The lichen genus *Pertusaria* in Poland I. *P. multipuncta* and *P. ophthalmiza*

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The paper presents results of study on two morphologically very similar lichens in Poland, *P. multipuncta* (Turner) Nyl. and *P. ophthalmiza* (Nyl.) Nyl. (Pertusariales, Ascomycota). Previously, specimens were determined using only thallus characters and spot test reaction, with no data on lichen substances. This led to several misidentifications. After the revision of all available material of *P. multipuncta* from Poland it appeared to be reported correctly only from one locality in Gorce Mts. Most of other specimens belong to *P. ophthalmiza*, which has not been recorded in Polish lichenological literature until 2008.

Key words: Pertusariales, chemotaxonomy, neglected lichens, distribution

INTRODUCTION

In Poland 40 taxa of the genus *Pertusaria* DC. have been reported so far (see Hanko 1983; Fałtynowicz 2003; Kossowska 2008). Some species were considered to be rather common (e.g., *P. albescens* (Huds.) M. Choisy & Werner, *P. amara* (Ach.) Nyl.), but some were very rarely reported (e.g., *P. melanochlora* (DC.) Nyl.). Taxonomic status of few species is still very poorly known (e.g., *P. inaequalis* Erichsen), and they need revision of type collections.

Up to now there has been no detailed study of the genus *Pertusaria* in Poland. Many species are often sterile, and produce only soredia or isidia; these taxa are often superficially rather similar and cause difficulties in the identification. In such cases, lichen substances play a very important role in the determination of species. Secondary chemistry is also very important in non sorediate, fertile taxa, which sometimes differ only in the chemical content (see Dibben 1980; Hanko 1983; Archer 1997; Chambers et al. 2009). However, so far only very few species have been confirmed by thin layer chromatography (TLC) in the country. Previously, specimens were determined using only thallus characters and spot test reaction, and very rarely the ascus type was studied. This could have led to many misidentifications.

Based on morphology and TLC-data, the present paper focuses on two taxa, namely *P. multipuncta* (Turner) Nyl. and *P. ophthalmiza* (Nyl.) Nyl., which are very similar in morphology, but have different chemistry. Both produce ascomata, which are covered with soredia-like granules, and thus resemble soralia. *P. multipuncta* was reported in Poland more often than *P. ophthalmiza*, as it could have been easily determined with the aid of the key by Nowak and Tobolewski (1975). Until recently *P. ophthalmiza* has been reported from Poland only by Hanko (1983), who cited single, old collection. That record was not included in the recent Polish checklist of lichens and lichenicolous fungi (see Fałtynowicz 2003), and its presence in the country has remained unnoticed until the paper by Kukwa et al. (2008). These authors reported also new Polish localities of the species.

As preliminary study suggested that the name *P. multipuncta* might have been misapplied in Poland for the material of *P. ophthalmiza*, we decided to revise all available specimens of both taxa. The aim of this paper is to present the results of study on the chemistry, morphology, habitat requirements and distribution of *P. multipuncta* and *P. ophthalmiza* in Poland. This paper is the first of a series of articles devoted to a revision of *Pertusaria* in Poland.

MATERIAL AND METHODS

The present study is based on the specimens deposited in the Polish lichen herbaria: GPN, KTC, KRAM, LBL, LOD and UGDA. The morphology of the specimens was studied using a stereomicroscope. The thickness, morphology and colour of thallus, colour, shape and size of apothecia were noted. Secondary metabolites were identified by thin layer chromatography (TLC) according to the methods of Orange et al. (2001). The chromatograms were developed in solvent C. Localities of all Polish material examined are mapped according to the ATPOL grid square system (Zając 1978; modified by Cieśliński and Fałtynowicz 1993; see also Kukwa et al. 2002).

CHARACTERISTIC OF THE GENUS PERTUSARIA

The genus *Pertusaria* (Pertusariales, Ascomycota; see Schmitt et al. 2006) includes corticolous, saxicolous, and terricolous crustose taxa, most of which do not grow on pure limestone or other strongly calcareous substrata (Fałtynowicz 2003; Chambers et al. 2009). Many species occur in dry, open habitats, but some prefer humid localities (Nowak, Tobolewski 1975; Fałtynowicz 2003; Chambers et al. 2009). The genus is cosmopolitan and its members are found from the Arctic and Antarctic to the tropics in both Hemispheres (e.g., Dibben 1980; Hanko 1983; Archer 1997; Chambers et al.

