# Three species of the *Cladonia pyxidata-chlorophaea* complex new to Latvia

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**Abstract:** The study of the *Cladonia pyxidata-chlorophaea* complex in Latvia is based on the revision of 276 specimens from the two largest lichen herbarium collections in Latvia. Nine species belonging to the *Cladonia pyxidata-chlorophaea* complex were determined using TLC and morphological studies – *C. chlorophaea*, *C. cryptochlorophaea*, *C. fimbriata*, *C. grayi*, *C. humilis*, *C. merochlorophaea*, *C. novochlorophaea*, *C. pocillum* and *C. pyxidata*. Of these, three species are reported as new to Latvia – *C. cryptochlorophaea*, *C. merochlorophaea*, *C. merochlorophaea*, and *C. novochlorophaea*. Information regarding the morphology, secondary chemistry, ecology, and localities of the newly reported species is provided.

Keywords: Cladoniaceae, lichens, thin layer chromatography

#### INTRODUCTION

The estimated number of lichen and allied fungi species is 690 that have been identified in Latvia by 2021. In the last ten years, more than 100 lichen species have been reported as new in Latvia (Abolina et al., 2015; Moisejevs, 2015, 2017, 2022; Motiejūnaitė et al., 2016; Moisejevs & Degtjarenko, 2017; Moisejevs et al., 2019; Degtjarenko & Moisejevs, 2020; Yatsyna et al., 2021). This lack of knowledge on local lichen biota can be mainly explained by the low number of active lichenologists in Latvia (Moisejevs, 2022). Most lichen taxa have never been studied using thin layer chromatography (TLC) in Latvia, including the complex Cladonia pyxidatachlorophaea. Studies conducted in neighbouring countries and in Hungary demonstrated that this complex includes a larger number of species that are already known in Latvia (Kowalewska et al., 2008; Tsurykau & Golubkov, 2015; Matwiejuk, 2017; Farkas et al., 2023).

The *Cladonia pyxidata-chlorophaea* complex is represented by morphologically and chemically similar taxa, characterized by squamulose primary thallus, scyphose podetia, and brown apothecia (Ahti et al., 2013). At least 14 chemotypes have been identified in this complex, comprising about 35 secondary metabolites (Culberson et al., 1985). In Latvia lichens of *Cladonia pyxidata-chlorophaea* complex typically grow in sun-exposed, well-lighted habitats, like dry heathlands, sand dunes, peat bogs, and forests on mossy granite rocks, tree bases and deadwood. They can also be found in humanaffected and anthropogenic habitats like clearcuts, roadsides, quarries, etc. (Åboliņa et al., 2015). The determination of species within the *Cladonia pyxidata-chlorophaea* complex in Latvia was previously conducted using spot test reactions and microscopic examinations of specimens. The present study aims to obtain new data about the *Cladonia pyxidatachlorophaea* complex based on available herbarium materials and standardized TLC.

#### MATERIALS AND METHODS

This study was carried out using herbarium material collected in Latvia from 1953 to 2023. In total, 276 samples were analyzed, representing all regions of Latvia and a wide range of habitats (e.g., bogs, meadows, forests, clear-cuts, dunes, sandstone outcrops, boulders, etc.), and substrate types (e.g., peat, soil, sand, deadwood, bryophytes, tree bark, rubber, etc.). The materials from the two largest herbarium collections were examined: the Herbarium of the University of Latvia (RIG) and the Daugavpils University lichen herbarium collection (DAU). In addition, specimens collected by the first author (AK) throughout the territory of Latvia in 2023 were included in this study. The collected specimens are deposited in DAU. The morphology of the Cladonia pyxidata-chlorophaea complex was studied using Nikon SMZ745T stereomicroscope, and lichen substances were identified by TLC in solvent system C (Orange et al., 2001).

## **RESULTS & DISCUSSION**

Of the 276 analyzed specimens, nine species were identified in this study: *Cladonia chlorophaea*, *C. cryptochlorophaea*, *C. fimbriata*, *C. grayi*, *C. humilis*, *C. merochlorophaea*, *C. novochlorophaea*, *C. pocillum* and *C. pyxidata*. Of these three species, *C. cryptochlorophaea*, *C. merochlorophaea* and *C. novochlorophaea*, are reported as new for Latvia. However, two species of the *Cladonia pyxidata-chlorophaea* complex (*C. asahinae* and *C. conista*) are still not detected in Latvia, even though they are known in neighboring Belarus, Lithuania, and Poland (Tsurykau & Golubkov, 2015; Motiejūnaitė, 2017; Matwiejuk, 2017).

### New species for Latvia

#### CLADONIA CRYPTOCHLOROPHAEA Asahina

The primary thallus squamulous, which are persistent and mid-sized (1-3 mm). Squamules thin and with a greenish-grey color on top and white underneath. Podetia up to 1.4 cm tall, greenish-grey, with regular scyphi up to 5 mm wide, with proliferations, including granular soredia that are up to 80 µm thick, phyllidia, and microsquamules. Macrosquamules absent. Apothecia not seen, dark pycnidia on margins of scyphi.

Cryptochlorophaeic, paludosic, and fumarprotocetraric acids have been detected in sampled material. Species is morphologically similar to *C. grayi, C. merochlorophaea* and *C. novochlorophaea*. However, *C. cryptochlorophaea* contains cryptochlorophaeic and paludosic acids.

Records represent terricolous, corticolous and saxicolous specimens. All specimens were found in open, sun-exposed habitats. Two specimens were collected in pine forests, while one was found in a raised bog. The species is already known to occur in neighboring Estonia (Randlane et al., 2023), Lithuania (Motiejūnaitė, 2017), and Belarus (Tsurykau & Golubkov, 2015).

