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Notes on the *Ramalina pollinaria* group (*Ramalinaceae*, *Ascomycota*) in Poland, with the first records of *R. arsenii* from the country

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Abstract. The *Ramalina pollinaria* group is comprised of three species in Poland: *R. arsenii*, *R. europaea* and *R. pollinaria*. Their presence in the country is confirmed by sequencing the nuITS rDNA barcode marker. *Ramalina arsenii* is reported from Poland for the first time and this is the first corticolous record of this species. All species are characterized and compared morphologically.

Key words: fruticose lichens, lichenized fungi, taxonomy

Introduction

The family *Ramalinaceae* C. Agardh consists mostly of genera with crustose thalli. However, four genera are fruticose. Three of these are endemic to the Pacific coastal deserts of the New World (*Niebla* Rundel & Bowler and most species of *Vermilacinia* Spjut & Hale) and the Atlantic coast of south-western Africa (*Namibialina* Spjut & Sérus. and one *Vermilacinia* species). The fourth genus, *Ramalina* Ach., contains around 230 species. It is more widely distributed and is considered subcosmopolitan (Spjut 1995; Spjut et al. 2020; Sérusiaux et al. 2021).

Species of *Ramalina* are often highly variable in terms of both morphology and chemistry, and can exhibit significant variation even within the same locality. This has led to the description of numerous species or intraspecific taxa (Motyka 1962), many of which are now regarded as chemotypes or morphotypes of a single species (e.g., Nimis 2016; LaGreca et al. 2020; Martellos et al. 2023). So far, fourteen species have been reported from Poland (Fałtynowicz et al. 2024). This number includes *Ramalina pollinaria* (Westr.) Ach., a sorediate species with shiny thallus surface, which produces usnic and evernic acids (Smith et al. 2009). This species also showed quite a lot of variability in morphology, and has recently been divided into four distinct species that differ in morphology and geographical distribution. These are *R. arsenii* Sérus., van den Boom & Magain, which is considered exclusively saxicolous, *R. europaea* Gasparyan, Sipman & Lücking *R. pollinaria* s.str., which has a Eurasian distribution, and *R. labiosorediata* Gasparyan, Sipman & Lücking, which is known only from North America (Gasparyan et al. 2017; Sérusiaux et al. 2021; Kashiwadani et al. 2023; Sharifi et al. 2025). The latter three species can grow on tree bark and rock surfaces (Gasparyan et al. 2017; Sharifi et al. 2025). So far, only *R. europaea* and *R. pollinaria* have been reported in Poland (Fałtynowicz et al. 2024; Szymczyk et al. 2024), but their identification has not been confirmed by sequencing the nuITS rDNA, a barcode marker which allows the separation of these species with certainty. This paper presents the first molecular evidence to confirm the presence of *R. europaea* and *R. pollinaria* in Poland. Additionally, we present the first records of *R. arsenii* in Poland, which are also the first corticolous records for the species.

Materials and methods

Taxon sampling

Fresh specimens were collected during fieldwork in Poland by M. Kukwa and R. Szymczyk. The specimens have been deposited in the lichen collection at the University of Gdańsk (UGDA). Morphology was examined using a stereomicroscope Nikon SMZ800N. Secondary substances were studied using thin-layer chromatography, following the protocols presented by Orange et al. (2001).

DNA extraction, PCR amplification and sequencing

We successfully obtained molecular data from seventeen specimens of the *Ramalina pollinaria* group. DNA

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was isolated using a modified CTAB method (Guzow-Krzemińska & Węgrzyn 2000). The primers used for DNA amplification were ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). PCR amplification followed the protocol outlined in previous studies (Ossowska et al. 2018, 2019). The sequencing was performed by Macrogen (http://www.macrogen.com). The sequences were aligned in Auto Assembler v. 1.4.0 (Parker 1997). The newly obtained nucITS rDNA sequences of *Ramalina* spp. were deposited in the GenBank database (http://www.ncbi.nlm.nih.gov/genbank), and their accession numbers are listed in Table S1.

