

Two lichen species, *Bacidina caerulea* and *B. flavoleprosa* (Ramalinaceae, Ascomycota), new to Poland and notes on the taxonomy of *B. flavoleprosa*

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Abstract. Two species, *Bacidina caerulea* and *B. flavoleprosa*, are reported from Poland. DNA sequencing was performed on all Polish specimens of both species. The nuITS rDNA sequence of *B. caerulea* showed 100% similarity to the OP256853 sequence, with no differences in nucleotide substitutions between them. In the case of *B. flavoleprosa*, Polish specimens exhibit a high degree of morphological and anatomical similarity to the protologue of *B. flavoleprosa*, however, the number of nucleotide substitutions in nuITS rDNA sequences referred to as this species shows considerable variation. However, since the morphology of the Polish specimens agree with that of *B. flavoleprosa*, and the number of available sequences is limited, we refrain from describing a new species.

Key words: cryptic species, haplotype network, lichenized fungi, taxonomy

Introduction

The lichen genus *Bacidina* Vězda (Ramalinaceae) comprises mainly epiphytic or saxicolous species (Ekman 1996, 2023), however several species are lignicolous and several being truly foliicolous (Lücking 2008; Lücking et al. 2009). Members of the genus possess a crustose thallus, which can be continuous or consist of tiny, more or less connected granules made of photobiont cells surrounded by fungal hyphae. The thallus can be variously shaped, for example, as irregular crusts or as coralloid or squamulose (Ekman 1996, 2023). Apothecia are white, pale yellow to orange-brown, sometimes darker or nearly blackish, and biatorine (Ekman 1996, 2023; Cannon et al. 2021; Molenaar et al. 2023). The proper exciple (often described as paraplechtenchymatous) is composed of indistinctly or distinctly radiating hyphae with apically large cells having broadly ellipsoid, vesicle-like to almost globose lumina (Ekman 1996, 2023; Czarnota & Guzew-Krzemińska 2018). The ascospores are bacilliform to acicular, and always eight per ascus (Ekman 2023). The conidiomata are in the form of pycnidia, and conidia show considerable variation in shape, curvature, and septation – sometimes even within the same species (for more details see Ekman 1996, 2023; Molenaar et al. 2023).

The classification of *Bacidina* (e.g., Vězda 1983, 1991; Sérusiaux 1995; Hawksworth & Poelt 1996; Ekman

1996), as well as its phylogeny, has been debated by many authors (e.g., Ekman 2001, 2023; Czarnota & Guzew-Krzemińska 2012, 2018; Gerasimova et al. 2021, 2023; van den Boom & Alvarado 2025) and still some issues remain unresolved. A particularly salient question pertains to the taxonomic distinction between *Bacidina* and *Woessia* D. Hawksw. & Poelt (Ekman 1996, 2001). The genus *Woessia* was reinstated by van den Boom and Alvarado (2025). However, the relationships between *Bacidina*, *Woessia* and several other genera clustered in one clade obtained in the phylogenetic tree are not well resolved (van den Boom & Alvarado 2025). Also, there are no distinct morphological differences between both genera and some species (e.g., *B. modesta* (Zwackh ex Vain.) S. Ekman and *B. sulphurella* (Samp.) M. Hauck & V. Wirth considered synonymous with *B. modesta*, see Ekman 2001) seem to be placed twice in *Bacidina* and *Woessia* (van den Boom & Alvarado 2025). Therefore, the question whether *Bacidina* and *Woessia* should be kept as distinct genera remains, in our opinion, unresolved. Another issue is the discrepancy between the true and the reported number of *Bacidina* species (e.g., in Index Fungorum or MycoBank). Ekman (2023) highlights this issue by providing examples of *Bacidina* names at species levels that are actually synonyms. One example is *B. mendax* Czarnota & Guz.-Krzem., which Ekman (2023) considers to be a synonym of *B. assulata* (Körb.) S. Ekman. On the other hand, *Bacidina caerulea* (Körb.) S. Ekman, was described by Körber (1860), but for many years it was treated as a synonym or variety of *B. friesiana*

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(Hepp) S. Ekman. However, Ekman (2023) recently provided evidence that *B. caerulea* is a distinct species from *B. friesiana* and the name was reinstated.

