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Epiphyllous fungi from Miocene deposits of the Bełchatów Lignite Mine (Central Poland)

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Abstract

Fossil fungal fructifications were encountered during palynological investigation of the Miocene deposits of the Bełchatów Lignite Mine (Central Poland). Five fossil taxa similar to family *Microthyriaceae* (*Phragmothyrites* cf. *lutosus* (Dilcher) R.K. Kar & R.K. Saxena, *Phragmothyrites* sp. 1, *Phragmothyrites* sp. 2, *Trichothyrites* cf. *hordlensis* P.H. Smith, and *Trichothyrites* sp.) and one taxon (*Callimothallus pertusus* Dilcher) similar to extant anamorphic genus *Mycoleptodiscus* Ostaz. are reported. *Callimothallus pertusus*, *Phragmothyrites* cf. *lutosus*, *Phragmothyrites* sp. 2, and *Trichothyrites* cf. *hordlensis* are new for the fossil mycota of Poland. The presence of *Callimothallus pertusus* points to warm palaeoclimatic conditions which is confirmed by previously obtained data from plant macro and microremains.

Key words – fungal fructifications – Neogene – palaeoecology – Poland – taxonomy

Introduction

In the Bełchatów Lignite Mine situated in the Central Poland (51°15'46,4"N 19°18'49,2"E) south of town of Bełchatów occur lignite seams within tectonic depressions named the Kleszczów Graben. Palaeobotanical studies of the plant macroremains (leaves, fruits and seeds) and palynological investigations has allowed reconstruction of the vegetation of this area during early to late Miocene (Stuchlik et al. 1990, Worobiec & Lesiak 1998, Worobiec 2003b, 2014, Worobiec & Szykiewicz 2007, Worobiec et al. 2012). Among investigated remains, however, fossil fungi were rather scarce and were confined to remains of fungal fructifications represented by fossil-taxa *Rosellinites congregatus* (Beck) Mesch. and *Trematosphaerites lignitum* (Heer) Sacc. and to fructifications assigned as “*Microthyriaceae*” found in the Miocene deposits (Stuchlik et al. 1990). In the course of geological mapping of Neogene deposits in an outcrop of the Bełchatów Lignite Mine in 1995 an assemblage of mainly fossil leaves (catalogue number KRAM-P 218) was collected in the Miocene deposits. Comprehensive analysis of the palaeofloristics of the macroremains assemblage followed by palynological investigation of four samples taken from specimens of fossil leaves allowed reconstruction of the Neogene flora and vegetation (Worobiec & Szykiewicz 2016, Worobiec & Worobiec 2016). During pollen analysis, various fructifications of epiphyllous fungi were encountered. As epiphyllous fungi repeatedly reported from the fossil state (Hongsan et al. 2016) have a potential as palaeoclimatic proxy (Conran et al. 2016), the present investigations were aimed at detailed taxonomic and palaeoecological study of these fungal remains.

Material & Methods

Deposits with fossil plant macroremains assemblage KRAM-P 218 were left as abandoned channel fill in the floor part of clayey-sandy unit (I-P) of the Neogene deposits of the Bełchatów Lignite Mine (Worobiec & Szykiewicz 2016) considered to be late Miocene (Szykiewicz 2000). Geology of the locality were described in detail by Worobiec & Szykiewicz (2016).

Four samples from the KRAM-P 218 collection of plant macroremains from specimens (rock samples with leaf macroremains) number 85, 104 and 109 (two samples 109A and 109B) were taken. The samples were processed in the laboratory, using hydrochloric acid and sulfuric acid (Moore et al. 1991). Additionally, hydrofluoric acid was used to remove mineral matter and the residuum was sieved at 5 µm on a nylon mesh. From each sample five microscope slides were made, using glycerine jelly as a mounting medium. The specimens with leaf remains, palynological residues and slides are stored in the W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków (Poland).

The studied samples yielded rich palynological material consisting mainly of sporomorphs (pollen grains and spores). The results of palynological studies were described in detail (Worobiec & Worobiec 2016). During the current investigation, all these slides were re-examined for the presence of remains of epiphyllous fungi.

