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Taxonomic notes on the Polish *Tulostoma* species

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Tulostoma genus Pers.: Pers. is represented in Poland by five species (Wojewoda 2003). Only two of those: *Tulostoma brumale* Pers.: Pers. and *T. fimbriatum* Fr. can be considered as a relatively common while others such as: *Tulostoma kotlabae* Pouzar, *T. melanocyclum* Bres. and *T. squamosum* (Gmelin in L.): Pers. are very rare. Observations of macrofungi of xerothermic habitats at the Nida Basin (Central Poland), demonstrated the existance of many varieties of particular taxa until now not listed in the Polish mycological literature, and also new sites of all so far described *Tulostoma* species. Recording of lower taxa such as: *Tulostoma brumale* Pers. var. *longipes* (Czern.) J. E. Wright, *T. brumale* Pers. var. *pallidum* (Lloyd) J. E. Wright, and *T. fimbriatum* Fr. var. *campestre* (Morgan) Moreno are identified at the study area.

Key words: Basidiomycota, *Tulostomataceae,* species differentiation, distribution

INTRODUCTION

Species belonging to the genus *Tulostoma* has been investigated by many researchers and a variety of taxonomic approaches to these fungi have been adopted in monograph studies. The genus *Tulostoma* initially consisted of two sections: *Eutylostoma* and *Schizostoma* (Fries 1921; Fischer 1900, 1933; Petri 1909), distinguished by peristome morphology. A more precise classification was proposed by Pouzar (1958), who assigned the species to four sections: *Brumalia*, *Poculata*, *Fimbriata* and *Volvulata*, based on the morphology and the way the exoperidium breaks off, the morphology of the peristome and the stem. The system proposed by Pouzar was used by Wright (1987) in the current classification based on primary and secondary micromorphological characters. Primary characters include: the shape, size and colour of the endoperidium; the colour, persistence of the exoperidium and the way it

breaks off, peristome, spore size and episporium ornamentation. Secondary characters used to identify the species are: the height, colour and morphology of the stem surface, the thickness of capillitial threads, and the morphology of transverse septa produced by them.

During the field investigations, a great number of the genus *Tulostoma* fruitbodies were collected of which precise identification (especially to the lower taxa) was very difficult. The aim of this study was to compile keys to allow identification to the lower taxa such as for example varieties, within examined species. Keys to macro- and microscopic characters are given, which aim to underline intraspecific variability of selected species growing in Poland. Lower taxa can be fully determined and identified correctly based on the descriptions of macro- and micormorphological features.

MATERIAL AND METHODS

Mycological investigations were conducted in xerothermic habitats in the Nida Basin and the Chęciny District since October 2010. Studies will continue over the next three years. Communities of xerothermic vegetation distributed in protected areas such as Nature 2000 ecological sites, nature reserves and landscape parks have been examined. Preliminary results of studies are presented below.

Fruitbodies of *T. fimbriatum* were collected at localities in Kielce and in xerothermic psammophilous grasslands in Zajączków village in the Chęciny District.

Carpophores of the genus *Tulostoma* were collected each time during observations. The number of fruitbodies of a species and organoleptic features, i.e. the shape, size and colour of the endoperidium, the colour and breaking off of the exoperidium, and the stem colour and features, were recorded upon collection.

Laboratory studies were conducted using a light microscope and a scanning electron microscope. Threads of the capillitium, septa and spores whose morphology, size and shape are important taxonomic features were examined by LM. Slides were made from each fruitbody. Ten randomly selected spores were measured in each slide. Microscopic structures were observed using an immersion lens.

The following studies were used for taxonomic identification: Pouzar (1958), Moreno (1980), Wright (1987), Rudnicka-Jezierska (1991). The nomenclature of the taxa is given after Wright (1987) and Wojewoda (2003).

Interspecific relationships in the taxonomy of the species were described while determining the species based on the above studies. Notes on the intraspecific differentiation of taxa were based on the material collected by the authors.

The dry material is deposited in the Fungal Herbarium (KTC) of the Faculty of Mathematics and Nature, Jan Kochanowski University, Kielce (Poland).

RESULTS

Lower taxa previously not reported in the Polish mycological literature on the fungi of the genus *Tulostoma* are presented. New localities of the species described to date are also reported (Tab. 1).

Micromorphological features characteristic of two species, *Tulostoma brumale* and *T. fimbriatum*, and their varieties, are presented.

Key to varieties of ***T. brumale*** Pers.: Pers.