Another challenge is the presence of ‘morphs’ within *Bacidina*, i.e., specimens that differ from the type specimens in terms of their morphology, anatomy and/or chemistry (Ekman 1996, 2023). For example, *B. neosquamulosa* (Aptroot & Herk.) S. Ekman has pink-buff to flesh-colored apothecia (Cannon et al. 2021), as well as a morph with pale apothecia (Ekman 2023). Such differences may probably suggest the presence of cryptic species within *Bacidina*; however, without molecular evidence, it is impossible to distinguish between them. Ekman (2023) also reported such a morph in *B. flavoleprosa* Czarnota & Guzew-Krzem., where the pigmented form is represented by the type of *B. flavoleprosa* (GenBank sequence acc. no. JN972443; see Czarnota & Guzew-Krzemińska 2012), while the pigment-deficient form was named *B. delicata* (Larbalestier ex Leight.) V. Wirth & Vězda (GenBank acc. no. AF282097). However, this was later reclassified as *B. flavoleprosa* (Ekman 2023).

Also, the distribution of several species is incompletely known, especially of these, which often occur in a sterile state or were recently distinguished. This is the case of *Bacidina caerulea* and *B. flavoleprosa*. So far, *B. caerulea* has only been reported from Sweden, Norway, and the Netherlands (Ekman 2023; Molenaar et al. 2023) and *B. flavoleprosa* from the Czech Republic, Colombia, Germany, Sweden and United Kingdom (Czarnota & Guzew-Krzemińska 2012; Cannon et al. 2021; Ekman 2023). Here, we present, the first records of these species from Poland with molecular data and the discussion on their anatomy and morphology, as well as the genetic variation in sequences referred to as *B. flavoleprosa*.

Materials and methods

Taxon sampling

Fresh material was collected during fieldwork in Poland by M. Kukwa. The specimens are deposited in the lichen collection of University of Gdańsk (UGDA). Morphology and anatomy were examined under a stereo- and light microscope using standard protocols.

DNA extraction, amplification, sequencing and haplotype network analysis

Genomic DNA was isolated using a modified CTAB method (Guzow-Krzemińska & Węgrzyn 2000). The PCR amplification and DNA purification protocols were the same as those described in our previous papers (Ossowska et al. 2018, 2019, 2023; Szczepańska et al. 2024; Szymczyk et al. 2024). Sequencing was performed by the MacroGen sequencing service.

Both the forward (F) and reverse (R) strands were assembled and edited using BioEdit 7.0.9 (Hall 2011). The newly obtained nuITS rDNA sequences were analyzed using a BLASTn search (Altschul et al. 1990). To create a haplotype network, we used the sequences identified by BLAST as being the most similar to our specimens.

The alignment was prepared in Seaview (Galtier et al. 1996; Gouy et al. 2010), after which the terminal ends were cut off. The TCS network (Clement et al. 2002) was plotted using Pop Art software (<http://popart.otago.ac.nz>) and modified in Inkscape (<http://inkscape.org>). The new sequences of *B. caerulea* and *B. flavoleprosa* have been deposited in GenBank (see the Taxonomy section for their accession numbers).

Results and discussion

As a result of our study, we obtained three nuITS rDNA sequences (GenBank accession numbers are presented below in specimens examined sections). The first showed 100% similarity to the sequence of *B. caerulea* (GenBank acc. no. OP256853) deposited by Ekman (2023), with no differences in nucleotide substitutions between them. The morphology and anatomy of Polish specimen is also consistent with the description of *B. caerulea* presented in Ekman (2023). The species was found on the bark of *Sambucus nigra* in eutrophicated vegetation with young trees and bushes. It was accompanied by *Athallia cerinella* (Nyl.) Arup, Frödén & Söchting, *Lecania cyrtella* (Ach.) Th. Fr., *L. naegeli* (Hepp) Diederich & van den Boom, *Lecidella elaeochroma* (Ach.) M. Choisy, *Physcia* spp. and *Xanthoria parietina* (L.) Th. Fr. Similar habitat requirements were reported by Ekman (2023).

