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Revision of the genus *Daldinia* in the KRAM herbarium collection: *Daldinia childiae*, *D. loculata* and *D. loculatoides*, three new species for Poland

Introduction

The fungi from the genus *Daldinia* have been neglected for a long time. The genus was found by two mycologists (Cesati, De Notaris, 1863) and named in honor to the monk, Agostino Daldini. The first important monograph was prepared in 1932 (Child, 1932) and after that, important elaborations appeared (You et al., 1997; Stadler et al., 2014) that enabled progress in the taxonomy of this genus. The species of the genus *Daldinia*, according to Cesati and De Notaris (1863), are conspicuous because of their relatively large stromata. Most of them possess internal alternating zones. The genus comprises ca. 50 species that occur on angiosperm hosts. Only *D. lloydii, D. eschscholtzii* and *D. childiae* occasionally were noted on gymnosperm hosts (*Pinus* and *Cryptomeria*). *Daldinia hawksworthii* is known only in its anamorph state (Stadler et al., 2014). However, anamorph states of other species can occur in a wide range of angiosperms as endophytes.

The taxonomy of this genus is based on morphological characters, colour of stromatal pigments, secondary metabolites, anamorph structures and molecular methods (Stadler et al., 2001, 2014; Guidot et al., 2003; Stadler, 2011; Helay et al., 2018). The anamorphic structures are most often referred to as the genus *Nodulisporium* Preuss that produces conidia from percurrent proliferating conidiogenous cells (Petrini, Müller, 1986; You et al., 1997). Conidia are formed on young stromata or on invaded wood. Stromata attached to the tree branches can actively discharge ascospores for more than 100 days (Ingold, 1965) and finish their discharge in late autumn. Fungi from the *Daldinia concentrica* complex produce volatile antibiotics that are active against plant pathogenic nematodes (Liarzi et al., 2016a, b) and *D. hawksworthii* is an insect-associated endophytic species that can produce volatile, small polyketide dalsymbiopyrone (Pažoutová et al., 2013).

Mycelia of *Daldinia* have been characterised as "early colonisers." They are present in the tissue of the host plants for a very long time without causing any symptoms of parasitism (Stadler et al., 2014).

For a long time, *D. concentrica* has been the only species reported from Poland (Mułenko et al., 2008). However, in light of recent taxonomic revisions of the genus *Daldinia* it appears that this species does not occur in Poland! Almost all Polish collections cited by Mułenko et al. (2008) were possibly wrongly determined and it is necessary to check and revise all Polish collections of this species. Apart from the doubtful collections of *D. concentrica*, only five species have been reported from Poland: *D. decipiens* (Karasiński, 2009; Ruszkiewicz-Michalska et al., 2015; Gierczyk et al., 2017), *D. petriniae* (Wojewoda et al., 2013; Stadler et al., 2014), *D. pyrenaica* (Karasiński, 2009), *D. vernicosa* (Stadler et al., 2014; Gierczyk et al., 2017) and *D. lloydi* (Stadler et al., 2014). The aim of the present study was the revision of the part of the richest Polish collection of *Daldinia* species, preserved in the fungal collection of the herbarium of the W. Szafer Institute of Botany, Polish Academy of Sciences, in Kraków (KRAM F).

Material and Methods

Morphological characters of stromata were examined under a stereo microscope Nikon SMZ 1500. Ascospores were examined in water, glycerol and lactophenol under a light microscope Nikon Eclipse 80i with an oil immersion lens. Twenty spores of each specimen were measured. Also the colour of stromatal pigments in 10% potassium hydroxide (KOH) was used to determine the fungal material. Fungi presented in this article are deposited in KRAM F. Information about the localities and habitats follow those on the original labels.

Results

***Daldinia childiae* J.D. Rogers & Y. -M. Ju,**
Mycotaxon 72: 512–513, 1999.

Stromata subglobose and turbinate, measuring up to 2 cm in diameter. The KOH-extractable stromatal pigments yellowish or yellow-brown in colour (Fig. 1A). Ascospores with narrowly-rounded ends, 13–14 × 6.3–7 µm (Fig. 2C). The transverse striation of episporium mostly loosely distributed (Fig. 3).

