

# Fungal endophyte *Cryptomycina pteridis* (Rebent.) Syd. on the native fern *Pteridium aquilinum* (L.) Kuhn in Poland

Elżbieta Zenkteler<sup>1\*</sup>, Zbigniew Celka<sup>2</sup>, Piotr Szkudlarz<sup>2</sup> & Piotr Grzegorzek<sup>3</sup>

<sup>1</sup>Department of General Botany, Faculty of Biology, Adam Mickiewicz University in Poznań, Uniwersytetu Poznańskiego 6, 61-614 Poznań, Poland; ORCID: <https://orcid.org/0000-0002-8514-4825>

<sup>2</sup>Department of Systematic and Environmental Botany, Faculty of Biology, Adam Mickiewicz University in Poznań, Uniwersytetu Poznańskiego 6, 61-614 Poznań, Poland; ORCID: ZC <https://orcid.org/0000-0002-5344-8939>; PSz <https://orcid.org/0000-0002-4915-2721>

<sup>3</sup>Museum in Chrzanów, Mickiewicza 13, 32-500 Chrzanów, Poland

\* corresponding author (e-mail: [elzbieta.zenkteler@amu.edu.pl](mailto:elzbieta.zenkteler@amu.edu.pl))

**Abstract.** *Cryptomycina pteridis* (Ascomycota, class Sordariomycetes) is a highly host-specific fungus, infecting only a native, fern *Pteridium aquilinum*. This endophyte is usually overlooked, although it is easy to recognise due to its characteristic symptoms on bracken fern fronds, especially advanced narrowing and shrivelling of pinnae and pinnules. Infested plants have pinnae and pinnules curling upwards, and dark stromata along the veins on the undersides of pinnules. The fungus is an obligate systemic endophyte, which disturbs fern growth. The paper presents some old and new records of *C. pteridis* in Poland.

**Key words:** *Cryptomycina pteridis*, host-specific fungus, new record, *Pteridium aquilinum*, bracken fern

## 1. Introduction

*Pteridium aquilinum* (L.) Kuhn is a bracken fern occurring in temperate and subtropical regions in both hemispheres. Although it is native to Eurasia and North America, the extreme lightness of its spores has led to it achieving a cosmopolitan distribution. It is a prolific and abundant plant in Poland, where it is limited to altitudes below 600 m (GBIF 2023a; Hassler 2023; POWO 2023).

This fern species is relatively rarely infected by pathogenic fungi due to its strong self-defence created by high concentrations of secondary metabolites: cyanogenic glycosides, ptaquiloside (norsesquiterpene glucoside), phenols, sesquiterpenes, and tannins (Castrejan-Varela *et al.* 2022). The surface of *Pteridium* leaves can also be colonized by rusts, including *Puccinia* spp., *Uredinopsis macrosperma* (Ckc.) Magn., and *Uredinopsis pteridis* Diet. & Holw., which in forests have been observed on neighbouring trees, i.e. *Abies* and *Picea* (McTaggart *et al.* 2014). Other saprophytic fungi, including *Ascochyta pteridis* Bres. & Irvine, *Cladosporium grumosum* Pers. ex Lk., *Mycosphaerella pteridi-*

*cola* Dearn & House, *Phoma aquilina* Sac. & Penz., and *Taphrina* spp., show low pathogenicity towards *Pteridium* (Cody & Crompton 1975). However, a few fungal endophytes can occur on *P. aquilinum*. The most interesting is *Cryptomycina pteridis* (Reb. ex Fr.) Hohn (Ascomycota, class Sordariomycetes, syn. *Sphaeria pteridis* Rebent.), described by Johann Friedrich Reben-tisch in 1804 (Bache-Wiig 1940). It is also known under the synonyms *Cryptomycina filicina* (Fr. Ex Fr.) L. & K. Holm and *Cryptomyces pteridis* Sydow 1921 (Sydow 1923). The fungus is linked to anamorphic *Cryptomycella pteridis* (Rebent.). It is highly host-specific, infecting only the genus *Pteridium*. Beside *P. aquilinum*, it was recorded also on *P. latiusculum* (Desv.) Hieron in the USA and Canada (Cody & Crompton 1975) and on *P. esculentum* (G. Forst.) Cockayne in Australia and New Zealand (Holm & Holm 1978). Its occurrence on *P. aquilinum* was recorded in Northern Europe – Norway, Sweden, Finland, Latvia, Estonia – as well as in West and Central Europe – e.g. Austria, Germany, Romania, and Poland (Ellis 2001-2022). Single records of *C. pteridis* are also known from South Africa and Asia (GBIF 2023b).

