



New and rare lichens of the family *Megasporaceae* discovered in Poland

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Abstract

Based on a revision of historic herbarium material and recently collected specimens, six lichenized fungi of the family Megasporaceae have been identified. Four of them, *Aspicilia verrucigera*, *Oxneriaria supertegens*, *Sagedia mastrucata* and *S. zonata* are reported for the first time from Poland. Also, we present new localities for two species, *Aspicilia goettweigensis* and *A. polychroma*, that have been rarely reported in the country. The characteristics of all these species, as well as their ecology and geographical distribution, are presented and briefly discussed. In addition, nucITS rDNA and mtSSU rDNA were sequenced for some specimens to confirm their identifications.

Keywords: barcoding, lichenized fungi, taxonomy

Introduction

After several years of scientific discussion concerning the phylogeny of Megasporaceae Lumbsch, Feige & K. Schmitz (Miadlikowska *et al.* 2006; Schmitt *et al.* 2006; Lumbsch *et al.* 2007), this lichen-forming fungal family was ultimately recognized as monophyletic within the order Pertusariales M. Choisy ex D. Hawksw. & O. E. Erikss. (Nordin *et al.* 2010). The first proposed taxonomy of the family included five genera – *Aspicilia* A. Massal., *Circinaria* Link, *Lobothallia* (Clauzade & Cl. Roux) Hafellner, *Megaspora* (Clauzade & Cl. Roux) Hafellner & V. Wirth and *Sagedia* Ach. (Nordin *et al.* 2010). In the course of further studies, three other genera – *Teuvoa* Sohrabi & S. Leavitt (Sohrabi *et al.* 2013), *Aspiciliella* M. Choisy (Zakeri *et al.* 2017) and *Oxneriaria* S.Y. Kondr. & Lőkös (Haji Moniri *et al.* 2017) – were distinguished. Most representatives of the family Megasporaceae are saxicolous lichens with crustose to placodioid thalli and characteristic aspicilioid (crater-like, urceolate) or less often lecanorine apothecia. Characteristic features of the family also include asci with a nonamyloid tholus, the ‘Caesiocinerea-green’ pigment (Meyer & Printzen 2000) present in the epihymenium, simple and hyaline ascospores, and branched, anastomosing paraphysoids (Lumbsch *et al.* 1994).

Because of the crustose thallus with rather variable morphology depending on the habitat conditions, the recognition of species within this family is quite difficult and must be based on a combination of several features, such as ascospore size, number of ascospores in the ascus, length of conidia, secondary lichen metabolites and habitat preferences.

Despite many years of study, Megasporaceae is still considered a taxonomically very difficult family, with some taxa possibly still representing a complex of unrecognised species, or their taxonomic concepts remaining unclear (Zakeri *et al.* 2019). There is also a lack of contemporary information on the taxonomy, diversity and geographical distribution of the representatives of this family in Poland.

In this paper, we present new localities for two species that have been very rarely reported in Poland, and we also report, for the first time, four further species recently found to occur in the country. All the studies were based on either

available material stored in Polish herbaria or fresh specimens collected during fieldwork that has been conducted since 2013. In addition to the morphological and chemical characteristics of the species, we also provide the accession numbers for the newly obtained nucITS rDNA, considered to be a barcoding marker for fungi (Xu 2016), including lichens (Kelly *et al.* 2011; Zheng *et al.* 2011; Leavitt *et al.* 2014; Dal Forno *et al.* 2022), and additionally, for some specimens, the mtSSU rDNA sequences.

Material and methods

This study was based on collections deposited in herbaria, including the GBN, KRA, KRAM, KRAP, KTC, SLTC, UGDA and WRSL, as well as the private collections of Dr Maria Kossowska (Hb. Kossowska) and the first author of the manuscript (Hb. Szczepańska). The descriptions and chemistry of species are based on the authors' observations. The morphology and anatomy of the species were studied using dissecting and light microscopes, following routine techniques; for the light microscopy, hand sections were made with a razor blade and mounted in water. The hymenium and conidia measurements were taken in water, with the ascospore measurements being made in 10% potassium hydroxide (KOH). The presence of the epihymenium pigment 'Caesiocinerea-green' was detected by colour reaction after application of 50% nitric acid (HNO₃), as well as KOH (Mayer & Printzen 2000). The TLC analyses were performed in solvent systems A and C using the standardized method of Culberson (1972) and following Orange *et al.* (2001).

Additionally, for some specimens, sequences of nucITS rDNA and mtSSU rDNA were obtained. The genomic DNA was extracted using a CTAB method, according to the standard protocol (Doyle & Doyle 1987), or the modified CTAB method (Guzow-Krzemińska & Węgrzyn 2000). The primers used for DNA amplification were ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990) for the nucITS rDNA and mrSSU1 and mrSSU3R (Zoller *et al.* 1999) for the mtSSU rDNA. The polymerase chain reaction (PCR) conditions followed Szczepańska *et al.* (2020) for the nucITS rDNA and Guzow-Krzemińska *et al.* (2016) for the nucITS rDNA and mtSSU rDNA markers. Amplification products were separated in 1% agarose gel, photographed and compared with the DNA mass ruler (Thermo Fisher Scientific Waltham, MA, USA). Bands corresponding to the amplified region were excised from the agarose gel and then purified by ethanol precipitation, or alternatively, using Clean-Up Concentrator (A&A Biotechnology). Purified amplicons were sequenced by Macrogen Europe (Amsterdam, the Netherlands). All of the newly obtained nucITS rDNA and mtSSU rDNA sequences (Table 1) were analysed using the Basic Local Alignment Search Tool (BLAST) (Altschul *et al.* 1997) before being deposited in GenBank. Best hits from BLAST analyses for newly generated sequences are reported in the text, while additional data for the most similar sequences for each species are given in Table 2.

The species

Aspicilia goettweigensis (Zahlbr.) Hue

Nouv. Arch. Mus. Hist. Nat., Paris, 5 ser. 2(1): 112. 1910 ≡ *Lecanora goettweigensis* Zahlbr., Ann. Naturhist. Mus. Wien 20: 345. 1905. Fig. 1A.