The two other sequences obtained from two sorediate specimens were 99% similar to the sequence of *Bacidia* sp. (GenBank acc. no. KX098339), which was deposited by Mark et al. (2016). Phylogenetic analyses suggest that this sequence is nested in a sister clade to *Bacidina flavoleprosa* (Mark et al. 2016; van den Boom & Alvarado 2025), although with no support. Furthermore, according to BLAST, this sequence is 97% similar to *B. flavoleprosa*. The second-best BLAST hit revealed that the Polish sequences are ~96% similar to the sequence obtained from the type specimen of *B. flavoleprosa* (GenBank acc. no. JN972443).

One of the specimens (UGDA L-65319) is fertile and matches morphologically and anatomically the description of *Bacidina flavoleprosa*: the thallus is sorediate, partly with delimited soralia or in part almost completely leprose, apothecia are beige to pale brown, with discs darker than excipulum, the hymenium is colorless, the hypothecium is brown (Arnoldiana-brown present) and the ascospores are acicular, measuring $\sim 25\text{--}28 \times 1.5 \mu\text{m}$ (Czarnota & Guzew-Krzemińska 2012). A similar set of traits is also characteristic of *B. arnoldiana* (Körb.) V. Wirth & Vězda and *B. adastr*a (Sparrius & Aptroot) M. Hauck & V. Wirth [both in *Woessia*, according to van den Boom & Alvarado (2025)]. However, our sequence shows only 95% and 94% similarity to *B. arnoldiana* and *B. adastr*a, respectively. These species also differ from *B. flavoleprosa* in terms of morphological characteristics (Czarnota & Guzew-Krzemińska 2012; Cannon et al. 2021; Ekman 2023). *Bacidina arnoldiana* has a granular, non-sorediate, green thallus, whereas *B. adastr*a produces pale pink to dark blue-black apothecia with a colorless to slightly straw-colored hypothecium (Czarnota

& Guzow-Krzemińska 2012; Cannon et al. 2021). The second specimen (UGDA L-67206) is sterile, but differs only in one nucleotide substitution from the fertile one (Fig. 1).

Due to an unresolved outcome in BLAST, we created a haplotype network comprising all sequences of *Bacidina flavoleprosa* deposited in GenBank, together with the sequence originally submitted as *B. delicata* (GenBank acc. no. AF282097); however, according to Ekman (2023), this sequence represents a pigment-deficient morph of *B. flavoleprosa*. We also included the sequences of *Bacidia* sp. (GenBank acc. no. KX098339), which show high similarity to *Bacidina flavoleprosa*, as well as the genetically similar *B. paradoxa* Palice (Vondrák et al. 2023). The sequence of the Colombian specimen deposited as *B. flavoleprosa* (GenBank acc. no. MT852413) was excluded, as it is considerably shorter (287 bp). The three sequences of *B. flavoleprosa* (GenBank acc. nos: JN972443 from the type specimen, OQ717718, and MT852413) represent three haplotypes. The sequence obtained from the type differs by 11 nucleotide substitutions from sequence no. AF282097, and differs by 18 nucleotide substitutions from sequence no. OQ717718 (Fig. 1), originating from a specimen collected in the Czech Republic (Vondrák et al. 2023). The newly obtained sequences from the Polish specimens differ by 15 and 16 nucleotide substitutions

from the type, suggesting that they may represent *B. flavoleprosa*. Similar differences are found between Polish sequences and sequence no. AF282097 (19–20 nucleotide substitutions), but an additional 12 substitutions separate them from the sequence obtained from the Czech specimen (no. GenBank acc. no. OQ717718). The situation becomes even more complex when the sequences are compared to sequences of *B. paradoxa*, which differ from the Polish sequences by 13–14 substitutions and by 18 substitutions from the sequence obtained from the type of *B. flavoleprosa*.