2009). Nevertheless, there are still substantial gaps in the distribution of many taxa in various regions of the world. In many cases, published data should be treated with caution, since the determination of species is often based mainly on morphological characters, but not secondary chemistry, which is an important systematic character set (e.g., Dibben 1980; Hanko 1983; Archer 1997; Chambers et al. 2009).

The taxonomy of *Pertusaria* and the delimitation from other phylogeneticaly related genera are still not satisfactorily settled. Traditionally, members of the genus *Pertusaria* are characterized by rather large (sometimes up to 4 mm in diameter) apothecia with hemiangiocarpous type of development, with open or almost closed, perithecia-like disk, hamathecium of branched and richly anastomosing, lax paraphysoids, thick-walled, amyloid asci with 1–8 spores, and thick spore wall. Thallus is crustose, moderately thick or \pm immersed, continuous to rimose-cracked, fissuredareolate or warted. In many species soralia and/or isidia are produced. In some taxa apothecial disk can be occluded with granular soredia and appearing soralium-like. Secondary lichen metabolites are very diverse in *Pertusaria*, and these can be divided into four chemosyndromes, each consisting of either xanthones, fatty acids, depsides or depsidones (e.g., Dibben 1980; Hanko 1983; Archer 1997; Chambers et al. 2009).

After the recent molecular studies by Schmitt and Lumbsch (2004), Pertusaria appeared as polyphyletic in its current circumscription and can be divided into three well supported groups, Pertusaria s.str.-group, Varicellaria-group and Variolariagroup. The first one is characterized by amyloid, 2-8-spored asci with distinctive ocular chamber, non-amyloid hymenial gel, thick or thin walled spore wall, and the presence of chlorinated xanthones, gyrophoric and planaic acids. Apothecia can be open with plane disc or closed, and then resembling perithecia. Members of the Varicellaria-group has strongly amyloid, 1-2-spored asci with no recognizable apex structures, strongly amyloid hymenial gel, \pm thick spore wall, disciform apothecia, production of lecanoric acid and absence of chlorinated xanthones. At present 3 taxa of Pertusaria are known to belong in this group, P. hemisphaerica (Flörke) Erichsen, P. lactea (L.) Arnold and P. velata (Turner) Nyl., and they are close to the monotypic genus Varicellaria Nyl. (with V. rhodocarpa (Körb.) Th. Fr.). The third, Variolariagroup, is characterized by strongly amyloid, 1-spored asci with no recognizable apex structures, weakly or not amyloid hymenial gel, thin spore wall, open apothecia, production of picrolichenic and thamnolic acids, and absence of chlorinated xanthones (Schmitt and Lumbsch 2004). However, since that time no taxonomical segregation has been proposed yet, therefore all taxa are still kept in *Pertusaria*.

RESULTS AND DISCUSSION

Pertusaria multipuncta (Turner) Nyl., Lich. Scand. (Uppsala): 179 (1861).

Syn. Variolaria multipuncta Turner, Trans. Linn. Soc. London 9: 137 (1806)

CHARACTERISTIC OF THE SPECIES. P. multipuncta is distinguished from superficially similar species by the white, well-delimited, scattered, sorediate warts containing

apothecia, 1-spored *asci* and the chemistry (physodalic acid and often protocetraric acid) (Hanko 1983; Chambers et al. 2009). *Thallus* of the species can be very variable; on sheltered, smooth bark it is often thin, continuous and even, but in some exposed habitats it is more robust and the surface is coarsely warted (Chambers et al. 2009). *Thallus* and *soralia* of *P. multipuncta* are K+ yellow, C-, KC+ yellow, PD+ orange-red, UV- or faintly glaucous (Chambers et al. 2009). In Polish specimen physodalic and protocetraric acids were detected.

AFFINITIES. In Europe *P. multipuncta* can be mistaken for the morphologically similar *P. ophthalmiza*. Both species produce similar soralium-like *apothecia*, but they can be easily separated by spot test reaction PD and thin layer chromatography: *P. multipuncta* reacts PD+ orange-red and contains physodalic acid, often together with protocetraric acid, and *P. ophthalmiza* is PD– and has fatty acids (Hanko 1983; Chambers et al. 2009). They differ also morphologically. *P. multipuncta* has fertile thallus warts measuring 0.5–1.5 mm diam., wide apothecial disc, and continuous and \pm even exciple; in *P. ophthalmiza* the fertile warts are smaller (0.3–0.8 mm diam.), disc is up to twice narrower, and the exciple irregular and crenate (Chambers et al. 2009).

