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MM, MR, and TS carried out the fieldwork and wrote the manuscript; WP performed mycological investigations

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No competing interests have been declared.

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ORIGINAL RESEARCH PAPER

Population characteristics, habitat, and conservation status of *Rhododendron ferrugineum* L. (Ericaceae), a glacial relict new to Poland

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Abstract

The first reliable information on the occurrence of *Rhododendron ferrugineum* in the Karkonosze Mts (excluding spots of directly acknowledged anthropogenic origin) was provided by A. Boratyński in 1983, but the status and origin of the plants were unknown. A recent phylogeographical study proved the natural character and relict status of the aforementioned population, which makes it the northernmost and most isolated site within the whole distribution of the species. In this study, we characterized the basic aspects of the ecology and conservation status of the population and, more specifically, focused on assessing the size of the population, general health of individuals, generative propagation ability, habitat conditions, and potential threats for the species. The population persists in the Sowia Dolina (east part of the Karkonosze Mts), in a microtopographically controlled, treeless microrefugium. Shrubs of *R. ferrugineum* are part of an acidophilous dwarf-heath plant community, similar to those occurring in the Alps and the Pyrenees, although less species-diverse. The plant community in the Karkonosze Mts has been preliminarily classified into the *Genisto pilosae-Vaccinion* alliance. The *R. ferrugineum* population consists of 68 individuals: 57 fully grown and juvenile and 11 seedlings. In 2017, 10 individuals flowered, seven of which developed fruits, while in 2018, 15 individuals produced flowers and eight developed mature fruits. Seeds collected in 2017 germinated in high numbers. Plants in the Karkonosze population hosted some fungal parasites typically found in *Rhododendron* species, but no intense disease symptoms strongly influencing plant fitness were observed. A combination of significant isolation, genetic distinctness, and high genetic diversity implies a high conservation priority for the *R. ferrugineum* population in Karkonosze. Despite the theoretical threats, including stochastic risks, the *R. ferrugineum* population seems to have been stable for a long time and, importantly, it is composed of individuals of different ages, from large flowering plants to seedlings.

Keywords

Karkonosze; glacial relict; population characteristic; refugium; Sudetes

Introduction

Until recently, the genus *Rhododendron* L. was thought to have been represented in Poland by two native species, *Rhododendron luteum* Sweet [1] and *R. tomentosum* Harmaja (formerly *Ledum palustre* L.) [1,2]. Many other species of rhododendrons have been cultivated in parks and gardens as ornamental plants [3,4]. Some of them are able to propagate naturally [5,6], but none became naturalized in Poland [7,8]. Moreover, there are known cases of introductions of *Rhododendron* species in the Polish and Czech mountains in the nineteenth and early twentieth centuries. Planted individuals were able to survive over a long time, but expansions from the sites of introduction have never been observed [9–11]. Rhododendrons were usually planted close to tourist trails, mountain shelters, or viewpoints as they were supposed to increase the landscape value.

A recent phylogeographical study based on AFLP genotyping and plastid DNA sequencing demonstrated the natural and relict character of an enigmatic population of the alpine rose (*Rhododendron ferrugineum* L.) in the Sowia Dolina in the eastern Karkonosze Mts, the highest mountain range of the Sudetes range, which is located at the Polish and Czech border [12]. Here, we provide the first comprehensive description of this newly recognized relict population of *R. ferrugineum* in Poland. We characterized the basic aspects of the ecology and conservation status of this population and, more specifically, focused on assessing the size of the population, general health of individuals, generative propagation ability, habitat conditions, and potential threats for the species.

