Folia Cryptog. Estonica, Fasc. 47: 97–99 (2010)

Some sterile *Caloplaca* crusts identified by molecular data from the Leningrad region (Russia)

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Abstract: Four samples of sterile *Caloplaca* crusts (*Teloschistaceae*, lichenized fungi) were determined on the basis of their ITS nrDNA sequences. The samples, collected in NW Russia, mainly from Kotlin Island, Baltic Sea, belong to three species, *C. dichroa*, *C. obscurella* and *C. phlogina*, the first and last species being new to north-western European Russia and to Leningrad region.

Kokkuvõte: Mõned steriilsed kuldsamblikud (perekond *Caloplaca*) Leningradi regioonist määratud molekulaarsete andmete abil

Neli steriilset kuldsambliku (sugukond *Teloschistaceae*, lihheniseerunud seened) eksemplari identifitseeriti nrDNA ITS-i sekventside alusel. Loode-Venemaalt, peamiselt Kotlini saarelt kogutud eksemplarid osutusid liikideks *Caloplaca dichroa*, *C. obscurella* ja *C. phlogina;* neist esimene ja viimane on uued liigid Loode-Venemaale ja Leningradi regioonile.

INTRODUCTION

Identifying sterile crusts is a problem for most lichenologists dealing with lichen biodiversity. Although some major works focus on sterile crusts (e.g. Tønsberg, 1992; Fryday & Coppins, 1997), their identification remains problematic. The predominantly crustose genus *Caloplaca* (*Teloschistaceae*) is a good example, where some lineages (phylogenetic species) may be phenotypically indistinguishable, especially when sterile; this genus contains vegetatively reproducing, sorediate/blastidiate lineages which tend to be sterile (e.g. in *Caloplaca citrina* group; Vondrák et al., 2009a).

According to molecular data (ITS nrDNA) it was possible to identify four samples of sterile sorediate *Caloplaca* crusts (Tab. 1) from the Leningrad region, Russia. These specimens could not be determined by phenotypic characters with certainty.

METHODS

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). The most similar known sequences were found using the BLAST search in the GenBank. New sequences were aligned along with similar GenBank sequences using BioEdit.

RESULTS

Caloplaca dichroa Arup

When fertile, this species usually has thick-walled ascospores ("hour-glass type", see Arup, 2006) as a diagnostic character; it also occurs in two colour variants, orange vs. yellow thallus (Arup, 2006). However, when it is sterile, it can be present in the yellow colour variant only and then it may be easily confused with other sorediate species, e.g. *C. citrina* (Hoffm.) Th. Fr., *C. limonia* Nimis & Poelt and *C. phlogina* (Ach.) Flag. The species occurs throughout Europe and has only once been recorded from Russia,

Table 1. Voucher specimens and GenBank accession numbers of their ITS sequences

Species	Collection data	GenBank Accession Number
Caloplaca dichroa	Russia, Leningrad Region, Saint-Petersburg, Kronstadt District, NW part of Kotlin Island, at fort Shantz, 60°01'31"N, 29°40'24"E, fort wall, on concrete, 21.07.2007, L. Konoreva & I. Stepanchikova (LE L7398, previous identification – *Caloplaca citrina*)	GU942738
Caloplaca obscurella	Russia, Leningrad Region, Saint-Petersburg, Kronstadt District, NW part of Kotlin Island, at fort Shantz, 60°01'39"N, 29°40'15"E, seashore, bark of old *Salix* sp., 05.07.2007, E. Kuznetsova & I. Stepanchikova (LE L7399)	GU942737
Caloplaca phlogina	Russia, Leningrad Region, Saint-Petersburg, Kronstadt District, NW part of Kotlin Island, at fort Shantz, 60°01'31"N, 29°40'24"E, ruin in a meadow, on concrete near seashore, 12.07.2007, L. Konoreva & I. Stepanchikova (LE L7400, previous identification – *Caloplaca chrysodeta*)	GU942736
Caloplaca phlogina	Russia, Leningrad Region, Lodejnopolsky District, Nizhnesvirsky State Reserve, c. 2 km NW of Lakhta village, 60°40'41"N, 33°01'05"E, pine forest, remains of small building, on brick, 23.08.2001, E. Kuznetsova & D. Himelbrant (LE L7401, previous identification – *Caloplaca chrysodeta*)	GU942735

Azov Sea coast (Vondrák et al., 2009a). It is new to north-western European Russia and the Leningrad region.

The ITS sequence of the sample from the Leningrad region (Kotlin Island in the Baltic Sea) was aligned with nine *C. dichroa* sequences from the GenBank; alignment of 477 base pairs was variable in 19 nucleotide positions, but two sequences from Sweden (DQ173230 & DQ173232) were identical with our sequence. After the addition of a sequence of the closely related *C. limonia*, the number of variable positions increased to 40. Morphologically, the specimen from the Leningrad region agrees well with yellow thallus variants in other European populations.

Caloplaca obscurella (Körb.) Th. Fr.

Fertile material of this species is characterized by the brown apothecia devoid of anthraquinones, but commonly sterile thalli may be confused with *C. ulcerosa* Coppins & P. James which also has crater-like soralia. *C. ulcerosa* tends to have paler areoles (whitish) than *C. obscurella* (greyish), and *C. ulcerosa* is typically maritime (Vondrák et al., 2009b), while *C. obscurella* also occurs inland. Both species are extremely unrelated thus their thallus morphology is an evidence for convergent evolution.

The material from the Leningrad region (Kotlin Island in the Baltic Sea) was sterile with pale areoles and crater-like soralia, and it was collected in maritime conditions. Thus we wavered in determination between both mentioned species. We aligned our sequence with two *C. obscurella* sequences from the GenBank, both from Italian material; in 525 base pairs, our sequence differed in two positions from AY313977 and in 12 positions from AY313976. *C. obscurella* forms a remote clade within the genus and no closely related sequences are available in GenBank. It is possible that the *C. obscurella* clade consists of more species; e.g. *C. sarcopisoides* (Körb.) Zahlbr. discussed by Arvidsson & Martinsson (1993) requires further investigation.

Caloplaca phlogina (Ach.) Flag.

The species, even when fertile, is sometimes indistinguishable from *C. citrina* and can be confused with other yellow sorediate *Caloplaca* crusts. Although it has the *C. citrina* morphology, it is phylogenetically far from *C. citrina* clade (Arup, 2006). Distribution of *C. phlogina* is confirmed by ITS data from coastal Europe, Canada and Chile (Vondrák et al., 2010) and it is also known from the Russian Black Sea coast

(Vondrák et al., 2009a). It is new to north-western European Russia and the Leningrad region.

Our two samples from the Leningrad region (Kotlin Island in the Baltic Sea and the Nizhnesvirsky State Reserve) were aligned with three Scandinavian *C. phlogina* sequences; alignment of 535 base pairs was variable in five positions only. After the addition of the closest known relative sequence to *C. phlogina* (a *Caloplaca pyracea*-like species, GU080301), the number of variable positions increased to 36. Specimens from the Leningrad region are finely sorediate (soredia c. 20–40 µm in diam.) but they fall within the variability of *C. phlogina* (Vondrák et al., 2010).

ACKNOWLEDGEMENT

Prof. Mark Seaward kindly provided linguistic corrections.

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