Early surveys made by Bache-Wiig (1940) showed that the fungus is systemic and perennial. The typical symptoms of 'leaf roll' disease are limited to fronds developed from systemically infected branches of the rhizome mat. Symptoms occur on fully expanded fronds and include characteristic stiffness and curling of pinnae and pinnules; followed by the eventual development of abundant, regularly distributed stromatic areas on the underside of pinnules. The fungus enters the host by sending a penetration tube through the wall of an epidermal cell. The tubes are absent in the apical cells; however, scattered infection sites occur in rhizomes. The fungus overwinters as inter- and intracellular mycelium in the leaf buds (Bache-Wiig 1952). The cited author made the first attempt to control *Pteridium* using *C. pteridis*. In her opinion, this endophyte proved to be compatible with the host and caused symptoms, but it did not kill the whole plant (Bache-Wiig 1952).

The relationship between *P. aquilinum* and fungi is better recognized thanks to later research on biological control of bracken with endophytes as mycoherbicides. In the Dartmoor National Park in Devon (UK), 21 endophytes were isolated from the fronds and rhizomes of *P. aquilinum* in 8 sites. A few isolates with the highest pathogenicity towards bracken were selected for potential application in biocontrol (Petrini *et al.* 1992). Among them, the most active were isolates of *Aureobasidium pullulans* (De Bary & Löwenthal) Amaud, especially in spring and autumn. *Sordaria fimicola* (Roberge ex Desm.) Ces. & De Not. and *Stagonospora* sp. were active only in spring, whereas *Ilyonectria destructans* (Zinssm.) Rossman, L. Lombard & Crous and *Phoma* sp., only in autumn. For biological control of *P. aquilinum*, chiefly *Aureobasidium pullulans* and *Stagonospora* sp. were recommended (Petrini *et al.* 1992).

In north-western South Dakota (USA), dispersal of *C. pteridis* by macro- and microconidia was analysed in a population of *P. aquilinum* (Gabel 1993). Macroconidia, dispersed from stromata on overwintering dead fronds, inoculated young fronds in spring. Conidia of this type were hyaline, unicellular, cylindrical (within rounded ends), found on fresh or dried cirrhi produced by mature stromata. The length of small macroconidia was 11.8–16.1 µm, while large macroconidia were 20.1–28.0 µm long (Gabel 1993). The poorly recognized discontinuous distribution pattern and dispersal strategies of *C. pteridis* remain unexplained (Gabel *et al.* 1996). Despite these difficulties, another pathogenic endophyte from *Pteridium* was isolated as *Cylindrosporium aquilinum* (Pass.) J. C. Gilman & W. A. Archer (syn. *Septoria aquilina* Pass.) and applied as a mycoherbicide. The cited authors sprayed bracken with *Cylindrosporium* conidial suspension, with significant and promising results. Inoculated bracken fronds became wilted, ne-

crotic, and died. Among all the above-mentioned fungi, *Cylindrosporium* is particularly promising as a biological agent, which works by producing toxic compounds that dissolve the cell walls of targeted plants. Unlike traditional herbicides, mycoherbicides can reproduce themselves and linger in the soil for many years to destroy noxious weeds (Gabel & Salazar 1996).