Material and methods

Study species

Rhododendron ferrugineum is an evergreen, small shrub, growing up to 60–80 cm high. Leaves are narrowly elliptic to elliptic, dark-shining green above and ferruginous beneath, with dense overlapping scales. Inflorescences contain numerous deep pink, rarely pale pink or white, flowers [13]. It occurs mainly in the Alps and the Pyrenees, with further detached populations in the Jura Mountains and the Northern Apennines [11,14]. Its presence and distribution in the Dinaric Mountains require confirmation [14]. *Rhododendron ferrugineum* grows in the subalpine zone of the mountains, rarely occurring below 1,600 m a.s.l., although there are known localities as low as at 700 m in Austria [11]. The distribution of the species is influenced by the bedrock and soil characteristics, occurring on acidic soils with siliceous rock substrates, poor in nutrients, clayey or peaty, and rich in moist humus [11,14,15]. *Rhododendron ferrugineum* is one of the main components of subalpine heaths with dwarf ericaceous shrubs. Phytosociologically, these communities are classified into the alliance *Rhododendro-Vaccinion* [16–19].

Field survey methods

We carried out field investigations during the vegetation seasons of 2017 and 2018. In the first step, we thoroughly explored the area of occurrence of *R. ferrugineum* mentioned in the literature – the Sowia Dolina. We counted all the individuals in the population and assessed the overall health of the plants. We also counted the number of flowers and developed fruits in 2017 and 2018. In 2017, we additionally collected seed samples and delivered them for germination to the Living Gene Bank of the Karkonosze National Park. Seeds were stratified at 5°C for 8 weeks and then germinated on garden soil in May 2018. Considering the purpose of this experiment was to qualitatively assess the viability of seeds, we did not perform detailed calculations of the germination rates.

To characterize the plant community of *R. ferrugineum*, we recorded all the vascular plants and cryptogams in a 10-m² plot representative for the whole population. We employed a 7-degree vegetation cover scale, according to the Braun-Blanquet method [20]. The used vascular plant nomenclature was according to Mirek et al. [1], mosses

according to Ochrya et al. [21], and plant communities according to Mucina et al. [22]. Diagnostic species for syntaxa were according to Chytrý [23].

Mycological analyses

During the two seasons, we assessed plant health every month from April to October in 2017 and twice (spring and summer) in 2018, and also collected leaves with disease symptoms for mycological analyses. Samples of leaves showing leaf blotch spots symptoms were taken from infected plants and sliced into inocula of ca. 0.2–0.3 cm. Potato dextrose agar (PDA, Biocorp) medium was used for the isolation of fungi. After incubation (22°C, 14–30 days, in darkness), they were subsequently determined to species level based on their morphology and using mycological keys [24,25].

In fall 2017, we carried out a mycological analysis of the seeds. Seeds of *R. ferrugineum* were divided into two parts. One group of seeds was surface-disinfected in sodium hypochlorite (1% available chlorine) for 10 s before transfer on 2% malt extract agar (MEA, Biocorp) in Petri dishes, while the other group of seeds was placed on 2% MEA in Petri dishes without the previous surface disinfection. The cultures on the Petri dishes were incubated at room temperature (22°C) for 10–14 days in darkness. After the incubation, fungi were identified by the morphology of their spores [26,27].



Fig. 1 Location of the population from the Karkonosze Mts (marked with the black dot) with the whole distribution range of *Rhododendron ferrugineum* in Europe indicated in dark gray (from Khela [14]).

Results

Localization, habitat, and plant communities

The population of *R. ferrugineum* is located in the upper part of the Sowia Dolina, approximately at 50°44' N, 15°46' E (Fig. 1; more detailed coordinates available upon request for conservation reasons), between 1,175–1,190 m a.s.l. The population covers a steep rock outcrop (slope 70–90°) on the west-facing slope of the valley, and the total covered area was estimated to be ca. 500 m². The outcrop is located below the tree line but, due to microtopographic constraints, it provides suitable treeless conditions, with only a few small saplings of *Sorbus aucuparia* L. and *Picea abies* (L.) H. Karst (Fig. 2). Shrubs of *R. ferrugineum* (Fig. 3) are part of an acidophilous dwarf-heath plant community. The dominant plants, excluding *R. ferrugineum*, are *Vaccinium myrtillus*

L., *V. vitis-idaea* L., and *Deschampsia flexuosa* L., accompanied by other acidophilous species such as *Huperzia selago* (L.) Bernh. ex Schrank., *Homogyne alpina* (L.) Cass., and *Calamagrostis villosa* (Chaix) J. F. Gmel. The moss layer is well developed and dominated by peat mosses, namely *Sphagnum girgensohnii* Russow, *S. capillifolium* (Ehrh.) Hedw., and the liverwort *Diplophyllum albicans* (L.) Dumort., which are indicators of a humid habitat. The plant community of *R. ferrugineum* was classified into the *Genisto pilosae-Vaccinion* alliance and was documented by the relevé below.

