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# Greenhouses of botanical gardens as a habitat of alien and native macrofungi: a case study in Poland

**Research Article** 

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Abstract: In 2010, 16 macrofungi species were found in greenhouses of the Botanical Garden in Warsaw-Powsin. These included 8 species of lepiotaceous fungi and a few species known from warmer areas. For 3 species identified, which are new to Poland (*Agaricus subrufescens, Leucocoprinus heinemannii, Marasmius teplicensis*), a description is given, with drawings of their microscopic features and photographs. Species composition of macrofungi is compared in 5 complexes of greenhouses (Bayreuth, Graz, Jena, Paskov, Warsaw) in 4 countries from Central Europe. The total number of species in these 5 studies is 206, including 27 (13.2%) lepiotaceous fungi. The smallest number of species identified was in Warsaw (16). More than twice this number was recorded in Jena (33) and Graz (34), while the richest fungal biotas were in Bayreuth (79) and Paskov (88). Of the 16 species found in Warsaw, 8 were also found in other greenhouses. In the group of 33 species recorded in at least two complexes of greenhouses, the most abundant were the lepiotaceous fungi (39.4%) and mycenaceous fungi (15.1%). *Leucocoprinus cepistipes* and *L. straminellus* were recorded most often, in 4 of the 5 studied greenhouse complexes. CCA analysis demonstrated that the highest impact on species diversity is the area of greenhouses.

Keywords: Basidiomycota • Ecology • Greenhouse fungi • Thermophilic species • Poland • Central Europe

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### 1. Introduction

Fungal species, both generally, and in respect to colonization of new areas and habitats outside the natural range of distribution, are much less studied than plants or animals [*e.g.* 1-3]. Greenhouses in botanical gardens, as well as commercial greenhouses, are favourable habitats for non-native fungal species coming from warmer regions [4-6].

The first records of macrofungi in greenhouses, mostly from West Europe, date back to the 19<sup>th</sup> century [7]. Greenhouses are relatively rarely sites of long-term research, although their role, *e.g.* as a source of expansion of alien fungi, may be important [5]. In recent years in Central Europe, systematic research on macrofungi in greenhouses was conducted in Austria [8,9], Czech Republic [10], and Germany [11-13]. In European greenhouses, new fungal species have been discovered recently, including *Conocybe karinae* [14], *Lepiota pseudorubella*, *L. rubrobrunnea* [15], *Leucoagaricus caldariorum* [16], *L. clavipes* [11], *Marasmius skalae* [17], and *M. teplicensis* [18], as well as species new to the Old World, e.g. *Melanotus flavolivens* [8,9], *Hydropus fluvialis*, and *Mycena neospeirea* [12]. Some tropical species have been first introduced into greenhouses, some of them later spreading in the wild, e.g. *Leucocoprinus birnbaumii* or *Gymnopus luxurians* [8,9,19].

Although the first data about macrofungi found in greenhouses in the area of Poland come from the late

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19<sup>th</sup> century [20-23], mycobiota of greenhouse fungi have not been studied systematically and the available information about them is fragmentary. In greenhouses of Polish botanical gardens, 28 fungal taxa were recorded before this study, both native and introduced from warmer regions (Table 1). A few published reports, about single species, come from commercial greenhouses [24] and from abandoned greenhouses [25].

The aims of this study were: (i) to assess the species composition of macrofungi in greenhouses of the Polish Academy of Sciences Botanical Garden – Centre for Biological Diversity Conservation in Warsaw-Powsin;

(ii) to evaluate the role of greenhouses as habitats for alien and native species; (iii) to compare the species composition of macrofungi in this study with results of similar studies in Central Europe, and thus undertake a preliminary meta-analysis of fungal life in greenhouses in our climatic zone.

# 2. Experimental Procedures

### 2.1 Description of the investigated greenhouses

The collection of greenhouse plants in the Botanical Garden – Centre for Biological Diversity Conservation

No.	Species	References
1	Antrodia vaillantii (DC.) Ryvarden	[112]
2	Auricularia mesenterica (Dicks.) Pers.	[113, 114]
3	Bjerkandera adusta (Willd.) P. Karst.	[114]
4	Chlorophyllum rachodes (Vittad.) Vellinga (as Macrolepiota rachodes (Vittad.) Singer)	[51, 115]
5	Coprinus disseminatus (Pers.: Fr.) Quél	[112]
6	Cystolepiota fumosifolia (Murril) Vellinga	[24]
7	Cyathus stercoreus (Schwein.) de Toni	[112]
8	*Derminus vittaeformis Fr. (as Agaricus vittaeformis Fr. = Ag. campanulatus Schaeff)	[21, 23]
9	Dentipellis fragilis (Pers.) Donk	[51]
10 11	Entoloma apiculatum (Fr.) Noordel. (as Agaricus apiculatus Fr.) Hemimycena cucullata (Pers.) Singer	[21] [51]
12 13 14	Hericium coralloides (Scop.) Pers. (as Hydnum coralloides Scop.) Hydnangium carneum Wallr. Hymenogaster albus Berk.	[51, 116] [22] [23]
15	Hyphodontia quercina (Pers.) J. Erikss. (as Hydnum fallax Fr.)	[21, 23, 51]
16	Inocybe cincinnata (Fr.) Quél. 1872 (as Agaricus cincinnatus Fr.)	[21]
17	Laetiporus sulphureus (Bull.) Murrill	[114]
18	Leucocoprinus birnbaumii (Corda) Singer	[48, 51, 114, 115]
19	L. cepistipes (Sowerby) Pat. (as Agaricus cepaestipes Sow. = Lepiota cepaestipes Sow.)	[20, 21, 23, 48]
20	L. straminellus (Bagl.) Narducci & Caroti (as Agaricus denudatus Rabh. = Lepiota denudata Rabnh. = Lepiota cepaestipes Sowerby = Agaricus luteus Withering)	[20-23, 48]
21	Lentinus sp.	[112]
22	Lepiota clypeolaria (Bull.) P. Kumm.	[21]
23	Lepiota rubella Bres.	[24]
24	Melanophyllum haematospermum (Bull.) Kreisel	[51]
25	Mycena osmundicola J.E. Lange.	[48, 114, 115, 117-119]
26	Postia floriformis (Quél.) Jülich (as Oligoporus floriformis (Quél.) Gilb. & Ryvarden)	[51, 115]
27	Psilocybe inquilina (Fr.) Bres. (as Naucoria inquilina Fr.)	[22, 51]
28	Volvariella volvacea (Bull.) Singer (as Volvaria volvacea Bull.)	[22, 51, 120]