Specimens examined:

1) Poland: the Sudetes, the Bystrzyckie Mts., the Topieliska Nature Reserve near Zieleniec, on a branch of *Betula* sp., 29 August 1984, leg. A. Chlebicki, KRAM F 40392 (as *D. concentrica*).

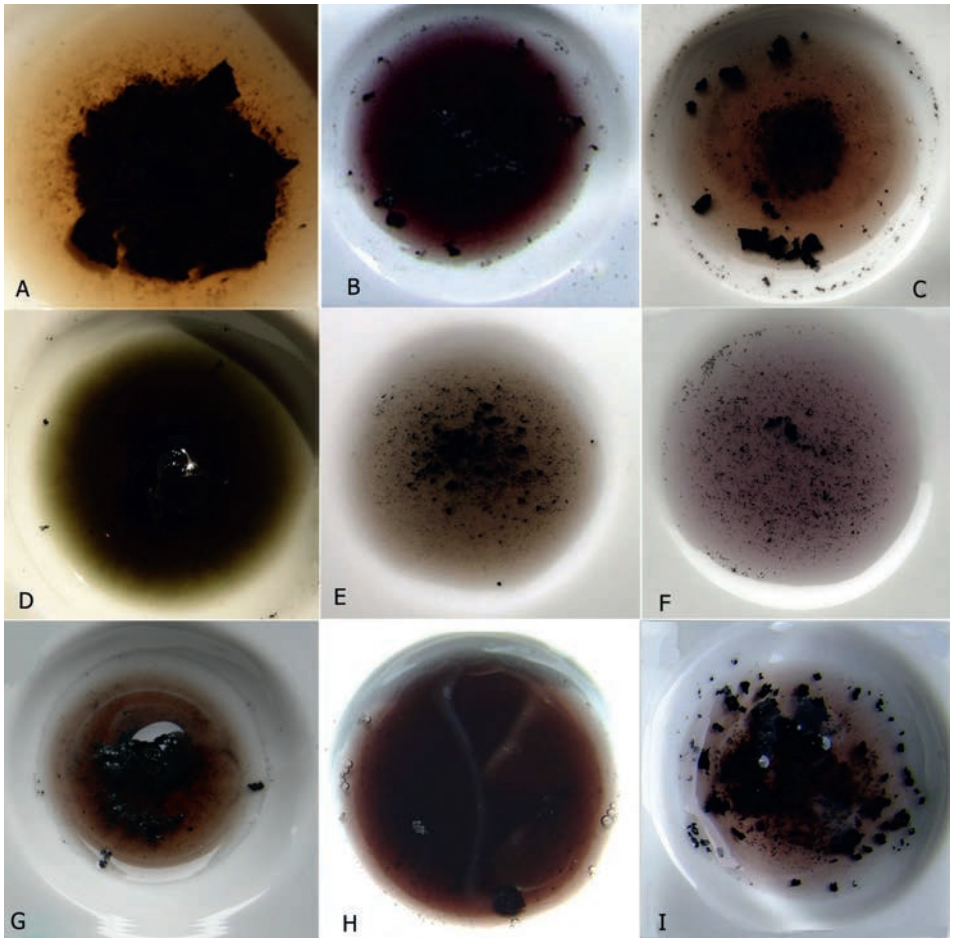

Fig. 1. KOH-extractable stromatal pigments: *Daldinia childiae* – A, *D. concentrica* – B, *D. eschscholtzii* – C, *D. lloydii* – D, *D. loculata* – E, *D. loculatoides* – F, *D. decipiens* – G, *D. vernicosa* – H, *D. petriniae* – I (Photo. A. Chlebicki)

2) Poland: Western Carpathians, Orawa-Nowy Targ Basin, near the road from Rogoźnik to Stare Bystre, ca. eight km southwest of Nowy Targ, ca. 620 m a.s.l., on a trunk of *Salix* sp., 7 June 1970, leg. Z. Heinrich, KRAM F 27202 (as *D. concentrica*).
3) Poland: Western Carpathians, the Pieniny Mts., the Skalice Range, in the forest near Łapsze Niżne, 18 km southeast from Nowy Targ, ca. 600 m a.s.l., on dead wood (possibly *Salix* sp.), 1 June 1979, collector unknown, KRAM F 31870 (as *D. concentrica*).
4) Poland: Western Carpathians, the Bieszczady Zachodnie Mts., Nasiczne, on a dead trunk of *Alnus incana* (L.) Moench, 26 December 2011, leg. A. Wilczek, KRAM F without number.