1. Stem above 45 mm in length .....
Fruitbodies slender, exoperidium thinly membranous, peristome mouth small, stem long, distinctly different by size from the typical variety *Tulostoma brumale*. Spores 4-6 μm, delicately verrucose, verrucae low, not coalescing. Occurs in sun-exposed places among herbaceous vegetation.
Tulostoma brumale var. ***longipes*** (Czern.) J. E. Wright (Fig. 10 A, B, C)

1*. Stem up to 45 mm in length 2

2. Spores with sparse fine verrucae. Capillitium hyaline, external surface covered with fine crystals, broadened or even strongly swollen at septa .....
Endoperidium ochraceous-white, sometimes with rusty brown spots, also yellowish brown. Mouth shortly tubular, zone around mouth distinguished by darker pigmentation, sometimes dirty brown or greyish brown. Stem 14-40 x 1,5-4 mm, straight, ochraceous fawn or fawn tawny. Spores 4-6 μm in diam., with sparse fine verrucae. Capillitium hyaline, thick-walled, external surface covered with crystal. Occurs mostly among grasses and mosses, on dry sandy or calcareous soils, in sun-exposed places.
Tulostoma brumale var. ***brumale*** (Fig. 11 A, B, C)

2*. Spores with sparse thick verrucae +/– coalescing and forming small crests. Capillitium hyaline, without crystals, slightly broadened at septa .....
Endoperidium pale to light ochraceous, usually not darkly pigmented and lacking a dark collar around peristome. Stem rather slender and thin. Spores 4.5-6 μm. Spore ornamentation more distinct than in *T. brumale* var. *brumale.* Under SEM appearing as thick verrucae that may form small crests. Capillitium hyaline, thick-walled, densely septate, external surface not covered with crystals. Among grasses, on dry, sandy or calcareous soils, in sun-exposed places.
Tulostoma brumale var. ***pallidum*** (Lloyd) J. E. Wright (Fig. 12 A, B, C)

Key to varieties of ***T. fimbriatum*** Fr.

1. Peridium over 15 mm wide, spores 5.5-6.5 μm .....
Exoperidium thin, white, only at base ochraceous-greenish. Endoperidium, smooth, whitish to ochraceous, slightly greenish-brown. Mouth typically fimbriate, more commonly denticulate. Stem 20-100 x 3-8 mm; straight but sometimes also curved, light brown, ending in a bulb at base. Spores globose or subglobose, 5.5-6.5 μm, densely verrucose, verrucae coalescing forming a distinct net. Capillitium hyaline, septate, with visible lumen, slightly coloured at septa.
Tulostoma fimbriatum Fr. var. ***campestre*** (Morgan) Moreno (Fig. 13 A, B, C)

1*. Peridium less than 15 mm wide, spores (5) 6-8 μm .....
Exoperidium thin, white. Endoperidium, smooth, dirty grey or ochraceous grey. Mouth plane, fibrillose, fimbriate, sparsely denticulate. Stem up to 50 x 3-5 mm, straight, dark brown, not ending in a bulb at base. Spores globose, (5-) 6-8 μm, with dense verrucae, rarely coalescing. Capillitium hyaline, slightly broadened at septa.
Tulostoma fimbriatum var. ***fimbriatum*** Fr. (Fig. 14 A, B, C)

