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The lichen-forming fungi of the *Xanthoparmelia pulla* group (Parmeliaceae, Ascomycota) in Poland

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Abstract

The paper presents the results of studies of *Xanthoparmelia pulla* group in Poland. The morphological and chemical analysis of herbarium materials confirmed the presence of four species of this group reported from Poland before. The study however, revealed considerable changes it the partiuclular species distribution. *X. verruculifera*, so far considered the most endangered species in the country, turned out to be the most frequent taxon. *X. loxodes*, regarded as common, has much less known localities than previously thought. This species was usually confused with *X. verruculifera*. *Xanthoparmelia pulla* is the rarest species and should be considered critically endangered in Poland. Most specimens of *X. pulla* collected in the country belong to *X. delisei*, which so far had only two historical records in Poland. All these taxa are characterized in terms of morphology, the content of secondary metabolites, ecology and distribution.

Keywords: parmelioid lichens; *Xanthoparmelia delisei*; *X. loxodes*; *X. pulla*; *X. verruculifera*; chemotaxonomy; ecology; distribution

Introduction

The genus *Xanthoparmelia* (Vain.) Hale, comprising ca. 800 species of worldwide distribution belongs to the largest family of lichenized fungi – Parmeliaceae [1,2]. The distinguishing features of the genus are: hyphal cell walls' polysaccharides with *Xanthoparmelia*-type lichenan [3–5], small ascospores with an arachiform vacuolar body [6], lack of pseudocyphellae, presence of a pored epicortex, eperforate apothecia, the presence of bifusiform conidia and usually simple rhizines [5]. This genus is further characterized by a considerable variation in cortical chemistry of individual species. Most taxa of this genus occur on the siliceous rocks in dry and well-sunlit places, in periarid, arid, semiarid and Mediterranean climates, mainly in the southern hemisphere [5].

Originally the genus *Xanthoparmelia* included exclusively the species containing atranorin and usnic or isousnic acids in the upper cortex, causing the yellowish tinge of thallus [7,8]. Lichens currently included to *Xanthoparmelia pulla* group but not containing these substances, with brown thalli and upper cortex stained by HNO_3 to blue-green, were initially grouped in the *Neofusca* subgenus (within the *Parmelia* genus) [9], which was then set up as a separate genus *Neofuscelia* [10]. Molecular studies have shown, however, that the genus *Neofuscelia* was polyphyletic, with its clades scattered within *Xanthoparmelia*. Consequently, the species of the genus *Neofuscelia* have been included in the *Xanthoparmelia* [11].

Xanthoparmelia pulla group includes about 25 taxa dispersed throughout the world, seven of which occur in Europe [12]. Due to the frequent absence of apothecia and the lack of obvious differences in their structure, these taxa are traditionally distinguished on the basis of morphological features of the thallus, such as the color of lower cortex, shape of lobes and the presence or absence of vegetative propagules [12]. However, chemical characteristics play the most important role in the identification of taxa [9,13]. Within the species of the genus *Xanthoparmelia*, ca. 90 secondary metabolites were identified, mainly phenolic compounds such as depsides, depsidones, antraquinones and monocilic compounds, as well as aliphatic acids [8,14], 15 of which were present within *Xanthoparmelia pulla* group [13].

So far four species of the *Xanthoparmelia pulla* group were reported from Poland: *X. delisei*, *X. loxodes*, *X. pulla* and *X. verruculifera* [15]. One of them, *X. loxodes*, was considered to be common throughout the country, while the *X. pulla* and *X. verruculifera* were admitted as more or less rare and endangered [16]. *X. delisei* in general was not distinguished by Polish lichenologists and its only records from present Polish territory were of historical nature [17].

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Proper identification of these species is relatively difficult and requires application of chemotaxonomic methods, e.g. thin layer chromatography (TLC) in addition to standard microscopic methods. Since these have not been widely used in the study of this group of lichens in Poland, data on the distribution, ecology and a status of threat for individual taxa are incomplete and require rewording. Therefore, the authors undertook a detailed review of herbarium material from Poland in order to verify data on lichens in this group.