Terminology for the morphology of fungal fructifications follows Korf (1958) and Wu et al. (2011). The method of measuring the size of fungal structures depends on their shape; we used diameter measurements for regular, round or broadly elliptical structures, and length and width for quadrangular structures.

Bright field microphotography of the fossils was done using a NIKON Eclipse E400 microscope fitted with a CANON A640 digital camera.

Results

The classification of fossil and recent fungi follows Kalgutkar & Jansonius (2000) and Wu et al. (2011).

Fungi incertae sedis

Callimothallus Dilcher, Palaeontographica B 116 (1–4): 13 (1965)

Synonyms: *Pseudosphaerialites* Venkatach. & R.K. Kar, Palaeobotanist 17: 180 (1969)

Siwalikiathyrites R.K. Saxena & H.P. Singh, Geophytology 12: 294 (1982)

Ratnagiriathyrites R.K. Saxena & N.K. Misra, Palaeobotanist 38: 268 (1990)

Callimothallus pertusus Dilcher, Palaeontographica B 116 (1–4): 13 (1965)

Fig 1, a–b

Fruiting body (sporodochium) ± orbicular, ca. 62 µm in diameter, margin entire to finely sinuate. Sporodochium composed of both isodiametric (central part) and slightly elongate (margin) cells (*textura angularis/prismatica*), up to 10 µm long and 3.5–5 µm wide. Cell walls straight to rounded. Most cells of the sporodochium are conidiogenous with a single pore oriented towards the centre of sporodochium, ca. 1–2 µm in diameter. Ostiole absent. Conidia not observed.

Material examined – Slide KRAM-P 218/104(5). One specimen.

Notes – The fossil-genus *Callimothallus* includes fungi possessing characteristic fruiting bodies in which predominate porate cells. Other fossil-genera with porate cells as *Pseudosphaerialites* and *Siwalikiathyrites* (Kalgutkar & Jansonius 2000), but also *Ratnagiriathyrites* (G. Worobiec & E. Worobiec, pers. obs.) should be considered synonymous with the earlier described *Callimothallus*. All of them most probably represent fungal remains of the same fossil-genus differing only in their state of preservation. Dilcher (1965) assigned *Callimothallus* into family *Microthyriaceae*. However, fungal fructifications with porate cells are not observed in the extant members of the family *Microthyricaceae* (Wu et al. 2011). Dilcher (1965) compared *Callimothallus* with recent epiphyllous chlorophycean algae from the genus *Phycopeltis* Millardet and considered that fruiting bodies of *Callimothallus* differ from *Phycopeltis*

in cell size and abundance of porate cells. Lange (1976) and Germeraad (1979) found recent anamorphic fungal genus *Mycoleptodiscus* Ostaz. (anamorphic of *Omnidemptus* P.F. Cannon & Alcorn, Wijayawardene et al. 2012) as extant equivalent to fossil-genus *Callimothallus*. *Mycoleptodiscus* with 17 extant species is characterised by more or less circular, flattened sporodochia (aggregation of hyphae bearing conidia) composed of porate, conidiogenous cells (phialides) that produce conidia (Whitton et al. 2012). Extant *Mycoleptodiscus terrestris* (Gerd.) Ostaz. seems rather similar to fossil *Callimothallus* due to similar porate cells and shape of fruiting body. *Mycoleptodiscus terrestris* has a wide distribution and a broad host range as parasite, and is found also on leaves (Hofstra et al. 2009). Other species of *Mycoleptodiscus* found on various substrates (e.g. Seephueak et al. 2011) could occur on living and decaying leaves (Ellis 1976, Bills & Polishook 1992, Whitton et al. 2012). Thus, fossil-genus *Callimothallus* could be considered as anamorphic fungus closely related or identical to extant *Mycoleptodiscus*, presumably associated with living or dead leaves. *Callimothallus* is continuously reported from the Eocene (Kalgutkar & Jansonius 2000) to Holocene (Padmalal et al. 2011) deposits. Besides Miocene Brassington Formation, Great Britain (Pound et al. 2012), Bełchatów Mine is the youngest known occurrence of *Callimothallus pertusus* in Europe.

