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Research

Diversity of epiphytic lichens and allied fungi on *Pinus heldreichii* and *P. peuce* in Bulgaria

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The study presents all available information for epiphytic lichens and allied fungi on *Pinus heldreichii* and *P. peuce* in Bulgaria. The trees are respectively, subendemic and endemic for the Balkan Peninsula. Comments on distribution and diversity of species are provided. A total of 78 species are reported. Two lichen species are reported for the first time from Bulgaria, *Blastenia monticola* and *Hypogymnia laminisorediata*. *Lecanora cadubriae* is confirmed from Bulgaria. The following species are reported for the first time for the Pirin Mountains: *Cladonia glauca*, *Lepraria elobata*, *Melanelixia glabratula*, *Micarea prasina*, *Nephromopsis chlorophylla*, *Strangospora moriformis*, while *Lichenoconium erodens* is new for the Vitosha region and *L. lecanorae* for the Rila Mountains.

Keywords: Balkan endemic, Bulgarian lichen mycota, climate change, coniferous forests, new records

Introduction

Pinus peuce Griseb. and *P. heldreichii* Christ are respectively, Balkan endemic and subendemic coniferous trees, distributed in most Balkan countries, while *P. heldreichii* is known also from Calabria, south Italy (Panayotov et al. 2016).

In Bulgaria, both species form the upper forest boundaries in the highest Bulgarian mountains. Natural stands of *P. heldreichii* are known only from the Pirin and Slavianka Mountains on marble, while *P. peuce* is more widely distributed on different siliceous substrates in the Pirin, Rila, the Balkan range and the Rhodopes floristic regions. The communities of *P. heldreichii* and *P. peuce* are included as vulnerable (VU) and endangered (EN) in the Red Data Book of the Republic of Bulgaria (Biserkov et al. 2015). The epiphytic lichen communities on both trees were not studied and included in the Red Data Book of the Republic of Bulgaria. However, many lichenological studies in Bulgaria report lichen species from *P. heldreichii* and *P. peuce*, as a result of fieldtrips in the Pirin and Rila Mountains (Suza 1929, Szatala 1930, Zhelezova 1956b,

Mayrhofer et al. 2020). In this study we present all available information about lichen diversity on both species. The information is gathered from literature, specimens deposited in SOMF, and fieldwork during the period from 2019 to 2022.

The role of *P. heldreichii* and *P. peuce* in forming the highest-elevation forest belts makes them important for studies of the effects of global warming on epiphytic lichens. Within the frame of the project 'Structural and functional characteristics and perspectives for diverse use of endemic relict coniferous forest communities in Bulgaria in state of climate change' we studied six sites (Fig. 1) in forests of *P. heldreichii* and *P. peuce*, where the total diversity of epiphytic lichens was recorded and permanent plots were established for future analyses of changes in lichen communities and cover due to climate change.

Two species are reported for the first time from Bulgaria, namely *Blastenia monticola* Arup & Vondrák and *Hypogymnia laminisorediata* D.Hawksw. & Poelt. Comments on lichen diversity and distribution are presented.

Material and methods

The sampling sites are in the Balkan range, Vitosha region, Pirin and Rila Mountains. Permanent plots were established in six sites, where a total diversity of epiphytic lichens was recorded (Fig. 1). Each site has dimensions of 100 × 100 m with homogeneous forest.

Studied specimens were deposited in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research, Sofia (SOMF). Specimens collected by B. Zhelezova, A. Vězda, J. Motyka, A. Atanassova, H. Mayrhofer and S. Nikolova from *P. heldreichii* and *P. peuce* were revised. All information about species distribution is presented, following the floristic regions of Bulgaria and their numeration (Denchev et al. 2022). Localities from old sources are given separately if there was an indication of altitude or other important information for the distribution. Several localities are from the area of the Banderitsa Chalet, and if there is no other information, the Banderitsa Chalet is considered as one locality.