Thallus lichenized, crustose, squamulose-areolate, olive-brown to olive-grey, matt. Areoles thin, flat and smooth at the margin of the thallus to thick and forming small squamules in the centre. Squamules irregular, white pruinose on the edges, partially erected and overlapping, convex and ear-shaped with medulla visible from underneath, 0.5–0.8 mm in diam. Prothallus rarely visible, fibrous, dark olive-grey. Apothecia rare, immersed, 1 to 3 per areole, 0.2–1.0 mm in diam., thalline margin same colour as the thallus, thin, white pruinose and radially incised on the inside edge, disc black, rounded, flat and matt. Hymenium colourless, 80–100 µm tall with submoniliform paraphyses (3–4 globose apical cells), epihymenium green-brown to olive-brown, N⁺ green intensifying, K⁺ yellowish brown (Caesiocinerea-green), hypothecium colourless. Asci 8-spored, ascospores hyaline, simple, narrowly ellipsoid, 18–25 × 8–12 µm. Conidia filiform, 14–20 × 1 µm.

Chemistry: According to Paukov *et al.* (2016), *A. goettweigensis* contains stictic acid (major) with norstictic and conorstictic acids (both minor) in the medulla. One Polish specimen lacked secondary substances (Hb. Szczepańska 1333), but the BLAST analyses of the nucITS rDNA sequence undoubtedly identified it as *A. goettweigensis* (see below).

Ecology and distribution: *Aspicilia goettweigensis* is a rare and poorly known species that has been recorded from only a few European countries, including Austria, the Czech Republic, France, Germany, Hungary, Italy and

Switzerland (Paukov *et al.* 2016; Nimis *et al.* 2018; Nimis & Martellos 2022). It occurs on basic and ultramafic rocks, including basalt, serpentinite, syenite and porphyry (Paukov *et al.* 2016). In Poland, the species has been reported from the Sudety Mts in the vicinity of the town of Jelenia Góra (Nowak & Tobolewski 1975) and in the Karpaty Mts in the Orawsko-Podhalańskie Depression (Bielczyk 2003).

TABLE 1. GenBank accession numbers of the newly-obtained nucITS rDNA and mtSSU rDNA sequences of specimens presented in the manuscript.

Species	Isolate no.	Locality	Voucher specimens	GenBank no. nucITS mtSSU
<i>Aspicilia goettweigensis</i>	150	Poland, Sudeckie Foothills	K. Szczepańska 1092	OP602297
<i>Aspicilia goettweigensis</i>	337	Poland, Sudety Mts	K. Szczepańska 1318	OP602304
<i>Aspicilia goettweigensis</i>	339	Poland, Sudety Mts	K. Szczepańska 1320	OP602306
<i>Aspicilia goettweigensis</i>	350	Poland, Zachodniosudeckie Foothills	K. Szczepańska 1333	OP602307
<i>Aspicilia verrucigera</i>	73	Poland, Kaszubskie Lakeland	M. Kukwa 2916	OP602295
<i>Aspicilia verrucigera</i>	19950	Poland, Bory Tucholskie	M. Kukwa 19950	OP700455 OP737751
<i>Aspicilia verrucigera</i>	19956a	Poland, Bory Tucholskie	M. Kukwa 19956a	OP700456 OP737752
<i>Sagedia mastrucata</i>	151	Poland, Sudety Mts	K. Szczepańska 1095	OP602298
<i>Sagedia mastrucata</i>	154	Poland, Izerskie Foothills	K. Szczepańska 1099	OP602299
<i>Sagedia mastrucata</i>	160	Poland, Izerskie Foothills	K. Szczepańska 1106	OP602300
<i>Sagedia mastrucata</i>	166	Poland, Sudety Mts	K. Szczepańska 1116	OP602301
<i>Sagedia mastrucata</i>	176	Poland, Sudety Mts	K. Szczepańska 1186	OP602302
<i>Sagedia mastrucata</i>	226	Poland, Izerskie Foothills	K. Szczepańska 978	OP602303
<i>Sagedia mastrucata</i>	338	Poland, Sudety Mts,	K. Szczepańska 1319	OP602305
<i>Sagedia zonata</i>	F13	Poland, Sudety Mts	K. Szczepańska 41	OP602290
<i>Sagedia zonata</i>	44	Poland, Karpaty Mts	KRAM L-42336	OP602291
<i>Sagedia zonata</i>	48	Poland, Karpaty Mts	KRAM L-42339	OP602292
<i>Sagedia zonata</i>	59	Poland, Karpaty Mts	J. Kiszka s.n. (KRAP)	OP602293
<i>Sagedia zonata</i>	60	Poland, Karpaty Mts	J. Kiszka, R. Kościelniak s.n. (KRAP)	OP602294
<i>Sagedia zonata</i>	132	Poland, Sudety Mts	K. Szczepańska 924	OP602296

All of the new *A. goettweigensis* localities presented in this paper are located in the area of low mountains in Lower Silesia (south-western Poland). An additional record was found after revision of the Stein's specimen stored in the WRS� herbarium, which was originally identified as *Aspicilia gibbosa* (Ach.) Körb. All of the recorded specimens were found growing on silicate rocks in exposed locations, influenced by high insolation and rather low moisture at altitudes of up to 500 m.

Notes: *Aspicilia goettweigensis* can be confused with other members of the genus, especially *A. laevata* (Ach.) Arnold and *A. verrucigera* Hue, due to the very similar sizes of the ascospores and conidia, and the secondary chemistry. The most important distinguishing feature of *A. goettweigensis*, however, is the appearance of the thallus, which consists of characteristic overlapping and convex squamules. In the literature, these squamules are described as hollow, cracking into "popcorn-like tufts", and showing white medulla in well-developed specimens (Paukov *et al.* 2016). In addition, unlike *A. goettweigensis*, *A. laevata* prefers shady and moist habitats, close to water or located in forests (Wirth 1995, Fletcher *et al.* 2009).

TABLE 2. The most similar sequences obtained from BLAST analyses of the newly generated nucITS rDNA sequences from specimens analysed in this work. GenBank accession numbers, localities, voucher data and references are given.