Sequence alignment and phylogenetic analyses

The newly generated nuITS rDNA sequences, together with selected representatives of *Ramalina* spp., were aligned in SeaView v. 4 (Galtier et al. 1996; Gouy et al. 2010) using the algorithm MUSCLE v. 3.5 (Edgar 2004). The final alignment consisted of 55 ITS rDNA sequences and 832 characters. Three sequences of *Ramalina fastigiata* were used as an outgroup, including one generated in this study. The dataset was subjected to IQ-TREE analysis to find the best-fitting nucleotide substitution models (Nguyen et al. 2015; Kalyaanamoorthy et al. 2017; Hoang et al. 2018) and the TN+G4 model was used for the phylogenetic analysis under the maximum likelihood criterion and followed with 1,000 bootstrap replicates (Nguyen et al. 2015; Kalyaanamoorthy et al. 2017; Hoang et al. 2018).

A Bayesian analysis was carried out using the Markov Chain Monte Carlo (MCMC) method, in MrBayes v. 3.2.7a (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) on the CIPRES Web Portal (Miller et al. 2010). Two parallel MCMC runs were performed, each using four independent chains and ten million generations, sampling every 1,000th tree. Posterior probabilities (PP) were determined by calculating a majority-rule consensus tree after discarding the initial 25% trees of each chain as the burn-in. Convergence between runs was also verified using the Potential Scale Reduction Factor (PSRF) with all values equal or close to 1.000. Phylogenetic trees were visualized using FigTree v. 1.4.3 (Rambaut 2009) and further modified in Inkscape (https://inkscape.org/). PP ≥ 0.95 and BS ≥70 were considered to be significant and are shown near the branches on the phylogenetic tree.

Results and discussion

The final DNA sequence alignment included sequences from 6 species, resulting in a total of 832 characters, 245 distinct patterns, 67 parsimony-informative sites, 48 singleton sites and 717 constant sites. The sequences of the *Ramalina pollinaria* group are divided into four distinct and well-supported lineages. These lineages represent four species: *R. arsenii*, *R. europaea*, *R. labiosorediata*, and *R. pollinaria* (see Fig. 1). Additionally, some sequences uploaded to GenBank as *R. pollinaria* were included in our phylogeny. The sequence of *R.* aff. *pollinaria* from China (GenBank accession no. JF923612) was also included by Gasparyan et al. (2017). In both our study and their own, this sequence does not group with any species with high support (Fig. 1). Perhaps the material belongs to an undescribed species. Four sequences (GenBank accession nos. JF923602, MN954836, MN006794 and OK577934) cluster with two sequences of *R. intermedia* (Delise ex Nyl.) Nyl. (Fig. 1), however, their relationship to that species requires further study.

We generated 16 new sequences from specimens belonging to the *R. pollinaria* group collected in Poland. Eight of these sequences belong to *R. europaea*, as they cluster together with sequences from this species, including the type specimen. Two sequences fit in the *R. arsenii* clade (together with the sequence obtained from the type) and six in the *R. pollinaria* clade.

The species

For the key to the species of the *Ramalina pollinaria* group, see Gasparyan et al. (2017) and Sérusiaux et al. (2021).

Ramalina arsenii Sérus., van den Boom & Magain, The Lichenologist 53(6): 434. 2021. (Fig. 2)

Notes. For a detailed description see Sérusiaux et al. (2021).

In the *Ramalina pollinaria* group the species is characterized by irregularly lip-shaped, rather large soralia that develop on the underside of the lobes, usually small thalli (up to 3 cm long) and the ITS barcode (Sérusiaux et al. 2021). According to Sérusiaux et al. (2021) and Sharifi et al. (2025), it can also be distinguished within the group by its saxicolous habitat, as it has only been reported on calcareous rocks. However, Polish specimens were found on the bark of trees, meaning that this character is no longer discriminatory. The Polish samples were initially identified as *R. europaea* because they had more or less oval soralia and few short branchlets producing small soralia (Fig. 2B, C), which resembled Fig. 2 in Gasparyan et al. (2017). However, some lip-shaped soralia, which are characteristic of *R. arsenii*, were also present (Fig. 2C–F). Nevertheless, similar (sub)terminal soralia were also found in *R. europaea* (Fig. 3A, F), but these were always accompanied by numerous small branchlets bearing punctiform soralia (Fig. 3A–D) or with additional more or less vaulted (more or less helmet-shaped) soralia (Fig. 3E, F) reported also by Gasparyan et al. (2017). Based on the morphology and substrate preferences of the specimens sequenced for this paper, we conclude that the determination of specimens without numerous branchlets bearing soredia and vaulted soralia should always be confirmed through ITS barcoding.