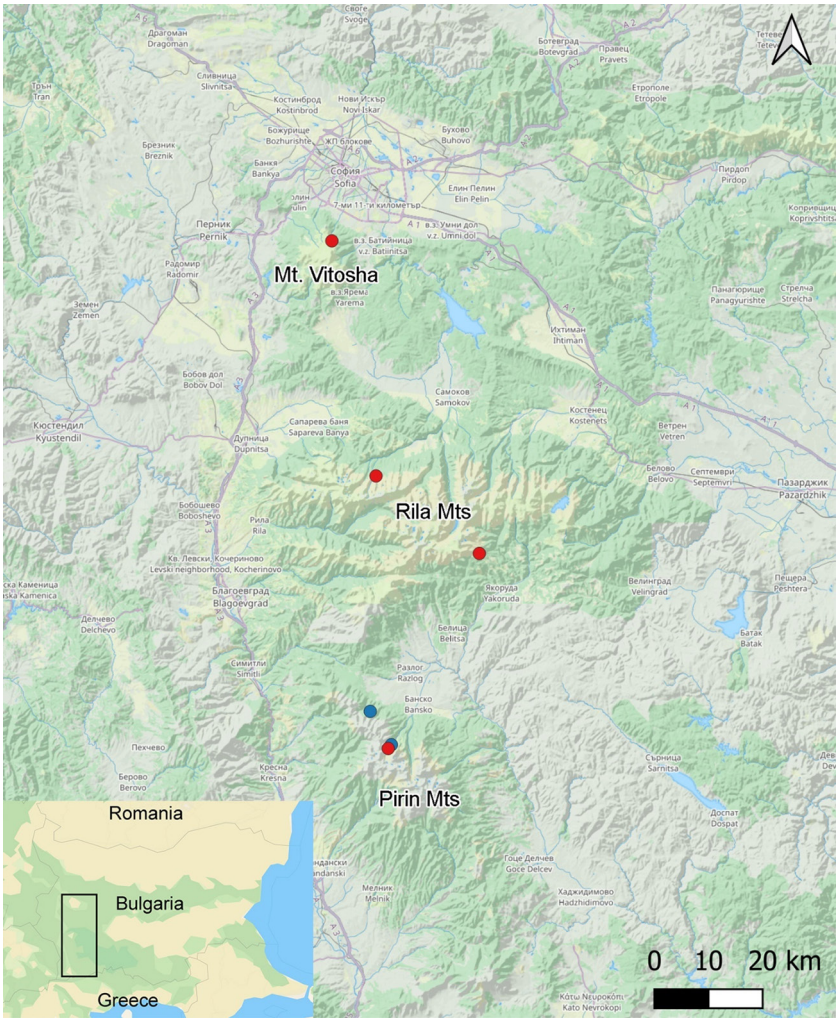

Figure 1. Map of Bulgaria with six sites where a complete inventory of species was made and permanent plots were established. Red dots indicate sites with *Pinus peuce*, while blue ones indicate *Pinus heldreichii*.

The main sources for identification of lichen specimens were Wirth (1995), Randlane et al. (2009), Smith et al. (2009), Arcadia (2022) and Nimis (2023). Chemical reactions were made following Orange et al. (2010). The taxonomic concept follows Denchev et al. (2022), whereas the new records for Bulgaria follows the protologues (Hawksworth 1973, Vondrák et al. 2020).

List of localities and sampling sites

Balkan range

5-1. Site Petrohan Pass, 43.11428°N, 23.13345°E, 1472 m.

Vitosha region

8-1. Site Goli Vrah, 42.59197°N, 23.29262° E, 1814 m.

Pirin Mountains

14-1. Blagoevgard Province, 41.76333°N, 23.42028°E, 1875 m (Mayrhofer et al. 2020).

14-2. Near Banderitsa Chalet, 29.VII.1954, B. Zhelezova (SOMF).

14-3. Banderitsa Chalet, B. Zhelezova (SOMF) (Motyka and Zhelezova 1962, Zhelezova 1963, Atanassova and Mayrhofer 2012).

14-4. Banderitsa Chalet, 1850 m (Zhelezova 1956b).

14-5. Site Vihren Chalet, 41.76192°N, 23.41695°E, 1969 m.