P. albescens and *P. amara* can also resemble *P. multipuncta* in morphology, but they are usually sterile, soredia are coarse (very often forming consoredia) and produced in true soralia; they posses also different lichen substances: *P. albescens* contains fatty acids, whereas *P. amara* picrolichenic acid, often accompanied by protocetraric acid (Tønsberg 1992; Chambers et al. 2009).

HABITAT. *P. multipuncta* is a typical epiphytic lichen species, but it can rarely grow also on siliceous rocks (Chambers et al. 2009). In Poland it was found on bark of *Fagus sylvatica* in beech forest.

DISTRIBUTION. In Poland *P. multipuncta* was reported from northern and southern part of Poland, but rather scarcely (e.g., Nowak and Tobolewski 1975; Fałtynowicz 2003 and literature cited therein; Motiejūnaitė et al. 2004). After the revision of all available material from Poland it appeared that most records were based on misidentifications. Only one specimen from Gorce Mts appeared to belong to this species. Most of other specimens belong to *P. ophthalmiza*, which has not been recorded in Polish lichenological literature until 2008 (see Kukwa et al. 2008, also below). Some few additional specimens identified as *P. multipuncta* were rather small and difficult to identify, however they certainly do not belong either to *P. multipuncta* or *P. ophthalmiza*.

P. multipuncta species was reported from many countries, however, as this name was often misapplied in the past (see Hanko 1983), several records are doubtful. Confirmed records of *P. multipuncta* appears to be confined to western and southern Europe, where it has been so far reported from Belgium, British Isles, Denmark, France, Germany, Italy, Luxembourg, Slovenia, Spain, Sweden (Hanko 1983; Suppan et al. 2000; Søchting and Alstrup 2002; Nimis and Martellos 2008; Diederich et al. 2009). According to Chambers et al. (2009) it occurs also in Macaronesia, Africa and Asia.

SPECIMEN EXAMINED. POLAND. Ge 21 – Gorce Mts, Gorce National Park, area of Wspólny Potok stream, in Kamienica stream valley, alt. 920 m, on *Fagus sylvatica*, 9 June 1997, *leg. P. Czarnota* (GPN 1583/94).

SPECIMEN EXAMINED FOR COMPARISON. NORWAY. Hordland, prov. Asköy par., S of Lake Askevatnet, 60°29'N, 05°11'E, alt. 20 m., on branches of *Sorbus aucuparia*, 11 June 1984, *leg. T. Tønsberg 884 & Moberg*, Lich. Sel. Exs. Upsal. 135 (KRAM-L 43334).

Pertusaria ophthalmiza (Nyl.) Nyl. - Flora 48: 354 (1865).

Syn. Pertusaria multipuncta var. ophthalmiza Nyl., Lich. Scand.: 180 (1861), Pertusaria multipuncta var. conferta Erichsen, Rabenh. Kryptog.-Flora von Deutschland, Österreich und der Schweiz, part IX, 5(1): 610 (1936) [note: this variety was described from Poland; see Hanko (1983)]

CHARACTERISTIC OF THE SPECIES. This species is anatomically and morphologically very similar to *P. multipuncta*, but it can be distinguished predominantly by the different chemical constituents; for the differences see under *P. multipuncta*. According to Hanko (1983) and Chambers et al. (2009), *P. ophthalmiza* contains 4 fatty acids, but in most Polish specimens 2–3 fatty acids were found. Only in three specimens 4 substances were found, but also 1 was detected in two samples. This variation is most probably caused by rather low quantity of material taken for the analyses, as most specimens were quite small.

AFFINITIES. *P. ophthalmiza* can be mistaken for chemically and, when young, also morphology similar *P. albescens*. Both species produce fatty acids, but they predominantly differ in thallus characters: *P. albescens* produces true soralia with granular soredia, whereas the sorediate warts of *P. ophthalmiza* contain apothecia (Hanko 1983; Tønsberg 1992; Chambers et al. 2009).