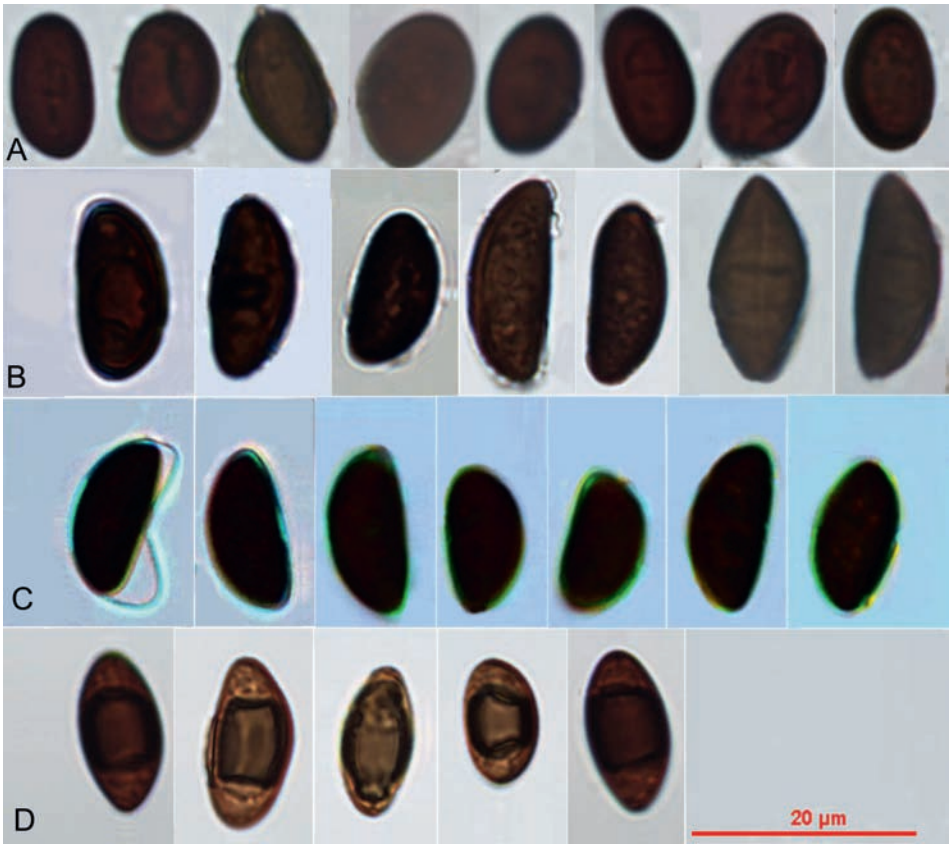

Fig. 2. Ascospores: *Daldinia loculata* – A, *D. decipiens* – B, *D. childiae* – C, *D. vernicosa* – D (Photo. A. Chlebicki)

Comments: The perfect state of *D. childiae* is host-limited to the angiosperm trees but its anamorph can inhabit a wide range of angiosperms as an endophyte (Stadler et al., 2014). It also occasionally occurs on gymnosperm hosts (i.e., *Cryptomeria*). The transverse striation of the spores of *D. childiae* is conspicuous and loosely distributed as compared to *D. concentrica* and *D. eschscholtzii* (Fig. 3).

In the autumn of 2011, an insect larva inside of *D. childiae* stromata was noted (Eastern Carpathians, Bieszczady Zachodnie Mts, Nasiczne). In the spring of 2012, the imago of a fly was found inside a box with *Daldinia* (Fig. 4). This fly possibly belongs to the old branch of wood-inhabiting Agromyzidae (T. Zatwarnicki and A. Palaczyk, pers. comm.).