14-6. Near Demyanitsa Chalet, 1900–2200 m (Zhelezova 1956b).

14-7. The path to Malak Kazan circus, 2100 m, 2.X.1962, B. Zhelezova (SOMF) (Zhelezova 1963).

14-8. Near Banderitsa Chalet, 1900 m (Zhelezova 1963).

14-9. Banderitsa River, 900 m (Zhelezova 1956b).

14-10. Demyanitsa Chalet, B. Zhelezova (SOMF) (Motyka and Zhelezova 1962).

14-11. Along the road from Banderitsa Chalet to Bansko, 41.77632°N, 23.43917°E, 11.VII.2007, H. Mayrhofer & S. Nikolova (SOMF).

14-12. Pirin Chalet, B. Zhelezova (SOMF).

14-13. Banderitsa Chalet, 29.VII.1954, Zhelezova (SOMF).

14-14. The region of Kazan circuses, 2.X.1962, B. Zhelezova (SOMF).

14-15. The region of Banderitsa Chalet, 14.VII.1967, A. Vězda (SOMF).

14-16. The valley of Banderitsa River, 1850 m (SOMF) (Zhelezova 1956b).

14-17. Along the road to Vihren Chalet near Baykusheva Mura, 41.76330°N, 23.42020°E, 10.VII.2007, H. Mayrhofer & S. Nikolova (SOMF).

14-18. Demyanitsa Chalet, 2000–2200 m (Zhelezova 1956b).

14-19. Below Bezbog Chalet (Zhelezova 1960, Pišút 1969, 1986, Shivarov et al. 2023).

14-20. Between Gotse Delchev Chalet and Bezbog Lake, 01.X.1957, B. Zhelezova (SOMF).

14-21. The valley of Banderitsa River, 1700 m (Zhelezova 1956b).

14-22. Demyanitsa Chalet, B. Zhelezova (SOMF).

14-23. Bayuvi Dupki–Dzhindzhiritsa Reserve, B. Zhelezova (SOMF).

14-24. Bezbog Lake, B. Zhelezova (SOMF) (Motyka and Zhelezova 1962).

14-25. Above Gotse Delchev Chalet (Motyka and Zhelezova 1962).

14-26. Vihren Chalet (Motyka and Zhelezova 1962).

14-27. Between Banderitsa Chalet and Vihren Chalet, 41.76333°N, 23.42028°E, 1875 m, 10.VII.2007, H. Mayrhofer & S. Nikolova (Mayrhofer et al. 2020).

14-28. Site Yavorov Chalet, 41.82300°N, 23.37727°E, 1716 m.

14-29. Site Banderitsa Chalet, 41.76805°N, 23.42392°E, 1876 m.

14-30. Yavorov Chalet (SOMF) (Zhelezova 1963).

14-31. The valley of Treta Reka River, above Pirin Chalet, 1750 m (Pišút 2001).

14-32. The trail to Vihren, 41.77278°N, 23.42639°E, 2011 m, 11.VII.2007, H. Mayrhofer & S. Nikolova (Mayrhofer et al. 2020).

14-33. Pirin Mts (Zhelezova 1956b, Motyka and Zhelezova 1962).

14-34. The trail towards Vihren Chalet, 1850 m (Pišút 1969, 1986).

14-35. Above Banderitsa Chalet near Kazanite, 2.X.1962, B. Zhelezova (SOMF).

14-36. Above Banderitsa Chalet towards Kutelo Peak (Pišút 1986, 2001).

14-37. Along the tourist road from Banderitsa Chalet to Kazanite circus; 41.77278°N, 23.42642°E, 1867 m, H. Mayrhofer & S. Nikolova (SOMF).

14-38. Razloshki Suhodol Circus, 2200 m (Zhelezova 1960).

14-39. Near Banderitsa Chalet, 41.76750°N, 23.42333°E, 1883 m, 10.VII.2007, H. Mayrhofer & S. Nikolova (SOMF).

14-40. Below Banski Suhodol circus (Cretzoiu 1937).

Rila Mountains

15-1. The valley of Skakavitsa River, 1800 m (Suza 1929).

