

***Pirottaea lychnidis* comb. nov. from the Bohemian
Switzerland National Park, Czech Republic**

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Chlebická M. and Konvalinková T. (2010): *Pirottaea lychnidis* comb. nov. from the Bohemian Switzerland National Park, Czech Republic. – Czech Mycol. 62(1): 19–32.

A find of *Pirottaea* from dead stems of *Silene dioica* collected in the Bohemian Switzerland National Park is described. The material possesses very well developed setae, is assigned to Nannfeldt's evolutionary lineage 2, and within the lineage differs from *Pirottaea pilosissima* known from stems of *Geranium sylvaticum* by shorter and up to 4-septate setae. *Peziza sphaerioides* var. *lychnidis* was revised and found to be conspecific with our material, but invalid. The earliest valid description of the taxon was found and a new combination is proposed. Results of a revision of several specimens of *Pirottaea* spp. published from the area of the Czech Republic are given.

Key words: *Peziza sphaerioides* var. *lychnidis*, *Pyrenopeziza lychnidis*, intraspecific variability, taxonomy, nomenclature.

Chlebická M. a Konvalinková T. (2010): *Pirottaea lychnidis* comb. nov. z národního parku České Švýcarsko. – Czech Mycol. 62(1): 19–32.

Je popsán nález rodu *Pirottaea* na odumřelých lodyhách silenky (*Silene dioica*) z národního parku České Švýcarsko. Tento nález s dobře vyvinutými setami je v rámci rodu zařazen do Nannfeldtovy evoluční linie 2. V rámci této linie se liší od druhu *Pirottaea pilosissima* známého z lodyh kakostu (*Geranium sylvaticum*) kratšími setami s maximálně čtyřmi septy. Na základě revize exsikátové položky je zjištěno, že taxon *Peziza sphaerioides* var. *lychnidis* je s naším nálezem konspecifický, ale neplatně popsáný. Je nalezen nejstarší platný popis pro tento taxon a je navržena nová kombinace. Jsou podány výsledky revize několika položek *Pirottaea* sp. div. publikovaných z území České republiky.

INTRODUCTION

Pirottaea Sacc. (Ascomycota, *Dermateaceae*, *Mollisioideae*, *Mollisia-Pyrenopeziza* complex) is a large genus of herbicolous discomycetes of which many species still remain undescribed (Nannfeldt 1985). Nannfeldt made an effort to study a large set of specimens from various substrates and found that species of *Pirottaea* have narrow host spectra. For example, Nannfeldt divided *Pirottaea gallica* Sacc. as delimited by Saccardo (1889) into three species. The species ac-

ording to Nannfeldt's work are specific to host genera or families, i.e. they are monophagous or oligophagous in his words. In the literature consulted (Saccardo 1889; Velenovský 1934; Petrak 1940; Kirschstein 1941; Graddon 1967, 1977, 1990; Dennis 1981; Nannfeldt 1932, 1985; Baral in Baral and Krieglsteiner 1985; Arnolds et al. 1995; Johnston 1998) we did not find any record of *Pirottaea* from *Silene*, the host of the species presented here, or from other *Caryophyllaceae*.

In the morphologically related genus *Pyrenopeziza* Fuckel also narrow host spectra (plant genera or families) occur among species, and Nannfeldt (1932) as well as Hütter (1958) reported only one species from *Caryophyllaceae*, *Pyrenopeziza lychnidis* (Desm.) Rehm.

Pyrenopeziza differs morphologically from *Pirottaea* by pale to light brown hairs with a distinctly thinner wall. The hairs in *Pyrenopeziza* are usually present only in the marginal part of the apothecium – this feature was well illustrated by Hütter (1958) and Nannfeldt (1932). Rarely, thick-walled light brown hairs spread over the apothecial flanks are present [*Pyrenopeziza escharodes* (Berk. et Broome) Rehm]. *Pirottaea* has very conspicuous, dark and thick-walled hairs, named 'hairs' according to e.g. Dennis (1981) or 'setae' according to Nannfeldt (1985). The term 'setae' is more appropriate: setae are thick-walled structures well separated from the ectal excipulum by a distinct, thick-walled, basal septum (Nannfeldt 1985).

In the literature, it was several times discussed whether *Pyrenopeziza lychnidis* can be a *Pirottaea* (e.g. Kirschstein 1936, Baral in Krieglsteiner 1999: 281). We also encountered this problem during the identification of a find of *Pirottaea* from the Bohemian Switzerland National Park. Based on a morphological study of Desmazières' specimen of *Peziza sphaerioides* var. *lychnidis* and several recent collections from dead stems of *Silene dioica*, the species known as *Pyrenopeziza lychnidis* is transferred to the genus *Pirottaea*.

METHODS

Dried material was measured and prepared for microscopy under an Olympus SZ 61 zoom stereo microscope and examined under an Olympus BX-51 light microscope (LM) with an oil immersion lens at a magnification of 1000×. Slides in 3 % KOH were used for drawings and measurements. Lugol's solution (IKI: 1 % iodine, 3 % KI in water) and Melzer's reagent (MLZ) were used to examine reactions of the ascospical ring, and 5 % KOH for obtaining the KOH-pretreated iodine reaction.

RESULTS

Pirottaea lychnidis (Desm. ex Sacc.) M. Chleb., **comb. nov.** – štětinatka silenková
Figs. 1–5

(MycoBank MB518906)

Basionym: *Pyrenopeziza sphaeroides* (Pers.) Fuckel [as ‘*sphaeroides*’] f. *lychnidis* Desm. ex Sacc., Syll. Fung. 8: 365, 1889.