Ramalina arsenii is very similar to *R. labiosorediata*, another member of the *R. pollinaria* group. The two species differ primarily in their distribution as *R. arsenii* occurs in Eurasia, while *R. labiosorediata* is only known from North America (Gasparyan et al. 2017; Sérusiaux et al. 2021). *Ramalina pollinaria* can sometimes produce labriform soralia, but this species is characterized by predominantly laminal soralia that are produced on the lower part of the lobe surfaces (and sometimes also on the upper part), as well as on the lobe margins or

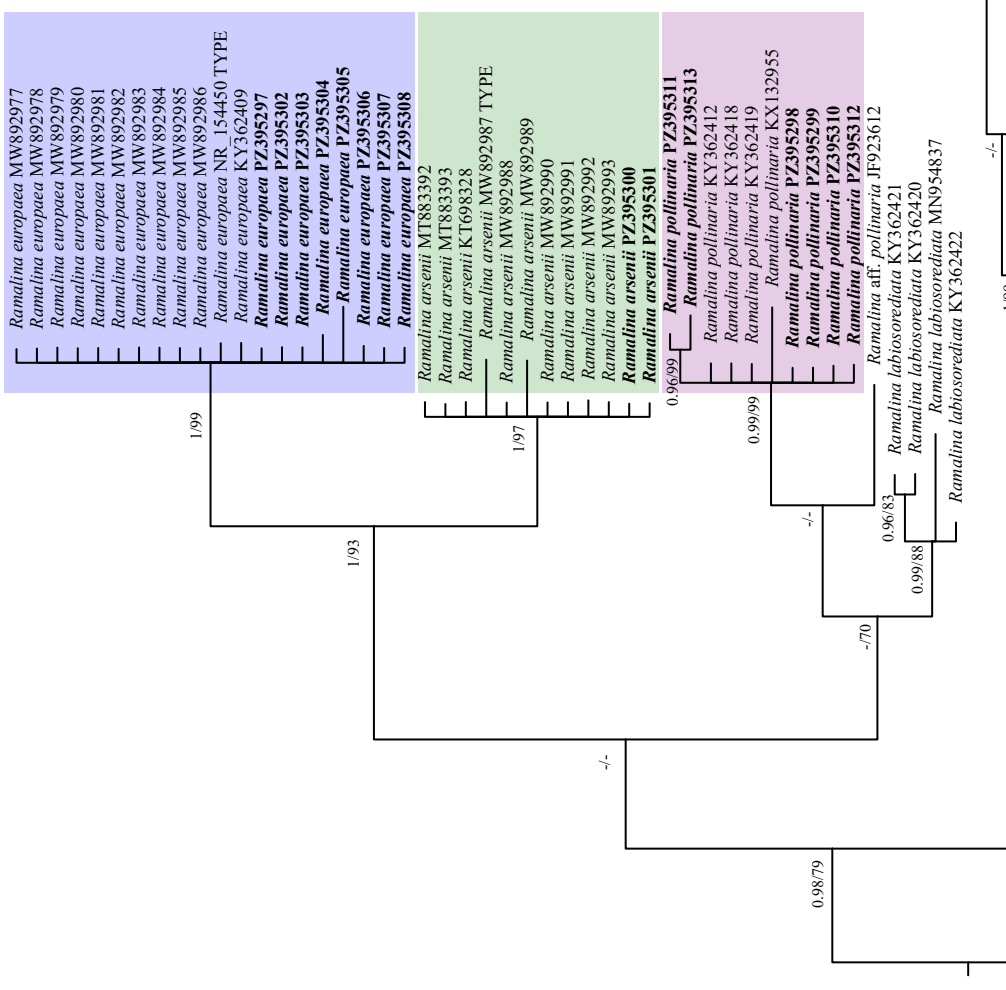

Figure 1. Majority-rule consensus tree resulting from MrBayes analysis of *Ramalina* subsp. based on nucITS rDNA data set with posterior probabilities (PP values ≥0.95) and bootstrap support values from IQ-tree (BS values ≥70) analysis presented near the branches. Sequences of *Ramalina fastigiata* were used as outgroup. Newly generated sequences from Poland are highlighted in bold. For details of samples, see Table S1.