Species	Locality	Voucher specimens	References	GenBank no. nucITS
<i>Aspicilia goettweigensis</i>	Austria	J. Vondrák 14026 (PRA, topotype)	Paukov <i>et al.</i> 2016	KX159289
<i>Aspicilia goettweigensis</i>	Russia	AGP20120513-03 (UPS,UFU)	Paukov <i>et al.</i> 2016	KX159292
<i>Aspicilia goettweigensis</i>	Czech Republic	Frolov 51 (herb. I. Frolov, dupl. UPS, UFU)	Paukov <i>et al.</i> 2016	KX159293
<i>Aspicilia goettweigensis</i>	Czech Republic	J. Vondrák 23397 (PRA)	Vondrák <i>et al.</i> 2022	OK332934
<i>Aspicilia goettweigensis</i>	Czech Republic	J. Vondrák 23713 (PRA)	Vondrák <i>et al.</i> 2022	OK332935
<i>Aspicilia verrucigera</i>	Czech Republic	J. Vondrák 24214 (PRA)	Vondrák <i>et al.</i> 2022	OK332936
<i>Aspicilia verrucigera</i>	Sweden	Tibell 22669 (UPS)	Nordin <i>et al.</i> 2007	EU057939
<i>Sagedia</i> aff. <i>mastrucata</i>	Czech Republic	Z. Palice 23193 (PRA)	Urbanavichus <i>et al.</i> 2020	MK778583
<i>Sagedia</i> aff. <i>mastrucata</i>	Czech Republic	Maliček 14741	Maliček 2022	OP730575
<i>Sagedia zonata</i>	Sweden	Nordin 6219 (UPS)	Nordin <i>et al.</i> 2007	EU057943
<i>Sagedia zonata</i>	Sweden	Nordin 5486 (UPS)	Nordin <i>et al.</i> 2007	EU057944
<i>Sagedia zonata</i>	Sweden	Nordin 5461 (UPS)	Nordin <i>et al.</i> 2007	EU057945
<i>Sagedia zonata</i>	Norway	Owe-Larsson 8942 (UPS)	Nordin <i>et al.</i> 2007	EU057946
<i>Sagedia zonata</i>	Sweden	Owe-Larsson H-254a (UPS)	Nordin <i>et al.</i> 2007	EU057947
<i>Sagedia zonata</i>	Sweden	Nordin 5998 (UPS)	Nordin <i>et al.</i> 2007	EU057949
<i>Sagedia zonata</i>	Sweden	Nordin 5932 (UPS)	Nordin <i>et al.</i> 2007	EU057950
<i>Sagedia zonata</i>	Sweden	Nordin 6035 (UPS)	Nordin <i>et al.</i> 2007	EU057951
<i>Sagedia zonata</i>	Sweden	Nordin 6006 (UPS)	Nordin <i>et al.</i> 2007	EU057952
<i>Sagedia zonata</i>	Sweden	Nordin 5949 (UPS)	Nordin <i>et al.</i> 2007	EU057953

The nucITS marker was sequenced from four specimens (Table 1), and the BLAST analyses showed 98% to 100% identity with the sequences of *A. goettweigensis* deposited in GenBank (Table 2, data for *A. goettweigensis*). Sequences of the best hits originated from Russia and the Czech Republic (KX159292 and KX159293, respectively).

Material examined: POLAND. Dolnośląskie province: Sudety Mts, Jeleniogórska Basin, Łomnickie Hills, Witosza Hill near Staniszków village, elev. 484 m, on granite rock, March 1882, *B. Stein* (WRSL-5791); Sudety Mts, Opawskie Mts, Karolinki rocks near Jarnołówka village, elev. 400 m, on natural outcrop of siliceous rocks in an oak grove, 18 February 2020, *K. Szczepańska 1318, 1320* (Hb. Szczepańska); Sudeckie Foothills, Strzegomskie Hills, Krzyżowa Hill, elev. 360 m, on basalt rock, 19 May 2013, *K. Szczepańska 1092, 1093* (Hb. Szczepańska); Sudeckie Foothills, Kaczawskie Foothills, Organy Wielisławskie quarry in Sędziszowa village, elev. c. 300 m, on porphyry rocks, 8 May 2020, *K. Szczepańska 1333* (Hb. Szczepańska).

Aspicilia polychroma Anzi Cat. Lich. Sondr.: 59. 1860.

≡ *Lecanora polychroma* (Anzi) Nyl., in Stizenberger, Ber. Tätigk. St. Gallischen Naturwiss. Ges.: 379. 1882.

(Fig. 1B)

Thallus lichenized, crustose, rather thick, irregularly cracked to areolate or verrucose-areolate, dark grey, brown-grey, often with olive or yellowish tinge, matt. Areoles flat to slightly convex, angular, smooth, 0.5–2.5 mm in diam. Prothallus rarely visible, fibrous, dark grey. Apothecia immersed, 1 to 5 per areole, 0.5–1.2 mm in diam., thalline margin same colour as the thallus or darker, thin, disc rounded to irregular, black, flat and matt. Hymenium colourless, 90–130 µm tall, with submoniliform paraphyses (3–5 globose apical cells), epihymenium green-brown to olive-brown,

N+ green intensifying, K+ yellowish brown (Caesiocinerea-green), hypothecium colourless. Asci 8-spored, ascospores hyaline, simple, narrowly ellipsoid, $18\text{--}23 \times 8\text{--}13 \mu\text{m}$. Conidia filiform $15\text{--}24 \times 1 \mu\text{m}$.