 Table 1. Macrofungi found in greenhouses of botanical gardens in Poland up to the year 2011.

\* currently this name is not used, no information about synonyms was found in current literature; Chelchowski [21] gives 2 synonyms: Agaricus vittaeformis Fr. and Ag. campanulatus Schaeff. However, they also do not enable identification of the species.

in Warsaw-Powsin has existed since 1982, however, the first greenhouses were opened to the public in 1990. Initially, the greenhouse collection was composed of about 500 taxa of vascular plants, but by 1994 the number had reached 1500, and by 2005, there was about 1800 taxa from 400 genera [26,27]. The whole complex of greenhouses, open to visitors, has an area of about 1900 m<sup>2</sup>. It is composed of 5 greenhouses connected with one another (Table 2). The tropical and subtropical parts include small ponds covering about 10 m<sup>2</sup> and 5 m<sup>2</sup>, respectively. Plants grow in the ground or in pots of various size (either ceramic or plastic). In the subtropical greenhouses (S1 and S2), a large part of the collection grows about 1.3 m below the floor level, in pits and silos with strengthened walls. In each greenhouse, climatic conditions are different, and plants specific to the given climate are grown there [26-31]. The weed flora of the studied greenhouses was earlier described [32].

#### 2.2 Material and methods

Investigations were conducted from January to December 2010. Usually twice a month, every 2 weeks or so, greenhouses were inspected in search of macrofungal sporocarps. The greenhouses were visited 24 times in total. September was the only month where no observations were made and no specimens were collected. The study area included all the greenhouses open to visitors. The records from each visit describe in which greenhouse and near which plant species the sporocarps were found. Plant species were named according to their labels (placed on or near the plants) or according to information from the staff of the greenhouses. Names of fungi follow those used by Robert *et al.* [33]. The material was studied according to standard methods used in the taxonomy of macrofungi. Microscopic structures were examined in dried material, mounted in Congo Red, Melzer's reagent or 10% ammonia, and in fresh material mounted in water or Melzer's reagent, using Bresser Bino Researcher or Nikon Eclipse E400 microscope. Drawings of microcharacters were made from micrographs taken with a Nikon Coolpix 950 Digital Camera. All measurements were made directly through the microscope under an oil immersion objective. The spore dimensions were established from measurements of 30 randomly selected, well-formed spores (exceptionally big or small as well as deformed or atrophied ones were excluded from the analysis). For basidia, cystidia, and pileipellis, the extreme size values are presented. For basidia and pileipellis hyphae, dimensions are based on measurements of 20 elements. The material collected is deposited in the private herbarium of Błażej Gierczyk (BGF) and in the fungarium of the Division of Mycology and Forest Phytopathology, Warsaw University of Life Sciences - SGGW (WAML).

Species composition of macrofungi in the studied greenhouses (labelled as PL) were compared with results of similar studies conducted in greenhouses of botanical gardens in Graz in Austria [8] (labelled as A), in Jena in Germany [12] (labelled as G1), in Bayreuth in Germany [11,13] (labelled as G2), and in a commercial greenhouse where cucumbers were grown, in Paskov near Ostrava [10] (labelled as CZ).

Spearman rank correlation coefficients were calculated to analyse the strength of the relationship between the analysed variables of greenhouses in botanical gardens (*e.g.* area, time of plant cultivation before the mycological study, and study period) and the number of taxa and species, number and percentage

Greenhouse	Year of erection	Area (m²)	Temperature in winter/summer (°C)	Humidity in winter/summer (%)	Major collections (genera)
Subtropical 1 (S1)	1992	320	10-12/ -ª	_b / _b	Citrus, Musa, Punica, Coffea, Camellia, Fuchsia, Bougainvillea, Brunfelsia, Olea, Piper
Subtropical 2 (S2)	1992	280	15/ -ª	_b / _b	Dicksonia, Cyathea, Cibotium, Fuchsia, Plumeria, Begonia, Agathis, Platycerium
Tropical (T)	2003	625	18-20/ -ª	- <sup>b</sup> / 70	Dicksonia, Cyathea, Cibotium, Cycadopsida, Ficus, Crinum, Hibiscus, Begonia, Cycas, Passiflora, Anthurium, Pellea
Succulents (S), including cacti	2003	370	12-14/ -ª	- <sup>b</sup> / 40-50	Aloë, Agave, Gasteria, Plumeria, Euphorbia, Crassula, Sansevieria, Cissus, Haworthia
Corridor (K)	2003	320	10/ -ª	_b / _b	Datura, Hydrangea, Nerium, Passiflora, Olea, Cissus, Jasminum, Hibiscus, Camellia, Bambusa

Table 2. Description of the investigated greenhouses of the Botanical Garden in Warsaw-Powsin, open to visitors.

