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Chemistry and morphology of *Chrysothrix candelaris* in Poland, with notes on the taxonomy of *C. xanthina*

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Abstract — The chemical and morphological variation of the lichen *Chrysothrix candelaris* s.l. as well as habitat requirements and distribution were studied in Poland. The species was found to be chemically variable, but as the chemotypes do not differ in granule size, habitat preferences, or distribution, they are treated as representing one chemically variable taxon. The size of the granules in Polish specimens is contrary to the previous reports for *C. candalaris,* but the measurements are very similar to those for *C. xanthina*, which differs only in distribution and habitat and is treated as a probable semi-cryptic species.

Key words — secondary chemistry, *Arthoniales*

Introduction

As recently circumscribed, *Chrysothrix candelaris* (L.) J.R. Laundon (Laundon 1981) is an epiphytic (rarely saxicolous) lichen occurring in Europe and characterized by a leprose, yellow, orange yellow, or green yellow thallus consisting of ecorticate granules and the presence of calycin (Kalb 2001, Harris & Ladd 2008, Kukwa & Knudsen 2011). According to Kalb (2001) the soredium-like granules (functioning as vegetative propagules) are large, 75–200 µm in diam., but Harris & Ladd (2008) reported them to be much smaller, 50–75 µm in diam.

The species is very similar to *C. xanthina* (Vain.) Kalb (Kalb 2001), which was resurrected from the synonymy of *C. candelaris* s.l., but differs in the production of pinastric acid, granules measuring 20–50 µm in diam. and occurrence in tropical and subtropical world regions and temperate North America (Kalb 2001, Harris & Ladd 2008, Kukwa & Knudsen 2011). Although both taxa appear easily distinguishable, some chemically deviating populations exist in the ranges of *C. candelaris* and *C. xanthina,* and these complicate delimitation

of both species. Kalb (2001) noted some European populations containing pinastric acid, therefore chemically similar to *C. xanthina* but morphologically indistinguishable from *C. candelaris*. At first they were considered as probably yet another species (Kalb 2001), but Harris & Ladd (2008) suggested that the material could represent a chemotype of *C. candelaris*. Also within the range of *C. xanthina* is material containing calycin and thus chemically identical to *C. candelaris* but with the granule diameter characteristic for *C. xanthina* (Kalb 2001). Laundon (1981) and Tønsberg (1992) also reported an additional chemotype of *C. candelaris* s.l. from Europe containing both calycin and pinastric acid, but that chemotype was not studied by Kalb (2001) or Harris & Ladd (2008).

Neither Kalb (2001) nor other authors provided studies of *C. candelaris* s.l. chemotypes occurring in Europe in terms of morphological variation, and the problem as to whether the European material represents one chemically variable taxon or a group of species remained unresolved. Although *C. candelaris* s.l. has been rather commonly reported in Poland (Fałtynowicz 2003), its chemistry and morphological variation have never been studied. As we had access to many specimens of this lichen, we studied whether Polish chemotypes differed in granule size, distribution, and habitat preferences to determine if Polish material of *C. candelaris* s.l. represented one taxon. This is particularly important as the species is listed as critically endangered to extinction (Cieśliński et al. 2006).

Material & methods

The material upon which the research was based consisted of 129 samples of *C. candelaris* s.l., all collected in Poland and deposited in KTC, KRAM, LOD, and UGDA. The samples were chemically analyzed with standard procedures of thin layer chromatography (TLC) (Culberson & Kristinsson 1970, Orange et al. 2001). Secondary metabolites were separated in solvent C and compared on each chromatogram with control extracts of calycin, pinastric and vulpinic acids from other lichens.

In order to determine the relation between chemotypes and morphological characters, simple granules were measured(aggregations disintegrating into smaller granules were not considered). For this purpose, granules from 15 specimens were measured (five of each chemotype): 51 granules from chemotype I, 57 from chemotype II, and 37 from chemotype III (chemotype numeration follows Tønsberg 1992; see also results). The measurements were taken in water with addition of ethanol, which reduced the hydrophobic properties of lichen metabolites present in granules and made the granules more easily visible; ethanol was selected, as it did not influence granule size (checked empirically). Because the data were normally distributed and the variance was constant, potential differences in granule sizes between chemotypes were tested by ANOVA F-test and examined by box whisker plots in the STATISTICA v.9.1 (StatSoft Inc. 2010), with abbreviations used in the text: N – total number of granules measured; df – degrees of freedom; p – probability.

All examined localities are mapped according to the ATPOL system in 10 × 10 km squares (Cieśliński & Fałtynowicz 1993; see also Kukwa et al. 2002, 2010, 2012).