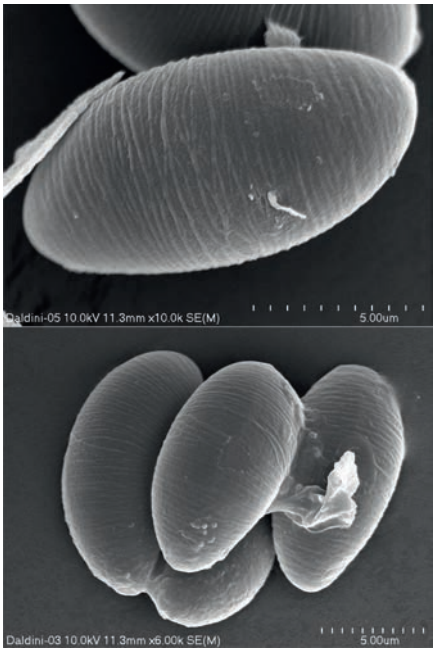

Fig. 3. Ascospores of *Daldinia childiae* (Photo. A. Chlebicki)

Fig. 4. Larva and imago of fly possibly from the family Agromyzidae, noted on old stromata of *Daldinia childiae* (Photo. A. Chlebicki)

***Daldinia concentrica* (Bolton: Fr.) Ces. & De Not.,**
Comm. Soc. crittog. Ital. 1 (no. 4): 197. 1863.

Stromata semi-spherical, sessile, widely attached to the substrate, smooth, up to 4.4 cm in diameter. The KOH-extractable stromatal pigments dark purple (Fig. 1B). Ascospores brown, ellipsoid, with narrowly rounded ends, 13.4–16.3 × 6.8–9 µm (Fig. 5).

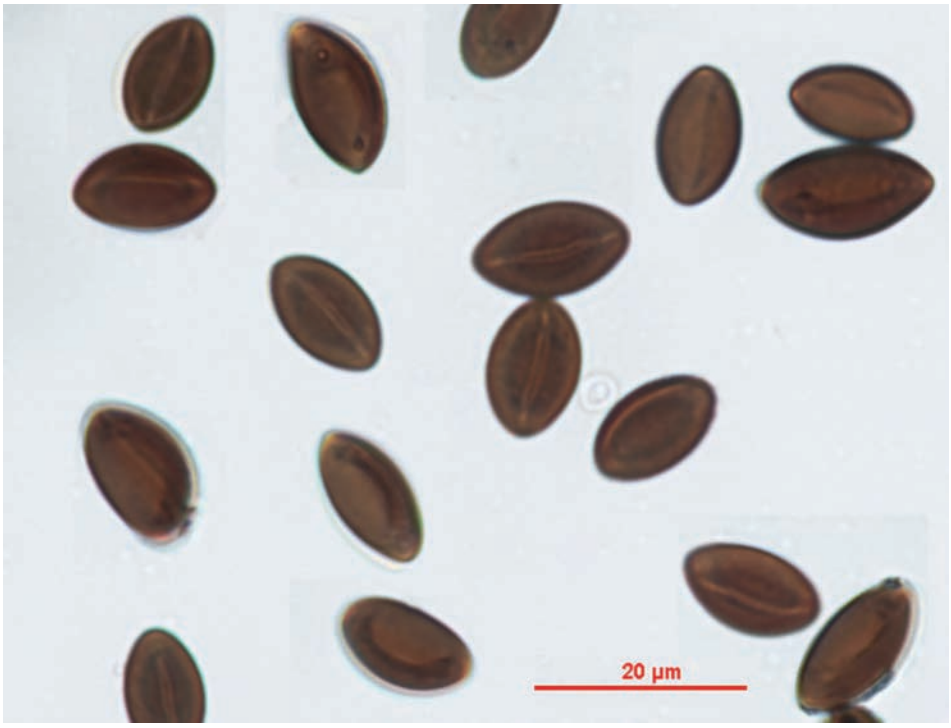

Fig. 5. Ascospores of *Daldinia concentrica* from Bulgaria, KRAM F 47531 (Photo. A. Chlebicki)

Specimens examined:

1) Bulgaria: Stranja Mts., the Ropotamo National Park, 5 km from an estuary, on *Acer campestre* L., 18 May 1981, leg. A. Chlebicki, KRAM F 47531 (as *D. concentrica*).

Comments: It is a the only specimen of *D. concentrica* in the KRAM F collection. Stadler et al. (2014) reported *D. concentrica* from the same locality in the Ropotamo National Park.