≡ *Pyrenopeziza lychnidis* (Desm. ex Sacc.) Rehm, Ascomyceten in Rabenhorst’s Krypt.-Fl. Deutschl., Oest. und Schweiz, 1/3: 1265, 1896.

= *Peziza sphaeroides* Pers. var. *lychnidis* Desm., Pl. crypt. N. France, nr. 174, 1826, nom. inval.

Description. Apothecia caulicolous, scattered, erumpent, round to slightly elongated, in dried state 320–600(–700) × 280–500(–600) µm large, black, setose, even when dry protruding beyond substrate and slightly or not exposing the disc, rehydrated apothecia with very pale buff discs slightly exposing in bigger apothecia and with dark brown to black patches on outer surface (composed of grana and/or setae). Ectal excipulum made up of a textura angularis, surface dark brown, otherwise hyaline, cells slightly elongated to isodiametric, (6.5–)11–15(–17) × (5.5–)7.5–11 µm, in margin slightly elongated towards marginal hairs and setae, not arranged in regular rows. Grana almost globose, 6–7.5 × 5–6 µm, thick-walled, their wall dark brown, refractive, 0.7–0.9 µm thick, lumen appearing dark to pale brown. Setae (45–)50–77 × 3–5.5 µm, with (2–)3–4 septa at intervals of 9–18 µm, upper cell 2.9–3.7 µm broad. Wall of setae dark brown, refractive, 0.5–0.6(–0.8) µm thick, septa of the same property but mostly slightly thinner, one septum in lower part of a seta sometimes two-layered (“double”), the seta constricted there and liable to break. Lumen appearing medium brown, in upper part of seta pale brown. Marginal hairs hyaline, 18–58 µm long, thin-walled, 0–4-septate, septa often constricted except for apical cell which is slightly pointed with obtuse apex. Asci (48–)55–69(–76) × (4.0–)4.4–5.9(–6.3) µm, 8-spored, arising from croziers, ascoapical ring with blue reaction in MLZ or KOH/MLZ, and blue or in several asci dark grey reaction in IKI, deep blue in KOH/IKI. Ascospores (11.5–)14–19(–26) × 1.8–2.3 µm, straight to slightly curved, non-septate, containing several small lipid bodies in each half. Paraphyses very slightly lanceolate, very slightly enlarged in apical part, with obtuse to subacute, rarely rounded tips, 2.8–3.7 µm broad, exceeding the asci by 7–14 µm.

Distribution. Europe (Great Britain, France, Germany, Czech Republic, Italy).

Specimen studied. Czech Republic: Northern Bohemia, National Park Bohemian Switzerland, S of Mezní Louka, valley of Kamenice, “Ve strži”, c. 170 m elev, on dead stems of *Silene dioica* (= *Melandrium rubrum*), 26 May 2006, leg. M. Chlebická et T. Konvalinková, host det. J. Hadinec, PRM 908389 et PRC.