Superficially *P. ophthalmiza* can resemble also *Ochrolechia turneri* (Sm.) Hasselrot. This species often produces regular and separate soralia, which may resemble fertile warts of *P. ophthalmiza*. Both taxa can be very easily separated by the C reaction of soralia and content of lichen substances. *P. ophthalmiza* is C negative and produces fatty acids, whereas soralia of *O. turneri* react C+ yellow and the species contains variolaric acid (Hanko 1983; Tønsberg 1992; Kukwa 2008).

HABITAT. Research on Polish material indicated that *P. ophthalmiza* is a corticolous species. It has been rather frequently found on *Tilia cordata* (13 specimens). Much less commonly it was found on *Alnus* spp. (4 specimens), *Carpinus betulus* (2 specimen) and *Fraxinus* (1 specimen), always in old, humid woodland. According to Chambers et al. (2009) this taxon can occur on acid bark (chiefly birches, oaks and coniferous trees) and overgrow epiphytic mosses, but in Poland it appears to be strictly corticolous on deciduous trees with more neutral bark.

DISTRIBUTION. *P. ophthalmiza* is much more widely distributed in Europe than *P. multipuncta*, and it is known, e.g., from Austria (Hafellner and Türk 2001), British Isles (Hanko 1983; Coppins 2002; Chambers et al. 2009), the Czech Republic (Hanko 1983), Estonia (Randlane et al. 2008), Fennoscandia (Hanko 1983; Santesson et al. 2004), Italy (Nimis and Martellos 2008), Slovenia (Suppan et al. 2000). The species has also been recorded from Macaronesia, North and South America and Asia (Dibben 1980; Chambers et al. 2009; Pišút 2009). In Poland this species is rather rare and it is here confirmed only from north-eastern part of Poland, the Carpathians and one old locality in the Sudetes (see Hanko 1983) (Fig. 1).

SPECIMENS EXAMINED. POLAND. **Bg 30** – Równina Augustowska plain, Puszcza Augustowska forest, W of Sucha Rzeczka village, forest section no. 199-200, on *Alnus glutinosa*, 11 Sept. 1986, *leg. S. Cieśliński* (KTC). **Bg 92** – Wysoczyzna Białostocka plateau, Puszcza Knyszyńska forest, Budzisk nature reserve, on *Fraxinus excelsior*, 5 Aug. 1994, *leg. S. Cieśliński* (KTC). **Cg 02** – Puszcza Knyszyńska forest, forest section no. 182, on *Carpinus betulus*, 9 Sept. 1987, *leg. I. & K. Toborowicz* (KTC). **Cg 03** – Puszcza Knyszyńska forest, Stare Biele nature reserve, on *Tilia cordata*, 4 Aug. 1994, *leg. S. Cieśliński* (KTC). **Cg 55** – Równina Bielska plain, Puszcza Białowieska forest, Białowieża National Park, forest section no. 256, on *Tilia cordata*, 18 Oct. 1989 and 5 June 1990, *leg. S. Cieśliński* (KTC); ibidem, on *Tilia cordata*, 10 May 1987



Fig. 1. Distribution of *Pertusaria multipuncta* and *P. ophthalmiza* in Poland in the ATPOL grid square system; empty circle – locality of *P. multipuncta*, black circles – localities of *P. ophthalmiza* based on revised specimens; square – historical record of *P. ophthalmiza* from Hanko (1983).

and 29 Oct. 2003, leg. K. Czyżewska (LOD–L 7504, 11836) and on Carpinus betulus, 29 Oct. 2003, leg. K. Czyżewska (LOD–L 11830); between forest sections nos 287D and 317B, on Tilia cordata, 1982, leg. S. Cieśliński & Z. Tobolewski (KTC); forest section no. 287, on Tilia cordata, 1982, leg. S. Cieśliński & Z. Tobolewski (KTC); forest section no. 341C, on Tilia cordata, 1984, leg. S. Cieśliński & Z. Tobolewski (KTC); forest section no. 341C, on Tilia cordata, 1984, leg. S. Cieśliński & Z. Tobolewski (KTC); forest section no. 400, on Tilia cordata, 27 March 2001, leg. M. Kukwa 210 (UGDA); forest section no. 317D, on Tilia cordata, 1982, leg. S. Cieśliński & Z. Tobolewski (KTC); **Cg 56** – Równina Bielska plain, Puszcza Białowieska forest, Białowieża National Park, forest section no. 246, on Tilia cordata, 1985, leg. S. Cieśliński, Z. Tobolewski (KTC); **Cg 64** – Puszcza Białowieska forest, forest section no. 342b, on Tilia cordata, 1979, leg. S. Cieśliński & Z. Tobolewski (KTC); vicinity of Topiło village, by Perebel stream, forest section no. 599, on Carpinus betulus, 12 May 2006, leg. M. Kukwa 5098 (UGDA); forest section no. 244B, on Tilia cordata, 1984, leg. S. Cieśliński & Z. Tobolewski (KTC); forest section no. 604B, on Carpinus betulus, 1982, leg. S. Cieśliński & Z. Tobolewski (KTC); Ge 50 – High Tatra Mts, Dolina Roztoki valley, by Roztoka stream, on Alnus sp., July1964, leg. J. Bystrek (LBL). Ge 51 – Rów Podtatrzański Depression, Łysa Polana settlement, on Alnus sp., 16 May 1965, leg. J. Bystrek (LBL, two specimens).