***Daldinia decipiens* H. Wollweber & M. Stadler,**
Mycotaxon 80: 168. 2001.

Stromata semiglobose and often subsessile. The KOH-extractable stromatal pigment vinaceous purple (Fig. 1G). Ascospores brown and amygdaliform with hyaline appendages at both ends, (16)17.5–20 × 7.5–9.6 µm (Fig. 2B).

Specimens examined:

1) Poland: the Ochojec Nature Reserve, on a *Betula* sp. branch, 12 July 2008, leg. D. Karasiński, KRAM F 5662 (as *D. decipiens*).
2) Poland: Wysoczyzny Podlasko-Białoruskie, in the Białowieża National Park, section 256, Square G4, in a *Peucedano-Pinetum* W. Mat. (1962) W. Mat. et J. Mat. 1973 forest, 27 October 1988, leg. A. Chlebicki, KRAM F 41088 (as *D. concentrica*).
3) Poland: Pojezierza Wschodniobałtyckie, in Puszcza Augustowska primeval forest, in a birch forest near Płaska Lake, on twigs of *Betula pendula* Roth, 2 May 2007, leg. A. Chlebicki, KRAM F 56344 (as *D. concentrica*).
4) Poland: Górny Śląsk, Wyżyna Śląsko-Krakowska, Jastrzębie Zdrój district, Wodzisław Śląski, in the forest on the branches of *Betula* sp., 3 March 1972, leg. K. Filipowska, KRAM F 12634 (as *D. concentrica*).

Comments: The species belongs to the *D. petriniae* group. It is characterised by the long spores (up to 20 µm) and purple stromatal pigment. It occurs mostly on burnt substrates. The species was already noted by Karasiński (2009), Ruszkiewicz-Michalska et al. (2015) and Gierczyk et al. (2017).

***Daldinia eschscholtzii* (Ehrenb.: Fr.) Rehm,**
Annals Mycol. 2(2): 175. 1904

Stromata turbinate and sessile. Surface without any conspicuous perithecial outlines and with slightly papillate ostioles. The KOH-extractable stromatal pigment vivid vinaceous purple (Fig. 1C). Ascospores brown, inequilateral with narrowly rounded ends, 11.5–13.4 × 5–6 µm.

Specimen examined:

5) Cameroon: in East Province, Department Lom et Djérem, between Koumé and Koundi, ca. 15 km northwest from Bertoua, ca. 710 m a.s.l., in the Guineo-Congolian tropical rainforest, on a fallen trunk of a deciduous tree, 15 December 2007, leg. J. Piątek and M. Piątek, KRAM F 59661 (as *Daldinia* sp.).

Comments: It is a common species in warmer climates, that occurs frequently in Africa and was also noted in Cameroon (Stadler et al., 2014).

***Daldinia lloydii* Y.M. Ju, J.D. Rogers & F. San Martín,**
Mycotaxon 61: 273. 1997.

Stromata fulvous with a surface cracked into polygonal scales. The KOH-extractable stromatal pigments olivaceous green (Fig. 1D).

Specimens examined:

1) Ukraine: Zhulkev Region, Dublany, the Botanical Garden, on handrail made of a birch wood (*Betula* sp.), August 1928, leg. K. Rouppert, KRAM F 1588 (as *D. concentrica*).

Comments: The species was reported from Poland by Stadler et al. (2014) from Myszyniec.

***Daldinia loculata* (Lév.) Sacc.**
Syll. Fung. I: 394 (1882).

Stromata hemispherical and sessile, from 2.5 up to 4 cm in diameter. The KOH reaction of stromatal materials mostly yields a dense purple pigment (Fig. 1E). Ascospores dark brown, with broadly rounded ends, (13)14–15(16) × 7–8.3 μm (Fig. 2A).

Specimens examined:

1) Poland: Niziny Sasko-Łużyckie, Zielona Góra voivodeship, Zasieki and Lubsko, near the Polish-German border, on burned trunks of *Betula* sp. April 1984, leg. B. Ginko, KRAM F 39306 (as *D. concentrica*).
2) Poland: Wyżyna Środkowomałopolska, Małopolska voivodeship, the Ojców National Park, Sępówka Valley, on a burned trunk of *Fagus sylvatica* L., 7 November 1964, leg. W. Wojewoda, KRAM F 13033 (as *D. concentrica*).
3) Poland: Pojezierze Wschodniobałtyckie, in Puszcza Augustowska Forest, Sosnowo Island in Serwy Lake, on a dead trunk of *Betula pendula*, 10 July 2003, leg. A. Chlebicki, KRAMF 55322, (as *D. concentrica*).

