

# Study of two fungal species of *Tulostoma* genus encountered for the first time in Morocco: *Tulostoma melanocyclus* Bres. and *Tulostoma kotlabae* Pouzar

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**Abstract**— In Morocco, the works on mushrooms are rare and no complete list that lists the species in a given region is not yet available. Surveys in the mobile dunes of Mehdia (North West) and Tifnit (South West Morocco) have allowed us to determine for the first time in Morocco two species of the genus *Tulostoma*, *Tulostoma melanocyclus* Bres., (1904) and *Tulostoma kotlabae* Pouzar (1958). This study is part of the contribution to the determination of the fungal diversity in Morocco that it remains incomplete.

**Keywords**— Mushrooms, Morocco, Mehdia, Tifnit, Dunes, *Tulostoma melanocyclus*, *Tulostoma kotlabae*.

## I. INTRODUCTION

The genus *Tulostoma* belongs to the Gasteromycetes, order Tulostomatales and family Tulostomataceae (Courtecuisse & Duhem, 2000; Sesli *et al.*, 2000; Gerault, 2005; Kirk, 2005; Karadelev *et al.*, 2006). However, some species of this genus are currently considered to have affinities with the family Agaricaceae (Agaricales, Agaricomycetidae, Agaricomycetes, Agaricomycotina, Basidiomycota, Fungi) (Kirk *et al.*, 2008).

The genus *Tulostoma* was proposed by Persoon (1794), its representatives have a worldwide distribution, with a common presence in temperate and sandy areas (Wright, 1987). According to Kirk *et al.*, (2008), this genus includes 267 species, characterized by stipitate basidiocarps (Courtecuisse, 2000), a stem inserted at the base of the endoperidium (Internal peridium) that opens at the top by a perforation, a well developed peristome (Roger, 1981; Romagnesi, 1995; Courtecuisse & Duhem, 2000 and Gerault, 2005), a septated capillitium (Baseia *et al.*, 2002) and verrucous spores.

In this work, two species of *Tulostoma*' genus (*Tulostoma melanocyclus* Bres. And *Tulostoma kotlabae* Pouzar) were studied. Similarly, the affinity of the Tulostomatales with the Agaricales was discussed.

## II. MATERIAL AND METHODS

Prospecting, carried out in the mobile dunes of Tifnit in August 2010 (Southwest of the Souss region) and Mehdia in January 2015 (North West of Morocco), allowed us to encounter two species of the genus *Tulostoma*. Specimens of these two species were collected and brought back to the laboratory.

The macroscopic descriptions are based on the morphological characteristics (shape, color, size, aspect, ...) as well as other peculiarities related to peridium and stipe (odor, flavor, ...). This study is completed by a microscopic description of the spores, capillitium and stipe. The dimensions of spores and capillitium are measured via a micrometer 10 × (18 mm) with a scale of 10 mm divided into 100 graduations (0.1 mm). Microscopic observations were made using an optical microscope (magnification × 400). The mounting liquid is tap water. The shape of the spores is obtained from the calculation of the quotient of Bas (1969) according to the following ratio,  $Q = \text{length (L)} / \text{width (l)}$ .

The identification of the species was carried out by consulting the work of Malençon (1952 & 1956); Malençon and Bertault (1955 to 1969); Roger (1981); Wright, (1987); Courtecuisse and Duhem (2000); Poumarat (2001 & 2003); Gerault (2005); Kirk *et al.*, (2008) and Outcoumit (2011).

## III. RESULTS

Two species (*Tulostoma melanocyclus* and *Tulostoma kotlabae*) are described for the first time in Morocco.

***Tulostoma melanocyclus*** Bres. (1904)

Terricultural species harvested on 28/01/2015 on sandy soil among the mosses under Juniper (*Juniperus phoenicea* L.) in the dunes of Mehdia.

**The head** is spheroidal, 1.2 cm in diameter, ochraceous white (Figure 1, A (1)). **The stipe** is very thin, 2.5 to 3 cm long and 0.3 cm wide, cylindrical, fistulose, its surface is brown ochraceous, with fine concolorous scales and whitish

color on the inside (Figure 1, A (3)). **The peristome** is placed in the center, circular 1 mm at margin a little toothed and surrounded by a brownish aureole (Figure 1, A (2)).

**The spores** are brownish, verrucous, globose to subglobose ( $1 < Q < 1.05$ ), of 4.66 to 6  $\mu\text{m}$  (Figure 1, D).

**The spore-print** is brownish. **The gleba** at maturity is converted into brownish dust (Figure 1, B). **The capillitium** is very thin, of 3.33  $\mu\text{m}$ , inflated at the level of bulkhead (9.99 $\mu\text{m}$ ), with presence of loops (Figure 1, C (1 and 2)).