Material and methods

A total of 304 herbarium specimens, deposited in the following herbaria: BSG, GPN, KRA, KRAM-L, KRAP, KTC, LOD-L, OLTC-L, POS-L, TRN, UGDA, WA, WRSL and private collections of M. Dimos-Zych, P. Grochowski, W. Gruszka, M. Kossowska, L. Lipnicki, K. Pietrzykowska and K. Szczepańska were investigated. Each specimen was analyzed for the presence of secondary metabolites in the thallus using thin layer chromatography (in solvent A and C), in accordance with the methods described by Orange et al. [18]. When the substance was present in all specimens in relative high concentration, it is marked (+), when it was only trace constituent (t), if it was absent in some samples (+/−) is used. In addition, the morphological features, especially the shape and size of lobes and isidia were investigated, using a stereoscopic microscope. Brief characteristics of each species are based on personal observations.

The list of examined specimens is available in Appendix S1. The names of physico-geographical mesoregions are given according to Kondracki [19]. The distribution of all species in Poland is shown on maps based on the ATPOL grid square system [20], modified by Cieśliński and Fałtynowicz [21].

Results

***Xanthoparmelia delisei* (Duby) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch**

Taxon 53(4): 967. 2004.≡*Parmelia olivacea* var. *delisei* Duby, Bot. Gall. 2: 602. 1830.≡*Neofuscelia delisei* (Duby) Essl., Mycotaxon 7: 50. 1978.

DIAGNOSTIC CHARACTERS. Thallus foliose, forming rosettes, loosely appressed to the substrate. The upper surface light yellowish-brown or slightly darker, often distinctly maculate, smooth or transversely wrinkled towards the center. Lobes irregular, flat or slightly convex, with overlapping edges, slightly shining at tips. Isidia absent. Apothecia usually present and numerous. Secondary metabolites detected by TLC: glomelliferic (+), glomellic (+), perlatolic (+), stenosporic (t) and gyrophoric (+/−) acids.

HABITAT. In Poland the species was recorded on siliceous boulders and stones in treeless, open places exposed to direct sunlight, often in areas used for agricultural purposes and on the roadsides.

GENERAL DISTRIBUTION. The species is widespread almost worldwide. It occurs in Africa, Australia, Asia, South America and Europe [12]. In Europe it was reported from Belgium [22], Germany [23], Great Britain and Ireland [24], Greece [25], Italy [26], Montenegro [27], Netherlands [28], Spain and Portugal [29], Sweden, Norway and Finland [30].

DISTRIBUTION IN POLAND. *X. delisei* has been considered an extremely rare species on Polish territory. It was only reported in historical works from the present Polish north and north-east [17,31]. This analysis showed that the taxon is relatively common and widespread in the lowland parts of the country, with a distinct concentration of localities in the north-east Poland (Fig. 1). The species reaches the highest altitude in the Sudety Mountains (about 600 m above sea level). In the Carpathians it has not been found to date.

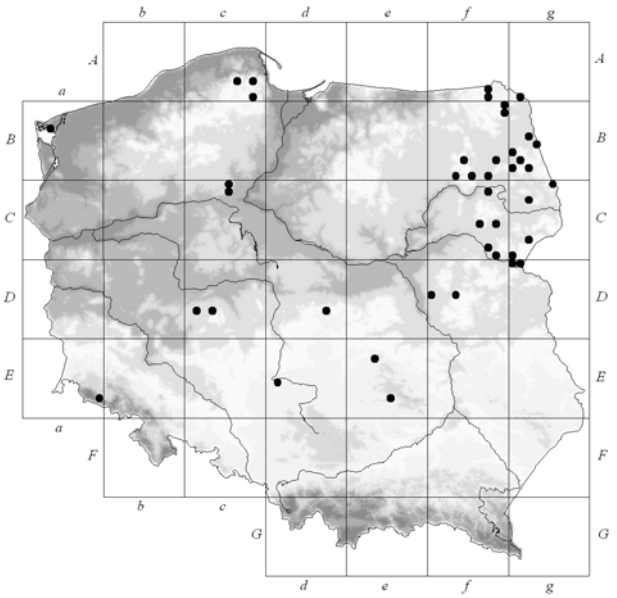

Fig. 1 Distribution of *Xanthoparmelia delisei* in Poland.