DISCUSSION

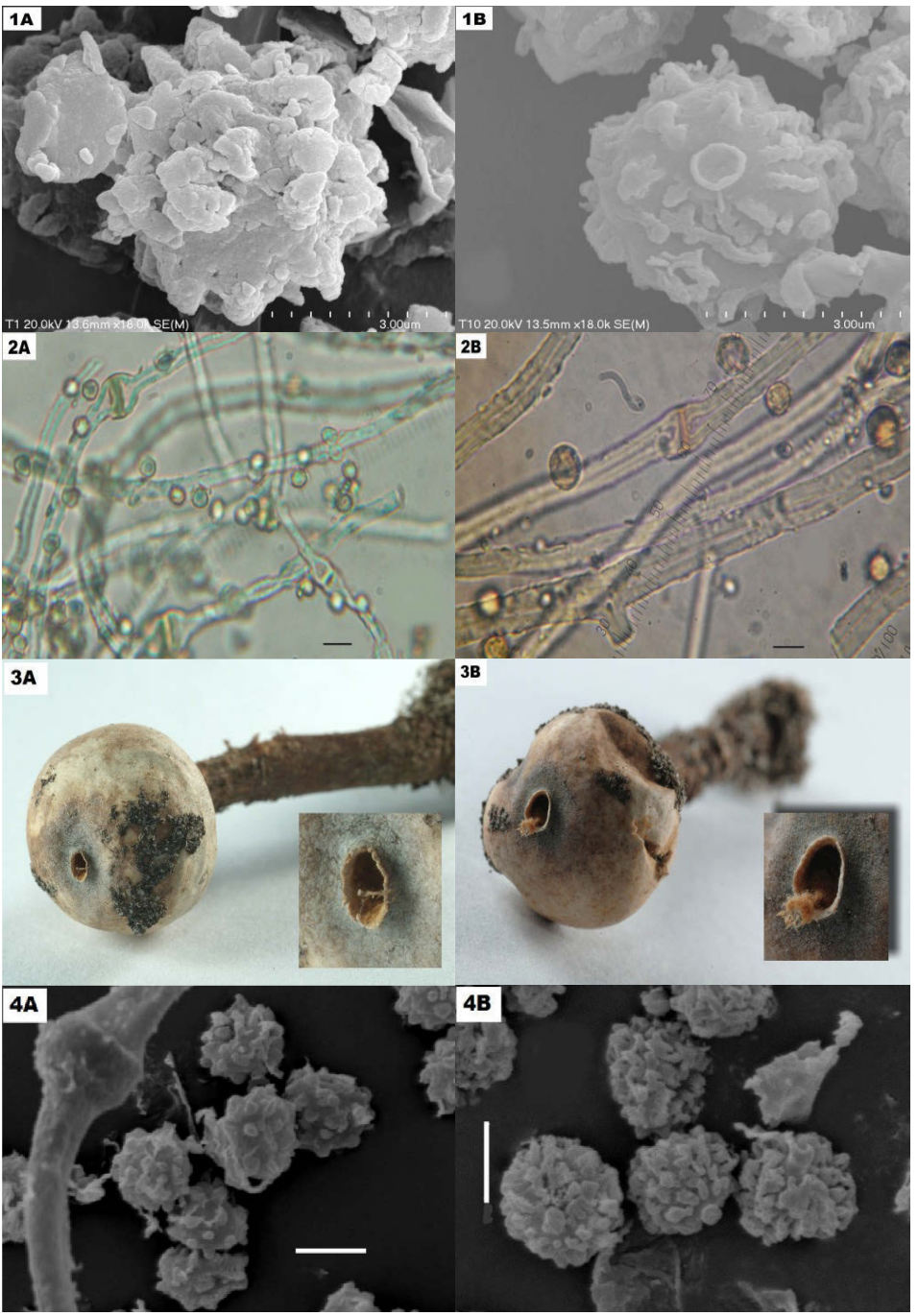
Tulostoma exhibit high morphological similarity between species, which causes several taxonomic problems. They are often similar in shape, size and colour of the endoperidium, the morphology and colour of the peristome, pigmentation, persistence and breaking off of the exoperidium, and the size, colour and morphology of the stem. The preservation of morphological features in specimens of individual species also affects correct identification of important diagnostic differences between fruitbodies as some traits become similar when fruitbodies mature and age. Features that are not persistent include exoperidium pigmentation, scaling on the stem, the shape and attachment of scales to the stem, the shape and the size of the mouth, and the pigmentation of the peridium and the stem.

Problems discussed in this chapter were especially challenging in the case of *T. brumale* var. *brumale* and *T. kotlabae*. The taxa are very similar and can be easily mistaken. They are also similar to other species. A list of similarities and differences is given in Table 2.