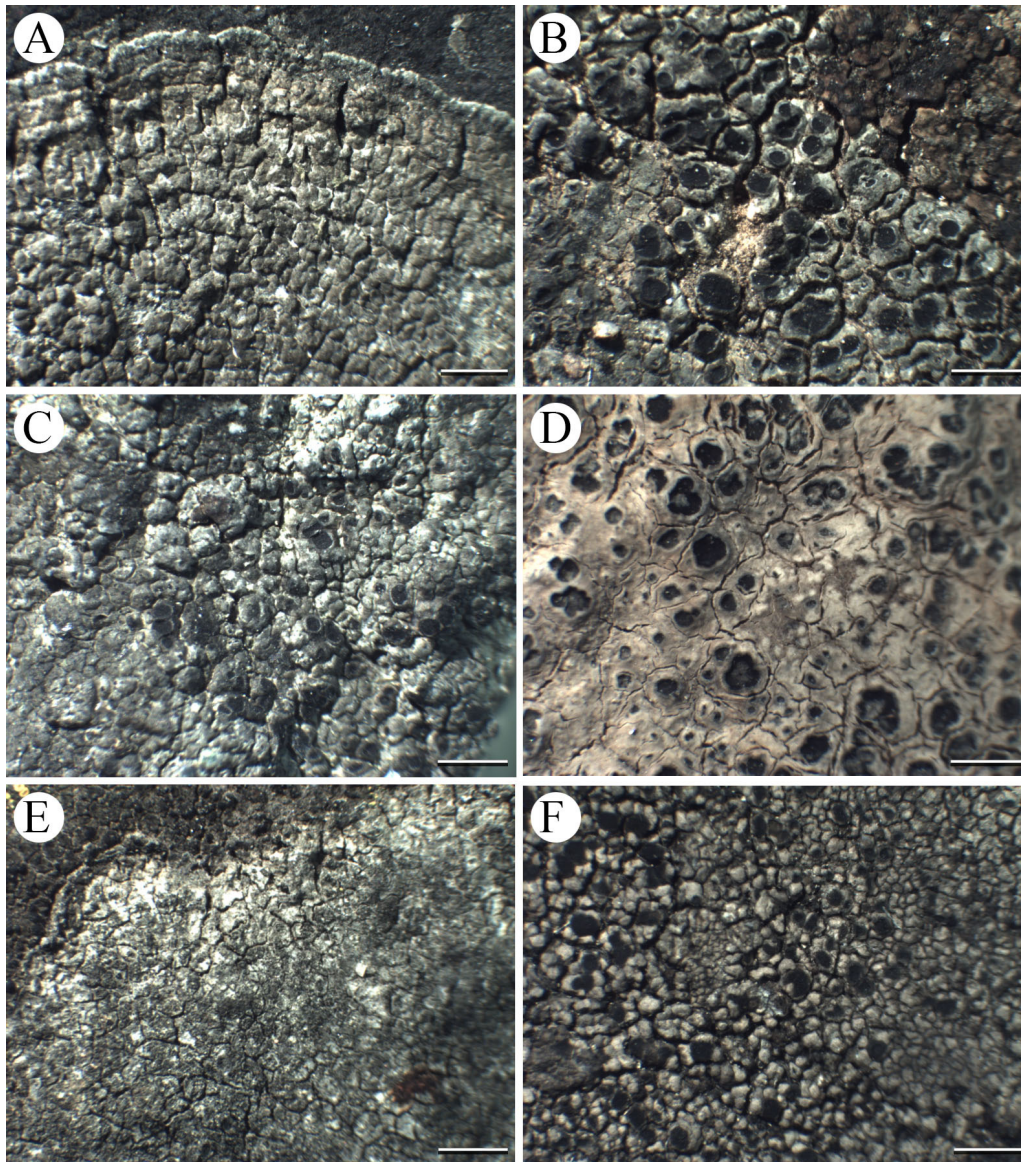


FIGURE 1. Specimens treated. A. *Aspicilia goettweigensis* (Hb. Szczepańska 1093). B. *Aspicilia polychroma* (Hb. Szczepańska 1022). C. *Aspicilia verrucigera* (Hb. Szczepańska 1272). D. *Oxneriaria supertegens* (KRAM L-34348). E. *Sagedia mastrucata* (Hb. Szczepańska 1186). F. *Sagedia zonata* (KRAM L-42336). Scales: 2 mm. Fot. K. Szczepańska.

Chemistry: No secondary metabolites were detected in the analysed material.

Ecology and distribution: *Aspicilia polychroma* is a rare, mountain species occurring mainly in Europe, especially in the Alps, the Karpaty Mts and the Pyrenees. It has been reported from Austria, Finland, France, Germany, Italy, Norway, Portugal, Slovakia, Spain and Switzerland (Vitikainen *et al.* 1997; Llimona & Hladun 2001; Lisická 2005; Roux 2012; Wirth *et al.* 2013; Flakus 2014; Nimis *et al.* 2018; Westberg *et al.* 2021; Nimis & Martellos 2022), as well as USA and Canada (Esslinger 2016). In Poland, only a variety *A. polychroma* var. *rubrireagens* Asta & Cl. Roux, in which the thallus reacts K+ red, has been reported from a single locality in the High Tatry Mts (Flakus 2007). The new records represent the typical variety without lichen substances.

Aspicilia polychroma prefers slightly calciferous, siliceous rocky habitats in open and well-lit locations. All newly recorded specimens were found growing at altitudes above 700 m, on boulders in neutrophilic habitats surrounded by pastures or meadows.

Notes: *Aspicilia polychroma* may be confused with other taxa of the Megasporeaceae family, especially *Aspicilia calcitrata* Cl. Roux & A. Nordin, *Circinaria gibbosa* (Ach.) A. Nordin, Savić & Tibell, *C. caesiocinerea* (Nyl. ex Malbr.) A. Nordin, Savić & Tibell and *Sagedia zonata* Ach., all of which also occur on siliceous rocks in open places,

and all with thalli showing no colour reaction with standard spot tests (Owe-Larsson *et al.* 2007; Fletcher *et al.* 2009; Roux *et al.* 2011; Wirth *et al.* 2013). The main distinguishing feature of *A. polychroma* is, however, the length of the conidia, which are clearly longer than in the mentioned taxa. In addition, *A. gibbosa* and *C. caesiocinerea* produce larger ascospores (20–26[–30] × 12–16[–18] µm) and, additionally, *C. caesiocinerea* contains aspicilin as a secondary metabolite in the thallus (Owe-Larsson *et al.* 2007; Fletcher *et al.* 2009; Wirth *et al.* 2013).

