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RESEARCH ARTICLE

STUDY OF SOME NEW SPECIES OF THE *INOCYBE* GENUS WITH GIBBOUS SPORE FOR THE MOROCCO'S FUNGAL FLORA

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The surveys between October 2010 and February 2011 have allowed us to identify five species of Inocybe (Fr.) Fr. 1863, four of them are new to the fungal flora of Morocco: Inocybe tigrina, I. rhodella, I. pallida and I. umbrina. Inocybe margaritispora was encountered for the first time in the forest of Mamora

ABSTRACT

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INTRODUCTION

The Inocybe genera presently belong to the Inocybaceae (Agaricales, Basidiomycota) (Cannon and Kirk, 2007), was first recognized as a distinct tribe of Agaricus by Fries (1821). This genus, is the larger of the two genera in the family and is one of the largest in Agaricales (Kirk et al., 2008). It is a highly diverse monophyletic group of ectomycorrhizal fungi that comprises between 500 and 700 species worldwide, but the number continues to increase due to new discoveries (Bresadola, 1980; Kirk et al., 2008; Matheny et al., 2009; Alvarado et al., 2010). The Inocybe species can be recognized by the following characters: small to medium-sized basidioma with a conical shape; spermatic, earthy, bitter almond, pelargonium, or fruit-like smell; radially fibrous to cracked cap; tobacco brown, smooth-walled, and lumpy-angular basidiospores (Solak et al., 2009). The Inocybe (Fr.) Fr. 1863 and Auritella Matheny & Bougher 2006 are currently placed in the family Inocybaceae (Matheny, 2005; Matheny et al., 2009.). However, Inocybe is often placed in the Cortinariaceae family (Matheny and Bougher, 2006). The molecular phylogenetic studies have shown that Inocybaceae is a sister clade of the Crepidotaceae family (Matheny 2005, Matheny et al., 2006). Matheny et al., (2006) also suggest that the ectomycorrhizal habit of Inocybaceae is one of several independent transformations to this nutritional habit and one that significantly separates it from Crepidotaceae.

In this study, new species of the Inocybe genus were identified.

MATERIELS ET METHODES

The surveys, carried out in the forest of Mamora's cork oak (Northwestern Morocco) between October 2010 and February 2011 have allowed us to identify five species of Inocybe. The specimens of these species were collected and brought to the laboratory. The macroscopic descriptions of the carpophores were focused on morphological characters (shape, color, size,

appearance...) and other features related to the pileus and stipe (smell, taste ...). This description is supplemented by a microscopic description of sporae and cuts at the level of the hymenium, cuticla, flesh and stipe. The dimensions of the basidiospores, cystidia , basidia and sometimes sterigmata are measured via a micrometric eyepiece large field $10 \times (18 \text{mm})$ to 10 mm divided scale graduations $100 \ (0.1 \ \text{mm})$. The microscopic observations have been realized using an optical microscope (magnification \times 400). The spore shape is obtained from the quotient calculation Bas (1969) in the following ratio, Q = length (L) / width (l). The mounting liquid was the rainny water.

The identification of species was based on the works of Malençon and Bertault (1970), Bon (1997-1998), and the identification keys of Courtecuisse and Duhem (2000) and of Lachapelle (2002).

RESULTATS

Five species of the Inocybe genus (Inocybe tigrina, I. rhodella, I. pallida, I. umbrina and I. margaritispora) were harvested on Stenotaphrum secundatum and under Quercus suber in the forest of Mamora (Northwestern of Morocco).

Inocybe tigrina R. Heim (1931).

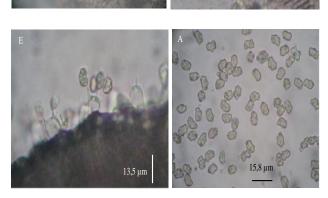
Humicolous species harvested on 12/20/2010 on Stenotaphrum secondatum in gardens of the Faculty of Sciences of Kenitra and under Quercus suber in the forest of Mamora.

The pileus (4.5 to 5 cm in diameter) is circular, convex to plano convex, hilly, squamulo-tabby, beige-yellowish brown and has a brownish cap. The flesh is more or less thin and beige to light brown. The margin is ridged and curved. The stipe $(3.5 \times 0.2 \text{ cm})$ is cylindrical, central, fibrillose, solid and beige. The lamellae are tight decurrent, unequal and beige-olive color, red to the margin. The lamellar edge is smooth (Figure 1 A).

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The basidia (33.3 \times 8.3 μ m) are hyaline, clavate and tetrasporic. The sterigmata measures 6.6 μ m (Figure 1 E). The basidiospores (6.6-7.9 \times 3.9-5.3 μ m) are gibbous, elliptical and present six to eight bumps (Figure 1 F). The pleurocystidia (66.6 \times 9.9 μ m) are closely fusiform, thick-walled, hyaline, cylindrical bottom and top attenuated (Figure 1 C and D) The weft is regular and parallel (Figure 1, B).





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Figure 1: Pileus and hymenophore (A), weft (B), pleurocystidia (C and D), basidia (E basidiospores (F) of *Inocybe tigrina*. (×400).

Inocybe rhodella Matheny, Aime & M.E. Sm. (2012).