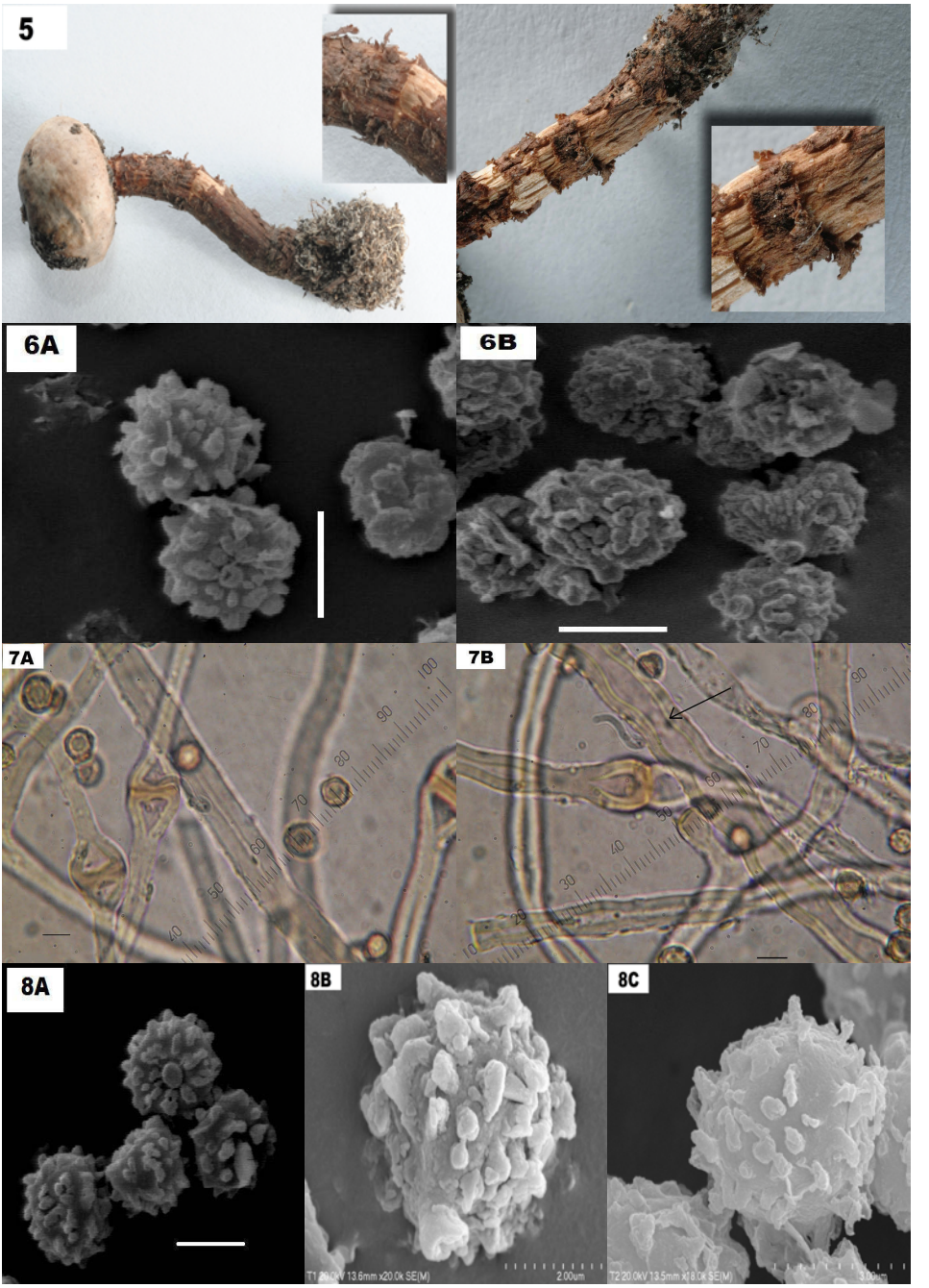
Many authors, including Wright (1987) and Pouzar (1958), have pointed out diagnostic problems between *Tulostoma brumale* var. *brumale* and *T. melanocyclum*. Authors encountered such problems while identifying fruitbodies of these species. The colour of the peristome is an especially important feature used to determine fruitbodies of *T. brumale* var. *brumale*. Like in *T. melanocyclum*, fruitbodies of *T. brumale* var. *brumale* have a darkly pigmented collar around the peristome. However, the peristome is light-coloured in *T. brumale* var. *brumale* while it is dark brown in *T. melanocyclum* similarly to the collar around the mouth (Fig. 3 A, B). The two species