Material examined: POLAND. Dolnośląskie province: Sudety Mts, Sowie Mts, Sokola Pass, elev. c. 800 m, on a siliceous boulder in the meadow, 11 April 2015, *K. Szczepańska 1022* (Hb. Szczepańska); Małopolskie province: Karpaty Mts, Beskid Sądecki Mts, Jaworzyna Krynicka Range, Zadnie Góry settlement, elev. c. 850 m, on sandstone rocks, 4 July 1989, *L. Śliwa* (KRA-396); Jaworzyna Krynicka Range, Zadnie Góry settlement, elev. c. 790 m, on sandstone rocks, 4 July 1989, *L. Śliwa* (KRA-397); Jaworzyna Krynicka Range, Sarnica Mt, elev. c. 920 m, on sandstone rocks, 4 Aug. 1989, *L. Śliwa* (KRA-398); Karpaty Mts, Beskidy Zachodnie Mts, Gorce Mts, by the black tourist trail in the Jastrzębie clearing at the edge of the Gorce National Park, elev. 960 m, on sandstone rock, 22 October 1997, *P. Czarnota, J. Kiszka* (GPN-1895/94).

Aspicilia verrucigera Hue Nouv. Arch. Mus. Hist. Nat. 5 sér. 2: 48. 1912.

≡ *Lecanora verrucigera* (Hue) Zahlbr., Cat. Lich. Univ. 5: 357. 1928.

(Fig. 1C)

Thallus lichenized, crustose, thick, areolate to verrucose, dark grey, rarely with dark brown or yellowish-brown tint, matt. Areoles flat to convex, irregular, very thick, with a partially squamulose or warted surface, 0.5–2.0 mm in diam. Prothallus usually indistinct, black. Apothecia immersed, 1 to 5 per areole, 0.3–1.0 mm in diam., thalline margin indistinct or thin, on the disc level, smooth, concolorous with thallus, disc rounded to irregular, black, flat to slightly convex, very rarely pruinose, matt. Hymenium colourless, 90–150 µm tall, with submoniliform paraphyses (3–4 globose apical cells), epihymenium green-brown to olive-brown, N+ green intensifying, K+ yellowish brown (Caesiocinerea-green), hypothecium colourless. Asci 8-spored, ascospores hyaline, simple, ellipsoid, 16–23 × 8–13 µm. Conidia filiform, 12–23 × 1 µm.

Chemistry: *Aspicilia verrucigera* contains stictic (major) and norstictic (minor) acids in the cortex (Owe-Larsson *et al.* 2007). However, in some Polish specimens (UGDA L-11099, KRA-443, Hb. Szczepańska 1272, 1273), cryptostictic, connorstictic and constictic acids (all minor) have also been detected.

Ecology and distribution: *Aspicilia verrucigera* is a rare species occurring in temperate to boreal and alpine areas of Asia, Europe and North America (Owe-Larsson *et al.* 2007, Shu-Xia *et al.* 2013). It has been reported from only a few countries, including Denmark, Finland, France, Germany, Netherlands, Norway, Sweden (Vitikainen *et al.* 1997; Aptroot *et al.* 1999; Søchting & Alstrup 2008; Roux 2012; Wirth *et al.* 2013; Westberg *et al.* 2021), USA, Canada (Esslinger 2016) and China (Shu-Xia *et al.* 2013). *Aspicilia verrucigera* has not previously been reported from Poland. However, after revision of the available *Aspicilia* material, a few specimens of this species were found in Polish herbaria, as well as newly collected specimens from northern and southern Poland. It is known from scattered localities, in both the lowlands and low mountain areas of the country.

This species occurs on siliceous rocks and boulders in open and well-lit locations.

Notes: The Polish specimens of *A. verrucigera* were usually incorrectly identified as *Circinaria caesiocinerea* (Nyl. ex Malbr.) A. Nordin, Savić & Tibell or, rarely, as *Aspicilia laevata* (Ach.) Arnold. *Circinaria caesiocinerea* and *A. verrucigera* may have been confused because of the similarity in their preferred habitats, as well as the appearance of the thallus, which is rather thick, areolate and grey. However, both taxa can be easily distinguished based on the secondary lichen metabolites: *A. verrucigera* always contains stictic acid, whereas *C. caesiocinerea* produces aspicilin (Owe-Larsson *et al.* 2007; Fletcher *et al.* 2009; Wirth *et al.* 2013). In the case of *A. laevata*, the main difference is the habitat because this species grows in shady and moist places, usually in mixed forests or close to streams and rivers. In addition, the thallus of *A. laevata* is thinner than in *A. verrucigera*, smooth and irregularly cracked rather than areolate, and has a greenish tint (Wirth 1995; Fletcher *et al.* 2009; Wirth *et al.* 2013).

The nuclITS marker was sequenced from three specimens (Table 1), which shared 99.79 or 99.65% identity with the sequences of *A. verrucigera* deposited in GenBank originated from the Czech Republic and Sweden (Table 2, data for *A. verrucigera*). In addition, two mtSSU rDNA sequences from specimens of *A. verrucigera* (Table 1) showed 100% identity with the sequence from the Czech Republic (OK465538).

Material examined: POLAND. Dolnośląskie province: Sudety Mts, Złote Mts, ruins of the castle Karpień, elev. c. 775 m, on gneiss rock, 25 October 2015, *K. Szczepańska 1036* (Hb. Szczepańska); Sudety Mts, Karkonosze Mts, Szklarska Poręba town, Marianki rocks, elev. 700 m, on granite rocks, 21 June 2018, *K. Szczepańska 1272, 1273* (Hb.