***Tulostoma kotlabae*** Pouzar (1958)

Terricultural species harvested on 04/08/2010 on sandy soil in the dunes of Tifnit.

**The head** is spherical, from 1.5 cm to 2 cm in diameter, pale to ochraceous white. **The stipe** is hard and tenacious, thin, striped 5 to 6 cm long and 0.4 to 0.6 cm wide, cylindrical, fistulous, bulbous and whitish in color (Figure 2, A). **The peristome** is central, oval of 2 to 6 mm and of light color (Figure 2, B).

**The spores** are globose ( $1 < Q < 1.05$ ), slightly verrucous, measuring 4-5.5  $\mu\text{m}$  and light brown in color (Figure 2, E). **The spore-print** is rusty brown. The gleba becomes a brownish dust at maturity (Figure 2, D). **The capillitium**, thick-walled, is light brown, thin, 3.33-6.66  $\mu\text{m}$ , swollen at bulkheads, unfilled (Figure 2, F).

#### IV. DISCUSSION

In Morocco, Malençon harvested in 1952 and 1956 two species of the genus *Tulostoma*, *Tulostoma tortuosum* Ehrenb., (1829) and *Tulostoma volvulatum* Kalchbr., (1881) which have been preserved in the National Herbarium of the Rabat Scientific Institute (El-Assfoury *et al.*, 2003). The *Tulostoma* genus is represented by 5 species (*Tulostoma brumale* Pers. (1794), *Tulostoma campestre* Morgan (1889), *Tulostoma mammosum* P. Micheli ex Fr. (1829), *Tulostoma tortuosum* Ehrenb. (1829) and *Tulostoma volvulatum* Kalchbr. (1881), I.G. Borshch. (1865)). Malençon and Bertault have reported *Tulostoma campestre* Morgan, (1889) and *Tulostoma mammosum* P. Micheli ex Fr., (1829), *Tulostoma tortuosum* Ehrenb., (1829) in the 7<sup>th</sup> list of Fungi of the Rif (1961a) and *Tulostoma brumale* Pers., (1794) in the 5<sup>th</sup> list of Fungi of Tangier (from 1955 to 1969) Middle Atlas, (1967). Similarly, these species are also cited in other bibliographic works (El Kholfy *et al.*, 2011; Ouabbou *et al.*, 2012; Haimed *et al.*, 2013 and Outcoumit *et al.*, 2014). Otherwise, *Tulostoma brumale* Pers., (1794) is the only species of the genus *Tulostoma* which was described in Morocco (Middle Atlas) by Larouz (2007).

The genus *Tulostoma* is little studied in Morocco, this requires important studies to be carried out concerning the ecological requirement, the time of push and carpophores

distribution mode in different localities and regions of Morocco.

The species of *Tulostoma* genus present a great morphological similarity, origin of several taxonomic problems (Fries, 1921; Fischer 1900, 1933; Petri, 1909; Pouzar, 1958 ; Wright, 1987; Courtecuisse & Duhem, 2000; Sesli *et al.*, 2000; Esqueda *et al.*, 2004; Gerault, 2005; Karadelev *et al.*, 2006; Calonge, 2007 and Tomaszewska *et al.*, 2011). Otherwise, various taxonomic approaches have been adopted in monographic studies on *Tulostoma* species (Luszczynski, 2000; Calonge, 2007 and Tomaszewska *et al.*, 2011). The latter is subdivided initially into two sections (*Eutylostoma* and *Schizostoma*), distinguished by the morphology of the peristome (Petri 1909; Fries 1921; Fischer 1900, 1933). Pouzar (1958) subdivided the genus *Tulostoma* into four sections (*Brumalia*, *Poculata*, *Fimbriata* and *Volvulata*), differentiated on the basis of morphology, rupture of exoperidium, and morphology of peristome and stipe (Calonge, 2007 and Tomaszewska *et al.*, 2011).

Mycologists have reported that the differentiation between *Tulostoma brumale* var. *brumale* and *T. melanocyclum* is done on the basis of peristome characteristics (Fries 1921; Fischer, 1900, 1933; Petri, 1909; Pouzar, 1958 ; Wright, 1987). A peristome comparison of these two species showed that *Tulostoma brumale* var. *brumale* is characterized by a light colored peristome. By cons, *T. melanocyclum* presents a peristome surrounded by a brownish aureole (Tomaszewska *et al.*, 2011). This description of peristome is identical to that given to our species, *T. melanocyclum* (Figure 1, A (1)). The latter species is also mainly recognized by similar macroscopic characteristics with *Tulostoma brumale* Pers. : Pers, but with different microscopic characteristics: capillitium colorless lightly branched, with thickening around partitions (Esqueda *et al.*, 2004), and echinulate spores (5-6,5  $\mu\text{m}$  diam) with large spines fused at the apex which are similar to the spore ornamentation of *T. squamosum* (Luszczynski, 2000).