The attempts to control *Pteridium*, presented above, are justified for the protection of agricultural crops (Cody & Crompton 1975). However, in the forest ecosystem, the presence of *P. aquilinum* has a positive significance. The rhizosphere of *Pteridium* is exposed to numerous soil microorganisms, including arbuscular mycorrhizal fungi (AMF). Their roots frequently develop symbiotic associations with the Glomerales, e.g. *Planticonsortium tenue* (Greenall) C. Walker & D. Redecker, syn. *Glomus tenue* (Greenall) I. R. Hall (Martinez *et al.* 2012; Lehnert & Kessler 2018; Lopez *et al.* 2022). The presence of AMF has been confirmed and their important protective function on *Pteridium* in Poland is emphasized (Turnau *et al.* 1993). Interestingly, *P. aquilinum* is particularly resistant to heavy metals by decreasing metal uptake, and mitigating the toxic effect of arsenic, cadmium, lead, and zinc. It acts as an ecological filter that influences regeneration of other plants and meets the demands for a good bioindicator to improve environmental control (Lopez *et al.* 2022).

The aim of the study is to direct the attention of botanists and foresters to the characteristic symptoms of infection by *Cryptomycina pteridis* on *Pteridium aquilinum*. Knowledge of the symptoms will help in broader data collection on new sites, resulting in an assessment on the status of the endophyte in Poland and Europe.

## 2. Material and methods

We collected *P. aquilinum* fronds with symptoms of *C. pteridis* infection – leaf roll and dark stromata on the lower side of the pinnules – in Budzyń Forest Range (Podanin Forest District, division 253; 52.9136672°N, 16.983552°E) on 28 September 2022. The stand was monitored again on 13 September 2023. Probably due to differences in weather conditions between the years, in 2023 the fronds of *Pteridium* were mostly withered or completely dry and the disease symptoms were much more evident. Symptoms of the disease on the freshly collected leaves were compared with those presented in the *Identification Handbook* (Ellis 2001–2022) and with *Pteridium* specimens from the collection of 'Fungarium' created by J. W. Szulczewski – an outstanding plant taxonomist, ethnobotanist, zoologist, physiographer, and educator (Dzięczkowski 1977). The collected specimens infested by *C. pteridis* are deposited in the Herbarium of the Department of Systematic and Environmental



Botany (formerly Department of Plant Taxonomy), Adam Mickiewicz University, Poznań, Poland (POZ).

### 3. Results and discussion

*C. pteridis* was found on a diseased *P. aquilinum* in Budzyń forest in 2022. The infected fronds showed

the leaf roll symptoms, including upwards curling. The characteristic underside symptoms on green pinnules are convex stromata arranged along the nerves and covered by dark cirrhi containing conidia (Fig. 1A). The infected plant was growing next to a forest path, in an old moderately moist mixed forest dominated by *Pinus sylvestris* L. with blackberry and raspberry bushes in



**Fig. 1.** External evidence of symptoms of *Cryptomycina pteridis*, on green (A) versus brown and dried pinnules (B) of *P. aquilinum* (photograph by Z. Celka). Sheets from 'Fungarium' of J. W. Szulczewski (C, D); dried adaxial side of *Pteridium* pinnae with symptoms of *Cryptomycina pteridis*, collected on the edge of Pożegowo Moraine in the Wielkopolska National Park in 1960 (photograph by P. Szkudlarz)



the undergrowth. In August 2022, no infections were spotted on fronds belonging to the population within 20 m<sup>2</sup> around the diseased plant. In contrast, during monitoring of the same site the following year (September 2023), on the dry leaves conspicuous symptoms of dark, drying stromata along the veins, and darkening

of pinnae and pinnules were observed (Fig. 1B). In the case, the underside of pinnules from Budzyń forest revealed the same symptoms as on pinnae of *Pteridium* found in the 'Fungarium' sheet of J. W. Szulczewski (Fig. 1 C-D). Those symptoms occurred commonly, on numerous fronds belonging to the same population in



**Fig. 2.** Comparison of *Pteridium aquilinum* frond appearance on 28 September 2022: (A) infected with *Cryptomycina pteridis* and (B) uninfected; and on 13 September 2023: (C) infected and (D) uninfected but completely dry. All found in Budzyń Forest Range (photographs by Z. Celka)



2023. The disease symptoms were more intense during frond browning in autumn than in summer (Fig. 2). The advanced narrowing and shrivelling of pinnules and the overhanging tips of the pinnules gave the fronds an openwork appearance that allowed us to distinguish the diseased plant from healthy ones (Fig. 2A-C).