Szczepańska); Śląskie province: Karpaty Mts, Beskidy Zachodnie Mts, Beskid Mały Mts, Bujakowska Mt, elev. c. 600 m, on the southern slope on the small wall, 23 August 1960, *J. Nowak* (KRAM L-7499); Karpaty Mts, Beskid Żywiecki Mts, Grupa Pilska Range, Żabnica Duża village, Tokarnia settlement, elev. 780 m, on a sandstone block in a loose wall, 28 September 1964, *J. Nowak* (KRAM L-16657); Beskid Żywiecki Mts, Młoda Hora settlement near Soblówka village, elev. 960 m, on piles of sandstone boulders, 26 September 1986, *J. Nowak* (KRAM L-30388); Małopolskie province: Karpaty Mts, Beskid Sądecki Mts, Radziejowa Range, Szczawnicka Rock, elev. 1160 m, on sandstone rocks in full sun, 1 September 1990, *L. Śliwa* (KRA-443); Świętokrzyskie province: Świętokrzyskie Mts, Świnia Góra Nature Reserve near Bliżyn village, on sandstone rocks in a forest clearing, 5 May 1959, *J. Nowak* (KRAM L-5394, 5363); Świętokrzyskie Mts, Klonowskie Range, Klonów village, on sandstone rock, 1980, *A. Kowalczyk, K. Toborowicz* (KTC); Świętokrzyskie Mts, Cisowskie Range, 0.7 km south of Września Mt, on siliceous rock, 1981, *S. Cieśliński, B. Łyś* (KTC); Lubuskie province: Kaszubskie Lakeland, near the Stone Circles (burial mounds) in Borcz village, on a granite stone in bright forest, 7 March 2004, *M. Kukwa 2916* (UGDA L-11099); Kaszubskie Lakeland, Kaszubski Park Krajobrazowy, south-west of Uniradze village, forest section no. 49, oak-beech forest, on stone, 11 August 2020, *M. Kukwa 21284, 21285, 21286, A. Kowalewska* (UGDA L-43555, 43556, 43557); Zachodniopomorskie province: Polanowska Upland, Baranowiec forest district, on a silicate rock by the road at the edge of the meadow, 19 October 1979, *I. Izydorek* (SLTC); Pomorskie province: Bory Tucholskie, Kręgi Kamienne Nature Reserve, north-west of Odry village, on erratic blocks in the circle, 12 June 2018, *M. Kukwa 19950, 19956a* (UGDA L-25370, 25383); Bory Tucholskie, Kręgi Kamienne Nature Reserve, north-west of Odry village, on erratic blocks in the circle, 19 June 2018 *M. Kukwa 19972, 19972a, 19979a* (UGDA L-25405, 25406, 25415).

Oxneriaria supertegens (Arnold) S.Y. Kondr. & Lőkös Acta Bot. Hung. 59 (3–4): 358. 2017.

≡ *Aspicilia supertegens* Arnold, Verh. K. K. Zool.-Bot. Ges. Wien 27: 567. 1877. ≡ *Lecanora supertegens* (Arnold) Zahlbr., Cat. Lich. Univ. 5: 354. 1928.

(Fig. 1D)

Thallus lichenized, crustose, thick, usually circular, smooth to irregularly cracked, creamy-white, yellowish-white to greyish-white, matt. Prothallus not visible. Apothecia immersed, 0.5–1.5 mm in diam., thalline margin concolorous with the thallus, thin to thick, disc rounded to irregular, black, flat and matt, sometimes with white pruina. Hymenium colourless, 120–150 µm tall, with submoniliform paraphyses (3–4 globose apical cells), epihymenium green-brown to olive-brown, N+ green intensifying, K+ yellowish brown (Caesiocinerea-green), hypothecium colourless. Asci 8-spored, ascospores hyaline, simple, ellipsoid, 18–24 × 8–15 µm. Conidia filiform, slightly curved, 14–25 × 1 µm.

Chemistry: No secondary metabolites were detected in the analysed material.

Ecology and distribution: *Oxneriaria supertegens* is a rare, circumpolar and arctic-alpine species (Thomson 1984), which has been reported from Austria, Finland, France, Germany, Italy, Norway, Portugal, Spain, Sweden and Switzerland (Vitikainen *et al.* 1997; Llimona & Hladun 2001; Roux 2012; Wirth *et al.* 2013; Nimis *et al.* 2018; Westberg *et al.* 2021; Nimis & Martellos 2022) and from outside Europe from USA and Canada (Thomson 1984; Esslinger 2016). Here, it is reported for the first time from Poland. Three specimens of this species, stored in the KRAM herbarium, were collected in the Tatry Mts in 1987, but remained unidentified.

Oxneriaria supertegens prefers specific, very moist habitats, especially siliceous boulders close to mountain brooks and rivers that are frequently sprinkled or periodically flooded with fresh water.

Notes: The species most similar to *O. supertegens* is *Aspicilia aquatica* (Fr.) Körb. Both taxa occur in similar, humid habitats and have a whitish, smooth to slightly cracked thallus. The main differences between the species are the length of the conidia (shorter in *A. aquatica*, 12–15 µm) and the size of the ascospores (larger in *A. aquatica*, 22–27[–35] × 14–20 µm) (Wirth *et al.* 2013).

Material examined: POLAND. Małopolskie province: Karpaty Mts, Tatry Zachodnie Mts, upper part of the Jarząbcza Valley, elev. 1360 m, on granite boulders in the bed of the Jarząbczy stream, on surfaces protruding above the water surface, 10 October 1987, *J. Nowak* (KRAM L-34324); Tatry Zachodnie Mts, upper part of the Jarząbcza Valley, elev. 1310 m, on granite boulders in the bed of the Jarząbczy stream, flooded periodically with water, 10 October 1987, *J. Nowak* (KRAM L-34348, 34332).

Selected specimens of *Aspicilia aquatica* examined. POLAND. Małopolskie province: Karpaty Mts, High Tatry Mts, Pańszczyca Valley, Czerwony Stawek pond, elev. ca. 1700 m, on granite rock in water, 8 July 1971, *J. Nowak* (KRAM L-19885); High Tatry Mts, Rysy Mt., elev. 2140 m, in water race, on siliceous rock, 8 Aug. 2003, *A. Flakus s.n.* (KRAM L-48329); High Tatry Mts, Rysy Mt., elev. 2160 m, 20 July 2004, *A. Flakus 2724* (KRAM L).

Sagedia mastrucata (Wahlenb.) A. Nordin, Savić & Tibell Mycologia 102: 1346. 2010.

≡ *Lichen mastrucatus* Wahlenb., Fl. Lapp.: 413. 1812. ≡ *Lecanora mastrucata* (Wahlenb.) Ach., Syn. Meth. Lich. (Lund): 148. 1814. ≡ *Aspicilia mastrucata* (Wahlenb.) Th. Fr., Nova Acta Regiae Soc. Sci. Upsal., ser. 3, vol. 3: 234. 1928.