-<sup>a</sup> no heating, indoor temperature depending on the outdoor conditions, during hot weather the system of shading blinds is used -<sup>b</sup> no fogging

contribution of lepiotaceous fungi, as well as between species number and the number and percentage contribution of lepiotaceous fungi. Correlations were considered significant if p < 0.05. The calculations were made with Statistica 10 software.

To determine which of the analysed parameters of greenhouses in botanical gardens (*e.g.* area, year of erection, time of plant cultivation before the mycological study, and date of observations) affected most strongly the similarity of species composition of the mycobiota of the analysed sites, canonical correspondence analysis (CCA) was used. It was based on the group of species that were recorded in at least two of the investigated greenhouses. The CCA diagram shows similarity of species composition of the greenhouses, reflected in distance between their labels, and importance of individual environmental factors, reflected in length of their vectors. The calculations were made in R software [34] using the vegan library [35].

### 3. Results

In greenhouses of the Botanical Garden in Warsaw-Powsin, 16 species of macrofungi were recorded. The largest variety of species (8) was found in the greenhouse with the collection of succulents (S), *i.e.* plants of arid climate. In the tropical greenhouse (T), 5 species were present, while in the subtropical ones (S2 and S1), 4 and 3 species, were found respectively.

In the part known as corridor (K), 3 species were also found. Two species dominated: *Cystolepiota fumosifolia* and *Agaricus subrufescens*. They were recorded at the largest number of sites and their basidiomes were the most abundant. Three species (*Agaricus subrufescens*, *Leucocoprinus heinemannii*, and *Marasmius teplicensis*) had never been recorded in Poland before. Four species (*Leucocoprinus birnbaumii*, *L. cepistipes*, *L. straminellus*, and *Chlorophyllum rachodes*) were previously reported in greenhouses from Polish botanical gardens (Table 1). The other 12 species were recorded for the first time in Polish greenhouses.

### 3.1 List of species of macrofungi recorded in greenhouses of the Botanical Garden in Warsaw-Powsin in 2010

Agaricus subrufescens Peck. (Figure 1, Figure 2): In S on the ground, under Acokanthera oblongifolia, Agava americana, Azurocereus hertlinga, Cereus jamacaru, Cylindropuntia rosea, Espostoa lanata, Haemanthus albiflos, Hippeastrum striatum, Laurus nobilis, Opuntia ficus-indica, Plumeria rubra, Tipuana tipu; March, April-August, October, November; in groups and singly.

Description and iconography [36-45].

Specimens examined. Pileus 11 cm in diameter, flattened, its centre glabrous, reddish-brown, at margins covered with distinct fibrillose squamules and flattened scales of the same colour, contrasting with whitish or pale brownish background. Stipe cylindrical with a clavate base,  $10 \times 1.5$  cm, pale cream-ochre, above



Figure 1. Agaricus subrufescens Peck. (phot. Andrzej Szczepkowski)



Figure 2. Agaricus subrufescens Peck. microcharacters: A – cheilocystidia; B – basidia; C – stipitipellis with veil elements; D – spores (scale bars – 10 μm)

annulus smooth, below minutely pruinose with a veil. Annulus superior, membranous, with small squamules underside, disappearing with age. Lamellae crowded, free, initially pale, whitish or pinkish, later dark brown. Flesh whitish, slightly yellowing in stipe base. Smell like bitter almonds, slightly sour in old specimens. Schaeffer reaction positive, yellow to orange; KOH reaction positive, yellow.

Spores broadly ellipsoid, 5.0-6.5 × 4.0-5.0 µm, without germ-pores. Basidia 4-spored, up to 25 × 9 µm. Cheilocystidia catenate, composed of globose, ellipsoid, ovoid to subcylindrical elements, up to 20 µm in diameter. Pleurocystidia absent. Pileipellis a cutis, composed of cylindrical hyphae (*ca.* 7 µm in diameter), intermixed with inflated elements up to 45 × 15 µm. Terminal elements somewhat ascending, clavate 25-38 × 20-25 µm. Veil composed of loosely arranged, ellipsoid to globular elements, 10-20 × 10-20 µm.

Species growing on rotting debris and wood-chips of deciduous trees in woods, parks and gardens, frequent in domesticated and human-disturbed habitats. It was discovered in the USA [46], where it is common [39,47]. According to Kerrigan [39], this species is identical to a very popular cultivated and medical mushroom *Agaricusblazei*Murrill(*sensu*Heinemann)=*A.brasiliensis* (an invalid name).

Agaricus xanthoderma Genev.: In S on the ground, under Aloe brevii, Dracena draco, Kalanchoe teretifolia; October, November; singly and in small groups; in T also on the ground, under *Ipomoea batatas*, Jatropha integerrima; April, May; in groups and singly. This species probably comes from warmer regions of Europe [48,49]. In Poland it was first reported in 1965 [48]. In the 1990s it was classified as rare (category R) in the Polish red list of fungi [50]. At present it is quite common, recorded in forests, thickets, parks, botanic gardens, meadows, at margins of small postglacial ponds, also on rehabilitated coal mine dumps [51,52].

Asterostroma cervicolor (Berk. & M.A. Curtis) Massee: In T on coir (*i.e.* coconut fibre) insulating a water pipe; December; singly.

Native but rather rare; in forests, on trunks of *Pinus sylvestris*, on wet coniferous and deciduous wood in wooden bridges, also in houses, in rooms and balconies, on wooden flower-pots, and masonry [51,53,54]. This report is the fourth finding of this species in Poland, classified as endangered (category E) [55]. It is a white-rot fungus; basidiome resupinate, smooth, whitish to ochre or cinnamon.