Our review of literature on this fungal endophyte has shown that in Poland *C. pteridis* was reported from 57 locations (Appendix 1). One of the earliest records of *C. pteridis* was made by Marian Raciborski in 1890 in the vicinity of Kraków (Wróblewski 1915). Most of the finds – as many as 22 stands – were documented by Schroeter (1908). Single sites of *C. pteridis* were

recorded in 1918-1978: three in Białowieża Forest, two in Wielkopolska, five in southern, and one in southwestern Poland. In 1992-2023, P. Grzegorzek found it in 12 stands (Appendix 1). One of the recent locations of *C. pteridis* (2008) includes the local bracken population of *P. aquilinum* ssp. *aquilinum* on the slopes of Strzałba Hill near the ‘Janina’ coal mine in Libiąż (southern Poland). Ferns were in poor condition (in a dry site, at the end of August), but despite this, evident linear sori were noticeable along pinnule margin, suggesting a readiness for spore release (Fig. 3A) (P. Grzegorzek, unpublished). Hitherto underestimated symptoms of *C. pteridis* are the drying and brittleness of the pinnule



**Fig. 3.** Crumbled dried leaflet tissue, and dark spots on the rachis (arrows) on pinnules of *Pteridium aquilinum* subsp. *aquilinum* from Libiąż in 2008 (A); and curling, browning, and drying of pinnules of *P. aquilinum* subsp. *pinetorum* from ‘Dolina Żabnika’ nature reserve in 2019 (B) (photographs by P. Grzegorzek). The most conspicuous symptoms of *Pteridium* disease in autumn (C) (photograph by Z. Celka)



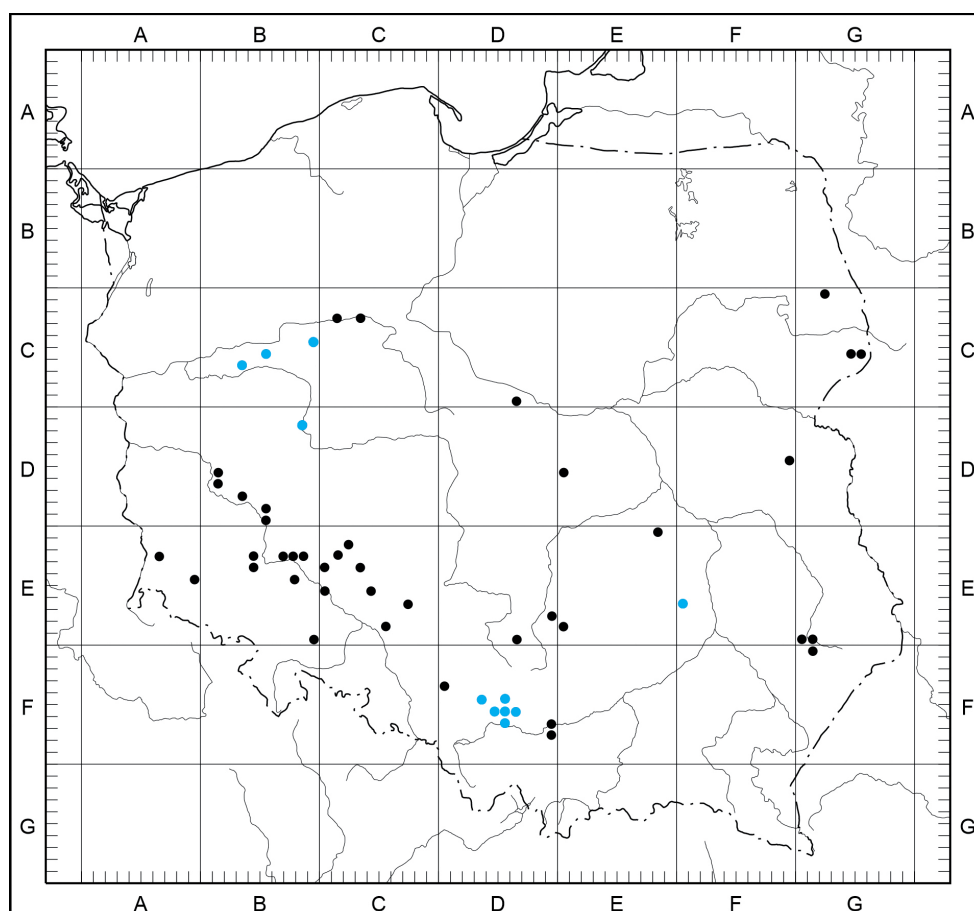
margins (lesions) seen on young and mature fronds. The fungus was also found on *P. aquilinum* ssp. *pinetorum* in southern Poland in nature reserve ‘Dolina Żabnika’ near Jaworzno (Silesia). The entire population of bracken was in good condition, in a wet location, in June 2019 (Fig. 3B). The above-mentioned data confirm the endophytic occurrence of *C. pteridis* on both subspecies of *P. aquilinum* growing in Poland (Zenkteler & Nowak 2019). Particularly the observations carried out in autumn show an increase in the symptoms of infection, making it easier to spot diseased individuals (Fig. 3C). The specimens collected by P. Grzegorzek – curator of the Museum in Chrzanów – are documented in his private herbarium (specimens from 1992-1994 and 1999) and available at <https://www.bio-forum> (Grzegorzek 2008-2019). Repeated observations of the recorded sites of *C. pteridis* have confirmed its survival e.g. in Libiąż and ‘Dolina Żabnika’ reserve (P. Grzegorzek, personal communication).