Specimens examined: Krāslavas region, Šķeltovas parish, 55°55'N, 27°0'E, dry pine forest, on *Pinus sylvestris*, 01.05.2023, leg. AK. (DAU); Krāslavas region, Šķeltovas parish, 55°55'N, 27°0'E, dry pine forest, on soil, 01.05.2023, leg. AK (DAU); Valmieras region, Dīkļu parish, 57°24'N, 24°56'E, raised bog, on pine stump, 23.06.2022, leg. M. Kalniņš (DAU).

#### CLADONIA MEROCHLOROPHAEA Asahina

Primary thallus squamulose, persistent, the squamules are thin, small, ascending, 1–2 mm long, 1–3 mm wide. Squamules are greenish grey above, white but turning dark brown near the attachment point beneath. Soredia absent. Podetia brownish grey, strongly melanotic at base, without squamules, up to 2.7 cm tall. Surface of podetia at first corticate-areolate, soon becoming verruculose, the warts giving rise to rough, up to 80 µm wide granules, schizidia, phyllidia and microsquamules present. True soredia on podetia absent. Apothecia and pycnidia not seen.

Merochlorophaeic, 4-O-methylcryptochlorophaeic acids and fumarprotocetraric acids have been detected in all studied material. Similar to *Cladonia cryptochlorophaea* and *C. grayi*, but greenish brown with relatively large cups, and producing merochlorophaeic and 4-O-methylcryptochlorophaeic acids.

The records represent terricolous and saxicolous specimens. In Latvia, *C. merochlorophaea* was found in various sun-exposed habitat: mead-ows, cut-over sites, roadsides, as well as pine forests. Three specimens were found on dead-wood – on coniferous tree stamps, on deadwood of conifer, and old well-decayed planks. Six specimens were found on the soil. Species is already known in neighboring Estonia (Randlane et al., 2023), Lithuania (Motiejūnaitė, 2017), Belarus (Tsurykau & Golubkov, 2015), and Russia (Himelbrant et al., 2017).

Specimens examined: Ventspils region, Usmas parish, strict nature reserve "Moricsala", 57°11'N, 22°08' E, on stump, 04.08.2023, leg. AK (DAU); Ventspils region, Usmas parish, strict nature reserve "Moricsala", 57°36'N, 21°19'E, on a log in the forest, 04.08.2023, leg. AK (DAU); Ventspils region, Usmas parish, strict nature reserve "Moricsala", 57°36'N, 21°19'E, near the house on well-decayed planks, 03.08.2023, leg. AK (DAU); Augšdaugavas region, Liksnas parish, 55°57'N, 26°28'E, cut-over site, on sun exposed soil, 27.04.2023, leg. AK (DAU); Krāslavas region, Ūdrišu parish, 55°57'N, 27°0'E, young pine forest on soil, 21.05.2023, leg. AK (DAU); Augšdaugavas region, Skrudalienas parish, nature park "Silene", on soil in a dry meadow, 07.07.2010, leg. I. Gavarāne (DAU); Dobeles region, Tērvetes "Kalna muižas sils", 09.09.2071, leg. A. Pīterāns (RIG LATV6378); Talsu region, Kolkas parish, roadside near study center of University of Latvia behind dune 25.07.1993, leg. A. Pīterāns (RIG LATV12653).

#### CLADONIA NOVOCHLOROPHAEA (Sipman) Brodo & Ahti

The primary thallus is squamulose, persistent, with thin, small, esorediate squamules that are 1–2 mm long and 1–3 mm wide. Podetia up to 2 cm tall, brownish (dark), with scyphi up to 0.8 cm wide, simple to proliferating from margins, surface of podetia and outer part of the scyphi corticate, squamulose, densely verrucose, inner part of the scyphi eroded, covered with cortical plates and schizidia, soredioid granules scares to absent. Apothecia dark brown, stalked, and up to 3 mm wide. Pycnidia are present on the scyphal margin.

Homosekikaic and sekikaic acids were detected. One specimen also contained fumarprotocetraric acid. Species similar to *C. merochlorophaea* and *C. homosekikaica.* However, *C. novochlorophaea* have very eroded scyphal margins, which reveal a darkened medulla and presence of homosekikaic and sekikaic acids.

Records represent terricolous, corticolous, and saxicolous specimens. In Latvia, *C. novochlorophaea* was found in sun-exposed habitats, on soil in dunes and young forests, on conifer deadwood, and pine bark within pine forests. Species is already known in neighboring Estonia (Randlane et al., 2023), Lithuania (Motiejūnaitė, 2017), and Belarus (Tsurykau & Golubkov, 2015).

Specimens examined: Limbažu region, Liepupes parish, 57°33'N, 24°26'E, young dry pine forest, on pine bark, 27.04.2023, leg. AK (DAU); Augšdaugavas region, Līksnas parish, 55°57'N, 26°28'E, sun exposed cut-over site, on soil, 27.04.2023, leg. AK (DAU); Jēkabpils region, Rubenes parish, nature preserve "Tīreļu purvs" 59°38'N, 29°18'E, bog woodland with pines (EU protected habitat 91D0\*), on pine stamp, 20.10.2023, leg. AK (DAU); Krāslavas region, Ūdrišu parish, 55°55'N, 26°59'E, dry pine forest, on root plate, 01.05.2023, leg. AK (DAU); Liepājas region, Nīcas parish, on sandy soils in dunes, 09.10.1994, leg. A. Pīterāns (RIG LATV12421); Limbažu region, Liepupes parish, 57°33'N, 24°26'E, young dry pine forest, on pine bark, 27.04.2023, leg. AK (DAU); Augšdaugavas region, Naujenes parish, 55°52'N, 26°45'E, in forest on deadwood, 16.07.2017, leg. A. Petrova (DAU).

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