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Gasteroid fungi – the morphological characteristics of selected endangered and rare species noted in Poland

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ABSTRACT

The aim of the work was to present the characteristics of selected species from *Disciseda, Geastrum* and *Tulostoma* genera which due to the small differences in morphology of their fruit bodies may pose some identification problems. The selected species of gasteroid fungi of these genera are described based on the materials collected during the course of our studies. All materials were gathered during the research into macromycetes in xerothermic habitats located in the Nida Basin. Taxa noted by us are considered to be very rare in the mycobiota of Poland and are highly endangered.

KEY WORDS: Gasteromycetes, thermophilic fungi, endangered species of fungi, Agaricaceae, Geastraceae, Lycoperdaceae

Introduction

The gasteroid fungi (formerly Gasteromycetes) are polyphyletic group of fungi which currently belong to different taxa in the *Agaricomycetes* class (Hibbett & Thorn 2001, Binder & Bresinsky 2002). Biological and morphological properties as well as the development, maturation and dispersal of spores are characteristic features of the *Gasteromycetes*. Gasteroid fungi are usually spherical, piriform and clavate. The hymenium is enclosed inside the fruit body until spores mature. Fruit bodies consist of three basic parts: peridium (the wall), gleba (the fertile area) and trama (sterile hyphae that form pseudoparenchyma; Pilát 1958, Rudnicka-Jezierska 1991).

Fruit bodies of gasteroid fungi develop underground (hypogeously) and are spherical. As they mature they emerge over ground becoming epigeous. Gasteroid fungi are mainly saprobionts that grow in woodless areas, xerothermic, sandy and steppe sites, in forests, but also in wet places and even on the moors (Pilát 1958, Rudnicka-Jezierska 1991).

Species that differ by small macro- and micromorphological characters of the fruit body's structure in individual genera were selected for morphological analysis. A scrupulous submicroscopic and molecular examination of the structure of

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similar taxa helps to identify fruit bodies correctly and reduces the risk of determination errors (e.g. Tomaszewska *et al*. 2011).

The aim of this study was to present the characteristics of selected species from *Disciseda, Geastrum* and *Tulostoma* genera, which due to the small differences in morphology of their fruit bodies may pose some identification problems.

Material and methods

Species were collected during the research into macromycete fungi in xerothermic habitats located in the Nida Basin (south of Poland) between 1991 and 2013. The studies were intensified from 2010 until 2013. The investigations were conducted in protected areas such as nature reserves (Krzyżanowice, Skorocice), landscape parks and Natura 2000 sites (Nida Landscape Park – PLH260003 Ostoja Nidziańska, Szaniec Landscape Park – PLH260034 Ostoja Szaniecko-Solecka, Kozubów Landscape Park – PLH260029 Ostoja Kozubowska). The examined plant communities are protected under the Habitats Directive. The investigations conducted in these respected areas also provided data about their functioning and the interactions between fungi and xerothermic vegetation.

The mycological investigations were conducted using permanent research plots and were supplemented with the route method. A total of 30 plots (each of them of 100 m^2) were established in six communities of xerothermic vegetation from *Festuco-Brometea* class, such as: *Adonido-Brachypodietum pinnati, Festucetum pallentis, Inuletum ensifoliae, Sisymbrio-Stipetum capillatae, Seslerio-Scorzoneretum purpureae* and *Thalictro-Salvietum pratensis* (names according to Matuszkiewicz 2012). The observations and collections of fruit bodies were carried out at intervals. The number of species fruit bodies, the organoleptic properties, i.e. the shape, size and colour of the endoperidium, the colour of the exoperidium and the manner in which it flakes, and also the pigmentation and the structure of the stem surface, were all noted during collections.

The laboratory examinations were conducted using light microscopy (LM) and scanning electron microscopy (SEM). The structure, size and shape of the capillitium and the spores were measured using standard reagents and light microscope. The measurements were performed using 400x and 1000x magnification. The episporium sculpture was investigated using SEM.

The material (gleba samples with spores) was mounted on an aluminium stub and coated with 24-carat gold (Karcz 1996, 2009). The electron micrographs were taken at the magnifications of: 3000, 5000, 10000 and 12000x. The studies with the help of the scanning electron microscope (SEM) were carried out in the Department of Environment Protection of Jan Kochanowski University in Kielce and in the Laboratory of Field Emission, Scanning Electron Microscopy and Microanalysis at the Institute of Geological Sciences of Jagiellonian University in Kraków.