On the one hand, the distribution of *C. pteridis* in Poland is significantly influenced by its narrow host range, which limits the number of its potential sites.

On the other hand, ignorance of the typical symptoms also determines the low number of the reports about new sites of *C. pteridis*. The attached map shows that they were found mainly in central and southern Poland (Fig. 4). The northern parts of the country (Szczecin and Gdańsk Pomerania) remain still unexplored. Those areas have large populations of both subspecies of *Pteridium* (Zajac & Zajac 2001, 2019), so observations and research on this subject should begin there.

*C. pteridis*, restricted to one fern species, is an example of rare and exceptional relationship between fungus and plant. The fungus should be included in the list of threatened species both in Europe and worldwide (Aarnæs J-O 2002; Nordén & Jordal 2015). Also in Poland, further research is needed to determine the status of *C. pteridis*.

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**Fig. 4.** Distribution of *Cryptomycina pteridis* in Poland

Explanations: black circles – literature data, blue circles – data of the authors of this article and herbarium specimens of J. W. Szulczewski



**Author Contributions:**

Research concept and design: E. Zenkteler, Z. Celka  
 Collection and/or assembly of data: E. Zenkteler, Z. Celka, P. Szkudlarz, P. Grzegorzek  
 Data analysis and interpretation: E. Zenkteler, Z. Celka, P. Szkudlarz, P. Grzegorzek

Writing the article: E. Zenkteler, Z. Celka  
 Critical revision of the article: E. Zenkteler, Z. Celka  
 Final approval of article: E. Zenkteler, Z. Celka

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**Appendix 1.** Chronological list of *Cryptomycina pteridis* records in Poland, with ATPOL square numbers (see Zajac 1978) after names of localities