15-2. The valley of Levi Iskar River, 1500–1700 m (Szatala 1930).

15-3. The valley of Levi Iskar River, 1500 m (Szatala 1930).

15-4. Site Treshtenik Chalet, 42.082167°N, 23.61803°E, 1915 m.

15-5. Site Malyovitsa River, 42.20897°N, 23.39003°E, 1760 m.

15-6. Near Skakvitsa Chalet (Zhelezova 1963).

Table 1. Species diversity and distribution on *Pinus heldreichii* and *Pinus peuce* in Bulgaria.

Species	*P. heldreichii*	*P. peuce*	Substrates	Localities and sites
Amandinea punctata	x		bark	14-28, 29
Anaptychia ciliaris	x	x	bark	14-29; 15-1
Arthonia mediella		x	bark	14-1
Arthonia radiata		x	bark	15-2
#Arthopyrenia analepta		x	bark	15-3
Athallia holocarpa	x		bark	14-29
Blastenia monticola	x	x	bark	14-2, 3, 4, 14-29, 30
Bryoria capillaris		x	bark	14-5
Bryoria fuscescens	x	x	bark	14-28; 15-4, 5
Buellia erubescens	x		bark	14-31
Buellia griseovirens	x		lignum	14-32
Calicium tigillare		x	bark	14-6
Caloplaca cerina	x	x	bark, twig	14-3, 14-7, 14-30
Candelariella xanthostigma	x		bark	14-3, 14-29
Cetraria sepincola		x	bark	14-40
Chaenotheca chrysocephala		x	bark	15-6
Chaenotheca furfuracea			log	15-6
Cladonia coniocraea	x		log	14-29
Cladonia digitata		x	bark	15-5
Cladonia glauca	x		log	14-29
Cladonia ochrochlora		x	log	14-9
Cladonia sulphurina	x		log	14-19
Evernia divaricata	x	x	bark	14-10, 11, 14-28; 15-1
Evernia prunastri		x	bark	15-5
Hypogymnia austerodes	x		bark	14-33
Hypogymnia farinacea	x	x	bark	14-3, 4, 14-34
Hypogymnia laminisorediata	x	x	bark	14-3, 14-12, 14-28
Hypogymnia physodes	x	x	bark	14-3, 14-12; 15-1, 4; 8-1
Hypogymnia tubulosa	x	x	bark	14-3, 14-29;15-1
Hypogymnia vittata		x	bark	15-4
Imshaugia aleurites	x		bark	14-3, 14-16, 14-28
Lecanora allophana		x	bark	14-13
Lecanora argentata		x	bark	14-14
Lecanora cadubriae		x	bark	14-15; 15-4
Lecanora mughicola		x	log	14-16; 15-1
Lecanora pulicaris	x	x	bark	5-1; 8-1; 14-3, 4, 14-28, 29; 15-3, 4, 5
Lecanora saligna	x		log	14-3
Lecanora subintricata	x	x	bark	14-5
Lecanora varia	x	x	bark, log	14-4, 5, 6, 14-17, 14-23, 14-28, 14-35
Lepraria elobata	x	x	bark	14-5, 14-28; 15-4
Letharia vulpina	x	x	bark, snag	14-5, 14-18, 19, 14-23; 15-7, 8, 9, 10
**Lichenoconium erodens*		x	*H. physodes*	8-1
**Lichenoconium lecanorae*		x	*L. pulicaris*	15-4
Melanelixia glabra	x	x	bark	14-34; 15-1
Melanelixia glabratula	x		bark	14-28
Melanohalea exasperatula		x	bark	14-4; 15-1
Micarea prasina		x	log	14-5
#Naetrocymbe punctiformis		x	bark	5-1
Nephromopsis chlorophylla		x	bark	14-17
Ochrolechia alboflavescens	x	x	bark	14-3, 14-17, 14-28, 29, 14-30, 14-36, 37; 15-4
Ochrolechia parella	x		bark	14-30
Parmelia saxatilis	x	x	bark	14-18, 14-29
Parmeliopsis ambigua	x	x	bark	8-1; 14-5, 14-17, 14-28, 29; 15-4
Parmeliopsis hyperopta	x	x	bark	14-5, 14-28, 29; 15-4
Peltigera canina	x		bark	14-11
Physcia aipolia		x	bark	15-1
Physcia stellaris	x			14-3
Physcia tenella		x	bark	15-1
Physconia distorta	x		bark	14-29
Physconia perisidiosa	x			14-3

(*Continued*)

Table 1. Continued.