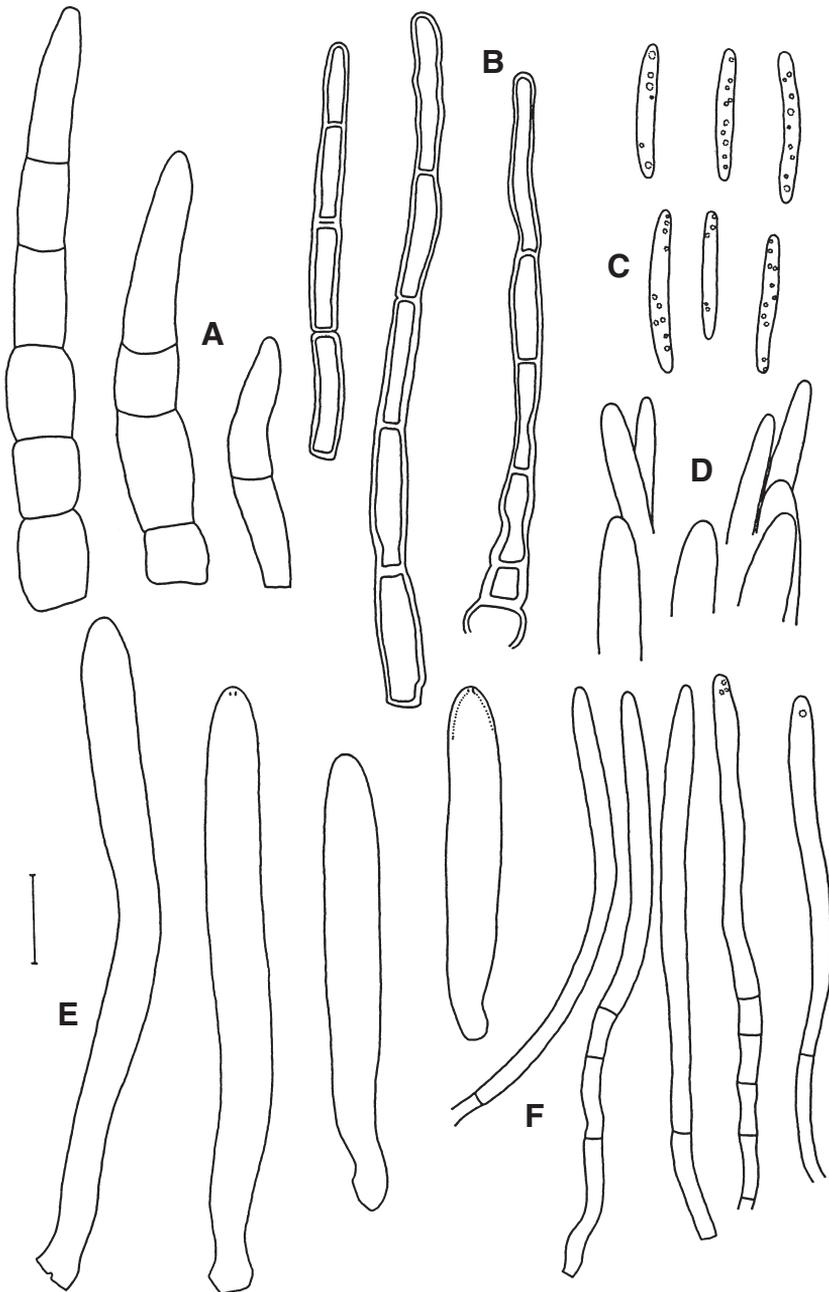


Fig. 1. *Pirottaea lychnidis*, PRM 908389. **A:** marginal hairs; **B:** setae (the brown colour of the setae is not evident from this