Chlorophyllum brunneum (Farl. & Burt) Vellinga [= Macrolepiota rachodes var. bohemica (Wichanský) Bellu & Lanzoni]: In S2 on the ground, under Cayratia wrayi, Cyathea cooperi, Dracena marginata; April; singly.

This species is known from Europe and North America. Our finding is its fifth documented record in Poland; earlier the species was reported from an urban park, forest patch among fields, a roadside, and a mixed forest [24,51].

Chlorophyllum rachodes (Vittad.) Vellinga [= Macrolepiota rachodes var. rachodes (Vittad.) Singer]: In S2 on a wheelbarrow with plant debris; May; singly. This species is native and very common in Poland [51,52].

*Coprinellus truncorum* (Scop.) Redhead, Vilgalys & Moncalvo [= *Coprinus truncorum* (Scop.) Fr.]: In K on/in a wooden pot with *Phoenix dactylifera*; March, April; in a group.

This species is native and not rare; it grows in forests, parks and cemeteries, usually on or near stumps or logs of decayed deciduous trees [51].

*Cystolepiota fumosifolia* (Murrill) Vellinga: In T on the ground, under *Acalypha wilkesiana, Acokanthera oblongifolia, Asplenium nidus, Cyathea smithii, Dioscorea macroura, Dieffenbachia* sp., *Ficus nitida, Fittonia verschaffeltii, Grevillea robusta, Hibiscus* sp., *Hoffmannia* sp., *Strelitzia regina, Zamia furfuracea;* March–May, August, October, November; usually in groups, sometimes of more than 20 basidiomes; in S on the ground, under *Aloe claviflora, A. verna, Haworthia glauca, Sansevieria parva;* April, May, August; in groups of up to 17 specimens; in S1 on the ground, under *Bougainvillea gabra;* November; in a group; in S2 on the ground, under *Impatiens niamniamensis* and *Ruscus hypophyllum;* April; in a group of 11 specimens.

Rare species, known from scattered locations in Europe (Denmark, Germany, Netherlands), North America, and the Indian subcontinent, where it was found in a deciduous forest on nitrogen-rich soil; known also from greenhouses [12,56-61]. First recorded in Poland in 2009 [24]. In the greenhouse investigated in this study it was first recorded in 2010, and reported in the cited study. Of its 4 known sites in Poland, one is located in the greenhouse, while 3 are in forests [24].

Henningsomyces candidus (Pers.) Kuntze: In T on a dead standing trunk of *Washingtonia filiera;* August; several groups.

A widespread species, known from Europe, North Africa, Asia, Australia, North America and South America [62]. It occurs in forests, on dead, often very rotten and moist wood of deciduous (*e.g. Betula* sp., *Salix* sp.) and coniferous trees [51,63-65], and also in greenhouses [62]. In Poland it is very rare. The present record is the fourth finding of this species in Poland.

*Lepiota lilacea* Bres.: In S1 on the ground, in a plastic pot with *Nerium oleander*; May; singly.

Species known from Europe, Asia, South America, North America, and Africa, occurring in tropical and temperate zones [59,66-70]. Not common but widespread, found in many countries, on rich soil in woods and parks, also in flower pots in houses and apartments [71] The present record is the seventh finding of this species in Poland [24,51,72]. For some time it was regarded as extinct in Poland [50,55,73]. Leucocoprinus birnbaumii (Corda) Singer: In K on the ground, in a plastic pot with Nerium oleander, and in 2 plastic pots with Wollemia nobilis; May, July, August; in groups; in S on the ground, under Kalanchoe teretifolia; July; singly.

It is a tropical species (Africa, South and Central America), which does not occur in the temperate zone. In Poland the first record of this species dates from 1960. It is known from a few localities: a greenhouse, a supermarket, a private apartment, and rooms of a university [48,51].

*Leucocoprinus cepistipes* (Sow.) Pat.: In S2 on the ground in a plastic pot with *Euonymus japonicus*; July; singly.

Cosmopolitan fungus, known from Africa, Australia, Asia, Europe and the Americas. A species of tropical origin, but the range of its natural distribution is not known. Found both indoors (greenhouse) and outdoors on rich soils, compost heaps, and wood chips [74-76]. In Poland known from 5 sites in greenhouses, one in a private apartment [48], and one outdoors in Gdańsk [24].

*Leucocoprinus heinemannii* Migl. (Figure 3, Figure 4): In S on the ground, under *Dichondra micrantha* and *Euphorbia milii;* July, August, October, November; in a group.

Description and iconography [12,71,75-80].

Specimens examined. Basidiome fragile, pileus up to 20 mm in diameter, first campanulate, plano-concave when old, sulcate in outer half, covered with blackish fibrils on white, whitish or yellowish background, at centre almost black. Stipe 15-50 × 1.5-2.5 mm, with a bulbous base, white to pale pinkish, covered with white fibrils. Annulus white, sometimes with brownishgray tint at margin, submembranous, persistent. Lamellae free, rather crowded, white to cream-coloured. Flesh white with fungal odour. Spores subellipsoid to oblong-amygdaloid, 6.0-8.0 × 3.0-4.0 µm, hyaline, smooth, without germ pores. Basidia 4-spored, 15-19 × 6.0-8.0 µm, surrounded with pseudoparaphyses. Cheilocystidia very variable. narrowly clavate. cylindrical to utriform, a few somewhat capitate, 20-30 × 8.0-10.0 µm. Lamella edge sterile. Pileus covering made from irregular short and long hyphae, up to 10 µm wide, pigment vacuolar, brown. Clamps absent in all basidiocarp parts.