NUMBER OF SPECIMENS EXAMINED – 55.

COMMENTS. All examined specimens of *X. delisei* were originally identified as *X. pulla*. According to Esslinger [9] the taxa are morphologically almost identical, although in some cases they can be separated on the basis of morphological features, such as the color of thalli (paler and yellowish to gray-brown in *X. delisei*, darker brown in *X. pulla*) and the appearance and shape of the lobes (broader, thicker and more strongly maculate in *X. pulla*). In addition, these species are different in the reaction of the medulla with KC, which is red to orange in *X. delisei* and pink-red to red in *X. pulla* [9,23]. These features, however, may not be sufficient for proper determination of the species. Both species differ much in the content of secondary metabolites in thalli, and therefore should be identified based on TLC analysis. *X. delisei* contains glomelliferic, glomellic, perlatolic, loxodellic, stenosporic, anziaic acids and occasionally gyrophoric acid [9,13], while in *X. pulla* were found stenosporic, divaricatic, perlatolic, 4-O-demethylstenosporic and oxostenosporic acids, sometimes with atranorin and gyrophoric or lecanoric acids [32].

The chemistry of *X. delisei* is identical as in case of *X. loxodes*. However, mature and well-developed thalli of the latter species produce isidia, visible on the upper surface

as cauliflower-like clusters. In the case of young and poorly developed specimens, where isidia may not be fully developed, the separation of these two species is not possible. Because of the identical chemistry of *X. loxodes* and *X. delisei*, some authors suggest that these two taxa may represent intergrading morphotypes of a single species [33]. In some sources *X. delisei* was treated as a chemotype of *X. pulla* and highlighted in a rank of variety [22,23,30].

***Xanthoparmelia loxodes* (Nyl.) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch**

Taxon 53(4): 968. 2004.≡*Parmelia loxodes* Nyl., Flora 55: 426. 1872.≡*Neofuscelia loxodes* (Nyl.) Essl. Mycotaxon 7: 51. 1978.

DIAGNOSTIC CHARACTERS. Thallus foliose, forming rosettes, loosely appressed to the substrate. Lobes rather flat, elongated and overlapping. The upper surface yellowish-brown to reddish-brown, smooth or wrinkled. Isidia present, coarse, more or less spherical and distinctly pustular. Apothecia rare. Secondary metabolites detected by TLC: glomelliferic (+), glomellic (+), stenosporic (+), perlatolic (t) and gyrophoric (+/−) acids.

HABITAT. *X. loxodes* is a species with a very wide range of habitat requirements. In the lowland part of Poland it was recorded on siliceous erratic boulders and stones, in well sunlit places on the edges of roads, fields and meadows. In mountain areas the species occurs on natural or artificially uncovered (e.g., abandoned quarries) rock outcrops of various chemistry and mineral composition (gneiss, sandstone, granite, basalt). Single records were also taken from anthropogenic calcareous substrates and from wood.

GENERAL DISTRIBUTION. The species is widespread, reported from North Africa, Asia, North America and Europe [12]. In Europe it occurs in Austria [34], Belgium [22], Czech Republic [35], Denmark [36], Germany [23], Great Britain and Ireland [24], Greece [25], Italy [26], Netherlands [28], Portugal [37], Spain [29], Sweden, Norway and Finland [30].

DISTRIBUTION IN POLAND. Until now *X. loxodes* was regarded as the most widespread and common member of the *X. pulla* group in Poland. In the checklist of Polish lichens [15] it was reported from numerous localities across the country. However, most of the 164 examined herbarium specimens of *X. loxodes* in fact represent another species, *X. verruculifera*.

