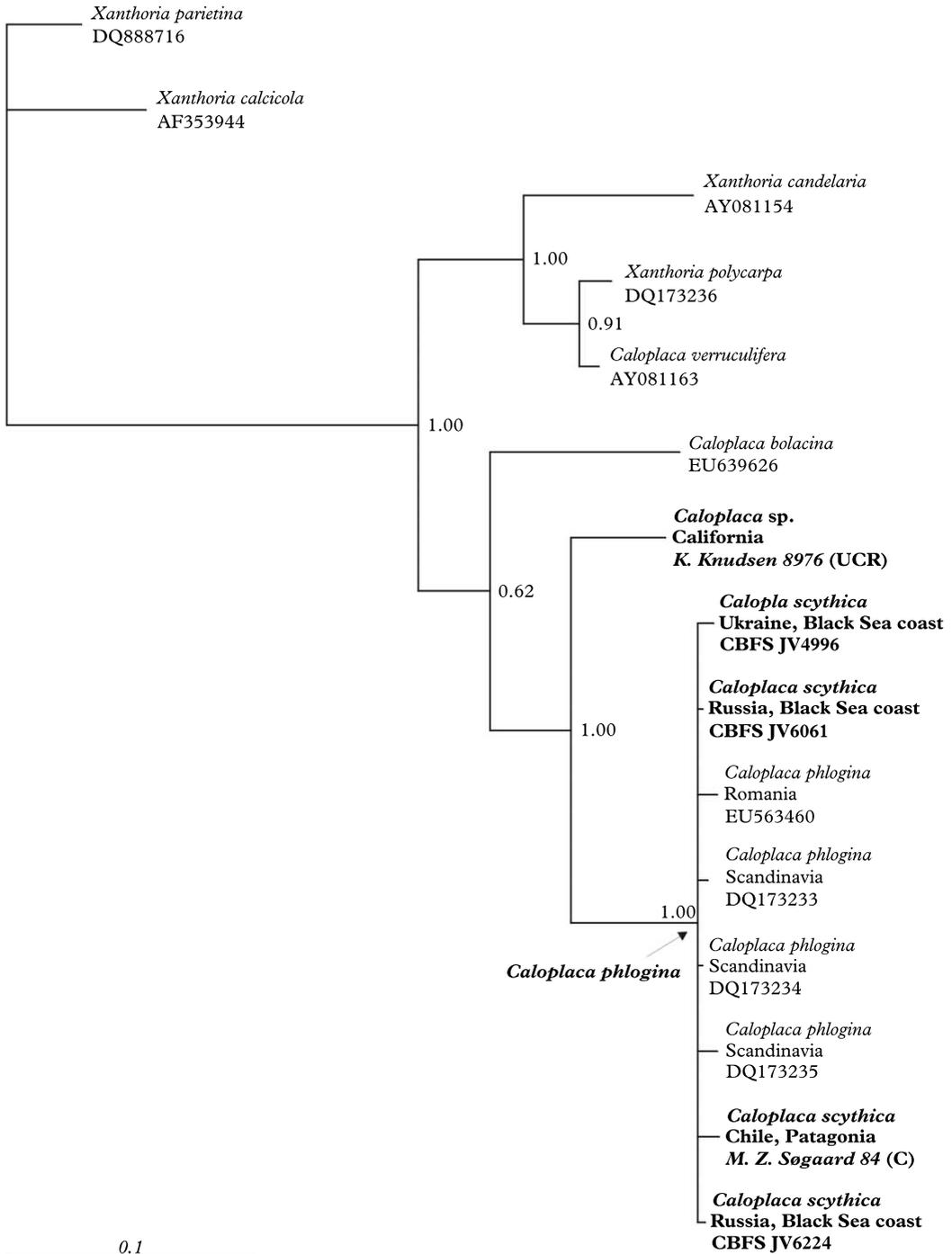


FIG. 2. Bayesian consensus phylogeny of the clade including *Caloplaca phlogina*. Node support values are Bayesian posterior probabilities. Newly obtained ITS sequences are in bold.

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

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

## Results

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

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

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

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

## Discussion

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

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

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

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

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

\*no. of measurements

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

### Founder effects in distant populations

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

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

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