The following studies were used for taxonomic identification: Pilát (1958), Wright (1987), Rudnicka-Jezierska (1991), Sarasini (2005) and Sunhede (1990). The nomenclature of the taxa is given after Index Fungorum (2014).

Results

Eight selected species of gasteroid fungi were examined. Macro- and micromorphological characters were used to describe the species. Species descriptions are based on material collected in our investigations. A list of similarities and differences between the described species is given in Tables 1, 2 and 3.

Disciseda bovista (Klotzsch) Henn.

Mature fruit bodies globose, rarely flattened. Exoperidium white, whitish yellow, mature brownish ashen. Endoperidium is rigid, pergameneous and nut-like coloured. Spores globose, (5–)6.5–7.8(–8.6) µm in diam. (according to Lizárraga *et al*. 2010: 4–7 µm in diam.), distinctly strongly verrucose, verrucae 1–1.5 µm, without sterigmata (Fig. 1a). Capillitium is light yellow, hyaline, quite thick-walled. Capillitium threads are wavy, brittle and 2.7–3.5 µm thick.

Disciseda candida (Schwein.) Lloyd

Mature fruit bodies are loaf-like. Exoperidium is dirty whitish yellowish, mature, earth brown. Endoperidium strong, leathery, matt, brown-grey to ashen in colour. Spores globose, punctate, delicately verrucose or glabrous, (3.8–)4.5–5 µm (according to Bates *et al*. 2009: 4.0–5.6(–6.4) × 4.0–5.6(–6.4) µm in diam.), without sterigmata (Fig. 1b). Capillitium light yellow, hyaline and thin-walled. Capillitium threads wavy, brittle and 2.5 µm thick.

Geastrum campestre Morgan

Exoperidium splits into 5 to 12 triangular segments that are hygroscopic or subhygroscopic. It is beige-coloured, grey-brown, light brown to dark brown and when expanded 3 to 5 cm in diam. on average. Endoperidium globose is 0.5 to 2 cm in diam. and only partially with apophysis. The granulately rough, farinaceous surface of the endoperidium is a characteristic feature of the species. Peristome cristate with delimited bulge, from 12 to 20 ridges (Fig. 2a). Gleba dark brown. Spores globose, finely verrucose, 4.8–7(–8) µm. It should be noted that there is a possibility of mistaking this species with *Geastrum berkeleyi* Massee which also has granulately surface of the endoperidium and cristate peristome.

Geastrum minimum Schwein.

Exoperidium splits into 6 to 12 not hygroscopic segments that reach 3 to 4 cm in diam. when expanded. Endoperidium globose is 0.4 to 1.2 cm in diam., grey-brown, ochraceous-brown or grey-white. A white layer of fine crystals of calcium oxalate on the endoperidium is the main diagnostic trait of the species. Peristome sericeous-fimbriate is lighter than the remainder of theendoperidium, with a collar delimited by a bulge (Fig. 2b). Gleba dark brown. Spores globose, 3.5–5.5(–7) µm in diam., minutely verrucose.

Geastrum schmidelii Vittad.

Exoperidium splits into 5 to 10 entirely not hygroscopic segments that reach 1-3 cm in diam. when expanded. Endoperidium globose is brown-grey to brown at the bottom, whitish at the top, especially in young fruit bodies. Peristome sulcate, from 10 to 19 ridges, delimited by a furrow, covered with farinose coating in young fruit bodies (Fig. 2c). Gleba dark brown. Spores globose, 4.7–7.5 µm (according to Sarasini 2005: (4–)4.2–4.8(–5.5) µm in diam.), distinctly thickly verrucose.

Tulostoma brumale Pers.

Exoperidium whitish, membranous, soon flaking away. Mature endoperidium ochraceous-white, sometimes with rusty-brown stains, also yellowish brown. Peristome tubular, mouth area darker

coloured, yellowish or dirty brown. Stem fibrillose, ochraceous-fawn, minutely and very delicately squamulose, straight (Fig. 3a). Spores (4.2–)4.7–5.64 µm in diam. (according to Sarasini 2005: (3.5–)4.2–4.7(–5.5) µm in diam.), globose, light yellow, minutely verrucose (Fig. 3b). Capillitium hyaline, thick-walled, lumen small, coloured at septa and swollen in septa, branched, external surface covered with fine crystals.