drawing); **C:** ascospores; **D:** paraphyses exceeding the asci; **E:** asci; **F:** paraphyses. Medium: 3 % KOH. Scale bar 10 μ m.

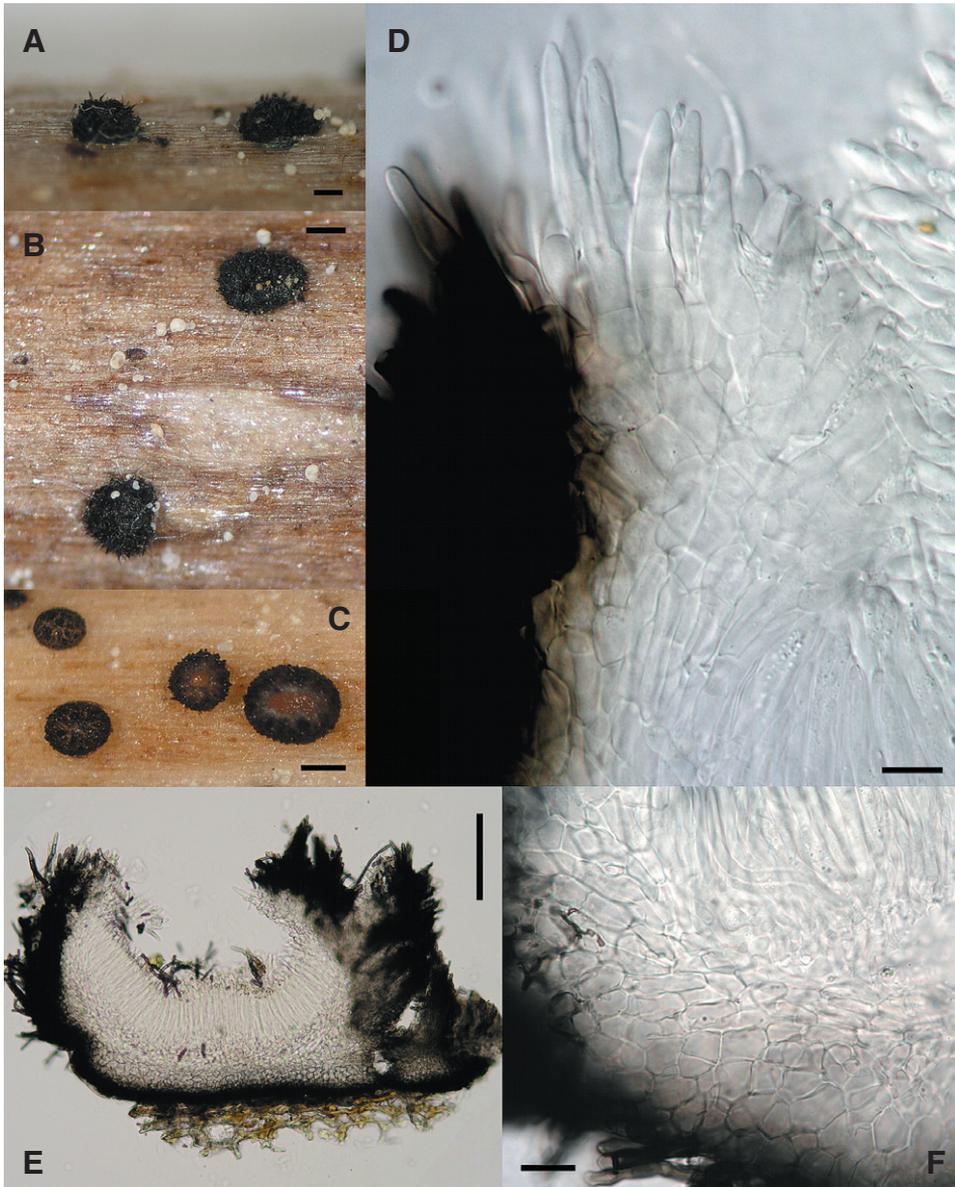
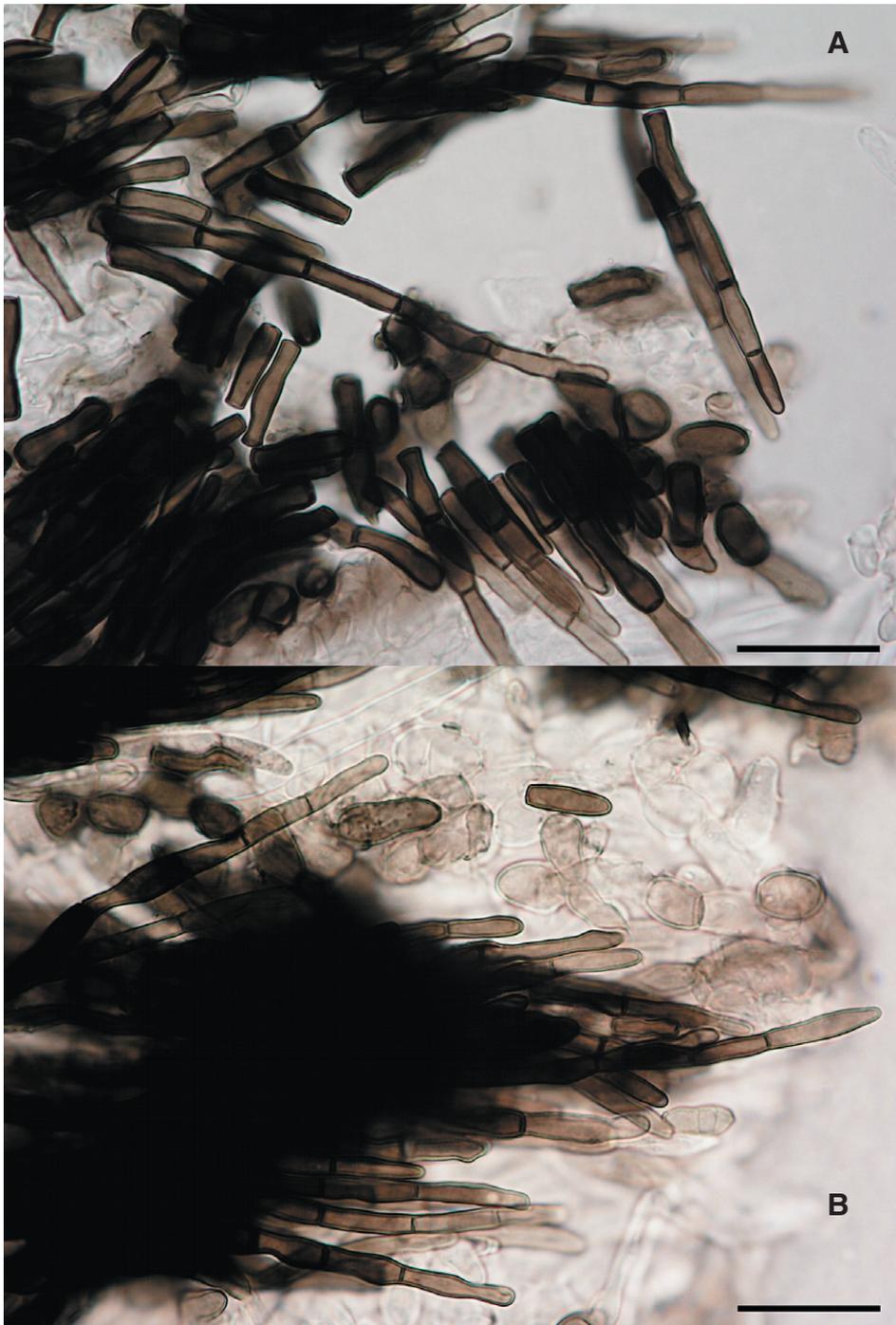