The records of *X. loxodes* are concentrated in the northern part of the country (Pojezierze Kaszubskie and Pojezierze Mazurskie lakelands), within the limits of the last glaciation, which left a large number of erratic boulders (Fig. 2). Individual records are also from Wielkopolska district, Wyżyna Wieluńska Upland and the Góry Świętokrzyskie Mountains. In the southern part of the country it was found only in the lower parts of the mountains and the foothills of the Sudetes and the Przedgórze Sudeckie Foreland, up to about 800 m above sea level. It was not recorded in the Carpathians so far.

NUMBER OF SPECIMENS EXAMINED – 72.

COMMENTS. *X. loxodes* is usually confused with another species producing isidia, namely *X. verruculifera*. Both taxa can, however, be distinguished on the basis of morphological features. *X. loxodes* develops larger and paller thalli, thicker

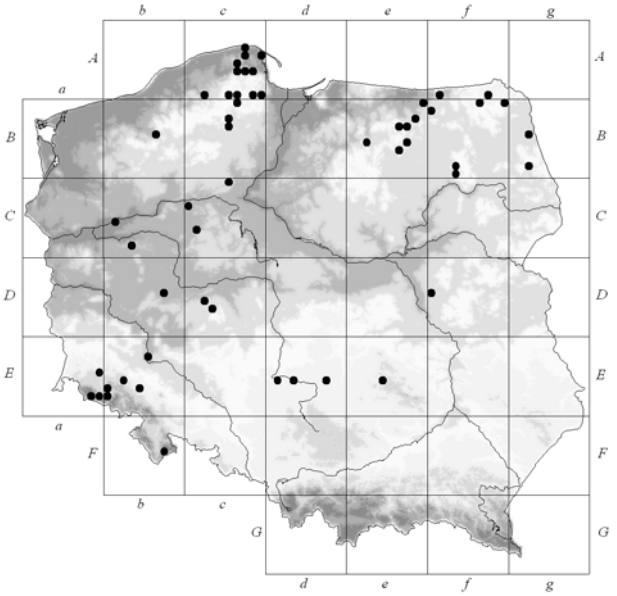

Fig. 2 Distribution of *Xanthoparmelia loxodes* in Poland.

and wider lobes and larger and more strongly pustular isidia [9]. These species also slightly differ in the reaction of medulla with KC, which in *X. loxodes* is red to orange, and in *X. verruculifera* pink and disappearing [9]. Both species have different chemistry of thalli and in case of any doubts the specimens should be analyzed for the content of secondary metabolites. *X. loxodes* contains glomelliferic, glomellic, perlatolic acids and occasionally anziaic, stenosporic, loxodellic or gyrophoric acids [9,12,13] while in *X. verruculifera* divaricatic, nordivaricatic, oxostenosporic, stenosporic, subdivaricatic, perlatolic and 4-O-demethylstenosporic acids were found. Gyrophoric and lecanoric acids may also be present as accessory compounds [26].

The chemistry of *X. loxodes* is very similar to the *X. delisei*, therefore, when the thalli are young and poorly developed (without distinct isidia), proper identification of these taxa may be impossible.

In the studied herbarium materials *X. loxodes* was also confused with *Melanelixia fuliginosa* (Fr. ex Duby) O. Blanco, A. Crespo, Divacar, Essl., D. Hawksw. & Lumbsch, which, however, develops thalli with distinctly shiny surface, covered by fine, cylindrical isidia and the medulla C+ turning red. Another species confused with *X. loxodes* is *Melanelia disjuncta* (Erichsen) Essl. The last one may be distinguished by generally smaller thalli, upper cortex not reacting with HNO_3 and isidia transforming into convex, distinctly globose soralia. It is worth to note that the taxonomic affinity of *M. disjuncta* is uncertain and requires further studies (e.g. [2,38]).

***Xanthoparmelia pulla* (Ach.) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch**

Taxon 53(4): 970. 2004.≡*Parmelia pulla* Ach., Syn. Meth. Lich.: 206. 1814.≡*Neofuscelia pulla* (Ach.) Essl., Mycotaxon 7: 52. 1978.