Tulostoma melanocyclum Bres.

Exoperidium whitish or light ochraceous, hyphal, persisting quite long. Endoperidium ashen-ochraceous or ochraceous-rusty. Peristome tubular, dark, dirty brown. Stem ochraceous-brown to dark brown, sulcate, covered with fine, adherent squamulae (Fig. 3c). Spores are globose and subglobose (Fig. 3d), brown, spinulose, (6.58–)7.52–8.46 µm (according to Hansen & Knudsen 1997: 4.5–5 µm, without the ornamentation). Capillitium subhyaline, thick-walled, well visible lumen, colourless and not thickened at septa, moderately branched, without crystals.

Tulostoma squamosum Gmelin

Exoperidium dark, sometimes whitish, thin-walled, persisting longer. Endoperidium pergameneously rigid, light yellow, white-ochraceous, becoming chestnut-coloured. Peristome tubular, mouth area concolourous with the head, stem cinnamon-coloured or brown-red, unevenly covered with protruding, sharp squamulae (Fig. 3e). Spores globose and subglobose, yellow-brown, small spiny, (4.5–)5.64–7.52 µm (Fig. 3f). Capillitium hyaline, thick-walled, lumen visible, slightly yellowish and often broader at septa, branched, without crystals.

Table 1. The comparison of selected morphological and anatomical characteristics in similar *Disciseda* species.

Feature	*Disciseda bovista*	*Disciseda candida*
Exoperidium	White, whitish yellow, mature brown pale grey	Dirty whitish yellowish, mature from brownish to earthy coloured
Endoperidium	Rigid, pergameneous, hazel nut like coloured	Strong, leathery, pergameneous, brown-grey
Peristome	Frayed	Frayed, frimbrillate
Gleba	Red brown	Brightly brown, rusty brown
Spores	(5–)6.5–7.8(–8.6) µm in diam. Distinctly strongly verrucose (verrucae 1-1.5 µm)	(3.8–)4.5–5 µm in diam. Smooth or puncticulate, very fine verrucose
Sterigmata	Absent	Absent
Capillitium	2.7–3.5 µm thick, wavy, fragile	2.5 µm thick, wavy, fragile

Discussion

The new information regarding many rare and endangered species of gasteroid fungi in Poland was collected in our study. Only one species, *Tulostoma brumale* has a larger number of localities, and for this reason it can be considered for more frequent. Other species are very rare and are considered as endangered (E) in Polish Red List (Wojewoda & Ławrynowicz 2006).

The aim of the study was to draw the attention to the selected features of fungi fruit bodies from the *Disciseda, Geastrum* and *Tulostoma* genera which due to their variability are difficult to identify. Full descriptions of the taxa are based on macro- and micromorphological characters recorded in field studies and laboratory examinations. The important differences between fruit bodies within genera and species may be noticeable but are strongly influenced by the sample size being collected and how well the morphology is being preserved. They also become less distinct as fruit bodies mature and become old. Consequently, our research has shown the need to conduct parallel complementary studies with the use of LM and SEM to carry out the correct determination of these fungi. The climatic and habitat factors can also impact phenotypic characters (Tomaszewska *et al*. 2012, 2014). The identification of taxa based only on observations of features by using the LM may contribute to erroneous determinations. Further complex investigations into gasteroid fungi are needed in order to verify fully the morphological structure and also to identify taxa correctly.

Table 2. The comparison of selected morphological and anatomical characteristics in similar *Geastrum* species.