Fig. 2. *Pirottaea lychnidis*, PRM 908389. **A–B:** apothecia; **C:** rehydrated apothecia; **D:** marginal hairs; **E:** apothecium in longitudinal section; **F:** ectal excipulum in longitudinal section. Medium: C: tap water, D–F: 3 % KOH. Scale bars: A–C: 200 μ m, D: 10 μ m, E: 100 μ m, F: 10 μ m.



Description of *Pirottaea lychnidis* according to specimen Pl. crypt. N. France, nr. 174 from herb. G, ut *Peziza sphaerioides* var. *lychnidis*

Dried apothecia black, rounded to broadly ellipsoid, mostly slightly elongated along the stem, but sometimes also elongated in other directions, (220–)300–510(–640) × (210–)280–420 µm. Rehydrated apothecia black, 510 × 440 µm, 640 × 540 µm. Areas of the stem bearing apothecia with a colour (lacking straw colours and having a slight vinaceous tint) different from areas without apothecia. Ectal excipulum composed of a thin-walled *textura angularis*, in surface layer coloured dark brown, otherwise hyaline to subhyaline, cell walls 0.5–0.7(–0.9) µm thick. Thick-walled cells (grana) scattered on outer surface of apothecium. Grana globose to ellipsoid, dark brown to pale brown, (5–)6.3–8.3(–9.4) × 4.5–6.7(–7.8) µm, thick-walled, wall (0.6–)0.7–1.1(–1.3) µm thick. Setae mostly flexuous and irregular in shape, 13–45(–72) × 4–6 µm, up to 4-septate, most frequently 2-septate, wall 0.6–1.0 µm thick in dark brown cells, 0.5–0.7(–0.8) µm thick in pale brown cells, and 0.4–0.6 µm thick in subhyaline cells. Cells of setae relatively often anastomosing. Some setae possess “double septa” in their lower, dark to pale brown, thick-walled parts. That part of setae possesses marked constrictions at septa, whereas the upper, paler part without “double septa” possess only moderate or no constrictions. Setae were rarely observed to have characteristic (deep) brown apical cells moderately tapered towards their rounded apices and with medium thick, refractive walls. Apical cells of setae mostly subhyaline with only slightly refractive walls. Some setae broken at the “double septum”, and terminal cells then lacking. Marginal hairs at base subhyaline, apically hyaline, thin-walled, 21–60 × 4–5 µm, 1–3(–4)-septate, slightly constricted at septa or non-constricted, apex most frequently convex, rarely pointed or rounded. Subhymenium composed of freely intertwined, (1.4–)1.6–2.5(–2.8) µm broad hyaline hyphae. Asci 41–48.5(–51.5) × 4.6–5.4(–5.7) µm, 8-spored, arising from croziers, ascopical ring with weak blue reaction in MLZ, deep blue in KOH/MLZ, and medium blue to weak blue, in several asci dark grey and in some other ones red in IKI, deep blue in KOH/IKI. Ascospores 12.3 × 1.9 µm, 19.6 × 2.4 µm, hyaline, non-septate, slightly curved, each with several small lipid bodies of c. 0.5 µm diam. Paraphyses hyaline, sublanceolate or only slightly tapered with rounded apex, 2.0–2.6 µm broad, exceeding the asci by 3–9 µm.

Specimen studied. France: [Lille? Lambersart?], “dans nos taillis” [in our coppice], on *Lychnis sylvestris* [= *Silene dioica*], G 274010 (specimen from the exsiccate collection Plantes cryptogames du nord de la France, nr. 174).

Fig. 3. *Pirottaea lychnidis*, PRM 908389. **A:** setae, including one with a double septum; **B:** setae. Medium: 3% KOH. Scale bars: 20 µm.

Further examined specimens of *P. lychnidis* showing the same type of setae as in specimen G 274010. Czech Republic: Praha-Běchovice, on *Silene* sp., 13 May 2009, leg. M. Chlebická, PRM 915610, ut *Pyrenopeziza lychnidis* [asci 54–66 × 5.0–5.9 µm, paraphyses sublanceolate (2.3–3.2 µm broad, exceeding the asci by 5.5–12.5 µm) or capitate (3.1–4.0 µm broad)]. – Czech Republic: Šumava Mts., Mt. Boubín near Zátoň, lawn at dam of lake “Boubínské jezírko”, on *Silene dioica*, 14 June 2010, leg. M. Chlebická, PRM [asci (45–)51–55 × 5.2–5.6 µm, paraphyses sublanceolate, 3.0–3.3 µm broad, exceeding the asci by 6–11 µm].

DISCUSSION

Nomenclature and taxonomy

Fascicles of the exsiccate collection Plantes cryptogames du Nord de la France were distributed in bound books containing 50 specimens per fascicle. Specimens are pasted on the pages one per sheet and on the same sheets also printed labels are pasted. The labels contain number, binomial, a reference to literature and sometimes synonymy, habitat, season, and descriptions. The books also contain 8 to 12 sheets at the beginning, and a page with an index at the end (Margadant 1968). According to Art. 30.4 of the International Code of Botanical Nomenclature, new taxa can be effectively published based on such an exsiccate collection. The book containing *Peziza sphaerioides* var. *lychnidis* is preserved in the FH herbarium. The name is, however, invalid according to Art. 32.1(d) of the Code, because it was distributed without a diagnosis, description or drawing. It was neither published by Desmazières in the botanical series of Annales des Sciences naturelles, like his other new fungal taxa. Also Hütter (1958) gives only reference to the specimen in the exsiccate collection. Other names from Hütter's synonymy of *Pyrenopeziza lychnidis* (*Peziza sphaerioides* Pers. from 1822 and *Peziza bongardii* Weinm. from 1836) represent species on *Valeriana* and *Melampyrum*. Cooke (1871) and Phillips (1887) published descriptions, but under Persoon's epithet *sphaerioides*. This epithet was also used in exsiccate collections: Desmazières (Pl. crypt. France, ed. 2, no. 124, 1838), Cooke (Fung. brit. exs., ser. I, no. 577, 1872), and Phillips (Elv. brit. exs., no. 34, 1874). *Micropeziza lychnidis* Fuckel described and illustrated by Fuckel in Heuglin (1874) from *Lychnis apetala* (= *Silene uralensis*) is a different fungus with plane, rounded apothecia with a crenate margin which is curved in dried state, and 4–5 µm wide, acute ascospores probably with an inconspicuous submedian septum. The earliest valid description for our fungus is then *Pyrenopeziza sphaerioides* (Pers.) Fuckel f. *lychnidis* Desm. ex Sacc., published in 1889.