Date: 2017-08-16; relevé area: 10 m²; slope 75°; aspect: W; cover: shrub layer (b) 5%, herb layer (c) 60%, moss layer (d) 50%; number of species: 16; diagnostic species for alliance *Genisto pilosae-Vaccinion*: *Vaccinium myrtillus* 2, *Deschampsia flexuosa* 2, *Calamagrostis villosa* +, *Homogyne alpina* +, *Vaccinium vitis-idaea* +, *Triantalis europaea* L. r, *Dicranum scoparium* Hedw. (d) +; accompanying species: *Sorbus aucuparia* (b) 1, *Rhododendron ferrugineum* 2, *Picea abies* (c) 1, *Huperzia selago* +, *Luzula luzuloides* (Lam.) Dandy & Wilmott +, *Sphagnum girgensohnii* (d) 2, *Sphagnum capillifolium* (d) 1, *Diplophyllum albicans* (d) 2, *Polytrichum strictum* Menzies ex Brid. +.



Fig. 2 General view of the rock outcrop and the population of *Rhododendron ferrugineum* in the Sowia Dolina, Karkonosze Mts (Poland).



Fig. 3 An individual of *Rhododendron ferrugineum* growing at the studied site.

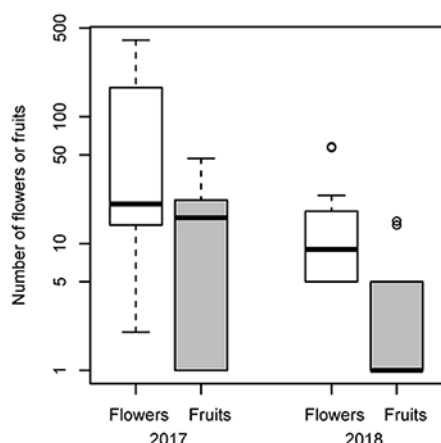


Fig. 4 Box-plots showing the number of flowers and developed fruits in 2017 and 2018 in the *Rhododendron ferrugineum* population in the Karkonosze Mts. The y axis is log-scaled, horizontal bar shows the median, boxes represent the range between the first and third quartile, whiskers represent observations in nonoutlier range, and dots represent outliers.

Demographic structure, characteristics, and condition of the population

The population of *R. ferrugineum* in the Sowia Dolina consists of 68 individuals: 57 fully grown and juvenile and 11 seedlings. In 2017, 10 individuals flowered, seven of which developed fruits. The total number of flowers was 1,101 (range: 1–401; *SD*: 141.3), and the total number of fruits was 154 (range: 0–46; *SD*: 14.7). In 2018, 15 individuals were flowering, but only eight were able to develop mature fruits. The total number of flowers was 230 (range: 4–57; *SD*: 17.7), and the total number of fruits was 45 (range: 0–14; *SD*: 4.6). We observed high variability in flowering intensity and the effectiveness of fruit development (Fig. 4). The largest shrubs, about 1.5–2 m in diameter, had most flowers but were also characterized by low effectiveness of fruit development. Individuals with the best light conditions were characterized by the highest percentage of fruit set and development of mature fruits. The seeds collected in 2017 germinated in high numbers (no specific data on germination rate were collected).

Health of the population

In 2017 and 2018, the first symptoms of fungal diseases were noted in May and June. We observed discolorations at the ends of older leaves, with dark spots – pycnidia of a fungus from the genus *Pestalotiopsis* Steyaert. All plants showed leaf blotch symptoms, with the following species isolated from the spots: *Alternaria alternata* (Fr.) Keissl (60% colonies), *Phoma levellei* Boerema & G. J. Bollen (20% colonies), and *Pestalotiopsis* spp. (10% colonies). On younger leaves, the spots appeared only after flowering. In July, on the lower side of the leaves located in the central part of the shoots, we observed characteristic leaf distortion typical for *Exobasidium rhododendri* (Fuckel) Cramer. The fungus was found in all individuals and it occurred on about 10–40% of the shoots.

