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ORIGINAL RESEARCH PAPER

# *Puccinia passerinii* (Pucciniales) on *Thesium ebracteatum* in the Biebrza National Park – new data on its distribution in Central Europe

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## Abstract
This work provides a morphological description of a parasitic fungus *Puccinia passerinii* Schroet. discovered in the Biebrza National Park on bractless toadflax *Thesium ebracteatum* Hayne, a host plant listed in Appendix II of the Habitat Directive and strictly protected in Poland. *Puccinia passerinii* was recorded in 1916 within the borders of current-day Poland, however, owing to a lack of herbarial materials it was not included into the checklist of Polish microscopic fungi. This work is the first report, after 100 years, on the appearance of this species in Poland, which enlarges the list of microfungi in this country and particularly in the Biebrza National Park. Moreover, a common parasite of rust fungi – *Sphaerellopsis filum* (Biv.) B. Sutton is reported for the first time on *P. passerinii*.

## Keywords
*Puccinia passerinii*; Pucciniales; rust fungi; *Thesium ebracteatum*; Biebrza National Park; *Sphaerellopsis filum*

## Introduction

The rust *Puccinia passerinii* Schroet. (Pucciniales), occurring in almost entire Europe and Asia, is an obligate parasite of plants of the genus *Thesium* L. So far, this fungus has been noted on *Thesium ebractetaum* Hayne in a few European countries: Denmark [1], Norway [2], Germany [3], Lithuania [4], Austria [5], Russia [6], and Estonia [7]. This species was also noted on other plants of the genus *Thesium*, i.e., on: *T. intermedium* Schrad. [1], *T. kernerianum* Simonk. [8], *T. kotschyanum* Boiss. [9], *T. longifolium* Turcz. [10], *T. refractum* C. A. Mey. [6,11], and *T. szowitsii* A. DC. [12].

The first information about the occurrence of *P. passerinii* on *T. ebracteatum* in the area of current-day Poland comes from Laubert [13], who in 1916 recorded this species from a town Różan, northeastern Poland. Due to a lack of morphological description of *P. passerinii* and herbarial documentation, this information was treated just as a note that needed to be confirmed [14] and, hence, this species was not included in the checklist of microscopic fungi of Poland [15].

The host of *P. passerinii* – bractless toadflax *T. ebracteatum* (Santalaceae) is under strict species protection in Poland and is listed in Appendix II of the Habitat Directive [16]. It is a European-continental species, and the area of its occurrence is limited to central and Eastern Europe. In Poland, the bractless toadflax has mainly been noted in the lowlands, but today almost all its localities are concentrated in the northeastern part of the country. *Thesium ebracteatum* occurs in national parks: Białowieża National Park, Kampinos National Park, Biebrza National Park, and Wigry National Park [17,18]. Among these, parasite microfungi were thoroughly elaborated in the Białowieża National Park [19] and partly in the Biebrza National Park [20,21].

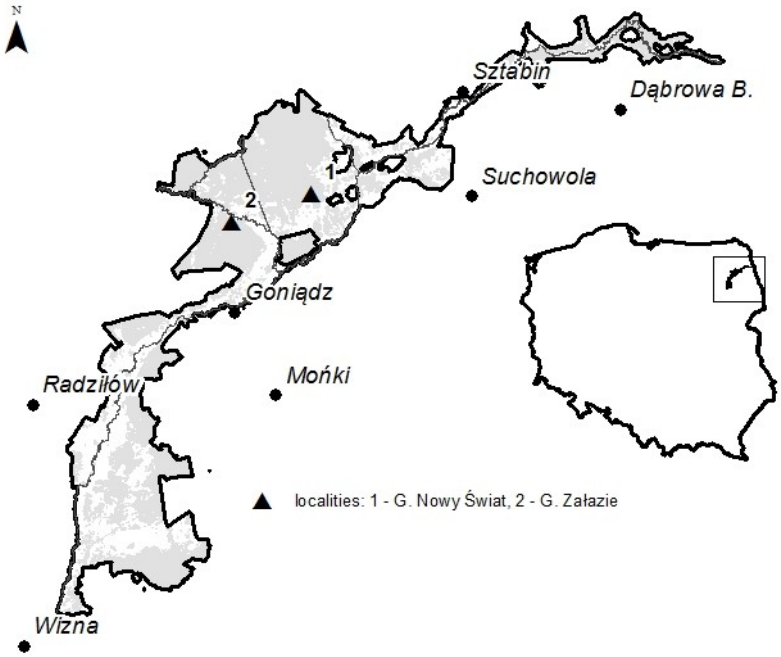


**Fig. 1** Localities of *Puccinia passerinii* on *Thesium ebractetatum* in the Biebrza National Park.

*Thesium ebracteatum* is under environmental surveillance in the framework of the National Environmental Monitoring program, which includes the evaluation of, among other things, health status of shoots at the locality [22].