The species *Pirottaea lychnidis* was previously reported in the literature by Rehm (1892: 614), Hütter (1958), Ellis and Ellis (1985), and Baral (in Krieglsteiner 1999: 281). We are not certain about the work by Nannfeldt (1932), who did not de-

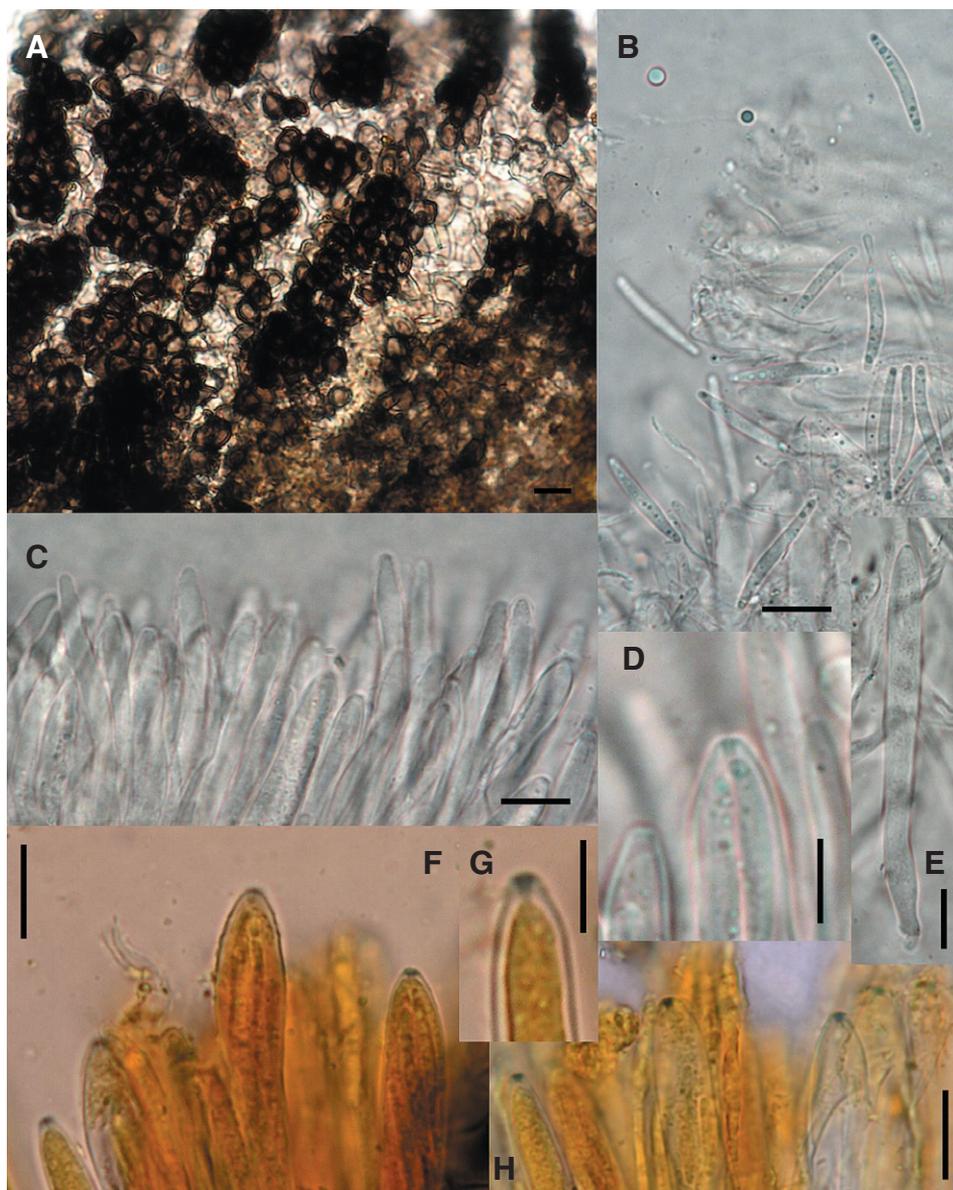


Fig. 4. *Pirottaea lychnidis*, PRM 908389. **A:** grana; **B:** ascospores; **C:** paraphyses exceeding the asci; **D:** ascus apex; **E:** ascus; **F-H:** ascus apices. Medium: A-E: 3 % KOH, F-H: IKI. Scale bars: A-C: 10 μ m, D: 5 μ m, E-F: 10 μ m, G: 5 μ m, H: 10 μ m.