Table 1
A list of taxa and their new localities

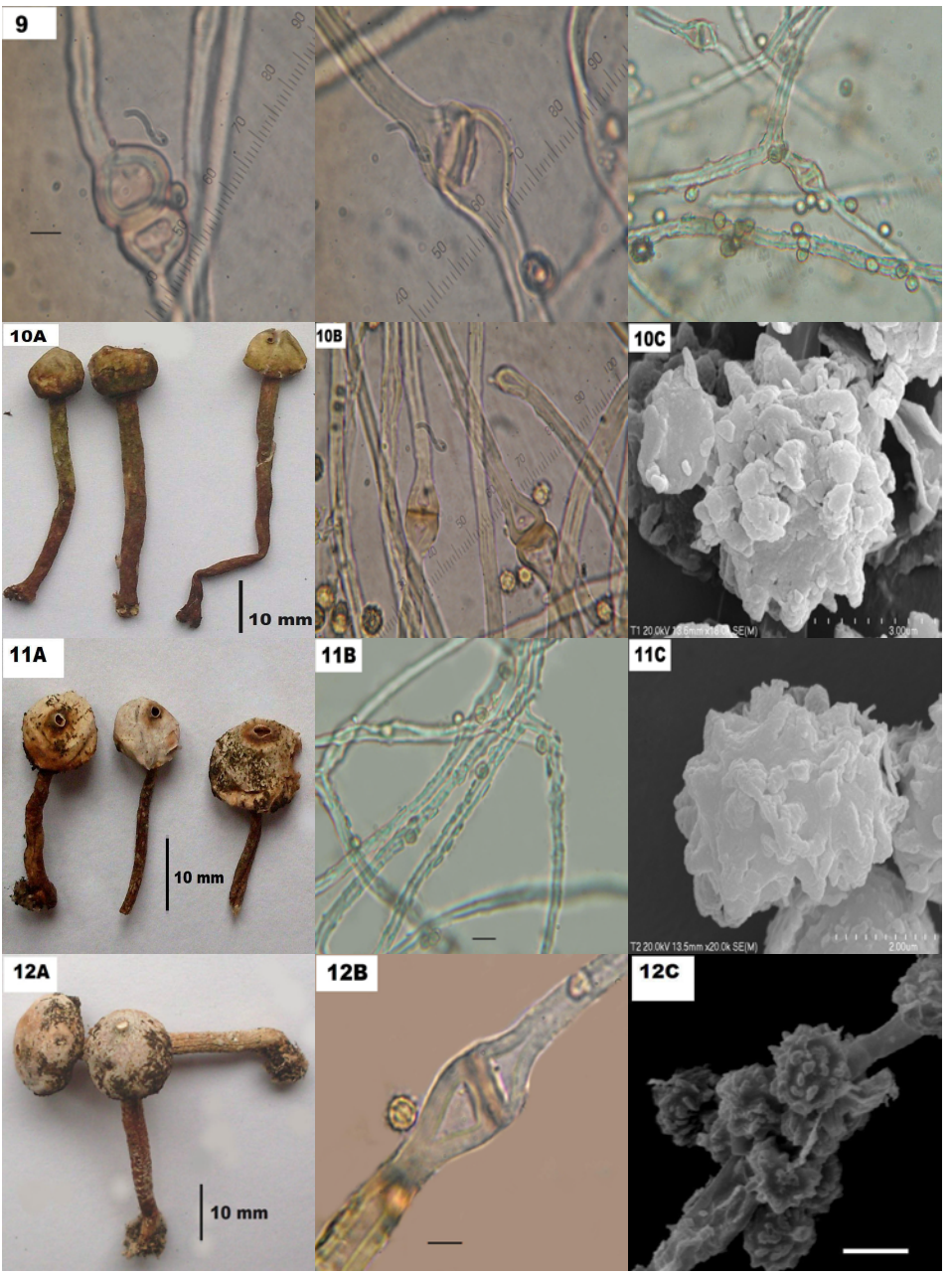
Lp.	List of taxa	Type of habitats	New localities	Number of specimens collected
1.	*Tulostoma brumale* var. *brumale* Pers.: Pers.	*Sisymbrio-Stipetum capillatae Koelerio-Festucetum rupicolae*	Wola Zagojska Górna n. Pińczów	16
2.	*Tulostoma brumale* Pers. var. *longipes* (Czern.) J. E. Wright	*Koelerio-Festucetum rupicolae*	Szaniec n. Busko-Zdrój	7
3.	*Tulostoma brumale* Pers. var. *pallidum* (Lloyd) J. E. Wright	*Koelerio-Festucetum rupicolae*	Wola Zagojska Górna n. Pińczów	5
4.	*Tulostoma fimbriatum* var. *fimbriatum* Fr.	*Spergulo-Corynephoretum*	Kielce	3
5.	*Tulostoma fimbriatum* Fr. var. *campestre* (Morgan) Moreno	border of sandy road	Zajączków (Chęciny District)	10
6.	*Tulostoma kotlabae* Pouzar	*Koelerio-Festucetum rupicolae*	Wola Zagojska Górna n. Pińczów	2
7.	*Tulostoma melanocyclum* Bres.	*Sisymbrio-Stipetum capillatae*	Krzyżanowice reserve, Wola Zagojska Górna n. Pińczów, Zwierzyniec n. Busko-Zdrój	13
8.	*Tulostoma squamosum* Gmelin: Pers.	*Festucetum pallentis*	Gacki, Wola Zagojska Górna n. Pińczów	18