In the literature, we find *T. fimbriatum* Fr. 1829, with fimbriated peristome, plan or slightly elevated, *T. fimbriatum* var. *campestre* (Morgan) G. Moreno 1980, with flattened peristome and *T. fimbriatum* var. *heterosporum* J.E. Wright 1987, with peristome forming a bead (Cheype, 2014).

Similarly, *Tulostoma brumale* var. *brumale* and *T. kotlabae* have very similar characteristics (Pale, white, beige or ochraceous endoperidium, yellow to light brown to squamous stipe). However, *T. kotlabae* is characterized by significantly smaller spores with small warts, capillitium without crystals and bulkheads not widened. By cons, *T. brumale* var. *brumale* Developed warts spores,

crystallized capillitium, and enlarged bulkheads (Tomaszewska *et al.*, 2011).

## V. CONCLUSION

Species of the genus *Tulostoma* are secotioides, that is to say, morphogenetic and ontogenetic type corresponding to gasteroides species manifest affinity to Agaricomycetideae (Courtecuisse and Duhem, 2000). They are characterized by the presence of a stipe, the glebe, the capillitium and the release of the spores is effected by an active action (Bessey, 1952 et Reijnders, 2000). The biomolecular studies recently carried out on the secotioid species have transferred these taxa according to their anatomical affinities with the Agaricales in the Agaricomycetideae (Moncalvo *et al.*, 2002 ; Justo *et al.*, 2004 and Vellinga, 2004).

The two species studied *Tulostoma kotlabae*, harvested in August 2010 on sandy soil in the dunes of Tifnit (Northeast of the central plateau) And *Tulostoma melanocyclus*, encountered under *Juniperus* (*Juniperus phoenicea* L.) in the dunes of Mehdiya in January 2015, were described for the first time in Morocco.

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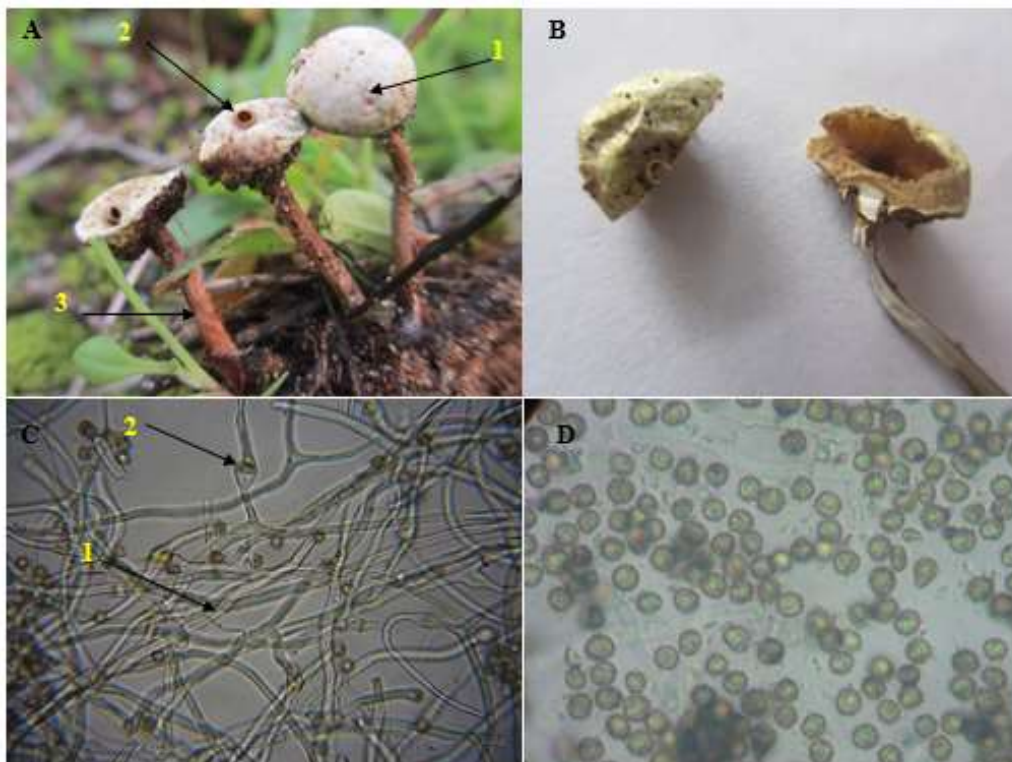


Fig.1: (1) Basidiocarps, (2) Peristome circular and (3) Stipe (A), Gleba and fistulous Stipe (B), (1) Capillitium (2) Knots (C) and Spores (D) of *Tulostoma melanocyclum*.