DIAGNOSTIC CHARACTERS. Thallus foliose, forming rosettes or irregular, weakly appressed to the substrate. Lobes slightly convex, rounded, rather thick. Upper surface olive-brown, yellowish-brown or slightly darker, distinctly wrinkled, slightly shining at tips of the lobes, without isidia. Apothecia frequent. Secondary metabolites detected by TLC: stenosporic (+), divaricatic (+), perlatolic (t), oxostenosporic (t), 4-O-demethylstenosporic (t), and gyrophoric (+) acids.

HABITAT. *X. pulla* was confirmed in Poland only from two sites till now, hence it is difficult to define its habitat preferences. However, it seems that this taxon requires much more xeric habitats then the other species of the *X. pulla* group. In Poland it has been recorded in warm, sunny and dry places, preferably with a southwestern exposure. It occured on the acidic or neutral volcanic rocks, overgrown by xerothermic grasslands with *Jovibarba sobolifera*, *Sedum maximum*, *Thymus* sp. etc.

GENERAL DISTRIBUTION. The species occurs in Africa, Australia, New Zealand and Europe [12]. In Europe it was recorded in Austria [31], Belgium [21], Cyprus [39], Czech Republic [32], Denmark [33], Germany [22], Great Britain and Ireland [20], Greece [23], Italy [24], Netherlands [26], Portugal [24], Spain [27], Sweden, Norway and Finland [28].

DISTRIBUTION IN POLAND. Up to date, the species was thought to be relatively common, with many localities across the country [15]. As shown by the chemotaxonomic revision of the available herbarium material, most of the 86 specimens previously labeled as *X. pulla* in fact represent *X. delisei*, and only two of them belong to *X. pulla*. The known localities of the species in Poland are situated in the southwestern part of the country, within the Sudetes and their foreland (Fig. 3).

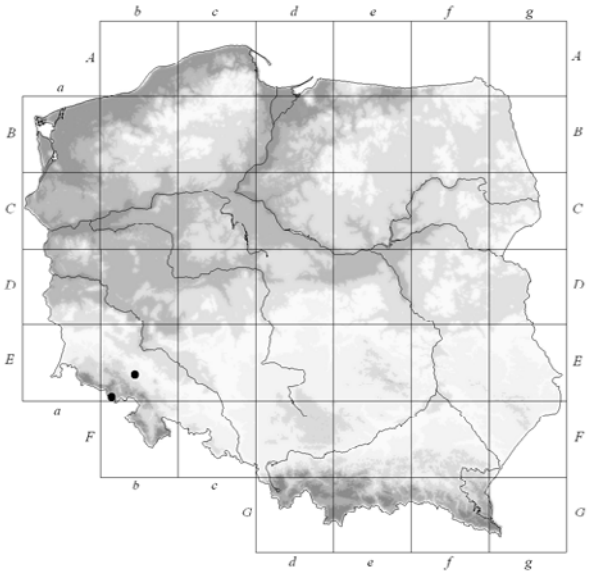

Fig. 3 Distribution of *Xanthoparmelia pulla* in Poland.

NUMBER OF SPECIMENS EXAMINED – 2.

COMMENTS. *X. pulla* is very similar to *X. delisei*. According to Esslinger [9], both species are slightly different in color, morphology of the lobes and reactions with KC (see comments on *X. delisei*). All morphological features, however, are so variable that they are not sufficient to separate these taxa properly. The identification of the species requires application of thin layer chromatography.

The chemistry of *X. pulla* is very similar to the chemistry of *X. verruculifera*. Therefore, morphological features of the thalli need careful examinations. In the case of young and poorly developed thalli without isidia or apothecia, the separation of these taxa can be problematic.