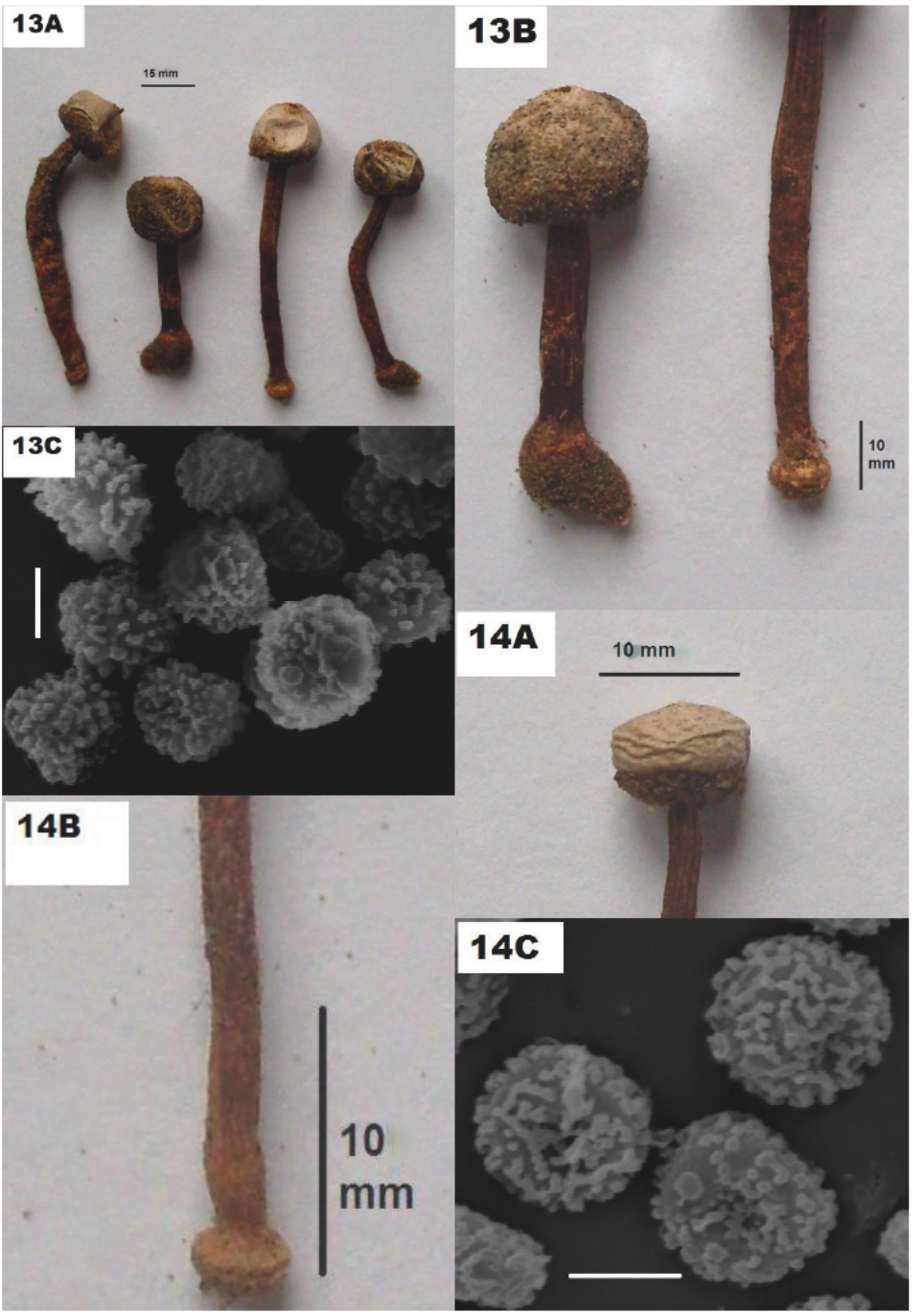
Figs 1–4. 1A. Spores of *T. brumale*, 1B. *T. kotlabae*. Scale bar = 5 μm SEM. 2. Morphology of the capillitium and septa: A – *T. brumale*, B – *T. kotlabae*. Scale bar = 5 μm LM; 3. Pigmentation and morphology of the peristome: A – *T. brumale*, B – *T. melanocyclum* (photo by G. Wołczyk); 4A. Spores of *T. brumale* and 4B. *T. melanocyclum*. Scale bar = 5 μm SEM.



Figs 5–8. 5. *Tulostoma squamosum*. Stem morphology (photo by G. Wołczyk). 6A. Spores of *T. brumale* and fruitbodies exhibiting similarity to 6B. *T. giovanellae.* Scale bar = 5 μm SEM. 7. A, B. Morphology of the capillitium in the fruitbodies exhibiting similarity to *T. giovanellae*. Scale bar = 5 μm LM. 8 A, B, C. Differentiation of spore morphology of *Tulostoma brumale*. Scale bars for: A = 5 μm; B = 2 μm; C = 3 μm.



Figs 9–12. 9. Differentiation of the morphology of capillitial threads and septa of *Tulostoma brumale*. Scale bar = 5 μm LM. 10. *T. brumale* var. *longipes*: A – mature fruitbodies (photo by A. Tomaszewska), B – capillitial threads. Scale bar = 5 μm LM, C – spores. Scale bar = 5 μm SEM. 11. *T. brumale* var. *brumale*: A – mature fruitbodies (photo by A. Tomaszewska), B – capillitial threads. Scale bar = 5 μm, C – spores. Scale bar = 5 μm SEM. 12. *T. brumale* var. *pallidum*: A – mature fruitbodies (photo A. Tomaszewska), B – capillitial threads. Scale bar = 5 μm LM, C – spores. Scale bar = 5 μm SEM.