Occurrence – Up to now 12 fossil-species of this fossil-genus have been described from Late Cretaceous (Maastrichtian) to Pliocene deposits (Kalgutkar & Jansonius 2000). To date this fossil-species have not been reported from the Cenozoic of Poland.

Phragmothyrites W.N. Edwards, Trans. British Mycol. Soc. 8 (1–2): 66 (1922)

Synonym: *Microthallites* Dilcher, Palaeontographica B 116 (1–4): 16 (1965)

Phragmothyrites* cf. *lutosus (Dilcher) R.K. Kar & R.K. Saxena, Palaeobotanist 23 (1): 9 (1974)

Fig 1, c–d

Fruiting body deformed, ± orbicular, preserved fragment ca. 67 µm in diameter, margin finely sinuate. Scutellum composed of both isodiametric (central part) and radiate (margin) nonporate cells (*textura angularis/prismatica*) up to 12.5 µm long and ca. 2.5–4.0 µm wide. Cell walls more or less straight. Marginal cells characteristically perpendicular divided to the edge of fruiting body. Ostiole absent.

Material examined – Slide KRAM-P 218/85(2). One specimen.

Notes – This specimen represents a fragment of probably immature fungal fruiting body of morphology typical of the fossil-genus *Phragmothyrites* with thin-walled, non-porate cells of scutellum and lacking ostiole. As regard the shape and cellular structure of fruiting body the specimen is similar to fossil-species *Phragmothyrites* cf. *lutosus* described from Eocene deposits of Western Tennessee, USA (Dilcher 1965, Kalgutkar & Jansonius 2000). Morphology of *Phragmothyrites* suggests affinity with representatives of the family *Microthyriaceae*. However, non-ostiolate fruiting bodies are very rarely found among extant members of *Microthyriaceae* (Wu et al. 2011). On the other hand, it is very probable that *Phragmothyrites* could represent a young stage (immature fructification) of ascomata of the *Microthyriaceae* without developed ostiole.

Occurrence – 13 fossil-species of this fossil-genus have been described from Early Cretaceous to Miocene deposits (Kalgutkar & Jansonius 2000). To date this fossil-species have not been reported from the Cenozoic of Poland.

***Phragmothyrites* sp. 1**

Fig 1, e–f

Fruiting bodies ± orbicular, 32–55 µm in diameter, margin finely sinuate. Scutellum composed of both isodiametric (central part) and radiate (margin) nonporate cells (*textura angularis/prismatica*), up to 12.5 µm long and 5.0–7.5 µm wide. Cell walls straight, rarely rounded. Ostiole absent.

Material examined – Slide KRAM-P 218/109B (4). Two specimens.

Notes – Considering the structure and small size of described above fruiting bodies they most probably represent immature fungal fructifications (ascomata), the smaller specimen (Fig. 1, f) even could represents so called “germling” of fruiting body.

Occurrence – In the territory of Poland fruiting body of *Phragmothyrites* sp. was reported from Miocene deposits of Legnica (Worobiec 2003a).

***Phragmothyrites* sp. 2**

Fig 1, g–i

Fruiting bodies ± orbicular, 32–55 µm in diameter, margin finely sinuate. Scutellum composed of both isodiametric (central part) and radiate (margin) nonporate cells (*textura angularis/prismatica*), up to 12.5 µm long and 5.0–7.5 µm wide. Cell walls straight, rarely rounded. Ostiole absent, opening visible in the centre of fruiting body of one specimen (Fig. 1, h–i) results most probably from cracking of the central cell during fossilization or sample preparation.

Material examined – Slide KRAM-P 218/85(1), 218/104(2). Two specimens.