Comments: This species is associated with *Betula* sp. but was also noted on Salicaceae Mirb. (Stadler et al., 2014). It differs from *D. vernicosa* by its sessile stromata and the granules in its stromatal morphology.

***Daldinia loculatoides* Wollw. & M. Stadler,**
Mycol. Res. 108(9): 1030. 2004.

Stromata semiglobose, sessile, black, up to 4 cm in diameter. The KOH-extractable stromatal pigment vinaceous purple (Fig. 1F). Ascospores dark brown, shaped like a Rugby ball, with broadly rounded ends, 15–17(20) × 7–8 μm (Fig. 6).

Specimens examined:

1) Poland: Wyżyna Środkowomałopolska, Małopolska Region, Kraków, Rakowicki cemetery, on a burned trunk of *Acer platanoides* L., 27 October 1993, leg. W. Wojewoda, KRAM F 34899 (as *D. concentrica*).

Comments: This is a rare species that differs from *D. loculata* by its larger ascospores and semiglobose stromata (Fig. 6).

Daldinia petriniae **Y.M. Ju, J.D. Rogers & F. San Martín,**
Mycotaxon 61: 275 (1997)

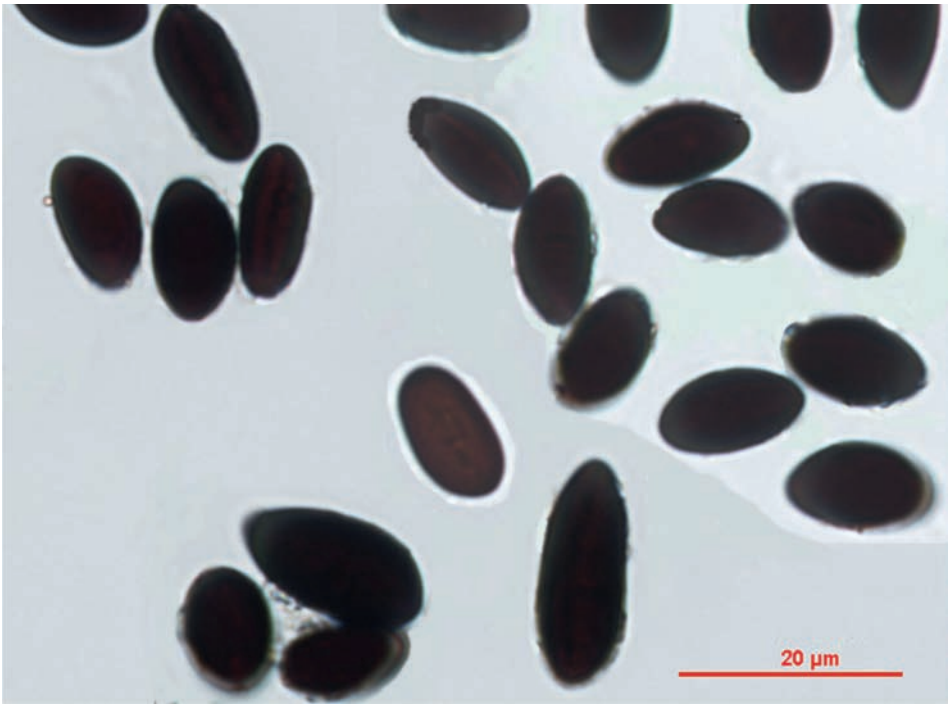

Fig. 6. Ascospores of *Daldinia loculatoides* (KRAM F 34 899) (Photo. A. Chlebicki)

Stromata semiglobose, aggregated and sessile. The KOH-extractable stromatal pigments vinaceous to vivid purple (Fig. 1I). Ascospores inequilateral, dark brown, 10–14 × 6–7 µm.