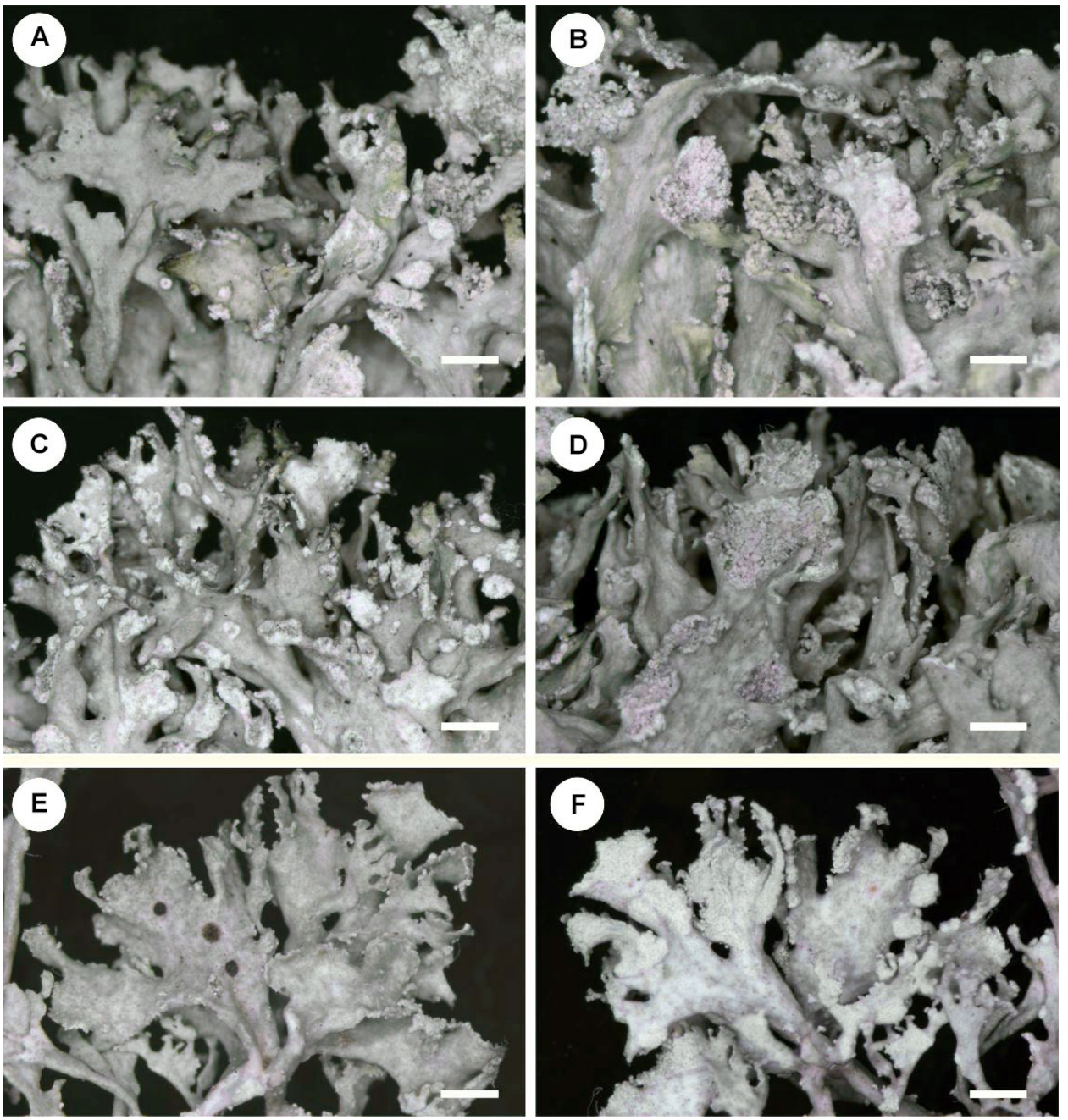

Figure 2. Morphology of *Ramalina arsenii*. A, E – upper surface of lobes; B–D, F – lower surface of lobes (A–D – UGDA L-66776; E, F – UGDA L-68770). Scales = 1 mm.

subterminally (Gasparyan et al. 2017; Sérusiaux et al. 2021; Sharifi et al. 2025).

Habitat. According to Sérusiaux et al. (2021) and Sharifi et al. (2025), *R. arsenii* is a saxicolous lichen. However, both specimens found in Poland were growing on the bark of solitary trees. These are the first records of the species on a substrate other than stone.

Distribution. So far, *Ramalina arsenii* has been reported from France, Germany, Italy, Spain and Switzerland in Europe (Sérusiaux et al. 2021; Martellos et al. 2023), and in Asia from Iran (Sharifi et al. 2025) and Russia (Altai Mts) (Kashiwadani et al. 2023). In this paper the species is reported from Poland for the first time. It was found in the Tatra Mts in the Carpathians in southern Poland and in the lowland area in the northern part of the country.