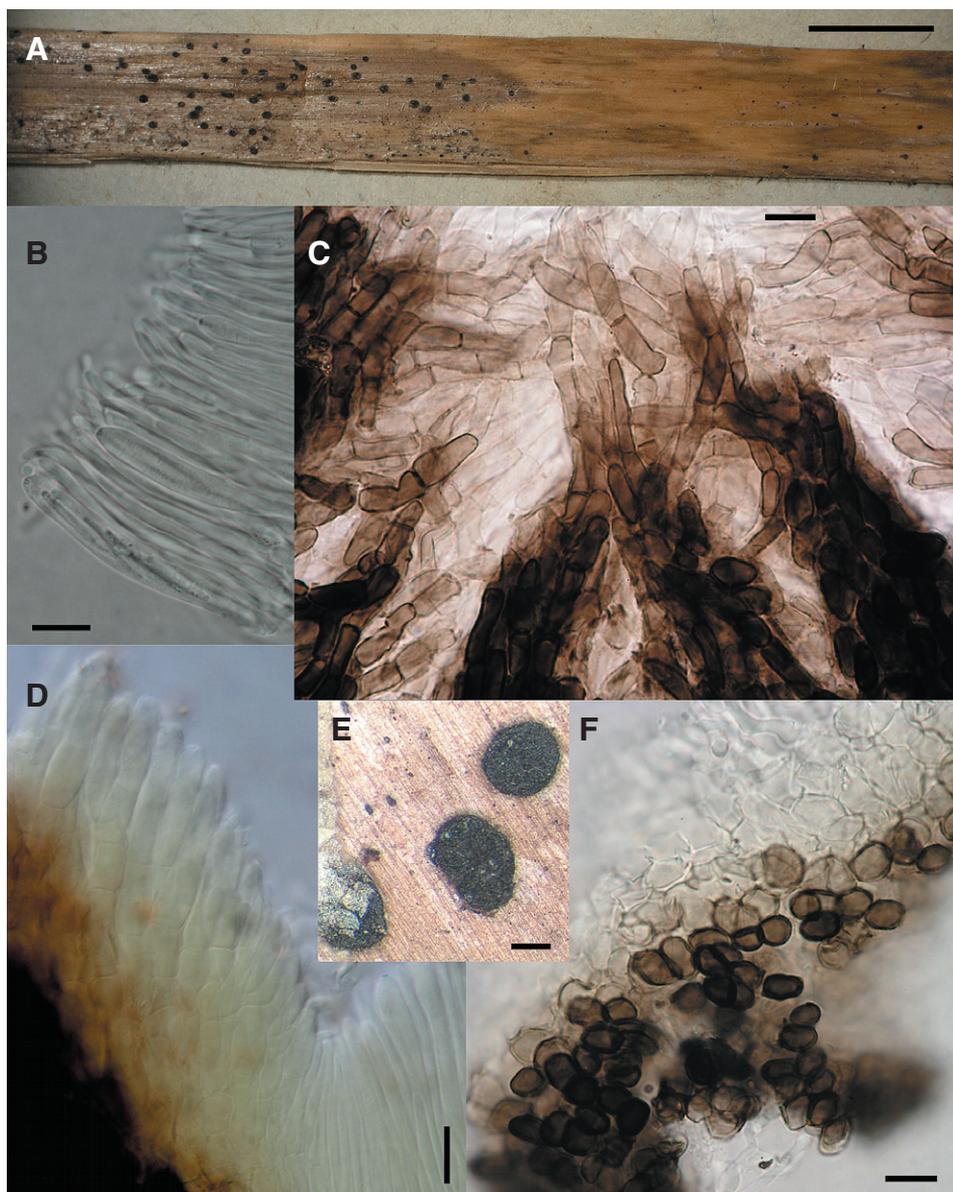


Fig. 5. *Pirottaea lychnidis*, G 274010. **A:** apothecia and colour of the stem; **B:** hymenium; **C:** setae; **D:** marginal hairs; **E:** apothecia (photo P. Clerc); **F:** grana. Medium: B–C: 3% KOH, D: MLZ after 3% KOH, F: 3% KOH. Scale bars: A: 5 mm, B–D: 10 μ m, E: 200 μ m, F: 10 μ m.

scribe setae and hymenial characters. Hütter (l.c.) described under *Pyrenopeziza lychnidis* a specimen with wide asci otherwise belonging to *Pirottaea lychnidis*.

Dennis (1981) noted that all species of *Pirottaea* are apparently uncommon. The season of 2006 (with a relatively wet and cold spring) as well as the canyon character of the locality “Ve strži” seem to have contributed to their fructification. The find from Praha-Běchovice, however, seems to signalise that the species is common, as was mentioned by Ellis and Ellis (1985).

All studied collections are considered as conspecific because of the same character of hymenial elements and absence of any clear difference in excipulum and setae. All excipular elements were also noted in the specimen G 274010 including few setae with free apices. We think that moisture conditions connected with hardness of the substrate may have had some influence on the development of the excipular characters. Nannfeldt (1984) also supposed influence of substrate properties on characters of the excipulum in the closely related *Hysteropezizella* complex (as delimited by Hein 1981).

The collection from Bohemian Switzerland National Park has very well developed setae, therefore it was used for comparison with other *Pirottaea* species. *Pirottaea lychnidis* is similar to *P. pilosissima*, which was described by Nannfeldt (1985) from *Geranium sylvaticum*. *P. pilosissima* has longer setae, (50–)80–100 µm, with 5–6 septa dividing a seta in 10–25 µm long cells, and differs also in other microcharacters and smaller apothecia. Nannfeldt (1985) did not present methods of his study. However, judging from the specimens studied and the arrangement of ascospores in the asci he apparently always studied dead herbarium material and his measurements are, therefore, fully compatible with our study.

According to Nannfeldt (1985), *Pirottaea pilosissima* has, in comparison with other *Pirottaea* species, an unusual feature: a large amount of textura prismatica in the ectal excipulum. A textura angularis-globulosa occurs, according to him, only in the surface layer of the basal excipulum, laterally transgressing into a textura prismatica. Nannfeldt (1985) did not illustrate the excipulum of the *Pirottaea* species studied. A similar structure of the excipulum is, however, delineated in his (Nannfeldt 1932: 143, 155) drawings of *Pyrenopeziza cotoneastri* (Starb.) Nannf. and *P. salicis* (Feltg.) Nannf. Hütter (1958) reported the presence of a textura prismatica in the margin of *Pyrenopeziza petiolaris* (Alb. et Schwein.: Fr.) Nannf. and illustrated it. *Pirottaea lychnidis* possesses elongated cells only in a thin layer below the subhymenium, slightly extending only towards marginal hairs (Figs. 2D, 2F), whilst the surface layer of the excipulum is overall composed of a textura angularis. In *P. pilosissima*, hyaline hypha-like endings in the marginal part of the apothecium are scarce and almost completely hidden by the fringe of setae (Nannfeldt 1985). The margin of *Pirottaea lychnidis* has a number of such hypha-like endings which we here name marginal hairs (Figs. 1A, 2D, 5D) in agreement with Baral (in Krieglsteiner 1999; in Baral and Marson 2005) and

corresponding with their pronounced hair shape. In *Pyrenopeziza*, Nannfeldt (1932) and Hütter (1958) used the term 'Randfasern' (= marginal fibres) for these structures concerning both the inner as well as outer part of the margin.