Figs 13–14. 13. *T. fimbriatum* var. *campestre*: A – mature fruitbodies (photo by A. Tomaszewska), B – stem morphology (photo by A. Tomaszewska), C – spores. Scale bar = 5 μm SEM. 14. *T. fimbriatum* var. *fimbriatum*: A – mature fruitbody (photo by A. Tomaszewska), B – stem morphology (photo by A. Tomaszewska), C – spores. Scale bar = 5 μm SEM.

Table 2
Selected species of the genus *Tulostoma* and other species recorded in Europe

Potential errors	Simlarities	Taxonomic differences
Tulostoma brumale var. *brumale* *T. kotlabae*	Endoperidium pale, white, beige or ochraceous; stem light yellowish, light brown, squamulose.	Microscopic features. Spores distinctly smaller in *T. kotlabae*. Ornamentation appears as sparsely scattered, low verrucae; more delicate than in *T. brumale* var. *brumale* (Fig. 1 A, B). Capillitial threads often encrusted with crystals in *T. brumale* var. *brumale*; without crystals in *T. kotlabae*. Septa straight and not broadened in *T. kotlabae* (Fig. 2 A, B).
T. kotlabae *T. albicans**	Endoperidium pale, whitish, beige; scales whitish or light brown, sloughing off disorderly. Capillitium threads hyaline, thick-walled, with straight, not broadened septa.	Spores minutely verrucose in *T. kotlabae*; *T. albicans*: spores smooth or only minutely asperulate.
T. brumale var. *brumale* *T. niveum**	Endoperidium pale; similar morphology and colour of the stem; spores verrucose; identical morphology and shape of transverse septa.	Peristome elongated and tubular in *T. niveum*, without a dark brown zone which is often present in *T. brumale* var. *brumale*.
T. fimbriatum *T. cyclophorum**	Mouth fimbriate, fibrillose; endoperidium and stem morphology.	Spores ornamentation appears as coalesced crests forming a distinct net over their entire surface in *T. cyclophorum* under SEM. Ornamentation appears as dense, uncoalesced verrucae in *T. fimbriatum* under SEM.

* based on: Pouzar (1958); Wright (1987); Moreno (1990); Jeppson (2005); Jordal and Johansen (2009)

also differ by the colour of the stem. The stem is intensively dark brown or dark chestnut in *T. melanocyclum* unlike *T. brumale* var. *brumale* which is distinguished by a light brown stem. They differ radically by spore size, morphology, shape and ornamentation. Spores in *T. melanocyclum* are larger than in *T. brumale* var. *brumale* and their ornamentation is visible not as verrucae but as small spikes appearing as coalesced columns (Fig. 4 A, B).

Another interesting species is *T. simulans*. The name refers to the fungus simulating other species. According to Wright (1987) the species can be confused with *T. brumale* var. *brumale*. However, only slight similarity between *T. brumale* var. *brumale* and *T. simulans* was observed in our studies. Both species may be mistaken due to similar microscopic features: verrucose spores and thickened transverse septa. The external structure allows the two species to be distinguished as appropriate.

More distinct morphological similarity was observed between fruitbodies of *T. simulans* and those of *T. squamosum*. The endoperidium in both *T. squamosum* and *T. simulans* is light ochraceous or light brown and both have a characteristic stem. The stem is covered with detached squamules in the two species; *T. simulans* differs by a light brown stem while it is brown reddish in *T. squamosum* (Fig. 5).

Another problem arose during the ongoing process of identification of *Tulostoma* sp. Fruitbodies initially determined as *Tulostoma brumale* var. *brumale* in the field are too large and too massive which is rarely observed in this species. The endoperidium ochraceous-coloured at base and a pale stem with distinct pale squamules were not consistent with the diagnosis of the species. SEM revealed high similarity to spores of *Tulostoma giovanellae*. The ornamentation appeared as tiny verrucae, fusing and forming fine chains especially conspicuous towards the apex (Fig. 6A, B).

This feature is not observed in spores in *T. brumale* var. *brumale*, but, as literature data show (Wright 1987; Altés et al. 1999), it is characteristic of *T. giovanellae* spores. These authors also report that capillitial threads are an important diagnostic trait that makes misdetermination of the species impossible. The walls of capillitial threads are conspicuously thick and they are distinctly irregularly undulate. This feature was observed in our studies; however, only individual threads were undulate while the majority were straight and thin-walled, like most species discussed here (Fig. 7). It is uncertain whether this variability of capillitial threads is probable for the species and further studies are required.