Species	*P. heldreichii*	*P. peuce*	Substrates	Localities and sites
Platismatia glauca	x	x	bark, twig	14-3, 14-8, 14-19, 14-28, 14-38
Pseudevernia furfuracea		x	bark	14-12, 14-20, 14-28, 29; 15-4
Pycnora praestabilis	x		lignum	14-39
Pycnora xanthococca	x		log	14-6
Ramalina farinacea	x		bark	14-28
Rinodina sophodes		x	bark	14-17, 14-37, 14-39
Strangospora moriformis		x	bark	14-5
Tephromela atra var. torulosa	x		bark	14-27, 14-37
Trapeliopsis granulosa		x	log	14-21
Usnea barbata	x	x	bark	14-20, 14-33, 15-12
Usnea cavernosa	x	x	bark	14-3, 14-22, 23, 24, 14-30; 15-14
Usnea czeczottiae	x		bark	14-3
Usnea dasopoga		x	bark	14-10, 14-23, 24, 25, 26
Usnea glabrescens	x	x	bark	14-28, 15-4
Usnea intermedia		x	bark	14-3, 14-29, 30, 15-4
Usnea substerilis		x	bark	14-27
Vulpicida pinastri	x	x	bark	14-17, 14-28, 29
Xylographa parallela		x	log	14-3, 14-5, 14-9, 14-37

Lichenicolous fungus – *, non-lichenized fungus – #.

15-7. Ribni ezera Chalet, 2200 m (Zhelezova 1956b).

15-8. Above Kirilova polyana towards Tiha Rila, 27.VI.2001, M. Gyosheva (SOMF).

15-9. Above Kirilova polyana towards Ribni ezera Lakes, IX.1961, B. Zhelezova (SOMF).

15-10. Semkovo, 30.VII.1972, B. Zhelezova (SOMF).

15-11. Above Kirilova polyana (Zhelezova 1956b).

15-12. Belmeken Chalet, route for Kostenets, II.1962, B. Zhelezova (SOMF).

15-13. Near Ribni ezera Chalet, 1900 m (Motyka and Zhelezova 1962).

15-14. Suhoto Lake, 20.V.1950, B. Zhelezova (SOMF).

Results and discussion

A total of 46 lichen species were recorded on *P. heldreichii* and 51 lichens, two non-lichenized, and two lichenicolous fungi on *P. peuce* (Table 1). Three species have conservation status in Bulgaria: *Anaptychia ciliaris* Flot. (NT), *Cetraria sepincola* (Ehrh.) Ach. (EN), and *Letharia vulpina* Hue (NT) (Shivarov et al. 2023). During the fieldwork, single thalli were found of *A. ciliaris* and *L. vulpina*, whereas *C. sepincola* was not found. The wolf lichen *L. vulpina* was known as common on *P. peuce* in the past (Zhelezova 1956a). Nowadays it is common only in the region of Bezbog Chalet, Pirin Mountains (14-19).

The most common epiphytic lichens are *Lecanora pulicaris* (Pers.) Ach., *Parmeliopsis ambigua* (Hoffm.) Nyl., and *P. hyperopta* (Ach.) Arnold. In some sites the dominant species on trunks can be *H. laminisorediata* (Fig. 2A–B) and *H. physodes* Nyl. The base of the observed trunks is usually covered with *Lepraria elobata* Tønsberg.

Physconia distorta (With.) J.R.Laundon, a species, that is widespread in lowlands in Bulgaria was found near Banderitsa Chalet at 1848 m. The occurrence of species with Mediterranean distribution at high altitudes indicate change in vertical distribution due to climate change. Moreover, the region of Banderitsa Chalet is well studied in the past by many lichenologists and this species has not been reported previously.