Notes – Similarly as in case of *Phragmothyrites* sp. 1, the discussed remains of fruiting bodies represent immature fungal fructifications. Cellular structure of rather elongated marginal cells is almost identical to fossil fructifications described as *Trichothyrites* sp. A. from upper Eocene/lower Oligocene deposits of Hordle Cliff, Great Britain (Smith 1980). It cannot be excluded that *Phragmothyrites* sp. 2 from Bełchatów represents in fact immature ascomata (without developed ostiole) of fossil-species *Trichothyrites* sp. A.

Occurrence – To date discussed morphotype of fossil-genus *Phragmothyrites* have not been reported from the Cenozoic of Poland. The similar cellular structure of marginal cells is observed in *Phragmothyrites eocaenicus* W.N. Edwards reported from Miocene deposits of Ścinawa (Ziemińska & Niklewski 1966).

Trichothyrites Rosend., Bull. Torrey Bot. Club 70 (2): 137 (1943)

Synonyms: *Notothyrites* Cookson, Proc. Linn. Soc. N.S.W. 72: 208 (1947)

Sphaerialites Venkatach. & R.K. Kar, Palaeobotanist 17: 181 (1969)

Trichothyrites* cf. *hordlensis P.H. Smith, Palaeontology 23 (1): 209 (1980)

Fig 1, j

Rather fragmentary remain of fruiting body, ca. 67 µm long (whole fruiting body is estimated to have ca. 80–90 µm in diameter). Scutellum composed of radiating rows of quadrilateral (*textura prismatica*) nonporate cells, up to 10 µm long and 2.5–4.0 µm wide. Cell walls usually straight. Ostiole central, roundish, ca. 15 µm in diameter. Collar distinct, ca. 10 µm wide, collar cells small, isodiametric with very thick and dark walls, ca. 2 µm in diameter.

Material examined – Slide KRAM-P 218/109B(3). One specimen.

Notes – The diameter of ostiole, structure of collar and scutellum cell size and arrangement are rather similar to the fossil-species *Trichothyrites hordlensis* described from upper Eocene/lower Oligocene deposits of Hordle Cliff, Great Britain (Smith 1980). Rather poor state of preservation, however, prevents unequivocal assignment to the discussed species. Similar fruiting bodies with roundish, central ostiole are usually found among members of the family *Microthyriaceae* (Wu et al. 2011).

Occurrence – Up to now 14 fossil-species of this fossil-genus have been described from Cenozoic deposits (Kalgutkar & Jansonius 2000). To date this fossil-species has not been reported from the Cenozoic of Poland. The similar fruiting body was described as *Trichothyrites* sp. from Miocene deposits from Legnica (Worobiec 2003a).

***Trichothyrites* sp.**

Fig 1, k–l

Fruiting body fragmentary, ca. 55 µm in size, probably ± orbicular, margin sinuate. Scutellum composed of radiating rows of quadrilateral (*textura prismatica*) nonporate cells, 3.7–7.5 µm long and 2.5–5.0 µm wide. Cell walls ± straight. Ostiole central, roundish, whole probably ca. 20 µm in diameter. Collar distinct, its cellular structure is almost invisible.