Saprotrophic on soil and litter, in greenhouses and indoors. Known from scattered localities in Europe (Netherlands, France, Italy, Germany and Hungary) and western North America. Species of tropical origin [76,81].

Leucocoprinus straminellus (Bagl.) Narducci & Caroti: In S on the ground, under *Ficus elastica*; singly; in S1 on the ground, among bark chips under *Camellia japonica*; July, August; in groups.



Figure 3. Leucocoprinus heinemannii Migl. (phot. Andrzej Szczepkowski)



Figure 4. Leucocoprinus heinemannii Migl. microcharacters: A - spores; B - basidia; C - cheilocystidia; D - pileus covering (scale bars - 10 µm).

A widespread species but in Europe known only from gardens, parks, greenhouses, and flower pots [7,66,74,77,78]. In Poland it has been recorded several times [20-23,48].

*Limacella* cf. *glioderma* (Fr.) Maire.: In S on the ground, under *Encephalartos middelburgensis*; November; 2 basidiomes.

The spores of the studied specimens of *Limacella* sp. are 4.5-5.5 × 3.5-5.0  $\mu$ m. Thus they are closer to the values reported for *L. illinita* (5-6 × 4-5  $\mu$ m) than those for *L. glioderma* (3.5-5 × 3-5.0  $\mu$ m), however, the basidiomes are more like those from *L. glioderma*, *i.e.* they have a darker colour and relatively short stem with reddish patches and scales without an annulus. The veil is poorly preserved on the collected basidiomes, but it is more fibrillose (like in *L. glioderma*) than formed of slime. Therefore, the specimens from Warsaw-Powsin have been determined as *L. cf. glioderma*.

*Limacella glioderma* is widely distributed in Asia, Europe, and North America. It grows solitary to gregarious in hardwood and coniferous forests on leaf or needle litter, preferentially on calcareous soil [82,83]. It has also been reported from greenhouses in Germany [8,9] and the United States [84]. In Poland it is known from several localities, mostly from forest communities, e.g. Ficario-Ulmetum campestris, Alnetum incanae, Potentillo albae-Quercetum, Pinetum mugo carpaticum, Polysticho-Piceetum, young forest with Sorbus aucuparia [51,85-88], primarily from protected areas (nature reserve "Wielka Kępa Ostromecka", Roztocze National Park, Pieniny National Park, Tatra National Park, Chęciny-Kielce Landscape Park). According to Łuszczyński [89], it is a montane and boreal-montane species in Poland.

*Mycenella lasiosperma* (Bres.) Singer: In K on the ground, in a plastic pot with *z Nerium oleander;* November; singly.

This species is native but rare in Europe [90]. In Poland it is only known from a few localities in riparian and hornbeam forests at natural sites: on a rotten mossy stump, on the ground, among litter and mosses, and also in a room in a building, on pieces of wood in a flower pot [51,52,91,92].

*Marasmius teplicensis* Antonín & Skála, (Figure 5, Figure 6): In S on roots, pieces of wood and bark, and on shoots partly buried in the soil, *e.g. Lampranthus* sp., as well as in a wound on the root crown of *Euphorbia tirucalli*; August; in groups.



Figure 5. Marasmius teplicensis Antonín & Skála (phot. Andrzej Szczepkowski)



Figure 6. Marasmius teplicensis Antonín & Skála microcharacters: A – spores; B – basidia; C – elements of pileipellis; D - cheilocystidia; E – pleurocystidia (scale bars – 10 μm)

#### Description and iconography [18,93,94].

Specimens examined. Pileus 11-21 mm, broad, hemispherical, slightly depressed at the centre, deeply radially striated, brown or reddish brown. Lamellae distant, L = 16-19, I = 0-1, free to nearly free, creamcoloured, up to 2 mm high, with darker, brown edges. Stipe 18-44 × 1.0-1.5 mm cream-coloured at apex, below dark brown to blackish-brown, smooth, hollow, white tomentose at base, and white rhizomorphs a few cm long. Context whitish, thin, without odour. Spores 17.0-21.0 × 3.5-5.0 µm, E = 3.9-5.3, Q = 4.6, narrowly clavate to elongate-lacrymoid. Basidia 25.0-33.0 × 6.5-9.0 µm, clavate, 4-spored, clamped. Cheilocystidia as broom cells of the Siccus-type, 10.0-20.0 × 6.0-9.0 µm, more or less clavate, with obtuse, slightly nodulose yellowbrown projections, 2.0-8.0 × 1.0-2.0 µm. Pleurocystidia 30.0-75.0 × 5.5-10.0 µm, narrowly cylindrical to fusiform, hyaline, clamped. Pileipellis hymeniform, made up of broom cells of the Siccus-type, 15.0-22.0 × 5.0-11.0 µm, clavate, yellow-brown, with nodulose projections, 2.0-12.0 µm long. Clamps abundant in all tissues.

Saprotrophic (probably) on roots of *Phoenix dactylifera* and on bark mixed with peat-loam. This species was first described by Antonín and Skála in a greenhouse in the botanical garden in Teplice (Czech Republic) [18]. Only known from the type-locality and

from a similar biotope in Liberec (north Bohemia, Czech Republic). Probably of tropical origin [94].

*Trechispora farinacea* (Pers.) Liberta: In T on a dead part of the trunk of *Cyathea australis*, and on a stump of a woody plant; November; singly.

This species is native and very common in all European countries. It is also known from Asia, Australia, North and South America [95,96]. In Poland it is found in forests, gardens, botanical gardens, on stumps, fallen dead trunks and branches of both deciduous and coniferous trees, and also on boards and on old basidiome of *Daedalea quercina* [51]. Basidiomes resupinate, with occasionally smooth, but usually farinaceous to grandinioid or nearly hydnoid hymenophore.