Based on the differences between the sequence obtained in the haplotype network, it is tempting to describe new species, particularly for the newly sequenced specimens from Poland. However, these differences may be due to the limited number of sequences available so far. Guzow-Krzemińska et al. (2019) created a haplotype network using 21 sequences of *Lepraria finkii* (de Lesd.) R.C. Harris. This species exhibited significant variation in the nucITS rDNA marker, with sequences differing by 1–36 nucleotide substitutions. The authors suggested that *L. finkii* may be comprised of cryptic species; however, since all specimens formed one clade and shared the same morphology, they maintained the single species classification. A very similar situation appears to be involved in *Bacidina flavoleprosa*. As the fertile material agrees with

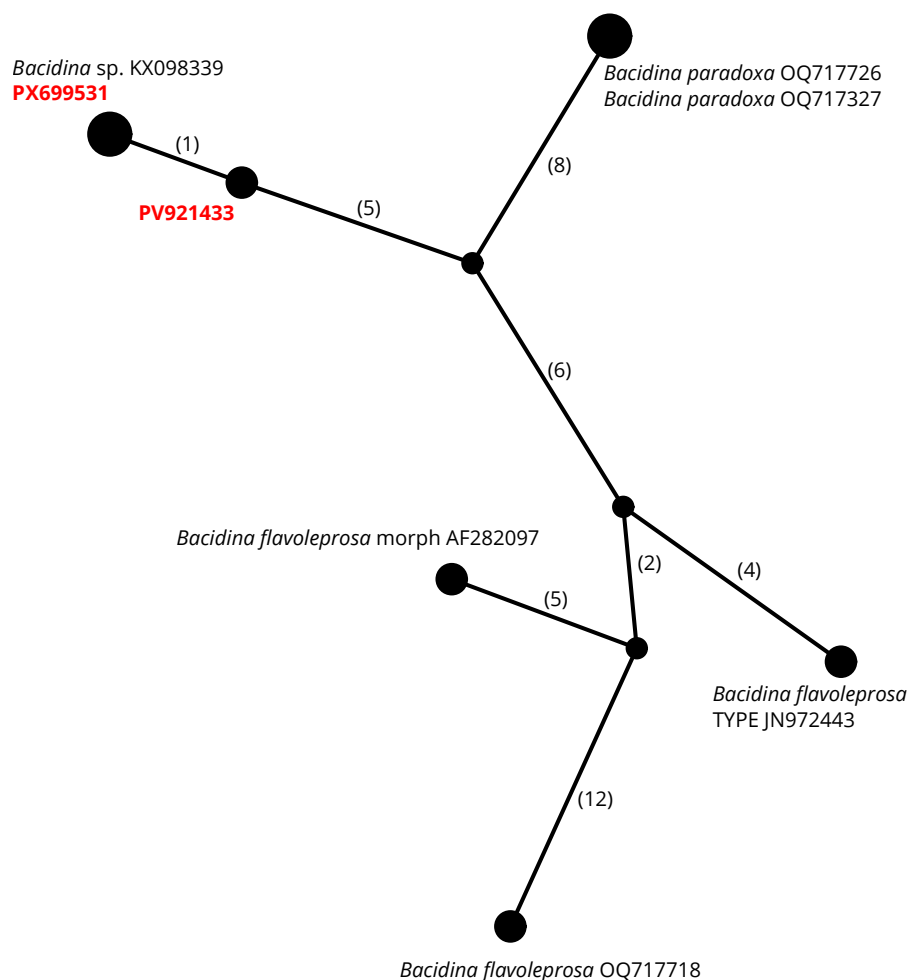


Figure 1. Haplotype network showing relationships between nuITS rDNA sequences of *Bacidina flavoleprosa*, *B. paradoxa* and *Bacidia* sp. (KX098339). The new sequences of *B. flavoleprosa* from Polish samples are marked in red.

the protologue, we prefer to use this name for the studied material, at least until more sequences are available. Further studies based on a larger number of specimens are also necessary to determine whether *B. paradoxa* is a distinct species or synonymous with *B. flavoleprosa*.