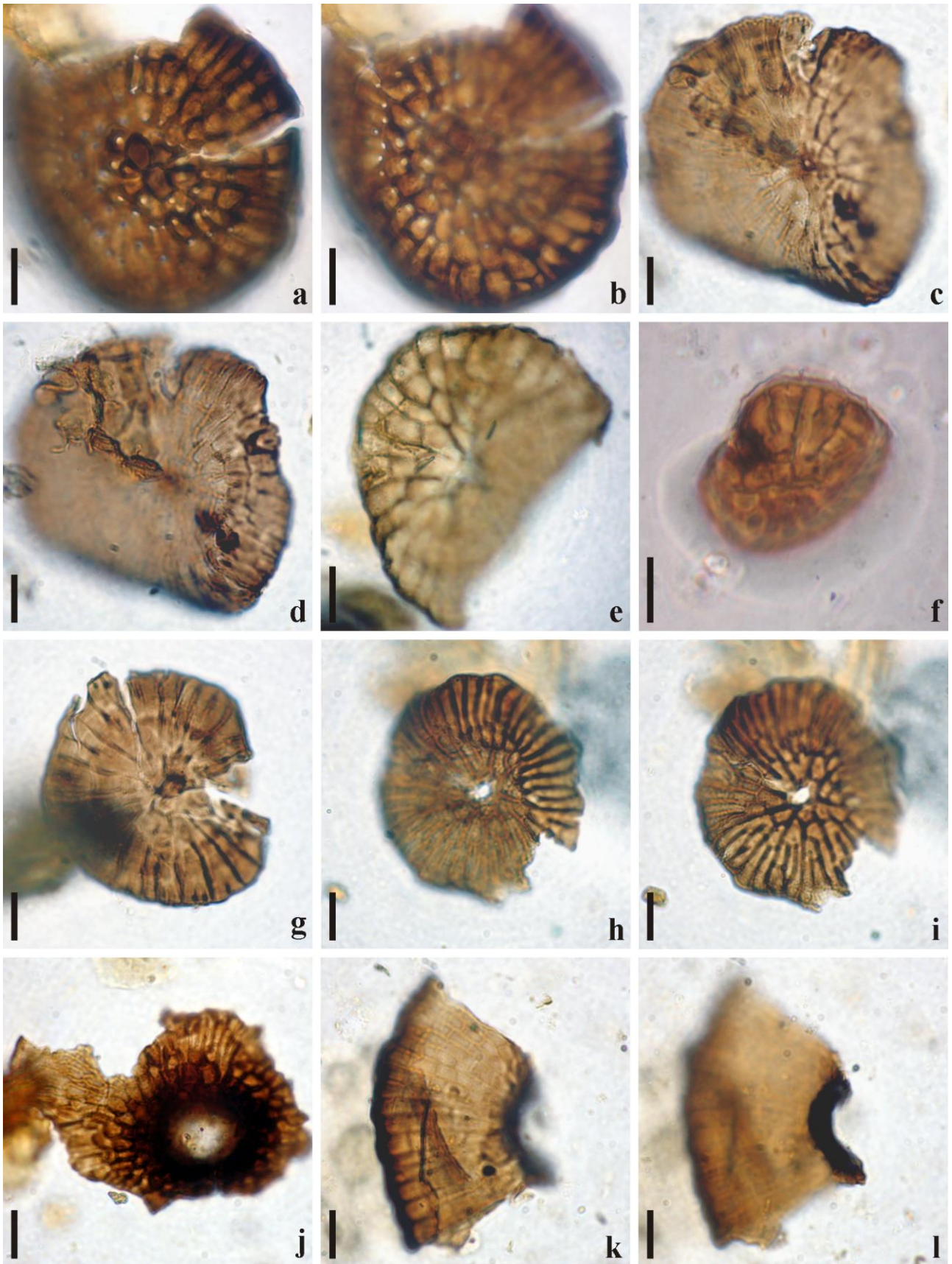


Figure 1 – Epiphyllous fungal fructifications from the Miocene of the Bełchatów Lignite Mine. **a, b** *Callimothallus pertusus* Dilcher. **c, d** *Phragmothyrites* cf. *lutosus* (Dilcher) R.K. Kar & R.K. Saxena. **e, f** *Phragmothyrites* sp. 1. **g–i** *Phragmothyrites* sp. 2. **j** *Trichothyrites* cf. *hordlensis* P.H. Smith. **k, l** *Trichothyrites* sp. Scale bar = 10 μ m.

Material examined – Slide KRAM-P 218/109A(1). One specimen.

Notes – This ostiolate fruiting body is typical for the fossil-genus *Trichothyrites* (Kalgutkar & Jansonius 2000). Poor state of preservation prevents assignment to any so far described fossil-species. Morphologically, most similar are ascomata of the extant genera *Lichenopeltella* Höhn. [syn. *Trichothyrina* (Petr.) Petr.] (Ellis 1977) and partly also *Arnaudiella* Petr. (Wu et al. 2011).