### 3.2 Comparison of species composition of macrofungi in greenhouses of Central Europe

The number of recorded species of macrofungi previously reported in greenhouses of Central Europe varied from 16 (PL) to 88 (CZ). The total number of species reported is 206 (195 of *Basidiomycota* and 11 of *Ascomycota*). One of the most highly represented groups were lepiotaceous fungi, whose contribution to the total number of species ranged from 11.4% (CZ)

Country (label), city	No. of taxa/ species of macrofungi	No. of species (and % contribution) of lepiotaceous fungi	Area (m²)	Study period (years)	Type of greenhouse	Year of erection (or reconstruction)	
Austria (A),	37/34	9	1570	3	Botanical	1989-1995	
Graz		(26.5)		(1998-2001)	garden		
Czech Republic (CZ),	05/88	10 110000		4	Cucumber	1086	
Paskov	33/00	(11.4)	110000	(1988-1991)	production	1300	
Germany (G1),	05/00	4	EZE	2	Botanical	1000 1000	
Jena	30/33	(12.1)	575	(2003-2004)	garden	1900-1903	
Germany (G2),	0.4/70	16	4500	7	Botanical	1978	
Bayreuth	64/79	(20.2)	4500	(2004-2010)	garden		
Poland (PL),	10/16	8	1000	1	Botanical	1000 0000	
Warsaw-Powsin	19/10	(50.0)	1900	(2010)	garden	1992-2003	

Table 3. Characteristics of the compared greenhouses in Central Europe.

to 50.0% (PL) (Table 3). In total, in 5 greenhouse complexes in 4 Central European countries, 27 species from this group of fungi were recorded. This number constituted 13.2% of all species of macrofungi found in the studied greenhouses.

The number of shared fungal species of any two of analyzed greenhouses ranged from 1 to 11. Species composition of the mycobiota of the Botanical Garden in Warsaw-Powsin included 4 species common to greenhouses in Austria and the Czech Republic, and 3 species common to both greenhouses in Germany (G1 and G2) (Table 4).

The total number of species recorded in at least 2 complexes of greenhouses was 33. These included 13 species (39.4%) of lepiotaceous fungi of 6 genera (Chlorophyllum, Cystolepiota, Lepiota, Leucoagaricus, Leucocoprinus, Melanophyllum) and 5 species (15.1%) of mycenaceous fungi, representing 2 genera (Mycena, Mycenella). None of the species occurred in all 5 of the analysed greenhouse complexes (Table 4). If greenhouses within botanical gardens alone are taken into account (4 localities, excluding the commercial greenhouse in Czech Republic), then 26 species were reported from at least 2 localities. These included 10 species (38.5%) of 5 genera (Chlorophyllum, Cystolepiota, Lepiota, Leucocoprinus, Melanophyllum), representing lepiotaceous fungi (Table 4). A comparison of species composition of fungi in greenhouses investigated in this study with results of the cited studies shows that 50% (8 species in total), mostly lepiotaceous fungi, were also found in greenhouses in Germany (6 species, i.e. 3 in G1 and 3 in G2), Austria (4 species), and Czech Republic (4 species). Two species of this group (Leucocoprinus cepistipes and L. straminellus) were recorded most often, i.e. in 4 of the 5 studied greenhouse complexes in 4 countries (Austria, Czech Republic, Germany,

and Poland; Table 4). The remaining 8 species found in greenhouses of the Botanical Garden in Warsaw-Powsin were not present in any of the other greenhouse complexes.

Spearman rank correlation coefficients indicate that study period significantly (R = 1.00, p = 0.000) affected the number of taxa and species. For the other variables analysed, no significant correlation was observed.

Canonical correspondence analysis (CCA) showed ordination of the compared biota along 3 axes. Eigenvalues of the axes were as follows: 0.4157, 0.3867, and 0.0824, and they explained 0.4698, 0.4371, and 0.0931 of the total variation, respectively. Since the eigenvalue of the third axis was markedly lower, the results are presented in the system of 2 axes. Axis 1 was most strongly correlated with the year of erection (or reconstruction) of greenhouses, axis 2 with greenhouse area, and axis 3 with time of plant cultivation before the mycological study (Table 5, Figure 7).

Taking into account the correlations of the analysed factors with axes, and the percentage of variation explained by individual axes, it can be concluded that similarity of species composition was most strongly affected by greenhouse size (area), less strongly by year of erection (erection), and only slightly by time of plant cultivation before this study (cultivation) (Figure 7). Greenhouse area seems to be positively correlated with the presence of species like Chlorophyllum brunneum, Leucocoprinus birnbaumii, and Limacella glioderma. The year of erection (or reconstruction) of greenhouses seems to be positively correlated with the presence of species like Conocybe crispella, Gymnopus luxurians, Lepiota cristata, L. elaiophylla, Mycena leptocephala, M. sanguinolenta, Psathyrella candolleana, and Schizophyllum commune (Figure 7).