Czarnota and Guzow-Krzemińska (2012) described *Bacidina flavoleprosa* based on a saxicolous specimen collected in a humid place, but Cannon et al. (2021) and Vondrák et al. (2023) reported it as an epiphytic species. Polish specimens were also epiphytic and found on bark of *Acer platanoides* and *Sambucus nigra* in deciduous forests and accompanied by *Lecania cyrtella*, *L. naegelii*, *Physcia* spp., *Phaeophyscia* spp. and *Xanthoria parietina*.

Taxonomy

Bacidina caerulea (Körb.) S. Ekman (Fig. 2)

For a detailed description and iconography see Ekman (2023) and Molenaar et al. (2023).

Specimen examined. POLAND. Pobreże Kaszubskie, Nadmorski Landscape Park, NE of Chłapowo, Dolina Chłapowska, 54.810193°N, 18.366123°E, on twigs of *Sambucus nigra*, 1 Oct. 2022, M. Kukwa 24065 (UGDA L-59098; GenBank acc. no. PV921434).

Bacidina flavoleprosa Czarnota & Guzow-Krzem.

For a detailed description and iconography see Czarnota & Guzow-Krzemińska (2012).

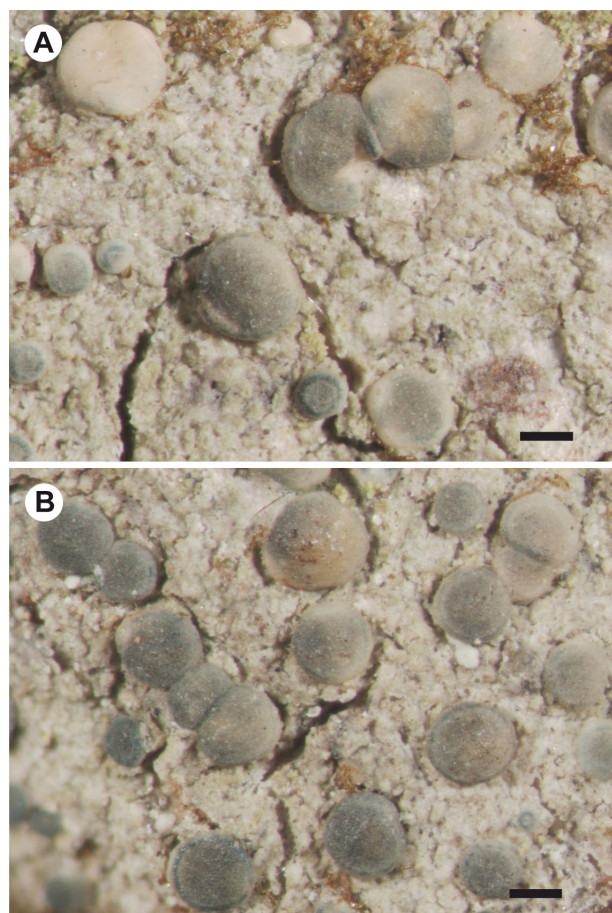


Figure 2. *Bacidina caerulea* – morphology of apothecia. Scales: A, B = 0.25 mm.

Specimens examined. POLAND. Wybrzeże Słowińskie, Słowiński National Park, NW of Kluki, Klucki Las forest, forest section no. 68, 54.700363°N, 17.307365°E, black alder forest with *Sambucus nigra*, on *Sambucus nigra*, 5 Jun. 2024, M. Kukwa 25867 (UGDA L-65319; GenBank acc. no. PV921433). Pojezierze Kaszubskie, Trójmiejski Landscape Park, E of Gdańsk Matarnia, forest section no. 152, 54.375197°N, 18.521453°E, deciduous forest, on *Acer platanoides*, 29 Oct. 2024, M. Kukwa 26691 (UGDA L-67206, GenBank acc. no. PX699531).

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