Specimens examined (all used for DNA sequencing; see Table S1). POLAND. Żuławy Wiślane, S of Szaleniec, 54.032463°N, 19.260499°E, alt. ~1 m, roadside trees, on *Fraxinus excelsior*, 30 Jul. 2024, M. Kukwa 26325 (UGDA L-66776). Tatry Reglowe, Tatrzański National Park, Dolina Kościeliska, alt. 970 m, trees on the glade, on *Tilia cordata*, 09 Sept. 2025, R. Szymczyk s.n. (UGDA L-68770).

Ramalina europaea Gasparyan, Sipman & Lücking, The Lichenologist 49(4): 306. 2017. (Fig. 3)

Notes. For a detailed description see Gasparyan et al. (2017).

Typical specimens of *Ramalina europaea* possess small, spine-like branchlets near the lobe tips, from which the soralia start out as punctiform, granular structures (later they can be more or less labriform), and granular

soredia (Fig. 3A–D). Sometimes vaulted (more or less helmet-shaped) soralia are also present (Fig. 3E, F). These characteristics clearly differentiate the species from *R. arsenii* and *R. pollinaria* (Gasparyan et al. 2017; Sérusiaux et al. 2021). The differences with *R. arsenii* are discussed under the latter species. *Ramalina pollinaria* has more irregular and larger soralia, which are produced on the lower side of the lobe surfaces (sometimes also on the upper side), along the lobe margins or subterminally. Additionally, the soredia are farinose (less than 50 µm in diam.) (Gasparyan et al. 2017; Sérusiaux et al. 2021; Sharifi et al. 2025). Gasparyan et al. (2017) pointed out that older soralia are rather similar and that some specimens (young, old or depauperate) can be intermediate in the thallus morphology and cannot be assigned with certainty to *R. europaea* or *R. pollinaria* without molecular data. Sharifi et al. (2025) also noted that the soralia can appear more or less labriform in both species. We sequenced some specimens with quite numerous branchlets in one part of the thallus, similar to *R. europaea* (one of them present in Fig. 4E, F), but on the other part of the same thallus, laminal, subterminal and marginal soralia were present as in *R. pollinaria* (Fig. 4D). The nuITS sequences placed such samples in *R. pollinaria*.

Helmet-shaped soralia are also found in *Ramalina baltica* Lettau and *R. obtusata* (Arnold) Bitter, which, like *R. europaea*, also produces evernic acid (Motyka 1962; Nowak & Tobolewski 1975; Sharifi et al. 2025). These two species can be easily differentiated by the absence of spine-like branchlets and the fact that their soralia are formed in the convex and hollow thallus lobes (Motyka 1962; Nowak & Tobolewski 1975; Sharifi et al. 2025).