(Fig. 1E)

Thallus lichenized, crustose, thin to thick, developing as small patches between other lichens, areolate, pale to dark grey, sometimes with greenish tint, matt. Areoles flat to convex, irregular, 0.25–2.0 mm in diam. Prothallus usually indistinct, pale-grey and shiny. Soralia on the surface of the areoles, irregular to rounded, flat to convex, 0.3–1.0 mm in diam., confluent in older parts of the thallus. Soredia greyish-black to greyish-green, usually darker than the thallus. Apothecia and pycnidia not seen in the analysed material.

Chemistry: Norstictic acid, sometimes additionally with stictic acids were detected in the Polish specimens, which is consistent with data from the literature (Wirth *et al.* 2013).

Ecology and distribution: *Sagedia mastrucata* is not a very common species. It has been reported mainly from Europe, including Austria, Finland, France, Germany, Italy, Norway, Portugal, Russia, Spain, Sweden and Switzerland, but also outside of Europe, from Turkey (Vitikainen *et al.* 1997; Llimona & Hladun 2001; Owe-Larsson *et al.* 2007; Roux 2012; Wirth *et al.* 2013; Nimis *et al.* 2018; Urbanavichus *et al.* 2020; Westberg *et al.* 2021; Nimis & Martellos 2022). Here, it is recorded from Poland for the first time, exclusively from the south-west part of the country, where it was found growing on basalt rocks in low mountain areas, under open, well-lit and usually warm and dry habitat conditions.

Notes: *Sagedia mastrucata* belongs to a poorly known complex of soredioid and usually sterile species that also includes *S. simoensis* (Räsänen) A. Nordin, Savić & Tibell and *Aspicilia grisea* Arnold. The characteristic features of these taxa are the vegetative structures that develop on the thallus surface, which are variously named in the literature as soralia, isidia, upwards, warted areoles, papilles, isidia-like papilles or papillate isidia (Nowak & Tobolewski 1975; Dietrich *et al.* 2005; Fletcher *et al.* 2009; Nordin *et al.* 2010; Wirth *et al.* 2013). The morphological differences between these species are not clear. Usually, it has been assumed that the main features of *S. mastrucata* and *S. simoensis* are the thick thallus and numerous, grouped isidia or papilles resembling isidia, which may develop soredia on their tops. *Aspicilia grisea* has been described as having a very thin, dark thallus and pale, circular soralia (Fletcher *et al.* 2009). The morphology of the Polish specimens partly fits the description of *A. grisea*, although, based on the BLAST search and data provided in Urbanavichus *et al.* (2020), all the specimens have been classified as *S. mastrucata*. It is clear that the taxonomy of this group of species require further study.

The nucITS rDNA marker was sequenced from seven specimens (Table 1) and showed *ca.* 99% identity with the sequences MK778583 and OP730575 of *Sagedia* aff. *mastrucata* deposited in GenBank (Table 2), both originating from the Czech Republic (Table 2, data for *S. mastrucata*). Other sequences labelled as *S. mastrucata* and deposited in GenBank were less similar to the newly generated sequences (*ca.* 95% of identity).

Material examined: POLAND. Dolnośląskie province: Izerskie Foothills, Szupiec rock close to Giebułtów village, elev. 746 m, on basalt rock in the meadow, 22 June 2013, *K. Szczepańska 978, 1099* (Hb. Szczepańska); Izerskie Foothills, Czubatka Hill near Platerówka village, elev. 357 m, on basalt rock, 16 March 2016, *K. Szczepańska 1106* (Hb. Szczepańska); Sudety Mts, Izerskie Mts, Odarte Skały rocks, northern slope of Urwista Mt, near Rebiszów village, in the closed basalt quarry, elev. 540 m, on basalt rock, 25 April 2016, *K. Szczepańska 1095, 1186* (Hb. Szczepańska); Sudety Mts, Złote Mts, Czarne Urwisko rock near Ułęże village, in the closed basalt quarry, 9 May 2016, *K. Szczepańska 1116* (Hb. Szczepańska); Sudety Mts, Opawskie Mts, Karolinki rocks near Jarnońtówek village, elev. 400 m, on a natural outcrop of siliceous rocks in an oak grove, 18 February 2020, *K. Szczepańska 1319* (Hb. Szczepańska).

Sagedia zonata Ach. Kongl. Vetensk. Acad. Nya Handl. 30: 165. 1809.

≡ *Lecanora zonata* (Ach.) H. Magn., Kongl. Svenska Vetensk. Acad. Handl., ser. 3 17(5): 139. 1939. ≡ *Aspicilia zonata* (Ach.) R. Sant., Lich. Sweden & Norway (Stockholm): 46. 1984.

(Fig. 1F)

Thallus lichenized, crustose, areolate, thin, pale-grey, greenish-grey or brownish-grey, matt. Areoles, flat to slightly convex, rounded to irregular, 0.5–2.0 mm in diam. Prothallus rarely visible, fibrous, dark-grey to black. Apothecia immersed, 1 to 4 per areole, 0.2–1.0 mm in diam., thalline margin thin, slightly raised, smooth, grey, usually darker than the thallus, disc black, rounded, flat and matt. Hymenium colourless, 90–110 µm tall with submoniliform paraphyses (3–4 globose apical cells), epihymenium green-brown to olive-brown, N⁺ green intensifying, K⁺ yellowish brown

(Caesiocinerea-green), hypothecium colourless. Asci 8-spored, ascospores hyaline, simple, ellipsoid, $16\text{--}22 \times 8\text{--}12 \mu\text{m}$. Conidia filiform $8\text{--}12 \times 1 \mu\text{m}$.

Chemistry: No secondary substances were detected in the analysed material.

Ecology and distribution: *Sagedia zonata* is not a very common species, occurring in scattered localities in only a few European countries, including Austria, Finland, France, Germany, Italy, Norway and Sweden (Roux 2012; Wirth *et al.* 2013; Nimis *et al.* 2018; Westberg *et al.* 2021; Nimis & Martellos 2022). It had not been previously reported from Poland; however, a few specimens of this taxon were stored in Polish herbaria under other names, including two samples collected by B. Stein in the Karkonosze Mts.