Feature	***Geastrum campestre***	***Geastrum minimum***	***Geastrum schmidelii***
Exoperidium	Splits into 5–12 triangular segments; hygroscopic or subhygroscopic; Beige-coloured, grey-brown, light brown to dark brown	Splits into 6–12 segments; not hygroscopic; Grey brown, ochraceous brown or grey white	Splits into 5–10 segments; not hygroscopic; Grey white to brown
Endoperidium	Globose, 0.5–2 cm in diam; Granulately rough, farinaceous surface	Globose, 0.4–1.2 cm in diam; Presence of crystals of calcium oxalate on the surface	Globose, 0.3–2.5 cm in diam; Smooth surface
Peristome	Cristate, with delimited bulge, 12–20 ridges	Sericeous-fimbriate, lighter than the remainder of the endoperidium, with a collar delimited by a bulge	Sulcate, delimited by a furrow, covered with farinose coating in young fruit bodies, 10–19 ridges
Gleba	Dark brown	Dark brown	Dark brown
Spores	4.8–7(–8) µm in diam, globose, finely verrucose	3.5–5.5(–7) µm in diam, globose, minutely verrucose	4.7–7.5 µm diam, globose, distinctly thickly verrucose

Table 3. The comparison of selected morphological and anatomical characteristics in similar *Tulostoma* species.

Feature	*Tulostoma brumale*	*Tulostoma melanocyclum*	*Tulostoma squamosum*
Exoperidium	Whitish, membranous, early flaking	Whitish or pale ochraceous, relatively long-term	Dark, sometimes whitish, thinly membranous, more durable
Endoperidium	White ochraceous, yellowish brown	Grey and ochraceous, cinereous ochraceous	White ochraceous
Peristome	Tubular, around the mouth dark coloured – yellowish or dirty brown	Tubular, dark, dirty brown	Tubular, around the mouth colored as the peridium
Gleba	Pale ochraceous	Pale ochraceous, ferrugineous	Pale ochraceous, ferrugineous
Stem	16–29 × 2–2.8 mm; fibrous, pale ochraceous, covered with tiny and very delicate scales	23–33 × 2–3 mm, ochraceous brown to dark brown, furrowed, covered with	18–24(–28) × 2.5–4.5 mm; cinereous or brown red, uniformly covered with coarse
Spores	(4.2–)4.7–5.64 µm in diam; globose, pale ochraceous, with small verrucae	(6.58–)7.52–-8.46 µm in diam; globose, brown, delicate spiny	(4,5–)5.64–7.52 µm in diam; globose or subglobose, yellow brown, small, spiny
Capillitium	Width of capillitium: 3.7–5.64 µm, width of septa: (6.11–) 8.64–9.4 µm. Hyaline, thick-walled with small lumen, swollen at the coloured septa, branched, external surface covered with crystalline plaques	Width of capillitium: (4.7–)5.64–6.58 µm, width of septa: 5.64 µm. Almost hyaline, thick-walled with a visible lumen, not swollen at the uncoloured septa, branched, without crystalline plaques	Width of capillitium: 4.7–6.58 µm, width of septa: 4.7–5.64 µm. Hyaline, thick-walled with a visible lumen, slightly swollen at the yellowish septa, branched, without crystalline plaques

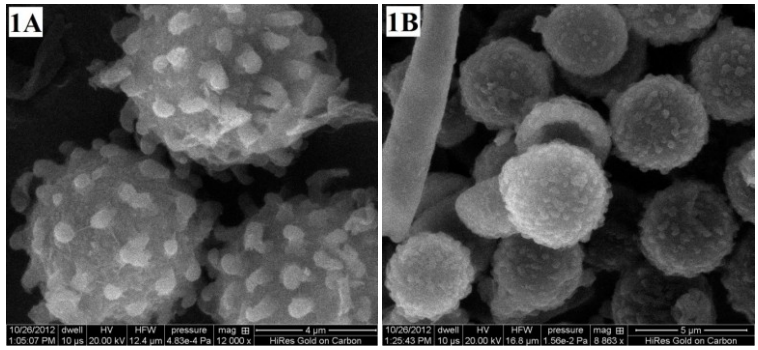

Figure 1. Spores of *Disciseda* species in SEM: A – *D. bovista,* B – *D. candida*.

Figure 2. The morphology of the peristome and the structure of the endoperidium surface in *Geastrum* species (photo by A. Tomaszewska): A–*G. campestre*, B–*G. minimum*, C – *G. schmidelii*. Scale bars = 5 mm.