Results

Chemotypic variation of *Chrysothrix candelaris* in Poland

Three chemotypes of *C. candelaris* s.l. were found in Poland, the first (I) with pinastric acid as major substance; the second (II) with calycin; and the third (III) containing both calycin and pinastric acid. In chemotypes I and II vulpinic acid was rarely also detected in minor or trace amounts. In our study we found additional variation within chemotype III; one of the substances, calycin or pinastric acid, was more concentrated on TLC plates than the other. That led us to suspect that samples representing chemotype III were only mixed collections of chemotypes I and II. Therefore, we analyzed several parts of thalli occurring on the same piece of substrate to detect if there were chemotypes I and II on the same bark piece, but we always got the same combination of substance concentrations on the TLC plates from all parts of the same specimen. This confirms that chemotype III is not a mechanical "hybrid" formed due to the co-occurrence of individuals containing pinastric acid (chemotype I) and calycin (chemotype II), but a naturally occurring chemotype producing both chemicals.

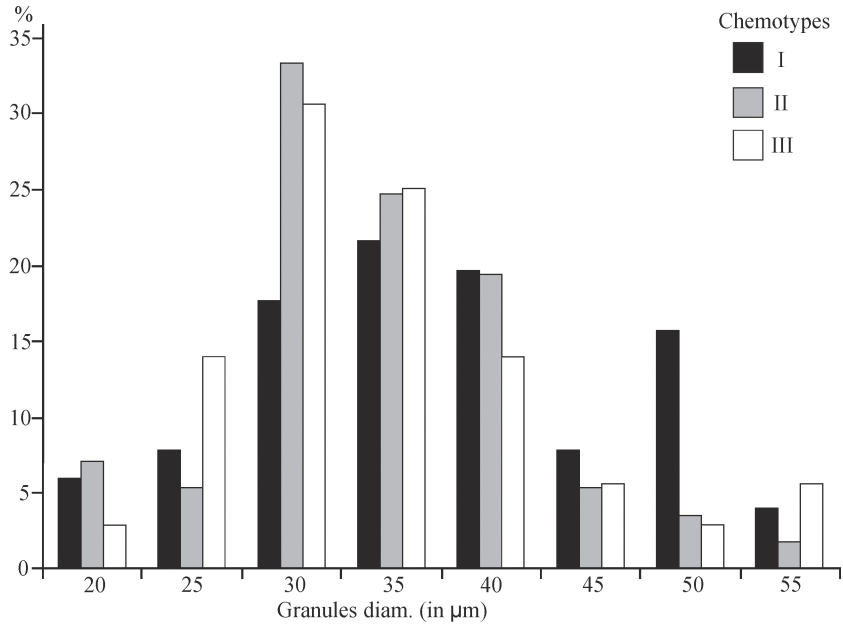

Plate 1. *Chrysothrix candelaris*. Percentage of granule diameter for chemotypes in Poland.

Morphological variation of *Chrysothrix candelaris* in Poland

The granules in 14 samples (including all three chemotypes of *C. candelaris*) were mostly 30–40 µm in diameter with only a few smaller and larger (Plate 1). Only in one sample of chemotype I were several larger granules (55 µm diam.) found. The differences in sizes of granules diameters were not statistically significant between all chemotypes (Anova, F = 2.33, N = 144, df = 2, p = 0.10). Granules in chemotypes II and III were almost of the same average size (34.12 µm versus 34.44 µm), whereas a slightly higher average value was found for chemotype I (37.35 µm) (Plate 2).

Distribution and habitat requirements of *Chrysothrix candelaris* in Poland

The three chemotypes differ in frequencies in Poland: chemotype I is the most frequent (94 samples), whereas II (20 samples) and III (15 samples) are