Specimens examined:

1) Poland: Western Carpathians, the Bieszczady Zachodnie Mts., Duszatyn, the Przełom Osławy Reserve, on a standing dead trunk (ca. 6 cm in diameter) of *Alnus incana*, 6 April 2012, leg. A. Chlebicki, KRAM F without number.
2) Poland: Western Carpathians, the Bieszczady Zachodnie Mts., Prełuki near Komańcza and Osławica River, on a dead, small trunk (7 cm in diameter) of *Alnus incana*, 9 April 2012, leg. A. Chlebicki, KRAM F without number.
3) Poland: Western Carpathians, the Bieszczady Zachodnie Mts., Nasiczne, on a dead trunk of *Alnus incana*, 26 December 2011, leg. A. Wilczek, KRAM F without number.

Comments: It was collected and determined by P. Drzewiecki in Mohle, Kujawsko-Pomorskie Region, on the trunk of a young alder (Snowarski, 2014). Karasiński (Wojewoda et al., 2016)

also noted this species in the Gorce Mts. and Domański et al. (1967) reported it from branches of *Alnus incana* (as *D. concentrica*) in the Bieszczady Zachodnie Mts. The fructification of the species begins in autumn. It is the most common species of *Daldinia* in KRAM F. This species differs from *D. concentrica* by its vivid purple pigments and smaller ascospores. *Daldinia petriniae* is similar to *D. eschscholtzii* but the latter species occurs in tropical zones.

***Daldinia vernicosa* Ces. & De Not.**
Comment. Soc. Crittog. Ital. 1: 198. 1863

Stromata subglobose, sessile, shortly stipitate to distinctly stipitate. The KOH-extractable stromatal pigment purple, vivid violet to vivid brown, or vinaceous purple (Fig. 1H). Ascospores dark brown with broadly to narrowly rounded ends: (10,5)12–14 × 6.2–7.3 µm (Fig. 2D).

Specimens examined:

1) Poland: Eastern Podkarpacie, Sandomierz Dell, Przemyśl, near the railway station in Przemyśl-Pikulice towards Sielec, near Wiar River, on dead wood, 14 September 1979, leg. W. Wojewoda, KRAM F 33838 (as *D. concentrica*).
2) Poland: Niziny Sasko-Łużyckie**,** in Zielona Góra voivodeship, close to Zasieki and Lubsko, near the Polish-German border, on burned trunks of *Betula* sp., April 1984, leg. B. Ginko, KRAM F 39306 (as *D. concentrica*).
3) Poland: Lakelands Wschodniobałtyckie**,** in Augustowska Forest, the Perkuć Nature Reserve, *Vaccinio myrtylli-Pinetum* Juraszek 1928 forest, on a lying *Betula* trunk, 16 September 1974, leg. W. Wojewoda KRAMF 32456 (as *D. concentrica*).
4) Poland: Lower Silesia, Sudety Mts., Przedgórze Sudeckie, Strzelińskie Hills, 1 km northeast of Biały Kościół, on a burned trunk of *Prunus spinosa* L., 26 March 1985, leg & det. A. Chlebicki, KRAM F 55346 (as *D. fissa* C.G. Lloyd).
5) Poland: in Biebrza Valley, the Biebrza National Park, Ciszewo, Brzeziny Ciszewskie Forest, on a *Frangula alnus* Mill. host, 17 August 1991, leg. & det. A. Chlebicki, KRAM F 43103 (as *D. fissa*).
6) Poland: Małopolska Region, Tarnów, near Lipie forest, on a dead trunk of *Betula pendula,* 31 June 1997, leg. M. Piątek, det. A. Chlebicki, KRAM F 56616, (as *D. fissa*).

Comments: The species was reported from Poland by Stadler et al. (2014) and Gierczyk et al. (2017). Chlebicki (2008) noted this species on *Prunus spinosa*, *Frangula alnus* and *Betula pendula* (as *Daldinia fissa*). Stromata of this species are reported also on fire-damaged trees (Stadler et al., 2014; Chlebicki, 2008). Stadler et al. (2014) recognised *D. fissa* as a synonym of *D. vernicosa* and suggested that *D. fissa* is an aberrant form with compressed stromata, damaged by insect larvae.