According to Nannfeldt's (1985) opinion, the genus *Pirottaea* does not form a monophyletic group, but evolved from several lineages of ancestors of *Pyrenopeziza*. A similar pattern of polyphyletic evolution can be found for example when evaluating the known ITS1-5.8S-ITS2 sequences (Benson et al. 2009) of *Diatrype* (8-spored) and *Diatrypella* (multispored). Baral (in Krieglsteiner 1999) considered *Pirottaea* an artificial genus which could be well unified with *Pyrenopeziza*. *Pyrenopeziza* and *Pirottaea* species have yet been sequenced only rarely. Thus, there are no molecular data which clarify whether the setae of *Pirottaea* originated or disappeared more times in evolution or not. We agree to use the term "evolutionary lineage" within the (well-separated) genus *Pirottaea*.

Nannfeldt (1985) recognised four evolutionary lineages in the genus *Pirottaea*. He mentioned the presence of "double" septa (Fig. 3A) as characteristic for lineages 2 and 4. Lineage 4 contains species with more thick-walled setae than lineage 2. Most of the species possess short setae, except for *P. adenostylidis* Nannf., *P. exilispora* Graddon, *P. malvae* (Fautr.) Nannf., *P. senecionis* Nannf., and *P. symphyti* Nannf., whose setae are up to 60 or 70 µm long. *Pirottaea lychnidis* belongs to lineage 2, characterised by long, soft, undulating setae and represented by only one species, *P. pilosissima*. Setae of *P. lychnidis* are less undulating, resembling in some aspects *Pirottaea saxonica* Nannf. from lineage 1, which differs, however, in 80–100 µm long, 5–8-septate setae gradually tapering to the top, and in the absence of "double" septa.

The subacute paraphyses present in *P. lychnidis* are unusual in *Pirottaea*. Unfortunately, Nannfeldt (1985) did not include paraphyses into his descriptions. However, all species illustrated by Graddon (1967, 1977, 1990) possess paraphyses with rounded tips. Baral (in Baral and Marson 2005) illustrated the subacute shape of paraphyses in specimens of an unidentified species of *Pyrenopeziza* from *Aegopodium podagraria* (H.B. 4081) and *Geum urbanum* (H.B. 4088) studied in living state, and for a herbarium specimen of *Pirottaea lychnidis* from *Silene dioica* (H.B. 5690, ut *Pyrenopeziza lychnidis*), and *Pyrenopeziza rubi* (Fr.) Rehm (PRM 666704, holotype of *Haglundia sarmentorum* Svrček).

Revision of published records of *Pirottaea* from the Czech Republic

New species described from the Czech Republic in the genus *Pirottaea* by Velenovský (1934) and Vacek (1950) belong to other genera (*Lachnum* s.l., *Haglundia*) according to Nannfeldt's opinion (Nannfeldt 1985). Further species reported by Velenovský are *P. gallica* Sacc. and *P. veneta* Sacc. et Speg. His specimen of *P. gallica* from Mnichovice (Central Bohemia) belongs to *Pyrenopeziza*

escharodes (Berk. et Broome) Rehm, according to our revision. Specimens from the Krkonoše Mts. (Sudetes) collected by A. Pilát (PRM 824987, PRM 148587) and a specimen collected there by K. Cejp (PRM 151868) belong to *Pirottaea gallica*, *P. cf. senecionis* Nannf. (+ *Colletotrichum* sp.), and *P. senecionis*, respectively, and their substrates are in fact *Cicerbita* (cf.), a dicot (+ *Senecio*), and *Senecio*. Velenovský's specimen of *P. veneta* from *Epilobium montanum* contains material similar to a fungus which Nannfeldt (1985: 9) mentioned as *P. aff. adenostylidis* from *Anthemis*. The studied material differs from *P. senecionis* by larger, non-septate ascospores and broader asci, but differs also from *P. adenostylidis* by slightly shorter and non-furcate setae. Its substrate was reidentified by Svrček (in herb.) as *Senecio* but, in our opinion, it may belong to *Achillea millefolium*. Petrak (Flora Boh. Mor. exsic. Nr. 1496) reported *Pirottaea veneta* (in fact *P. senecionis* in agreement with Svrček's note on herbarium sheet) from Hrabůvka near Hranice (Moravia). This means that, including our revision, there are 4 species of *Pirottaea* confirmed from the Czech Republic: *P. aff. adenostylidis*, *P. gallica*, *P. senecionis*, and *P. lychnidis*.

ACKNOWLEDGEMENTS

We would like to thank Jiří Hadinec for identification of the host plant. The Administration of Bohemian Switzerland National Park and Karel Prášil (Faculty of Science, Charles University), who organised the field research in the area, are gratefully acknowledged. We thank Hans Otto Baral for suggesting many small improvements in the manuscript. Our sincere thanks belong to Prof. Richard P. Korf and curators of the herbaria G, K, MPU, NY and FH for help in the search of the earliest valid description of *Pirottaea lychnidis*, and to Philippe Clerc for arranging a loan. The work was supported by grant of the Ministry of Culture of the Czech Republic no. MK00002327201.

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