Sagedia zonata occurs on silicate rocks, in high precipitation locations, in open places and usually in mountains (Wirth *et al.* 2013). In Poland, it has been exclusively reported from the Sudety and Karpaty Mts from relatively high altitudes (above 1000 m). In addition, after field studies conducted in 2006 (Kossowska *et al.* 2016) and the revision of material collected by B. Stein, it was confirmed, after 130 years, from a locality on a basalt vein in the Mały Śnieżny Kocioł cirque (Kleine Schneegrube) (Fig. 2A–C).

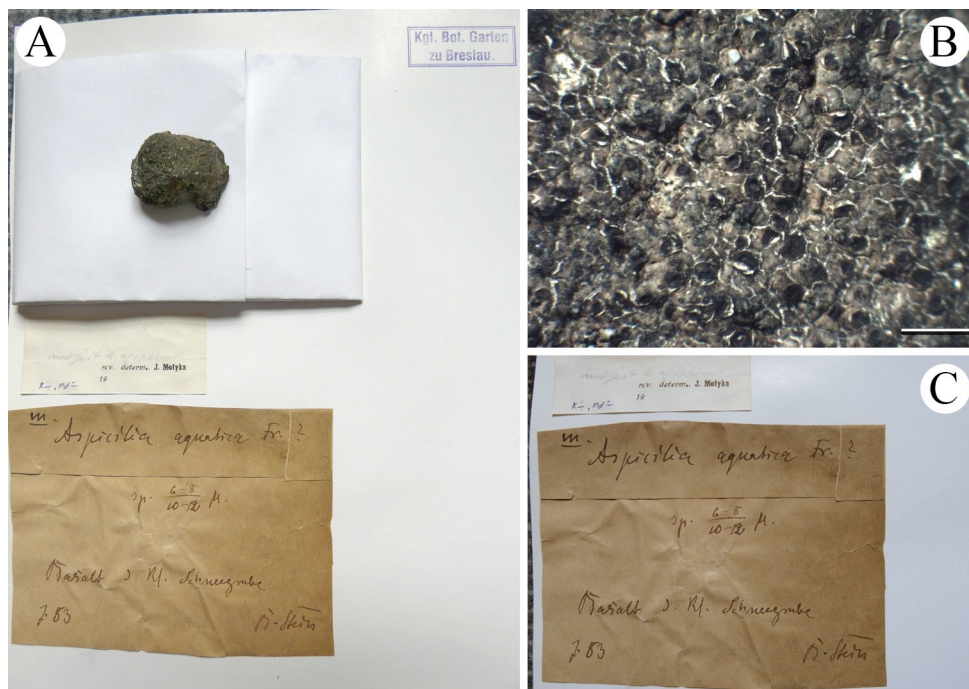


FIGURE 2. *Sagedia zonata* leg. B. Stein, rev. K. Szczepańska (WRSL-7755). A. Herbarium card. B. Specimen, scale: 2 mm. C. Herbarium labels with B. Stein signature and J. Motyka comment (“is not *A. aquatica*”) above. Fot. K. Szczepańska.

Notes: *Sagedia zonata* is a very often overlooked or incorrectly identified species because of the lack of obvious characteristic features. In Polish herbaria, it was usually labelled as *Circinaria caesiocinerea*. Indeed, these two taxa are similar, both in the terms of thallus morphology and the length of the conidia (Wirth *et al.* 2013). However, both species differ in ascospore size, which are larger in *C. caesiocinerea* ($20\text{--}26\text{--}30 \times 12\text{--}16\text{--}18 \mu\text{m}$) (Owe-Larsson *et al.* 2007; Fletcher *et al.* 2009; Nordin *et al.* 2010; Wirth *et al.* 2013). In addition, *C. caesiocinerea* usually avoids habitats with harsh climatic conditions in high mountains, being known in Poland mostly from lowlands. *Sagedia zonata* can also be confused with *Aspicilia aquatica* (Fr.) Körb., although the latter can be distinguished by the whitish colour of the thallus, the larger ascospores ($22\text{--}27\text{--}35 \times 14\text{--}20 \mu\text{m}$) and the occurrence in the vicinity of water (Owe-Larsson *et al.* 2007; Wirth *et al.* 2013). *Aspicilia polychroma* Anzi is similar in terms of the secondary chemistry, thallus appearance and ascospore size, but it can be distinguished by its longer conidia ($20\text{--}25 \mu\text{m}$) (Wirth *et al.* 2013).

The nucITS rDNA marker was sequenced from six specimens (Table 1) and showed a 98–99% identity with the sequences of *S. zonata* deposited in GenBank (Table 2, data for *S. zonata*), of which sequences of the best hits from BLAST analyses originated from Sweden.

Material examined: POLAND. Dolnośląskie province: Sudety Mts, Karkonosze Mts, Mały Śnieżny Kocioł cirque, on basalt rock, July 1883, B. Stein (WRSL-7755); Sudety Mts, Karkonosze Mts, Kocioł Wielkiego Stawu cirque, on granite rock, 21 July 1865, B. Stein (WRSL-7736); Karkonosze Mts, Mały Śnieżny Kocioł cirque, basalt vein, on basalt, August 2006, M. Kossowska, W. Fałtynowicz (Hb. Kossowska 383, 459, Hb. Szczepańska 41, 924); Karkonosze

Mts, Mały Śnieżny Kocioł cirque, basalt vein, on basalt, 18 July 2013, *K. Szczepańska 1102* (Hb. Szczepańska); Małopolskie province: Karpaty Mts, Tatry Zachodnie Mts, Chochołowska Valley, elev. 1140 m, on granite boulders in the Chochołowska clearing, 1 December 1995, *J. Nowak* (KRAM L-42336, KRAM L-42339); Podkarpackie province: Karpaty Mts, Bieszczady Mts, Wetlińska Meadow, south-western slope of the Roh Mt, elev. 1025 m, on sandstone rock, 11 September 2004, *J. Kiszka, R. Kościelniak* (KRAP L); Bieszczady Mts, Wetlińska Meadow, south-western slope of Roh Mt, close to the tourist trail, elev. 1190 m, on sandstone rock, 11 September 2004, *J. Kiszka, R. Kościelniak* (KRAP L); Bieszczady Mts, Wietlińska Meadow, north-eastern slope, close to mountain shelter, elev. 1160 m, on sandstone rock, 12 September 2004, *J. Kiszka* (KRAP L).

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