Discussion

The use of chemotaxonomic evidence, molecular phylogeny and anamorphic characters enable to divide the members of the genus *Daldinia* into five major groups (Stadler et al., 2014). The colour of stromatal pigments appears to be very useful in the identification of the specimens (Stadler et al., 2014) and it was successfully used to determine Polish collections from KRAM F.

After the revision, the exsiccata of the following *Daldinia* species are present in the KRAM F: *D. childiae* (the *D. childiae* group), *D. eschscholtzii* (the *D. eschscholtzii* group), *D. loculata*, *D. loculatoides* and *D. vernicosa* (the *D. vernicosa-D. loculata* group), *D. decipiens, D. lloidii* and *D. petriniae* (the *D. petriniae* group). From these species, three are reported here from Poland for the first time: *D. childiae*, *D. loculata* and *D. loculatoides*. Three species, which specimens are present in KRAM F, were gathered outside of Poland: *D. lloydii* from Ukraine, *D. concentrica* from Bulgaria and *D. eschscholtzii* from Cameroon. The most common species of the genus preserved in KRAM F appears to be *D. petriniae* noted on the *Alnus incana*. However, in the world *D. childiae* is the most commonly encountered species (Stadler et al., 2014).

In the case of one species from KRAM F, namely *D childiae*, a larva and an *imago* of a fly from the family Agromyzidae was found inside stromata. Insect-fungus interactions are known very well. They include dispersal, protection, nutrition and all kinds of symbiosis. Information about insects living on stromata of *Daldinia* is very scary. Alex Hyde (2020) noted *Platyrhinus resinosus* eating stromata of *D. concentrica* in Peak District National Park, Derbyshire, UK. Some insects, such as *Xiphydria* wood wasps, were noted inside stromata of *D. decipiens*. This insect can appear highly suitable as a vector for *Daldinia* (Šrutka et al., 2007; Pažoutová et al., 2013). Probably also the fly noted on stromata of *D. childiae* preserved in KRAM F can be considered as a vector for the fungus. Parasitic species can be used as aids in the host biogeography (Savile, 1975). However, our knowledge of the insects parasitising *Daldinia* is to scarce to draw any conclusions.

Acknowledgements

I would like to thank M. Stadler for his comments, suggestions and important article on secondary metabolites, A. Wilczek for collecting *Daldinia petriniae* specimens from the Bieszczady Zachodnie Mts. and T. Zatwarnicki and A. Palaczyk for suggesting that the fly could be Agromyzidae.

Conflict of interest

The author declare no conflict of interest related to this article.

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Abstract

The article presents the results of the revision of *Daldinia* (Ascomycota) specimens preserved in KRAM F (Kraków, Poland). The following species were identified: *Daldinia childiae*, *D. decipiens*, *D. loculata*, *D. loculatoides*, *D. petriniae* and *D. vernicosa*. Three of them were not reported from Poland so far: *D. childiae*, *D. loculata*, *D. loculatoides*. In addition to Polish speciments, there are also collections from outside Poland stored at KRAM F: one specimen of *D. concentrica* from Bulgaria, *D. lloydii* from Ukraine and *D. eschscholtzii* from Cameroon.

Key words: *Daldinia*, insect-associated fungi, stromatal pigments, wood-inhabiting fungi

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Rewizja okazów z rodzaju *Daldinia* w kolekcji zielnika KRAM: *Daldinia childiae*, *D. loculata* i *D. loculatoides*, trzy nowe gatunki dla Polski

Artykuł zawiera wyniki rewizji okazów z rodzaju *Daldinia* (Ascomycota), przechowywanych w zbiorach KRAM F. W kolekcjach stwierdzono następujące gatunki: *D. childiae*, *D. decipiens*, *D. loculata*, *D. loculatoides*, *D. petriniae* i *D. vernicosa*. Trzy z nich nie były do tej pory podawane z terenu Polski: *D. childiae*, *D. loculata*, *D. loculatoides*. W zbiorach KRAM F znajdują się również kolekcje spoza Polski: jeden okaz *D. concentrica* z Bułgarii, *D. lloydii* z Ukrainy i *D. eschscholtzii* z Kamerunu.

Słowa kluczowe: *Daldinia*, grzyby związane z owadami, barwniki podkładek, grzyby zasiedlające drewno

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