The aim of the study was morphological description of a parasitic fungus *P. passerinii* Schroet. discovered in the Biebrza National Park on bractless toadflax *T. ebracteatum* Hayne.

## Material and methods

Observations have been conducted on the area of the Biebrza National Park since 2009. The studied material included shoots of *T. ebracteatum* with noticeable signs of infestation, that were sampled in June and July of 2012 from two localities found in the Middle Basin of Biebrza River valley in the Biebrza National Park (BbPN) – Góra Załazie (53°34′13.1″ N, 22°43′57,5″ E) and Góra Nowy Świat (53°35′33.5″ N, 22°51′32.4″ E) – Fig. 1. At these localities, bractless toadflax grows on mineral elevations in dry grassland (Góra Załazie) and in the ecotone, for example near *Molinia* meadow (Góra Załazie) or on the forest edge (Góra Załazie, Góra Nowy Świat). In 2012, its population accounted for: 929 shoots at the locality Góra Nowy Świat, and ca. 17 800 shoots at the locality Góra Załazie, however, population of the species at Góra Załazie was estimated based on the extrapolation of results from test areas (data from the project of protective action plan for Natura 2000 area Biebrza Valley).

In the laboratory, the studied material was subjected to macro- and microscopic analyses. Observations were made under Olympus SZX9 stereoscopic microscope and Olympus BX41 light microscope equipped with the Nomarski contrast. Measurements were taken from 50 spores of each spore state – aeciospores and teliospores (15 spores in the case of uredospores) with the Cell Software (Olympus Soft Imaging Solutions GmbH, Germany). The photographic documentation was prepared with an Olympus camera. The rust species identification was made using the keys by Majewski [14] and Brandenburger [23]. The herbarial material was deposited at the Herbarium of the Department of Mycology, University of Warmia and Mazury in Olsztyn (Poland).

## Results

The macro- and microscopic analysis of shoots of *T. ebracteatum* revealed the presence of a parasitic fungus *P. passerinii* Schroet. (Fig. 2). The parasite was noted at two localities: Góra Załazie and Góra Nowy Świat. The percentage of infested fungi reached ca. 2.2% at the first and 17.5% at the second locality.

All developmental stages of *P. passerinii* [0, I, (II), III] were found in the analyzed material (Tab. 1 and Tab. 2). In the material sampled in June, numerous spermogonia and aecia and young developing telia were observed. In the material sampled in July, the mainly telial state in different developmental stages was present on the plant. Strong infestation of plants and deformation of shoots were observed as well (Fig. 2b–c). The morphological characterization of sampled specimens is given below.