Two lichen species are reported for the first time from Bulgaria, *B. monticola* and *H. laminisorediata*. The specimens collected near Banderitsa Chalet by B. Zhelezova on *P. heldreichii* and *P. peuce* published as *Caloplaca ferruginea* (Huds.) Th. Fr. (Zhelezova 1956b, 1963) belong to *B. monticola*. The latter is characterized by the presence of blastidia and isidia, which usually exceed 150 µm diam. in the studied Bulgarian specimens (Fig. 2C), whereas in *Blastenia ferruginea* (Huds.) A.Massal. vegetative diaspora absent and the species is with different ecology. *Blastenia monticola* occurs on bark and wood of subalpine/subarctic trees and is known from bark of *P. heldreichii* in the mountains of the Balkans (Vondrák et al. 2020). Here it is reported also on bark of *P. peuce*.

Hypogymnia laminisorediata was found at Yavorov Chalet (14-28) as a dominant species covering the trunks of *P. heldreichii*. The thalli were well developed and exceeded 10 cm in diam., with wide marginal lobes, and with rugose soralia in the central parts (Fig. 2A–B). The observed specimens deposited in SOMF from bark of *P. heldreichii* and *P. peuce* as *Parmelia bitteriana* Zahlbr. well agree with the description of *H. laminisorediata* in Hawksworth (1973). The species is known from Morocco, Italy, Serbia, Greece and Turkey (Arcadia 2022), and the occurrence in Bulgaria is expected. However, *H. laminisorediata* differs from *Hypogymnia farinacea* Zopf only by the wider lobes and thallus size (Hawksworth 1973). Further studies with molecular support are needed to confirm that the species is distinct from *H. farinacea*, as there are no DNA sequences available from *H. laminisorediata*.

Lecanora cadubriae (A.Massal.) Hedl. was excluded earlier from lichens occurring in Bulgaria (Denchev et al. 2022).

Figure 2. *Blastenia monticola* and *Hypogymnia laminisorediata*, general habit. A: *H. laminisorediata* on *P. heldreichii* in site Yavorov Chalet. B: *H. laminisorediata* SOMF 24313, locality 14-12, on *P. peuce*. C: *B. monticola* SOMF 31122, locality 14-3, on *P. heldreichii*. Scale bars: A and B = 1 cm, C = 2 mm.

However, a specimen of *L. cadubriae* collected by A. Vězda (14-15) was found, and the species was also collected during the fieldwork at the site Treshtenik Chalet (15-4).

The following species are reported for the first time for the Pirin Mountains: *Cladonia glauca* Flörke, *L. elobata* Tønsberg, *Melanelixia glabratula* (Lamy) Sandler & Arup, *Micarea prasina* Fr., *Nephromopsis chlorophylla* (Willd.) Divakar, A.Crespo & Lumbsch, *Strangospora moriformis* (Ach.) Stein, while *Lichenoconium erodens* M.S.Christ. & D.Hawksw. is new for the Vitosha region and *Lichenoconium lecanorae* (Jaap) D.Hawksw. for the Rila Mountains.

Although the lichen diversity is well-studied, especially in the Pirin and Rila Mountains, we can expect a considerably higher number of species to be found in remote and conserved forest. Studies with focus on *P. heldreichii* stands in Mt Slavyanka will reveal further species as this mountain is well conserved and not affected by the intensive tourism. High biodiversity can be expected on logs and snags of *P. heldreichii* and *P. peuce*, and more studies will be needed in such habitats.

Conclusion

The study presents all available data for epiphytic lichens and allied fungi on *P. heldreichii* and *P. peuce* in Bulgaria. Information on all 78 currently known epiphytic species of both trees is included. *Blastenia monticola* and *H. laminisorediata* are reported for the first time for the Bulgarian lichenized mycota. To our knowledge this is the first work with a focus on epiphytic species on *P. heldreichii* and *P. peuce*. The data for diversity and distribution will be useful in future analyses of changes in lichen communities in response to climate changes.

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Data availability statement

There are no additional data for this paper

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