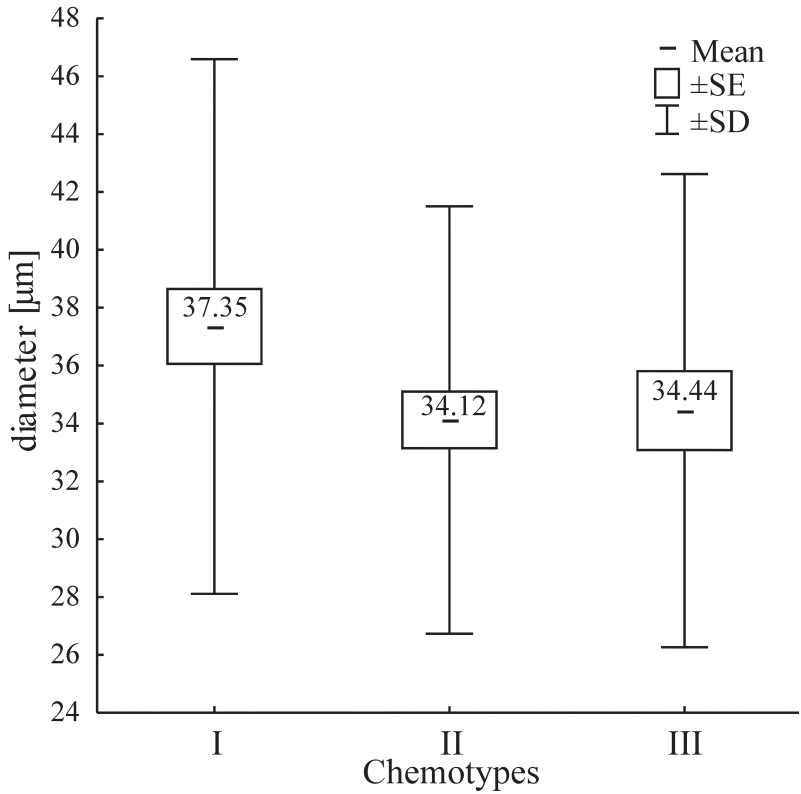

Plate 2. *Chrysothrix candelaris*. Box whisker plot of differences between granule size of chemotypes. Abbreviations: SD – standard deviation, SE – standard error.

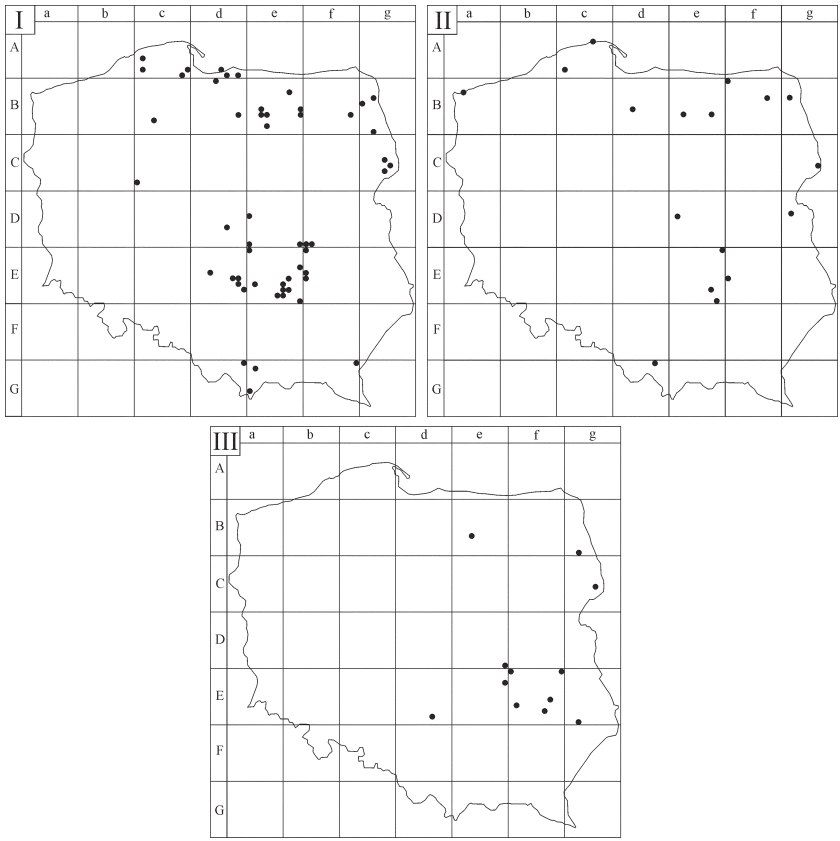

Plate 3. *Chrysothrix candelaris.* Distribution of chemotypes in Poland.

less frequent. Their distributions greatly overlap (Plate 3), and sometimes two different chemotypes were recorded in the same locality (but not growing side-by-side). Only chemotype III is more restricted in distribution, being absent from western Poland.

All chemotypes were most frequently collected on the rough bark of oaks (mostly *Quercus robur*), very often in shady old-growth broad-leafed forests, more rarely on other deciduous trees, conifers (two specimens), and wood (two specimens), and apparently do not differ in habitat preferences.

Discussion

The chemistry of the material referred to as *C. candelaris* in Poland is rather complex and our results are consistent with those of Laundon (1981) and Tønsberg (1992) and also partly of Kalb (2001) and Harris & Ladd (2008).

However, we found an additional, previously unrecognized variation within the third chemotype in which the ratio of the concentration of calycin and pinastric acid varied in different samples. Actually, all specimens can be organized to form a continuum with specimens in which only pinastric acid was found by TLC on one side, those with only calycin detectable on the opposite and samples with both substances in different concentrations in between. Therefore, the boundaries between all chemotypes seem to be less obvious than previously reported. The absence of calycin in chemotype I and pinastric acid in chemotype II can most probably be attributed to the method used for the analyses, and TLC may not be sensitive enough to detect traces of those substances.