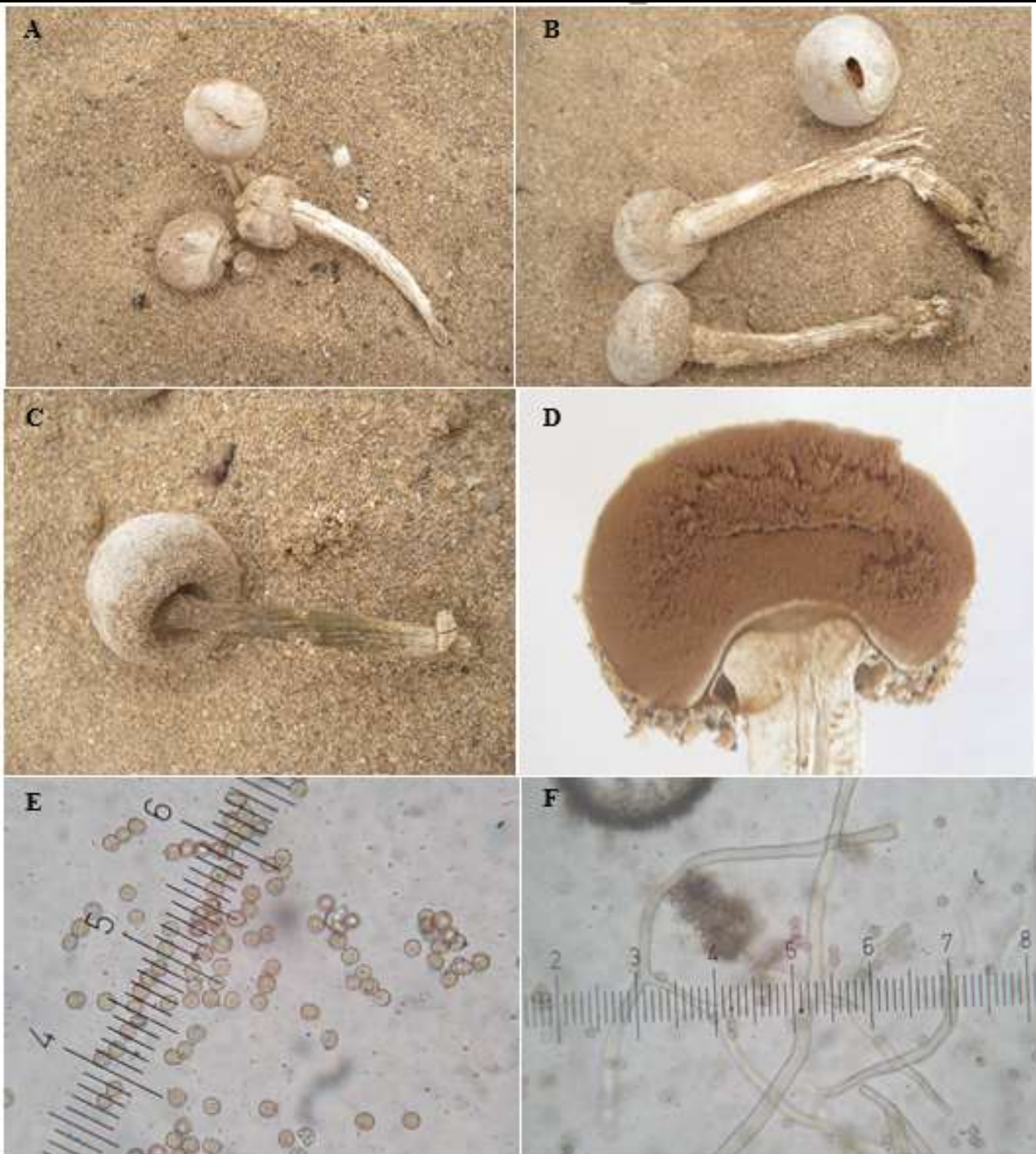


Fig.2: Basidiocarpes and stipe (A), Peristome (B), Insertion of the stipe (C), Gleba (D), Spores (E) and Capillitium (F) of *Tulostoma kotlabae*.