The seeds of *R. ferrugineum* were mostly colonized by *Trichoderma harzianum* Rifai, *T. hamatum* (Bonord.) Bainier, and *T. polysporum* (Link ex Pers.) Rifai. Single colonies of *Penicillium* sp. were also isolated. The species composition of fungi isolated from the seeds did not differ depending on the variant of seed decontamination procedure.

Discussion

The newly described population of *Rhododendron ferrugineum* is the northernmost and most isolated population of this subalpine shrub species. It is located approximately 350 km north from the nearest populations in the Austrian Alps [14]. The provenance and character of this population have long been enigmatic and, in general, it has not been accepted as a native element of the regional flora. There are known cases of *Rhododendron* introductions in the Polish mountains, mostly in the nineteenth century. For example, *R. hirsutum* in the Tatra Mts. [10] or *R. ferrugineum* and *R. caucasicum* in the Mały Staw Cirque in the Karkonosze Mts [9] and the Czech part of the Sudetes [11]. This is in line with other introductions of ornamental alien plants intended to increase the “landscape value” in Central Europe, which occurred in the nineteenth and twentieth centuries [28,29]. On the other hand, the oldest alleged mention about the *Rhododendron* occurrence in the Karkonosze Mts [30] may indicate that the first observation of this population was earlier than the introductions of alien ornamental plants, but this historical source is unclear. Schwenkfeld’s monograph [30] included a record of a species called “rosa alpina rubra,” which was later interpreted by Krockner [31] as *R. hirsutum* L. However, the information provided about the localization and species identity is unclear, and the latter author was unable to confirm the presence of this species in the field. Likewise, subsequent floristic studies [32–34] did not report any populations of *Rhododendron* in the Karkonosze Mts. Browicz and Jakusz [9] described

individuals of *R. ferrugineum* and *R. caucasicum* Pall. from the Kocioł Małego Stawu glacial cirque, but considered them anthropogenic. The rhododendrons were planted there by the owner of a local mountain shelter at the beginning of the twentieth century. Browicz and Jakusz [9] also mentioned a locality in the Sowia Dolina but were not able to find it. This locality was only confirmed by Boratyński [35], who discovered (or rediscovered) the population and noted 30 individuals and numerous seedlings in 1983. He further mentioned that the habitat conditions of the population were similar to those in natural localities of the species. Kwiatkowski [36] confirmed this information but did not provide any further details about the population.

Krocker's interpretation [31] of the observation provided by Schwenkfeld [30] should be considered cautiously because the latter work was published more than 150 years before the Linnean binomial species nomenclature was introduced [37]. It may be that his mention of “*rosa alpina rubra*” could refer to, for instance, *Rosa pendulina* L., which would fit the statement of the species' occurrence along the streams in the mountains (“*ad riuulos montium Iunio floret*”).

Despite the interpretation uncertainty of historical accounts, the inaccessible and remote location of the studied population supports its natural character because the known introduced populations were planted close to tourist trails, viewpoints, or other accessible places. Indeed, our detailed phylogeographical study [12] supported the natural character and relict status of this population, which persisted in the topographically controlled, treeless microrefugium. Moreover, the high genetic divergence of these plants indicates that the population from the Karkonosze Mts is probably a remnant of a former larger Central European lineage of the species.