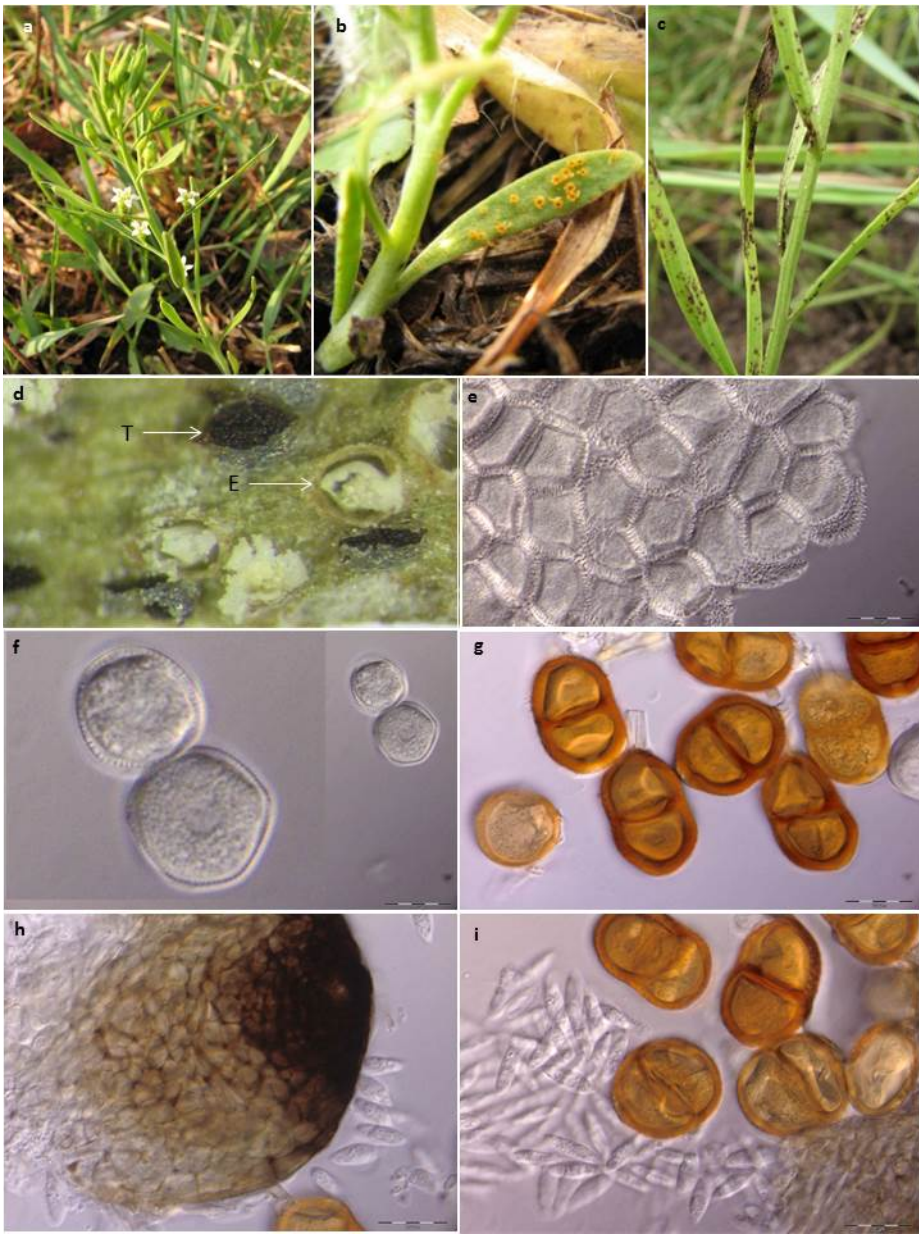


**Fig. 2** The developmental stages of *Puccinia passerinii* on *Thesium ebracteatum*. **a** Healthy plant. **b,c** Infected plants. **d** Aecia (E) and telia (T) on leaf. **e** The wall of aecial peridium. **f** Aeciospores. **g** Teliospores. **h** Sphaerellopsis filum – pycnidium. **i** Conidia of *Sphaerellopsis filum* and teliospores of *Puccinia passerinii*. Scale bars: 20 µm.

**Description of species.** Spermogonia numerous, amphigenous, well developed, 150–200 µm in size. Aecia very numerous on stems and amphigenous, orange-yellow (Fig. 2d); cup-like peridium with bended, ragged yellow rim, the outer walls up to 13 µm thick, the inner walls 2.5 to 4 µm thick and verrucose (Fig. 2e); aeciospores with spherical outline, bluntly polygonal, 17–20(–22) × 15–17(–18) µm, the wall up to 1.5 µm thick, verrucose (Fig. 2f). Urediniospores very rare, around telia, spherical or elongated, 24–26 µm in diameter. Telia initially rusty, then black-brownish, very numerous, on stems and amphigenous, elongated and spherical, spore-releasing, initially covered with epidermis (Fig. 2d); teliospores widely-ellipsoidal, (30–)33–38(–41) × (21–)23–28(–33) µm, rounded at both tips, sometimes slightly constricted at the transverse septum; the wall yellow-brown, 3–4 µm thick, covered with warts spaced 1–2 µm each other; pedicell short, breaking off, colorless, sometimes slightly yellow; a germ pore of the upper cell on the top half, and of the lower cell on the bottom half (Fig. 2g).

**Tab. 1** Comparison of descriptions of *Puccinia passerinii* Schroet.

| Spores | The size of spores (µm) | | |
|---|---|---|---|
| | this study | Majewski [14] | Brandenburger [23] |
| Spermogonia | 150–200 | 170–220 | 170–220 |
| Aeciospores | 17–20(–22) × 15–17(–18) | 15–21 × 14–16 | 15–21 × 14–16 |
| Urediniospores | 24–26 | 24–25 | 24–25 |
| Teliospores | (30–)33–38(–41) × (21–)23–28(–33) | 32–48 × 25–31 | 29–48 × 23–32 |

**Tab. 2** Comparison of descriptions of *Puccinia passerinii* Schroet., *P. thesii* Chaillet, and *P. mougeotii* Lagerh.