		Country				
No.	Species	А	CZ	G1	G2	PL
1	Agaricus bisporus		+		+	
2	Chlorophyllum brunneum*		+		+	+
3	Clitopilus passeckerianus		+		+	
4	Conocybe crispella	+			+	
5	Cystolepiota fumosifolia*			+		+
6	Galerina nana			+	+	
7	Gymnopus luxurians	+			+	
8	Hypholoma fasciculare	+	+	+		
9	Inocybe pusio			+	+	
10	Lepiota cristata*	+			+	
11	L. elaiophylla*	+			+	
12	L. rubella*			+	+	
13	Leucoagaricus leucothites*	+	+			
14	L. bresadolae*		+		+	
15	Leucocoprinus birnbaumii*	+	+			+
16	L. cepistipes*	+	+		+	+
17	L. cretatus*		+		+	
18	L. heinemannii*			+		+
19	L. straminellus*	+	+		+	+
20	Limacella glioderma	+				+
21	Melanophyllum haematospermum*	+		+	+	
22	Mycena alphitophtora			+	+	
23	M. leptocephala	+	+		+	
24	M. neospeirea			+	+	
25	M. sanguinulenta	+			+	
26	Mycenella trachyspora			+	+	
27	Pholiota gummosa		+		+	
28	Pluteus plautus		+		+	
29	P. podospileus			+	+	
30	Psathyrella candolleana	+			+	
31	Schizophyllum commune	+	+		+	
32	Trechispora farinacea			+		+
33	Xylaria arbuscula			+	+	

Table 4. List of species of macrofungi found in at least 2 of the 5 complexes of greenhouses compared in 4 countries of Central Europe.

\* lepiotaceous fungi

# 4. Discussion

Thus far, over the last 100 years, only 30 taxa of macrofungi have been reported from greenhouses of botanical gardens and commercial greenhouses in Poland. This paper presents results of the first Polish systematic observations (for 1 year) of macrofungi in greenhouses of the Botanical Garden in Warsaw-Powsin. A small number of fungal species were identified (16), nonetheless this increases the total number of taxa recorded in greenhouses in Poland to about 40. Additionally, some specimens (3 collections) from 3 genera (*Agaricus, Conocybe, Entoloma*) were not able to be identified to the species level, because of the limited

Variable	CCA1	CCA2	CCA3
Area	0.0318	0.9839	-0.1700
Year of erection	0.2616	-0.3015	-0.9188
Cultivation time	0.0610	0.5147	0.8586

 Table 5.
 Intercept correlation between environmental factors and canonical axes.

number of samples and poor condition of basidiomes. In total 19 taxa were recorded in greenhouses of the Botanical Garden in Warsaw-Powsin. All of the identified species belong to the phylum *Basidiomycota* and are



Figure 7. Canonical correspondence analysis (CCA) diagram for fungal species present in at least 2 complexes of greenhouses in the 4 compared botanical gardens: in Warsaw (PL), Jena (G1), Bayreuth (G2), and Graz (A), illustrating the relationship between species diversity and parameters of greenhouses: size (area), year of erection (*erection*), and time of plant cultivation before this study (*cultivation*).

saprotrophic fungi [97]. Nearly 1/3 (5 species) of the recorded taxa (*Leucocoprinus birnbaumii*, *L. cepistipes*, *L. straminellus*, *L. heinemannii*, and *Marasmius teplicensis*) are known from warmer climatic zones. The first three of them are considered as alien to Europe [5]. The next two, and especially the last taxon listed above (*M. teplicensis*), (also discovered recently in the Czech Republic), are probably alien to Europe, also [71,75,94].

Alien species, especially invasive ones, have becoming a growing problem on a global scale, primarily in relation to nature conservation but also in agriculture, forestry, and fish production [*e.g.* 98-103]. One of the sources of spread of alien species, including fungi, can be greenhouses [4,5], especially if the world climate is getting warmer. Nutrient-rich soils used in greenhouses, and the high temperatures necessary for plant cultivation, are favourable for many saprotrophic macrofungi. Moreover, in greenhouses of botanical gardens, diverse woody plants (trees and shrubs) create favourable conditions for development of some species of symbiotic (mycorrhizal) fungi. Fungal diaspores (propagules), including those of species coming from the subtropics and tropics, are probably introduced to greenhouses *via* soil, seeds, plant cuttings, tools, *etc.*, although there is no direct evidence confirming this [104]. They are found mostly in greenhouses and indoors (*e.g.* apartments, shops, swimming pools, glasshouses of zoo) [48,81,104-106]. In Poland some of them are also sporadically found outdoors, *e.g. L. cepistipes* [24]. In the Netherlands, this species was repeatedly recorded outdoors [76]. It should also be included in the list of species alien to Poland; currently the list contains 8 macrofungi [107].

The discovery of *M. teplicensis* in this study is the third time it has been reported in the world. All 3 locations where it has been identified are in greenhouses of Central Europe, in the Czech Republic and Poland. So far the species has been reported to grow on the roots of *Phoenix dactylifera*. We found it on new hosts/ substrates: *Lampranthus* sp. and *Euphorbia tirucalli*.

Half of the species recorded in this study (8) were lepiotaceous fungi. Nutrient-rich soils and sufficiently high temperature and humidity in greenhouses of botanical gardens ensure favourable living conditions, especially for lepiotaceous fungi.

In comparison with similar studies in other countries of Central Europe, the species diversity of fungi in greenhouses of the Botanical Garden in Warsaw-Powsin is relatively low, and the species composition differs strongly from those of other botanical garden greenhouses analysed, as shown by the results of CCA (Figure 7). Remarkable differences in the number of recorded species between our study and the earlier reports cited above certainly result from the difference in the area covered, as also shown by results of CCA (Figure 7).