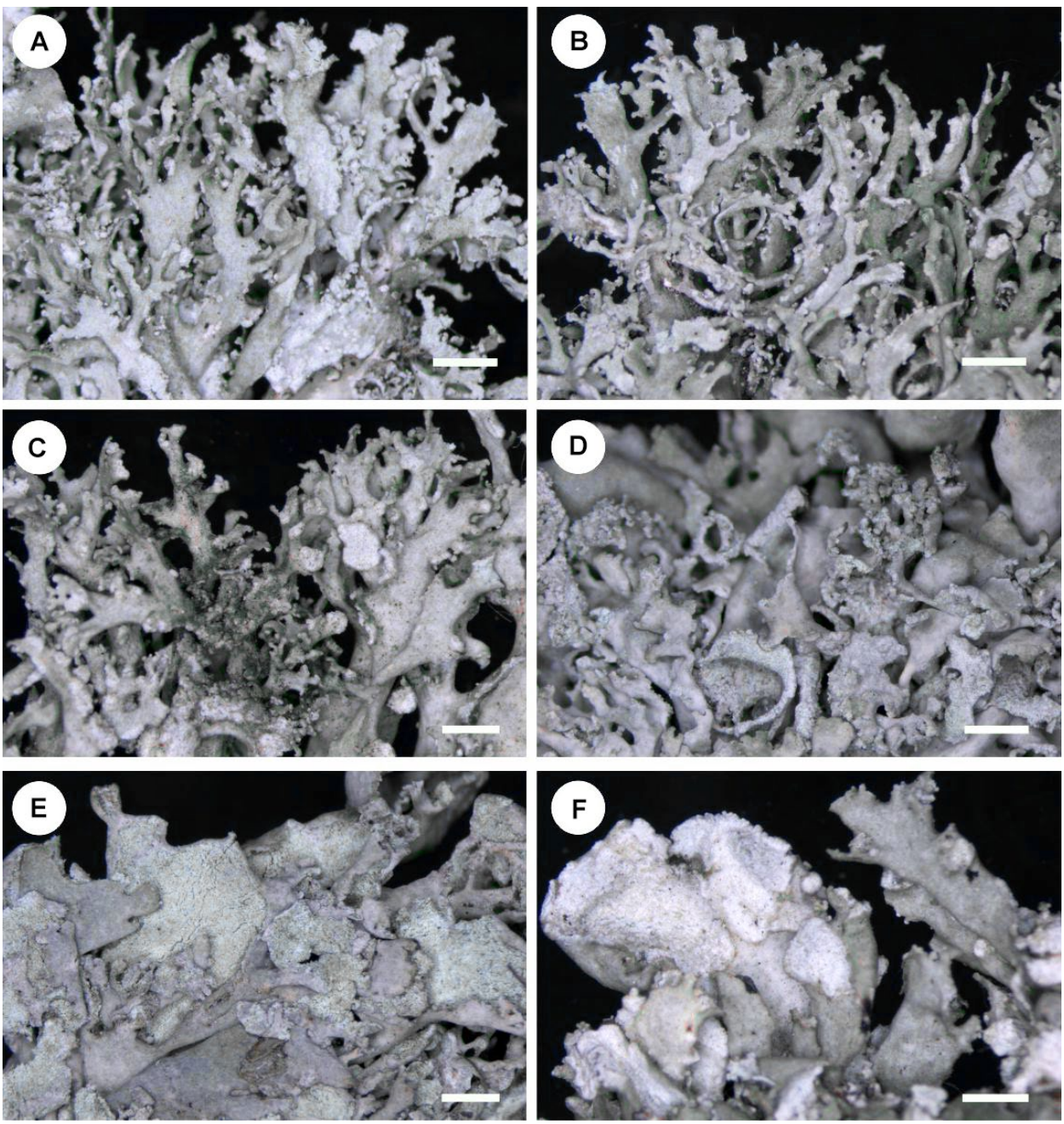

Figure 3. Morphology of *Ramalina europaea*. A, B, D – upper surface of lobes, with some of lobes turned over; C – lower side of lobes; E, F – lower side of lobes with vaulted (more or less helmet-shaped) soralia (A, B, D, E – UGDA L-65480; C, F – UGDA L-68768). Scales = 1 mm.

Habitat. According to Gasparyan et al. (2017) and Sharifi et al. (2025), *R. europaea* has a pretty wide habitat spectrum and can grow on various rocky substrata and various tree species. In Poland, the species was found mostly on the bark of trees, but also on brick.

Gasparyan et al. (2017) reported that the species was found in forests, but most of the Polish samples were found on roadside trees.

Distribution. Up to now *Ramalina europaea* is known from Austria, Belgium, Czechia, Finland, France, Norway, Latvia, Lithuania, Romania, the European part of Russia, Poland, Spain, Sweden, Switzerland and Ukraine in Europe (Gasparyan et al. 2017; Malíček et al. 2018; Dietrich et al. 2019; Khodosovtsev & Darmostuk 2020; Sérusiaux et al. 2021; Motiejūnaitė 2024; Szymczyk et al. 2024; Moisejevs et al. 2024) and Armenia, Iran, Russia (North Caucasus) and Turkey in Asia (Gasparyan et al. 2017; Sérusiaux et al. 2021; Sharifi et al. 2025).

So far, the species has only been recorded from the northern part of Poland, but unpublished observations suggest that it may be more widespread in the country. The distribution of the species in Poland will be discussed in an upcoming paper.