| Spores | Size of spores (µm) | | |
|---|---|---|---|
| | *Puccinia passerinii* (this study) | *Puccinia thesii* (Majewski [14]) | *Puccinia mougeotii* (Majewski [14]) |
| Spermogonia | 150–200 | 100–160 | Wanting |
| Aeciospores | 17–20(–22) × 15–17(–18) | 20–23 × 17–21 | 18 |
| Urediniospores | 24–26 | 20–28 × 20–25 | 20–23 × 17–21 |
| Teliospores | (30–)33–38(–41) × (21–)23–28(–33) | 40–57 × 23–28 | 30–43 × 16–21 |

A common rust parasite – *Sphaerellopsis filum* (Biv.) B. Sutton was noted on the developmental stages of *P. passerinii*. This hyper-parasite was noted in June and July. It formed numerous black pycnidia with numerous conidia, developing on aecia and telia of *P. passerinii*, thereby disturbing the development of telisopores (Fig. 2h,i). To our knowledge, *S. filum* has not been reported on *P. passerinii* until now.

## Discussion

New species of phytopathogenic fungi from the order Pucciniales as well as new hosts for this group of parasitic fungi have been increasingly noted in recent years in Europe, including Poland [24–33]. It results from the intensification of research addressing parasite microfungi and factors that facilitate the spreading of this group of plant pathogens onto new areas and new hosts. Reports concerning the parasite microfungi originate from both natural (national parks, reserves) as well as anthropogenic areas [34–38]. These investigations concern mainly common, wild and cultivable plants. In contrast, still little information is available on the parasite mycobiota of protected plants. This work is one of the few presenting such data.

The occurrence of parasitic fungi is determined by the presence and population number of host plants [39,40]. In some regions of Poland, localities of *T. ebracteatum* – a host of *P. passerinii*, disappeared completely or partly, for example in Lower Silesia and Great Poland regions. At some localities, an increase is observed in the population of this species even to a few hundreds shoots [17,41,42], whereas at the others – it is reported for the first time ever [41,42]. Nonetheless, the presence of *P. passerinii* on *T. ebracteatum* has so far not been confirmed in Poland. In our study, the parasite was observed in the Biebrza National Park, which harbor the most abundant known localities of *T. ebracteatum* in Poland, counting even tens of thousands of shoots. In turn, on most of the localities in Poland, its population number is low and occupies a few or up to 20 $m^2$. Although the monitoring of Natura 2000 program determines

the health status of plant shoots, it only provides information about the percentage of infested plants, deformations, or potentially about the presence of parasitic fungi with no information given regarding the species composition [22].

Comparing own description of *P. passerinii* with literature data [14,23], differences were noted in sizes of morphological structures (spermogonia and spores of the analyzed species). In our observations, the speromogonia were slightly smaller (150–200 μm) compared to literature data (170–220 μm), whereas aeciospores were slightly larger (Tab. 1). In turn, the length of teliospores was somewhat shorter (33–38 μm), whereas according to literature data their size ranged from 32 to 48 μm (Tab. 1).

Two other species of rust fungi may occur on plants of the genus *Thesium*, i.e.: *Puccinia thesii* Chaillet and *P. mougeotii* Lagerh., that may be mistaken with *Puccinia passerinii* (Tab. 2). These species were reported from few localities in Poland. *Puccinia thesii* was noted on *Thesium linophyllon* L. in the Kraków-Częstochowa Upland and at numerous localities of the Lublin Upland. *Puccinia thesii* on *T. ebracteatum* was reported in the Białowieża Primeval Forest. In this case, the material was not preserved, therefore the occurrence of the parasite on this host should be confirmed. *Puccinia mougeotii* was noted on *Thesium alpinum* L. in the Western Bieszczady and Tatras [14]. Reports on *P. thesii* and *P. mougeotii* at the mentioned localities are the only ones in Poland, hence both species could be considered as rare in this country.

Although many reports were published on the parasitic fungi of Poland, there are still missing data for many areas that are interesting from the floristic and phytosociological perspective. It also pertains to the Biebrza National Park, which before the year 2012 had not been under mycological surveillance. During short-term study conducted in this area in the years 2012–2013, analyses revealed as many as 480 species of microscopic fungi [20,21]. These investigations, however, did not include species of protected plants. Hence, the record of *P. passerinii* on *T. ebracteatum* in our study is not only a significant complement to the list of the microscopic fungi of Poland, including the Biebrza National Park, but also a valuable information for geobotanists and phytosociologists in the context of parasites of protected plants which by disturbing the health condition of the host may contribute to the reduction of its population.

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