Occurrence – In the territory of Poland *Trichothyrites*-like fungal fructifications were reported from Miocene deposits of Kłodnica (as *Trichothyrium fimbriatum* Speg., Macko 1957), Legnica (Worobiec 2003a), Góraźdże (as Microthyriales, Szulc & Worobiec 2012), and Nowy Sącz (as *Microthyriaceae*, Łańcucka-Środoniowa 1979).

Discussion

Five fossil-taxa of fungal remains morphologically similar to fructifications of the extant members of the *Microthyriaceae* family (*Phragmothyrites* cf. *lutosus*, *Phragmothyrites* sp. 1, *Phragmothyrites* sp. 2, *Trichothyrites* cf. *hordlensis*, and *Trichothyrites* sp.) and one fossil-species (*Callimothallus pertusus*) morphologically similar to extant anamorphic genus *Mycoleptodiscus* were found in the Miocene deposits of the Bełchatów Lignite Mine. *Callimothallus pertusus*, *Phragmothyrites* cf. *lutosus*, *Phragmothyrites* sp. 2, and *Trichothyrites* cf. *hordlensis* are new for the fossil floras of Poland.

Fructifications of epiphyllous fungi have been rarely reported from the tertiary deposits of Poland, usually as a result of palynological investigations (Macko 1957, Szafer 1961, Kita 1963, Ziemińska & Niklewski 1966, Łańcucka-Środoniowa 1966, 1979, Juchniewicz 1970, Jahn et al. 1984, Stuchlik et al. 1990, Worobiec 2003a, b, 2014, Worobiec & Gedl 2010, Szulc & Worobiec 2012). Only Worobiec (2003a) carried out detailed investigations on Neogene epiphyllous fungi and described an association of fungal fructifications (*Microthyriacites* sp., *Phragmothyrites* sp., *Plochmopeltinites* sp., and *Trichothyrites* sp.) from the middle Miocene lignite deposits from Legnica. Worobiec (2003a) also discussed ecology of extant and fossil epiphyllous microthyriaceous fungi. Fossil fungal material from Legnica and Bełchatów (this study) is most diverse (in terms of morphotypes) amongst all fossil epiphyllous fungal associations recorded from Poland.

The presence of the fruiting bodies of epiphyllous fungi is important for reconstructing palaeoclimate of the period of deposition of Neogene deposits of the Bełchatów Lignite Mine. Modern epiphyllous fungi show highest abundance and taxonomic diversity in warm and humid subtropical and tropical regions (Reynolds & Gilbert 2005, Thaug 2006, Hofmann 2010, Hosagoudar et al. 2011, Piepenbring et al. 2011). High annual rainfall and high air moisture are important for their growth (Selkirk 1975, Johnson & Sutton 2000, Limaye et al. 2007). Considering these facts, existence of epiphyllous fungi in the fossil state, especially microthyriaceous taxa is usually correlated with a humid, warm temperate to tropical climate (Dilcher 1965, Lange 1976, Elsik 1978, Kalgutkar & Jansonius 2000, Tripathi 2009, Conran et al. 2016). Epiphyllous fungi, however, do not require for their growth tropical to subtropical thermal conditions (Hofmann 2010) and some extant members of *Microthyriaceae* occur even in polar areas with wet climate (Lind 1928, Dennis 1968, Holm & Holm 1984). Contrary to microthyriaceous fungi, *Callimothallus pertusus* found in Neogene deposits of Bełchatów, seems to be important for paleotemperature inferences. As it was mentioned earlier, this fossil-species is recorded from fossil state from the Eocene (Kalgutkar & Jansonius 2000) to Holocene (Padmalal et al. 2011). However, Quaternary record of *Callimothallus pertusus* is confined to the area of subtropical to tropical, rather warm climate (mainly India, e.g. Padmalal et al. 2011). From Europe it is known from late Eocene of the Czech Republic (Knobloch et al. 1996) through Oligocene of Enspel, Germany (Poschmann et al. 2010) to late Miocene Brassington Formation, Great Britain (Pound et al. 2012). Palaeoclimatic conditions of the discussed period of Cenozoic in Europe range from near tropical (Eocene) to warm temperate (late Miocene) (cf. Mosbrugger et al. 2005). Considering all, the presence of fungal fructifications of *Callimothallus pertusus* in the fossil assemblages is a good indicator of warm climatic conditions in past. It is also confirmed by the results of Ding et al. (2011) and Du et al. (2012) which found