Although the comparative material of *Tulostoma brumale* and *T. fimbriatum* available for our study was quite extensive, intraspecific taxonomic identification of these taxa was very difficult due to high differentiation of episporium ornamentation, spore shape and size, and the morphology of capillitial threads. Co-occurring spores collected in a single sample of the spore mass of *T. brumale* differed by size, shape and ornamentation (Fig. 8). Great differentiation was also observed in capillitial threads. Individual hyaline threads were distinguished not only by thickness but also by transverse septa (Fig. 9). The species also varied considerably by external features of the fruitbodies. Some fruitbodies differed by the shape, size and colour of the endoperidium and the stem, peristome morphology, and the persistence and pigmentation of the exoperidium.

Two varieties of *Tulostoma brumale* were distinguished based on the variability and differentiation of the fruitbodies. In his world monograph of the genus *Tulostoma*, Wright (1987) lists a typical variety *Tulostoma brumale* var. *brumale* and two other varieties: *Tulostoma brumale* var. *pallidum* and *Tulostoma brumale* var. *longipes* exhibiting a long stem.

Three varieties of the species were distinguished in our material. Fruitbodies lacking dark pigmentation around the peristome mouth, characteristic of the typical variety *T. brumale* var. *brumale*, and having a lightly pigmented endoperidium and stem were recognized as *T. brumale* var. *pallidum*. Spores of this variety appear under SEM as rather thick verrucae and the external surface of capillitial threads is not covered with crystals which are present in *T. brumale* var. *brumale*. Fruitbodies having a long stem growing over 45 mm with spore ornamentation visible as low and fine verrucae were identified as the variety *T. brumale* var. *longipes*. Morphological features observed for lower taxa of the species agreed with the descriptions of the varieties of *T. brumale* var. *brumale* reported by Wright (1987).

Intraspecific differentiation of *Tulostoma fimbriatum* was approached in a similar way. The typical variety, *T. fimbriatum* var. *fimbriatum*, as well as a new variety, previously not reported in the Polish mycological literature and described by Moreno (1980), *T. fimbriatum* var. *campestre*, were observed in our studies. They mostly differed by the size and stoutness of the fruitbodies, the morphology, shape and colour of the stem, and, less importantly, the size and colour of the peridium. Fruitbodies of *T. fimbriatum* var. *fimbriatum* were conspicuously smaller and more delicate, which is rarely observed in *T. fimbriatum* var. *campestre*. The stem in *T. fimbriatum* var. *fimbriatum* is slender and can reach a maximum length of 50 mm, with the most usual colour dark brown, while the stem length can reach even 100 mm in *T. fimbriatum* var. *campestre*. It also forms a bulbous ending at base. *T. fimbriatum* var. *campestre* also differs from the typical variety by being slightly light brown.

FINAL REMARKS

Two species, *Tulostoma brumale* and *T. fimbriatum*, are especially interesting. Although they have been reported from relatively many sites in Poland and the comparative material is rich, their taxonomic interpretation is not clear. Further investigations into intraspecific differentiation of these fungi would help overcome problems arising during the determination of these taxa. Only the competent identification of their features at the level of macro- and micromorphological decreases the doubts of proper taxa identification, and makes it possible to correctly identify the species at the levels of intra- and interspecific. This in turn, gives the possibility to present the list of taxa in the complete taxonomic aspect, and results in giving signal to the presence of new species and lower taxa, which are extremely rare and valuable fungi of Polish mycobiota.

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Uwagi taksonomiczne do krajowych gatunków z rodzaju *Tulostoma*

Streszczenie

Wyniki przedstawione w niniejszej pracy są wstępnym rezultatem badań mikologicznych, prowadzonych w siedliskach kserotermicznych w Niecce Nidziańskiej i Okręgu Chęcińskim. Praca podkreśla główne trudności związane z oznaczaniem taksonów z rodzaju *Tulostoma*, wskazuje na ich przyczyny oraz przedstawia propozycję rozwiązań problemów pojawiających się w trakcie oznaczania taksonów. Trudności mnożą się zwłaszcza, gdy dysponujemy coraz większą liczbą okazów, a różnice określonych cech zaczynają się pogłębiać. Jedną z takich propozycji są klucze do identyfikacji gatunków według cech makro- i mikroskopowych. Zawarte w kluczach opisy cech budowy makro- oraz mikromorfologicznej odnoszące się do niższych od gatunku taksonów, dają możliwości poprawnego ich oznaczenia w pełnym aspekcie taksonomicznym.