Another species very similar to *X. pulla* is *X. perrugata* (Nyl.) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch, which has not been recorded in Poland so far. However, it may be possible to find this taxon in the country, because it was recorded in Central Europe [32]. Both species are slightly different in the chemistry of thalli. *X. perrugata* contains divaricatic acid as major and stenosporic acid as minor substances, in contrast to *X. pulla*, where the major substance is stenosporic acid [32]. In addition, *X. perrugata* has a markedly more rugose upper surface than the other members of *X. pulla* group [26].

***Xanthoparmelia verruculifera* (Nyl.) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch**

Taxon 53(4): 972. 2004.≡*Parmelia verruculifera* Nyl., Flora 61: 247. 1878.≡*Neofuscelia verruculifera* (Nyl.) Essl., Mycotaxon 7: 53. 1978.

DIAGNOSTIC CHARACTERS. Thallus foliose, medium or loosely appressed to the substrate. Lobes flat, short and rounded to elongated, contiguous or imbricate, slightly shiny at tips. Upper surface olive-brown, reddish-brown to dark-brown, smooth or slightly rough. Apothecia rare. Isidia present, pustular, densely arranged, forming branched-coralloid structures. Secondary metabolites detected by TLC: divaricatic (+), stenosporic (+), perlatolic (t), oxostenosporic (t), 4-O-demethylstenosporic (t) and gyrophoric (+/−) acids.

HABITAT. This species occurs in both lowland and mountainous areas, usually in open and well-lit places. It was recorded mostly on siliceous boulders and rocks, on the roadsides, fields and meadows and on natural rock outcrops (andesite, gneiss, sandstone, quartzite, serpentinite, sporadically also calcareous rocks) as well as on anthropogenic substrates, e.g., on bricks, stone tombs and walls.

GENERAL DISTRIBUTION. *X. verruculifera* occurs in North Africa, North America and Europe [12]. In Europe it was recorded in Austria [34], Belgium [22], Czech Republic [35], Denmark [36], Great Britain and Ireland [24], Germany [23], Italy [26], Netherlands [28], Spain [29], Sweden, Norway and Finland [30].

DISTRIBUTION IN POLAND. The species was considered rare and endangered in Poland, having just a few locations in the country [15]. The analysis of the available herbarium materials has shown that this taxon was rarely distinguished and had often been referred to *X. loxodes*. Thus, *X. verruculifera* proved to be the most common species of the *X. pulla* group in Poland.

This species occurs in almost all territory of Poland, from the lowlands to the lower mountain areas (Fig. 4). As the only representative of the *X. pulla* group it occurs in the Carpathians and is particularly abundant in the Gorce

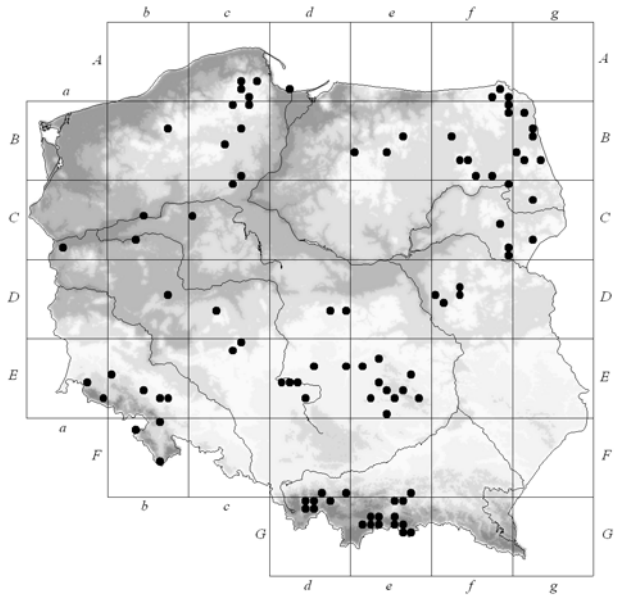

Fig. 4 Distribution of *Xanthoparmelia verruculifera* in Poland.

Mountains and the Beskid Sądecki Mountains. It reaches the highest altitude in the Pieniny Mountains at 760 m.

NUMBER OF SPECIMENS EXAMINED – 136.