- 1885: Owadów, EE08 – A. Zalewski in Wróblewski (1915).
- 1886: Krzywy Kołek near Skrwa, DC96 – A. Zalewski in Wróblewski (1915).
- 1890: Kobierzyn (currently part of Kraków), DF79; Przegorzały (currently part of Kraków), DF69 – M. Raciborski in Wróblewski (1925).
- 1902: Stołpno (currently part of Międzyrzec Podlaski), Moszczona, FD49 – Eichler (1902), Eichler (1907).
- 1904: Rothwasser (now in Polish: Czerwona Woda), SW, Hopfenberg, AE26 – R. Rakete; Staatliche Naturwissenschaftliche Sammlungen Bayerns, the Fungal Collection at the Senckenberg Museum für Naturkunde Görlitz. Occurrence dataset <https://doi.org/10.15468/fhmwzv> accessed via GBIF.org on 2023-03-22. <https://www.gbif.org/occurrence/441980514>
- 1908: Konotop, BD51; Borowiec, BD61; Głogów, Leśna Dolina, BD73; Karczowiska, BE23; Pątnów Legnicki, BE33; Lwówek Śląski, AE49; Góra, BD85; Górki (Naroczyce), BD95; Wołów, BE26; Rościszewice, BE27; Oborniki Śląskie, BE28; Stradomia, CE33; Goszcz, CE12; Byków, CE30; Dobroszyce, CE21; Kamienna, CE54; Mokra, BE47; Kotowice, CE50; Gromnik, BE99; Kluczbork, CE67; Kup, CE85; Gliwice, DF30 – Schroeter (1908).
- 1918: Konieczno near Włoszczowa, EE80 – Staatliche Naturwissenschaftliche Sammlungen Bayerns, the Fungal Collection at the Senckenberg Museum für Naturkunde Görlitz. Occurrence dataset <https://doi.org/10.15468/fhmwzv> accessed via GBIF.org on 2023-03-22. <https://www.gbif.org/occurrence/441980514>
- 1923: Zwierzyniec (Skierniewice), ED50 – Zwiegbaumówna (1925).
- 1926: Włoszczowa, DE79 – de Moesz (1926).
- 1966: Wielkopolska National Park, Pożegowo (currently part of Mosina), BD18 – J. W. Szulczewski, Herbarium POZ.
- 1969: Nakło, CC23; Osiek, CC21 – Michalski (1982).
- 1978: Roztocze National Park, Bukowa Góra, GE90; Jarugi GE91 – Chmiel (1978), Kozłowska *et al.* (2015, 2019a).
- 1990: Białowieża Forest, GC54 – M. Wołkowycki, Fungi Collection, Forest Research Institute, European Centre for Natural Forests. Occurrence dataset <https://doi.org/10.15468/5r5kcy> accessed via GBIF.org on 2023-03-22. <https://www.gbif.org/occurrence/1707012500>, also in Kozłowska *et al.* (2019b).
- 1991: Roztocze National Park, Czerkies, GF01 – Sałata (1991), Kozłowska *et al.* (2015, 2019a). Złoty Stok near Częstochowa, DE96 – M. Wołkowycki, Fungi Collection, Forest Research Institute, European Centre for Natural Forests. Occurrence dataset <https://doi.org/10.15468/5r5kcy> accessed via GBIF.org on 2023-03-22. <https://www.gbif.org/occurrence/1707012546> and <https://www.gbif.org/occurrence/1707012488>
- 1992: Krzemionki Opatowskie, north-west part of nature reserve, FE60 – P. Grzegorzek Herbarium.
- 1993: 3 km north of Jasionna, BC55 – P. Grzegorzek Herbarium.
- 1994: Northern shore of Lake Chojno, BC63 – P. Grzegorzek Herbarium.
- 1997: Białowieża Forest, *Pino-Quercetum*, *Peucedano-Quercetum*, GC55 – Chmiel (1997), Kozłowska *et al.* (2019b).
- 1999: South-east of Trzebinia, DF56 – P. Grzegorzek Herbarium.
- 2004: Cezarówka Dolna, Korzeniec Hill, DF55 – P. Grzegorzek Herbarium.
- 2005: Chrzanów Kościelec, DF56 – P. Grzegorzek Herbarium.
- 2008: Hill east of Kolorowa Street, Libiąż, Strzałba Hill, Libiąż, DF65 – P. Grzegorzek Herbarium.
- 2011: North-east of Jaworzno Ciężkowice, Płocka Droga (Żabnik–Kozie Brody), DF45 – P. Grzegorzek Herbarium.
- 2013: Góry Luszowskie, Trzebinia, DF55 – P. Grzegorzek Herbarium.
- 2019: Nature reserve ‘Dolina Żabnika’, DF45 – P. Grzegorzek Herbarium. Knyszyn Forest, GC02 – Kujawa *et al.* (2019).



- 2022: Budzyń Forest Range, Forest District Podanin, BC49 – leg. Z. Celka & E. Zenkteler, det. P. Szkudlarz, Herbarium POZ.
- 2023: Nature-landscape complex ‘Źródła Kłodnicy’, Katowice, DF43; forest between Imielin and Błędów, DF54 – P. Grzegorzek Herbarium.