Specimens examined (all used for DNA sequencing; see Table S1). POLAND. Kraina Wielkich Jezior Mazurskich, near Sztynort, 54.1379°N, 21.6736°E, roadside trees, on *Quercus robur*, 29 May 2024, R. Szymczyk s.n. (UGDA L-68769). Pojezierze Łasińskie, near Limża, 53.6101°N, 19.2091°E, roadside trees, on *Quercus robur*, 24 Sept. 2024, R. Szymczyk s.n. (UGDA L-65480). Pojezierze Olsztyńskie, Gietrzwałd, 53.7480°N, 20.2357°E, brick walls, on brick, 30 Oct. 2025,

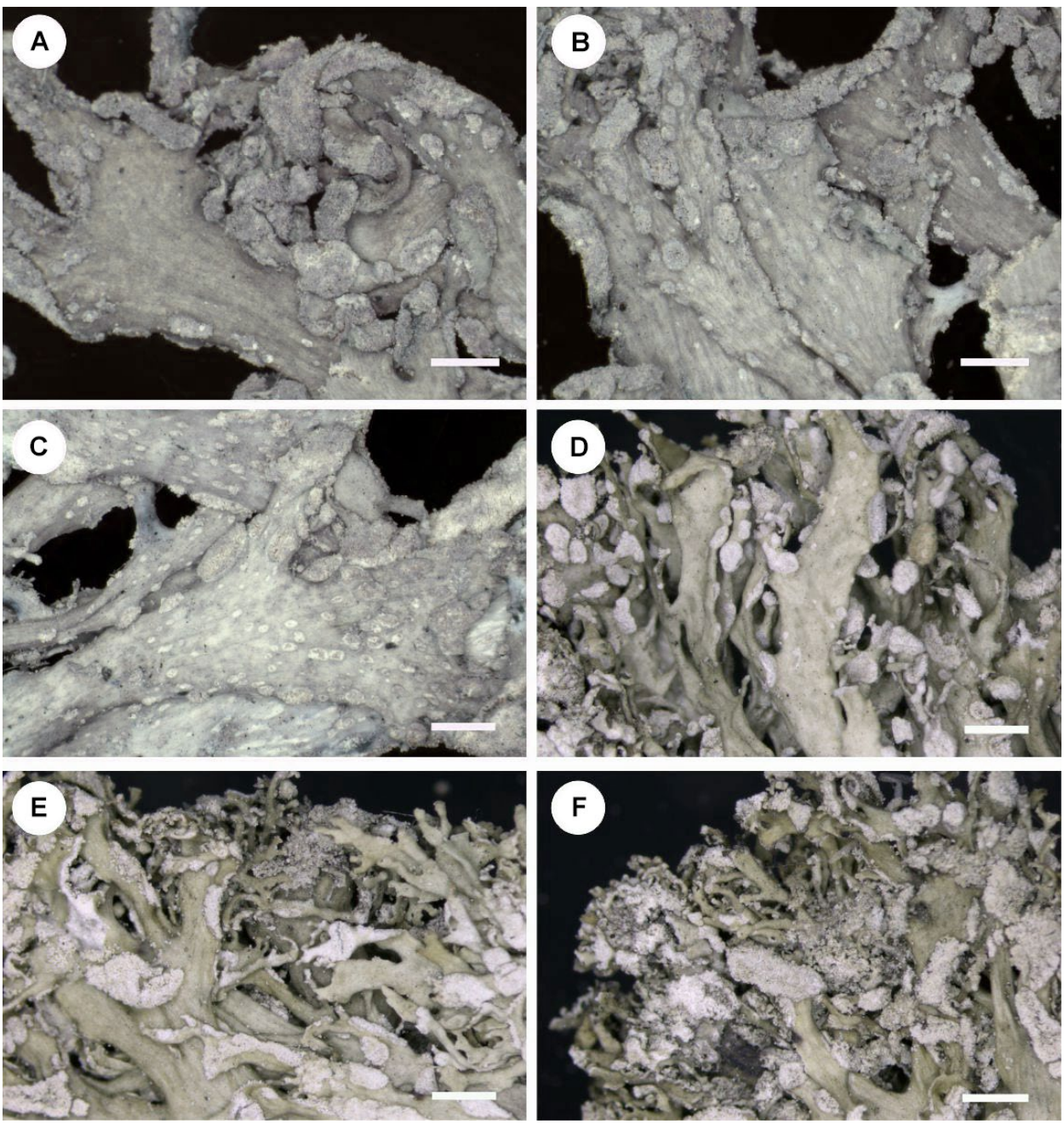

Figure 4. Morphology of *Ramalina pollinaria*. A, D, E – upper surface of lobes; B, C, F – lower side of lobes (A–C – UGDA L-68764; D–F – UGDA L-68772). Scales = 1 mm.