Exiccates examined: Nowak, Lichens Poloniae Meridionalis Exsiccati 90 (LOD-L, sub *Parmelia isidiotyla*).

COMMENTS. *X. verruculifera* is most often confused with *X. loxodes*. Both species produce isidia on thallus surface, but their morphology is different. The isidia of *X. verruculifera* can be defined as higher and tinier. In addition, Esslinger [9] pointed at the other morphological features that allow to separate these species (see comments on *X. loxodes*). However, the most important feature is the chemical content of the thallus.

The chemistry of *X. verruculifera* is very similar to *X. pulla* and *X. perrugata*. Therefore the special attention should be paid to the presence or absence of isidia. In the case of young and poorly developed thalli separation of these species may not be possible.

X. verruculifera can be confused with species belonging to the genera *Melanelia* and *Melanelixia*, especially *Melanelia disjuncta* and *Melanelixia fuliginosa*. These species are, however, relatively easy to distinguish from the species of *X. pulla* group due to the lack of reaction of the upper cortex with HNO_3 and a generally different chemical content of the thalli.

It is worth to note that the old name of *X. verruculifera* – *Parmelia verruculifera* Nyl. has sometimes been erroneously used for an epiphytic species – *Melanelixia subargentifera* (Nyl.) O. Blanco, A. Crespo, Divacar, Essl., D. Hawksw. & Lumbsch. During this study, among the examined herbarium specimens, we identified also specimens of this species. The taxonomy and nomenclature of *P. verruculifera* were discussed in detail by Esslinger [40].

Discussion

The examined herbarium specimens confirmed the presence of all four species of lichens belonging to the *Xanthoparmelia pulla* group reported from Poland before, however their distribution and ecology seem to be other than previously thought. *X. verruculifera*, so far considered the most vulnerable and classified to the EN (endangered) category on the red list of Polish lichens [16], proved to be the commonest species of the group. This taxon had been often confused with *X. loxodes*, especially in case of young specimens with poorly developed isidia. In view of the obtained results it should be excluded from the Polish red list.

According to previous data [15], *X. delisei* was considered to be an extremely rare species, having just two historical localities in Poland. The revision has shown that the taxon is much more frequent in the whole country. In fact, the rarest of the studied species is *X. pulla*. For a total of 86 herbarium specimens labeled as *Xanthoparmelia* (*Neofuscelia*) pulla only two specimens were ascribed to this species. Other represent mainly *X. delisei*, but also *X. loxodes* and *X. verruculifera*. *X. pulla* is thus the only species actually endangered in Poland, so the change in the status of the species within the red list of lichens from the NT (near treatment) to CR (critically endangered) needs to be taken into account.

As mentioned above, the propper identification of the species of the *X. pulla* group, requires not only a morphological analysis, but also chemotaxonomic studies. The most important diagnostic features, which enable to identify all of the taxa occurring in Poland, are presented in a Tab. 1.

Tab. 1 Overview of the distinguishing features in the *Xantoparmelia pulla* group in Poland.

Species	Isidia	Apothecia	Chemistry
X. delisei	absent	common	glomelliferic, glomellic, perlatolic, stenosporic and occasionally gyrophoric acid
X. loxodes	present	rather rare	glomelliferic, glomellic, perlatolic, stenosporic and occasionally gyrophoric acid
X. pulla	absent	common	stenosporic, divaricatic, perlatolic and occasionally gyrophoric acid
X. verruculifera	present	rather rare	stenosporic, divaricatic, perlatolic and occasionally gyrophoric acid

Acknowledgments

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Authors' contributions

The following declarations about authors' contributions to the research have been made: determination of the specimens: KS, MK; taxonomic analyses: KS, MK; writing of the manuscript: KS, MK; preparation of the distribution maps: MK.

Supplementary material

The following supplementary material for this article is available online at http://pbsociety.org.pl/journals/index.php/asbp/rt/suppFiles/asbp.2014.004/0:

1. Appendix S1: the list of specimens examined.

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