POORLY LOCALIZED SPECIMEN. Białowieża National Park, s.coll. (LBL).

ADDITIONAL SPECIMENS EXAMINED. AUSTRIA. Radstädter Tauren, Salzburg, an *Abies* in einem feuchten Bergwald zwischen Ober- und Untertauren, ± 1240 m, Oct.1963, *leg. Th. Schauer, Poelt & Steiner*, Lich. Alp. 222, sub *P. multipuncta* (KRAM-L 23765, LBL).

CONCLUSIONS

- The study has shown, that the secondary lichen metabolites are invaluable taxonomic characters, that helps to evaluate the occurrence and distribution of at least some lichens.
- *Pertusaria multipuncta* and *P. ophthalmiza* are morphologically very similar and visually almost indistinguishable, but they differ in secondary chemistry. The analyses of lichen substances proved that *P. multipuncta* was mostly misidentified, and at present it is known from only one locality in Gorce Mts; most of remaining records of this species belong to *P. ophthalmiza*.

• *P. ophthalmiza* appears to occur in well preserved natural ecosystems in North Eastern Poland and Tatra Mts. Surprisingly, it was not found in Bieszczady Mts, the mountain range with suitable habitats for this species.

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Porosty z rodzaju Pertusaria w Polsce. I. P. multipuncta i P. ophthalmiza

Streszczenie

Jak dotąd, rozpoznano w Polsce 40 gatunków z rodzaju *Pertusaria* DC. (por. Hanko 1983; Fałtynowicz 2003; Kossowska 2008). Na podstawie budowy morfologicznej i składu wtórnych metabolitów porostowych analizie poddano dwa gatunki z tego rodzaju, *P. multipuncta* (Turner) Nyl. i *P. ophthalmiza* (Nyl.) Nyl., które charakteryzują się podobną morfologią plech, ale różnią się chemizmem. *P. multipuncta*, podobnie jak *P. ophthalmiza*, cechuje się najczęściej cienką, gładką, niekiedy spękaną, biało-szarą plechą. Owocniki, zwykle pojedyncze, są zagłębione w brodawkach plechy przypominających soralia, a tarczki są płaskie lub lekko wypukłe i przyprószone ziarenkowatymi sorediami. Worki są jednozarodnikowe. Gatunki te różnią się chemicznie. *P. multipuncta* reaguje na czerwono z PD i zawiera kwas fysodalowy (często także protocetrariowy), natomiast *P. ophthalmiza* nie reaguje z PD i zawiera kwasy tłuszczowe (Hanko 1983; Chambers et al. 2009).

Dotychczas z terenu Polski częściej notowana była *P. multipuncta* (Fałtynowicz 2003), *P. ophthalmiza* zaś była podana tylko raz przez Hanko (1983). Gatunek ten jednak nie został ujęty na liście porostów Polski, a pierwsze dane o jego występowaniu na terenie kraju w polskiej literaturze lichenologicznej pojawiły się w pracy Kukwy et al. (2008). Przeprowadzone badania wykazały, że większość notowań *P. multipuncta* z Polski należy do *P. ophthalmiza* lub do innych gatunków porostów. *P. multipuncta* została potwierdzona na terenie naszego kraju tylko z jednego stanowiska w Gorcach. Rozmieszczenie obu gatunków w Polsce przedstawia rycina 1.

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