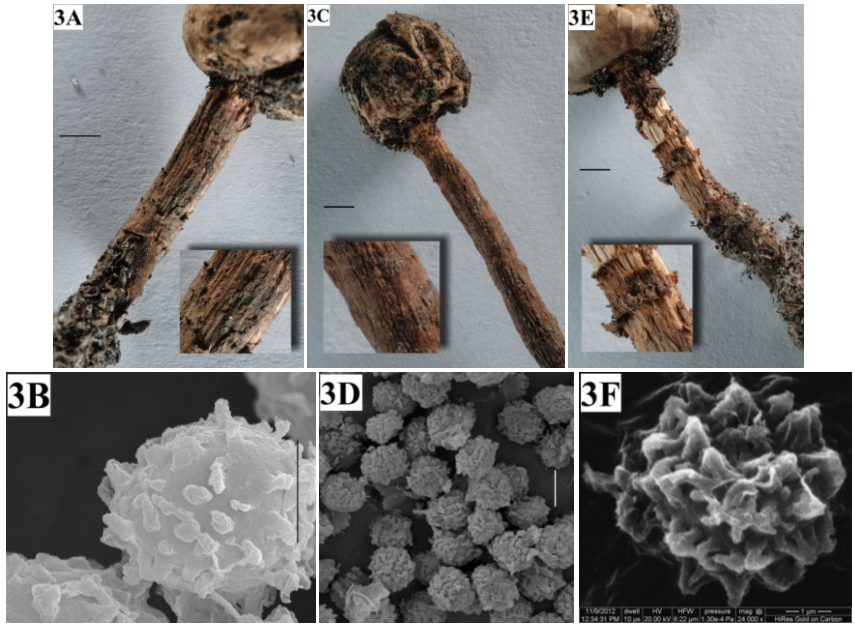

Figure 3. The morphology of the fruit body stem and the spore texture (in SEM) of *Tulostoma* species: A, B – *T. brumale*; C, D – *T. melanocyclum;* E, F – *T. squamosum*. Scale bars: A, C, E = 3 mm, B = 3 µm, D = 5 µm, F = 1µm, (photos A, C, E by G. Wołczyk).

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Streszczenie

W pracy opisano osiem wybranych gatunków grzybów gasteroidalnych należących do rodzajów: *Disciseda, Geastrum* oraz *Tulostoma.* Były to następujące gatunki: *Disciseda bovista, D. candida, Geastrum campestre, G. minimum, G. schmidelii, Tulostoma brumale, T. melanocyclum* oraz *T. squamosum*. Wszystkie gatunki zebrano podczas badań nad grzybami wielkoowocnikowymi na siedliskach kserotermicznych Niecki Nidziańskiej głównie w latach 2010-2013. Badania prowadzono w sześciu

zbiorowiskach roślinności kserotermicznej – *Adonido-Brachypodietum pinnati, Festucetum pallentis, Inuletum ensifoliae, Sisymbrio-Stipetum capillatae, Seslerio-Scorzoneretum purpureae* and *Thalictro-Salvietum pratensis* (nazewnictwo za Matuszkiewicz 2012). W fitocenozach tych zespołów wyznaczono trzydzieści powierzchni badawczych, na których prowadzono obserwacje w regularnych dwutygodniowych odstępach czasu.

Zanotowane grzyby należą do gatunków silnie zagrożonych w naszym kraju. Tylko jeden z nich – *Tulostoma brumale* – posiada większą liczbę stanowisk i z tego powodu można uznać go za częstszy. Pozostałe należą do grzybów bardzo rzadkich, a także do gatunków wymierających w Polsce, kategoria E (Wojewoda & Ławrynowicz 2006).

Do analizy morfologicznej wybrano gatunki, które w obrębie rodzajów wykazują niewielkie różnice w budowie owocników. Na podstawie cech makro- i mikromorfologicznych, które zaobserwowano podczas badań terenowych i laboratoryjnych, sporządzono pełne opisy zanotowanych taksonów. Istotne różnice w budowie owocników poszczególnych taksonów wynikają ze stopnia rozwoju i zachowania cech poszczególnych owocników (Tomaszewska *et al*. 2011). Cechy te zacierają się w miarę dojrzewania i starzenia się tych struktur. Na wykształcenie cech fenotypowych mogą także wpływać między innymi czynniki pogodowe i siedliskowe (Tomaszewska *et al*. 2012). Otrzymane wyniki wskazują na potrzebę dalszych badań tej grupy grzybów, w celu poznania pełnej zmienności cech budowy morfologicznej i poprawnej identyfikacji taksonów.