When our results of the granule measurements are compared with previous *C. candelaris* treatments, one can, however, see significant discrepancies. Much smaller granules were reported by Tønsberg (1992; (6–)12–25(–30) µm diam.) but much larger by Kalb (2001; 75–200 µm diam.) and Harris & Ladd (2008; 50–75 µm diam.). It is possible that the variation, at least in part, can be due to interaction with environmental factors prevailing in different geographical regions and habitats. To some degree, the earlier reports of large granules were due to the misinterpretation of aggregations as simple granules, as already pointed out by Kukwa & Knudsen (2011).

Kalb (2001) and Harris & Ladd (2008) suggested that *C. candelaris* might consist of more than one species. After studying the material of *C. candelaris* in Poland, we assert that it represents a morphologically uniform entity that is chemically variable. Also the distribution patterns and habitats in Poland do not differ between chemotypes. Under those circumstances, we do not agree that *C. candelaris* is a complex of taxa and that European material containing pinastric acid can represent separate species for which *Lepra citrina* Schaer. is the oldest available name, as was discussed by Harris & Ladd (2008) and Kukwa & Knudsen (2011).

On the other hand, the size of granules in Polish material of *C. candelaris* is consistent with data for *C. xanthina* (Harris & Ladd 2008, Kukwa & Knudsen 2011), which then is hardly distinguishable based on morphology and chemistry; as pointed out above, *C. xanthina* contains pinastric acid, but *C. candelaris* may produce that substance as well. One could regard them as synonymous as proposed by Laundon (1981). However, as *C. xanthina* differs in habitat preferences (e.g., smooth bark and fence-posts exposed to sun and rain, often eutrophicated habitats, burned oak woodlands and chaparral, redwood forests, bishop pine forests vs. *C. candelaris* usually on rough bark, rarely wood and rocks, in humid conditions) and is found in the tropics, subtropics, and temperate North America (vs. *C. candelaris* only in Europe; Kalb 2001, Harris & Ladd 2008, Kukwa & Knudsen 2011), *C. candelaris* and *C. xanthina* could be

considered semi-cryptic species, as Vondrák et al. (2009) proposed for some morphologically indistinguishable *Caloplaca* species with different distribution ranges and ecology. We prefer this solution, as there would be a great loss of information if the two taxa are prematurely and unjustifiably united. We therefore propose to keep them separate until more data from all continents, including molecular study, become available.

Selected specimens examined —

Chemotype I. **POLAND. Świętokrzyskie Mts:** Świętokrzyski National Park, Chełmowa Góra, ATPOL Ee–77, on *Abies alba,* 1965, S. Cieśliński (KTC); **Knyszyńska Forest.** Budzisk nature reserve, ATPOL Cg–02, on *Acer platanoides,* 1999, K. Czyzewska et al. (KTC); **Bielska Plain:** Białowieska Forest, forest inspectorate Hajnówka, forest section no. 602D, Myśliszcze range, ATPOL Cg–64, on *Quercus robur,* 1982, S. Cieśliński, Z. Tobolewski (KTC); **Radomska Plain:** Kozienicka Forest, Zagożdżon nature reserve, ATPOL De–99, on *Quercus robur,* 1968, S. Cieśliński (KTC); 0.75 km N of cross roads in Załamanek, ATPOL Df–91, on *Quercus robur,* 1978, A. Anusiewicz (KTC).

Chemotype II. **POLAND. Kraina Wielkich Jezior Mazurskich:** Mokre nature reserve, by Mamry lake, ATPOL Bf–00, on *Fraxinus excelsior,* 1988, S. Cieśliński (KTC); **Szydłowskie Foothills:** 4 km NW of Bogoria, Mostki forest district, forest section no. 48, Ee–98, on *Quercus robur,* 1986, M. Chyb, K. Toborowicz (KTC); **Kozienicka Forest:** Ponty-Dęby nature reserve, ATPOL Ee–09, on *Quercus robur,* 2003, S. Cieśliński (KTC); **Augustowska Plain:** Augustowska Forest, Perkuć, ATPOL Bg–31, on *Quercus robur,* 1986, S. Cieśliński (KTC); **Mazurska Plain:** Kruklanka range, by road from Marksewo to Babięta, ATPOL Be–67, on *Fraxinus excelsior,* 1989, S. Cieśliński (KTC).

Chemotype III. **POLAND. Bielska Plain:** Białowieska Forest, Białowieża forest inspectorate, forest section no. 396C, ATPOL Cg–55, on *Quercus robur,* 1983, S. Cieśliński, Z. Tobolewski (KTC); **Kozienicka Forest:** Ponty-Dęby nature reserve, ATPOL Ee–09, on wood, 2003, S. Cieśliński (KTC); **Radomska Plain:** Kozienicka Forest, Zagożdżon nature reserve, ATPOL De–99, on *Quercus robur,* 1968, S. Cieśliński (KTC).

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