R. Szymczyk s.n. (UGDA L-68093). Równina Bielska, Puszcza Białowieska, Chryzanów, 52.8143°N, 23.6310°E, open area, on twigs of *Quercus robur*, 10 Dec. 2024, R. Szymczyk s.n. (UGDA L-69071). Równina Warmińska, near Dobry, 54.1293°N, 19.9409°E, roadside trees, on *Quercus robur*, 25 May 2024, R. Szymczyk s.n. (UGDA L-68767 & 68768). Równina Warmińska, Surowe, 54.0375°N, 19.7694°E, roadside trees, on *Fraxinus excelsior*, 08.10.2024, R. Szymczyk s.n. (UGDA L-65484). Wysoczyzna Białostocka, Puszcza Knyszyńska, Sokołda, ~53.248°N, 23.472°E, roadside trees, on *Quercus robur*, 2024, R. Szymczyk s.n. (UGDA L-68762).

Ramalina pollinaria (Westr.) Ach., Lichenographia Universalis: 608. 1810. (Fig. 4)

Notes. For a detailed description see Gasparyan et al. (2017) and Sharifi et al. (2025).

Well-developed specimens of *Ramalina pollinaria* produce more irregular and larger soralia, which are situated on the lower side of the lobe surfaces (and sometimes also on the upper side), along the lobe margins or subterminally; they can sometimes become labriform. The soredia are farinose (less than 50 µm in diam.) (Gasparyan et al. 2017; Sérusiaux et al. 2021; Sharifi et al. 2025). The differences between this species and *R. arsenii* and *R. europaea* are discussed under the respective species.

Habitat. Although *Ramalina pollinaria* is usually epiphytic, it has also been reported from rocky substrate (Gasparyan et al. 2017; Sharifi et al. 2025). The specimens reported here were found on the bark and twigs of deciduous trees, typically in alleys or forests.

Distribution. *Ramalina pollinaria* has been reported in several regions worldwide; however, after the distinction of *R. arsenii* and *R. europaea*, these records require revision. So far, the species, as defined by Gasparyan et al. (2017), has been found in Armenia, Azerbaijan, Belarus, Iran, the Netherlands, Pakistan, Russia, Sweden and Switzerland (Gasparyan et al. 2017; Sharifi et al. 2025; literature cited therein). However, it is very probably more widespread.

Numerous records of the species from Poland exist (Fałtynowicz et al. 2024); however, many of these records belong to *R. europaea* (Kukwa, unpubl.). In this paper, we present the first Polish records of the species, that are confirmed by molecular data.

Specimens examined (all used for DNA sequencing; see Table S1). POLAND. Nizina Sępopolska, Michałkowo, 54.3184°N, 21.2963°E, roadside trees, on *Fraxinus exelsior*, 25 Oct. 2024, R. Szymczyk s.n. (UGDA L-68763, 68764 & 68765). Równina Warmińska, near Olkowo, 54.1039°N, 20.0335°E, roadside trees, on *Tilia cordata*, 10 Oct. 2024, R. Szymczyk s.n. (UGDA L-65482 & 65486). Wysoczyzna Elbląska, Wysoczyzna Elbląska Landscape Park, Dolina rzeki Grabianki valley, 54.2654°N, 19.5332°E, deciduous forest, on twigs of *Fraxinus excelsior*, 22 Sept. 2025, R. Szymczyk s.n. (UGDA L-69072).

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Data availability

The datasets generated and/or analyzed during the current study are available in the GenBank repository. The accession numbers are available in Supplementary materials (Table S1). The alignment was deposited in figshare.com (https://doi.org/10.6084/m9.figshare.32298300).

Author contributions

Conceptualization: M.K., R.Sz. and E.A.O.; methodology: M.K. & E.A.O.; formal analysis: E.A.O.; specimens collecting and determining: M.K. and R.Sz.; laboratory works: E.A.O.; morphological and chemical analyses of specimens: M.K. and R.Sz.; writing original draft preparation: M.K. and E.A.O. (material and methods); writing – review and editing: M.K., R.Sz. and E.A.O.

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Supplementary electronic materials

Table S1. Specimens of *Ramalina* used in molecular analysis with locality, voucher information, GenBank accession numbers and list of references. Sequences generated for this study are in bold. Note. Sequences of *R. arsenii* presented by Moon et al. (2016) and Kashiwadani et al. (2021) were deposited in GenBank as *R. pollinaria*. Download file

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