Callimothallus pertusus on fossil leaves of *Smilax tiantaiensis* Su-Ting Ding & Bai-Nian Sun and on needles of *Cunninghamia praelanceolata* Bao-Xia Du & Ban-Nian Sun respectively, both from Miocene deposits of Zhejiang, China. On the basis of fossil plant assemblages associated with *Callimothallus pertusus*, these authors considered warm (subtropical) and humid climatic conditions of this period. To conclude it would seem that on the basis of presence of *Callimothallus pertusus* in the investigated fossil fungal association, we can infer a warm and probably also humid climatic conditions of discussed period of Neogene of the Belchatów Lignite Mine. As *Callimothallus pertusus* in Cenozoic deposits of Europe is known from late Eocene to late Miocene, its presence in the Belchatów Lignite Mine coincides well with the middle/late Miocene age of investigated plant assemblage obtained from analysis of fossil leaves (Worobiec & Szykiewicz 2016) and pollen samples (Worobiec & Worobiec 2016).

The fungal fructifications from the plant assemblage KRAM-P 218 were accompanied by numerous well-preserved plant macroremains (*Acer*, *Dicotylophyllum*, *Fagus*, *Eucommia*, *Laria*, *Laurophyllum*, *Liquidambar*, *Pinus*, *Populus*, *Pterocarya*, *Quercus*, *Salix*, *Salvinia*, *Taxodium*, *Ulmus*, *Vitis*, and *Zelkova*) of riparian vegetation of bottomland hardwood forest. The floristic composition of the paleovegetation points to warm temperate climate with mild winters, comparable to Cfa climate type (warm temperate, fully humid with hot summer) in the Köppen-Geiger climate classification with presumable mean annual temperature of 13.5–16.5°C (Worobiec & Szykiewicz 2016).

The composition of the pollen spectra accompanying plant macroremains and fungal fructifications also shows a dominant role of wetland, predominantly riparian vegetation at the time of sedimentation. The riparian forests were probably composed of *Carya*, *Pterocarya*, *Celtis*, and *Ulmus*, accompanied by *Alnus*, *Acer*, *Fraxinus*, *Juglans*, *Liquidambar*, *Vitis*, *Zelkova*, and *Salix*. *Fagus*, *Quercus*, *Carpinus*, *Eucommia*, *Corylus*, Tilioideae and conifers as well as some thermophilous taxa (such as *Castanea*, *Symplocos*, *Reevesia*, Mastixiaceae, and plants producing pollen of fossil-species *Tricolporopollenites pseudocingulum*) probably grew in mixed forests. *Taxodium*, *Nyssa*, and presumably *Glyptostrobus* and *Alnus* were components of swamp communities that might have overgrown the neighbouring area with a higher groundwater level. Members of the families Ericaceae, Cyrillaceae, Clethraceae as well as *Myrica*, and probably also *Ilex* could be components of both swamp forests and bush swamps. The palynological results also suggest warm temperate and moderately wet climatic conditions (Worobiec & Worobiec 2016).

Thus, the climatic inferences from the analysis of fungal fructifications from the locality KRAM-P 218 from Belchatów Lignite Mine are confirmed by previously obtained data from plant macro- and microremains (Worobiec & Szykiewicz 2016, Worobiec & Worobiec 2016).

To conclude we must emphasize that fossil fungal remains could be rather important for calibration of the divergence time estimations in the phylogenetic trees obtained using molecular clock methods (Beimforde et al. 2014, Hongsanan et al. 2016, 2017, Liu et al. 2016, 2017, Mapook et al. 2016, Phukhamsakda et al. 2016, Samarakoon et al. 2016a, b, Hyde et al. 2017).

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