Our study lasted only 1 year, so it was much shorter than the studies reported in the cited works from other countries. The number of taxa was the highest (95 and 84) in a large-scale greenhouse plantation of salad cucumbers in the Czech Republic [10] and in the Botanical Garden of Bayreuth (Germany) [11]. These studies were performed over a longer study period (7 and 4 years, respectively) and larger area (11000 m<sup>2</sup> and 4500 m<sup>2</sup>, respectively). The study period likely has an effect on the number of species recorded. However, contrary to studies conducted in natural habitats, it seems that in greenhouses a shorter study time is enough to prepare an almost complete list of species occurring in a given object. The authors of the greenhouse studies cited here have claimed that during their research the species accumulation curves reached their maximum [Gminder, Pidlich-Aigner and Gubitz, personal communication]. Also in our study, the four control visits to the Warsaw-Powsin Botanical Garden greenhouses in the next year (2011) did not increase the number of species. However, because of non-uniform treatment of the period of study factor by various authors, we did not include it in CCA analysis. The species richness of the greenhouses in Paskov (CZ) was not only due to the large area and long research time, but also due to the addition of organic matter (straw, cow-dung, remnants of bark and bast) to the soil and nutrification because of the commercial

characters of plots. Moreover, cucumber greenhouses were heated [10]. The large number of species found in greenhouses of Bayreuth Botanical Garden may result from other factors, such as the stability of climatic conditions throughout the year and the relatively high diversity of the habitats in this object [13]. Lower numbers of taxa (35 and 37) were found in greenhouses in the Botanical Garden Jena (Germany) [12] and in the Botanical Garden of the Institute of Botany in Graz (Austria) [8,9]. They were of similar size as the greenhouses studied in Warsaw but were investigated over a longer period (2 and 3 years, respectively) (Table 3), and characterized by better (more stable) climatic conditions [8,9,12]. In the botanical gardens greenhouses compared, fungal species diversity could also be affected by diversity of plant collections. The small number of species in the greenhouses of the Botanical Garden in Warsaw-Powsin could also be due to the unstable climatic conditions (fogging occurs only in summer in tropical and succulents greenhouses, moreover the buildings are not heated during summer months). In addition the relatively short time of plant cultivation before the mycological study (about 10-20 years), and thus the young age of trees and shrubs, small amount of dead wood, and diligent care for the plant collection (regular removal of dead plant parts and whole plants, weeds as well as fungal sporocarps, and the use of agrochemicals) could limit the fungal species diversity.

As a consequence of the small number of botanical gardens greenhouse complexes compared (4) and the small number of species found in more than one of them (26), the presented comparison represents a preliminary meta-analysis of fungal life in greenhouses. The ambiguous relationships may indicate that many factors influence the occurrence of fungi in greenhouses. For this reason, undoubtedly, it is not only important to increase the number of study sites and extend the study period, but also to include in future analyses additional environmental factors, *e.g.* number of habitats, number of plant taxa in the collection and climatic conditions.

We assume that *Agaricus subrufescens* may appear outdoors in Poland in the near future if it has not appeared already. In the investigated greenhouses of the Botanical Garden in Warsaw-Powsin, basidiomes of this species were also found in 2011 and 2012 (A. Szczepkowski, unpublished data). This suggests that the greenhouses have been permanently colonized by *A. subrufescens*. In Europe this species is rare. It was first reported from the Netherlands in 1997 [36] as *A. rufotegulis*, and then collected in Great Britain [36,43], Portugal [38], Spain [108], and Belgium [40]. Kibby [43] noticed the spread of this species in southern England, where it is more and more common. In has also been found in Thailand [44], Brazil [109], Hawaii [110], Taiwan [111], and Israel [39]. It has been cultivated and consumed in the eastern states of the USA in the late 19th and early 20th century [51]. It is recognized as a medicinal mushroom, due to its possible anti-cancer properties, and widely used in many countries, *e.g.* Japan or Brazil [45]. It cannot be excluded that *L. cepistipes*, recently recorded in Poland for the first time outdoors [24], is a greenhouse escape, although in Europe it has already been found outdoors [76].

Out of the 16 fungal species recorded, 12 were reported from outdoor sites in Poland (*Agaricus xanthodermus, Asterostroma cervicolor, Chlorophyllum brunneum, Ch. rachodes, Coprinellus truncorum, Cystolepiota fumosifolia, Henningsomyces candidus, Lepiota liliacea, Limacella glioderma, Leucocoprinus cepistipes, Mycenella lasiosperma,* and *Trechispora farinacea*).

During the study, we recorded species that were very rarely found in Poland before, e.g. Henningsomyces candidus (fourth record in the country). According to Cooke [62], the taxon was recorded in a similar habitat, i.e. on a palm leaf sheath in the Berlin Botanical Garden's Palm House. Some of the species found in Polish greenhouses are classified as protected by law and/or threatened in our country [55]: Lepiota lilacea (category Ex); Asterostroma cervicolor, Cyathus stercoreus, Mycenella lasiosperma (E); Hericium coralloides, Limacella glioderma, Postia floriformis, Volvariella volvacea (V); Dentipellis fragilis, Melanophyllum haematospermum (R). Certainly for some of these, which are typically distributed in natural habitats, the conditions in greenhouses may be more favourable for survival. We believe that as a result of further research on macrofungi in greenhouses, it will be possible to distinguish a group of species whose populations will be maintained in artificial conditions, in greenhouses. This would be a form of ex situ conservation of fungi. It would be particularly important for very rare and threatened native species, which thus far could only be

preserved by protecting their natural habitats or storing their genetic resources.

To identify the mycobiota of greenhouses and determine their role in the spread of alien species, further and more complex research is needed in various parts of Poland and elsewhere on the continent. In future projects, molecular studies of the specimens collected in greenhouses are needed. Research in greenhouses also provides new data on ecological plasticity of native species and their ability to adapt to conditions shaped by human activity, while in the case of species associated with specific plant species (parasites, saprotrophs colonizing leaf litter and wood), research may show their ability to colonize some alien, non-European plant species.

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