Rhododendron ferrugineum is considered a typical species of higher parts of mountains (at and above the tree line), in the subalpine vegetation belt, rarely growing in mountain coniferous forests [11,13,14]. In many regions of the Alps and Pyrenees, it is a major component of the high-mountain shrub communities of the *Rhododendro-Vaccinion* alliance [11,16–19]. In these regions, *R. ferrugineum* occurs in the *Rhododendro-Vaccinietum* community, characterized by the occurrence of *Vaccinium myrtillus*, *Calamagrostis villosa*, *Empetrum hermaphroditum* Hagerup, *Homogyne alpina*, *Lonicera caerulea* L., *Vaccinium gaultherioides* Bigelow, *Melampyrum sylvaticum* L., *Pinus mugo* Turra (or *Pinus uncinata* Ramond, which substitutes *P. mugo* in the Pyrenees), *Deschampsia flexuosa*, *Diphasiastrum alpinum* (L.) Holub, *Huperzia selago*, and *Pyrola minor* L. The investigated population in the Karkonosze Mts occurs within the altitudinal belt dominated by acidophilous montane spruce forests, but only 50–100 m below the average tree line elevation in the massif [38]. The local topography, characterized by steep and rocky slopes, provides suitable conditions for the species growth, free of the competition of trees and shrubs. The plant community with *R. ferrugineum* in the Karkonosze Mts is similar to those occurring in the Alps and the Pyrenees, although more species-poor, with several of the typical accompanying species recorded: *Vaccinium myrtillus*, *Deschampsia flexuosa*, *Calamagrostis villosa*, *Homogyne alpina*, and *Huperzia selago*. In the Karkonosze, such communities are usually classified into the *Genisto pilosae-Vaccinion* alliance. According to Chytrý [23], it includes heathlands with *Calluna vulgaris* (L.) Hull, *Vaccinium myrtillus*, and *V. vitis-idaea* confined to sites without pronounced summer drought and with continuous snow cover in winter, which has a frost-protective effect. Like other heathlands, they occupy acidic, nutrient-poor soils. This alliance is typical for the Hercynian mountain ranges of Western and Central Europe. The presence of *R. ferrugineum* makes this vegetation similar to the *Rhododendro-Vaccinion* alliance, as both syntaxa share many species [17].

Like most of the species occurring above the tree line, *R. ferrugineum* is characterized by high light requirements, growing only in open or slightly shadowed places [15]. In the studied population, plants mostly grow in open, sun-exposed sites and only partially in shaded places. According to our observations, the individuals that were partially shaded by young *Picea abies* or *Sorbus aucuparia* individuals had lower seed set than those growing in open places. Ecologically, *R. ferrugineum* is characterized by growing on moderately-moist/moist and acidic/oligotrophic soils [15]. This is reflected in the studied population as all the healthy, flowering individuals grow in places with good water conditions, indicated by a well-established moss layer with a high number of peat mosses and liverworts. The co-occurrence of acidophilic species, such as *Vaccinium myrtillus*, *Deschampsia flexuosa*, *Calamagrostis villosa*, or *Trientalis europaea*,

points to the oligotrophic and acidic conditions of the study site [15]. Hence, the overall ecological conditions of the site are similar to those of the characteristic habitats of *R. ferrugineum* in the main part of its distributional range.

Plants of the Karkonosze population hosted some fungal parasites typically found on *Rhododendron* species [39]. Notably, the fungus *Exobasidium rhododendri*, a representative of a genus assembling obligatory pathogens of Ericaceae, which commonly occurs in *R. ferrugineum* throughout its range, represents the first report of the species in Poland [40]. The fungi of the genus *Pestalotiopsis* found in the studied plants are leaf pathogens that were also observed in other species of the genus *Rhododendron* and may affect plant fitness [41]. Furthermore, the observed seed infection by fungi from the genus *Trichoderma* could indicate possible future problems for ex-situ seed propagation [26,27], as the tested disinfection method did not bring positive results. Nevertheless, we observed abundant germination of the collected seeds and seedlings in the natural locality, which confirms that the population can efficiently reproduce generatively.

A combination of significant isolation, genetic distinctness, and high genetic diversity implies a high conservation priority for the *R. ferrugineum* population in the Karkonosze. The population is very small, both in terms of the number of individuals and spatial extent. Although the species occurs in a protected area, the small size can make it vulnerable to larger habitat disturbances, such as slope erosion. Moreover, shrub expansion could unfavorably change light conditions on-site, although this threat is remote as steep slopes and high site humidity naturally block tree expansion. Despite the theoretical threats, including stochastic risks, the *R. ferrugineum* population seems to have been stable for a long time and, importantly, it is built by individuals of different ages, from large flowering plants to seedlings.

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