Humicolous species harvested on 11/08/2010 under the Quercus suber in forest of Mamora

The pileus (2.5-3 cm) is circular, convex to plano-convex, cobblestone, and brown. The flesh is more or less thin and whitish. The margin is ridged and straight. The stipe (6×0.4 cm) is cylindrical, central, fibrillose, full color and reddish powder on top. The lamellae are adnate, crowded, unequal and brown-olive color. The lamellar edge is smooth and whitish (Figure 2, A and B).

The basidia (33.3×6.6 μm) are hyaline, clavate and tetrasporic. The sterigmata measures on 2.3 μm . The basidiospores (9-11 \times 6-6.6 μm) are gibbous, ellipsoids and present six to ten bumps (Figure 2 D).The pleurocystidia (80 \times 13.3 μm) are closely fusoïdes, subcylindrical, hyaline by crystals in the top and crown thick-walled (Figure 2 C).

Inocybe pallida Velen. (1920).

Humicolous species harvested on 10/20/2010 on Stenotaphrum

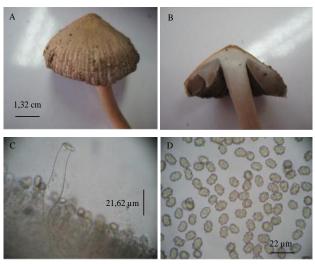


Figure 2: Pileus (A), longitudinal section of the fruiting body (B), pleurocystidia (C) basidiospores (D) of *Inocybe rhodella*. (× 400).

secondatum in gardens of the Faculty of Sciences of Kenitra and under Quercus suber in the forest of Mamora.

The pileus (2.5-5 cm) is circular, convex to plano convex, hilly and spiky white to beige. The flesh is more or less thick in the center and thin whitish to the margin. The margin is smooth and wavy. The stipe (3.5-4.5 \times 0.4 -0.6 cm) is cylindrical, central, and fibrous, full bulbous more or less notched and white to pinkish reflection. The lamellae are adnate, crowded, unequal and beige-olive color. The lamellar edge is smooth and whitish (Figure 3 A and B).

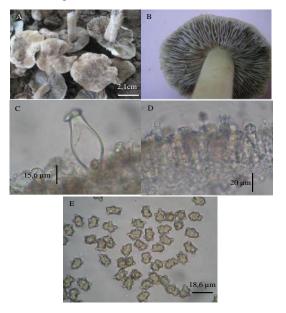


Figure 3: Pileus (A) lamellae insertion (B), pleurocystidia (C) basidia (D) basidiospores (E) of *Inocybe pallida*. (×400).

The basidia ($40\times10~\mu m$) are hyaline, clavate and tetrasporic. The sterigmata measures 4.3 μm (Figure 3, D). The basidiospores ($6.6\text{-}9.3\times5.3\text{-}6.6~\mu m$) are gibbous, ellipsoids and present six to ten bumps (Figure 3 E). The pleurocystidia ($63.3\times16.6~\mu m$) are closely fusoides, subcylindrical, hyaline, and obese in the center, crowned top of crystals and thick wall (Figure 3, C).

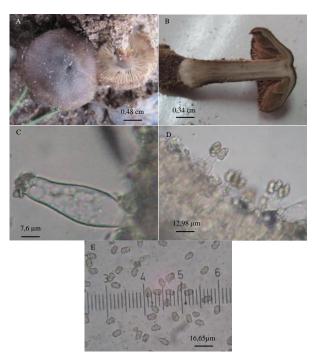


Figure 4: Pileus and lamellae insertion blades (A), the longitudinal section of carpophore (B), pleurocystidia (C) basidia (D) basidiospores (E) of *Inocybe umbrina*. (× 400).

Inocybe umbrina Bres. (1884) and Massee (1914)

Humicolous species harvested on 02/02/2011 and under Quercus suber in forest of Mamora.

The pileus (1.5-2 cm) is circular, convex to plano convex, hilly, streaked and dark brown. The flesh is more or less thin and white-beige. The margin is ridged and curled. The stipe (2 \times 0.5 cm) is cylindrical, central, fascicle (2 to 3 stipes) fiber, and full of light brown color. The lamellae are adnate, crowded, unequal and brown-olive color. The lamellar edge is smooth and whitish (Figure 4 A and B).

The basidia (30 \times 6.6 $\mu m)$ are hyaline, clavate and tetrasporic. The sterigmata measures 4 μm (Figure 4, D). The basidiospores (6.6-8.3 \times 5-3.3 $\mu m)$ are sub-gibbous, ellipsoids and wear four to six bumps (Fig. 4, E). The pleurocystidia (53.3 \times 11.6 $\mu m)$ are closely fusoïdes, subcylindrical, hyaline by crystals in the top and crown thick-walled (Figure 4, C).

Inocybe margaritispora (Berk.) Sacc. (1887).

Humicolous species harvested on 02/02/2011 and under Quercus suber in the forest of Mamora.

The pileus (3 cm) is circular, convex to plano convex, cobblestone, and brown striated. The flesh is more or less thin and beige-olive. The margin is simple and straight. The stipe $(2 \times 0.5 \text{ cm})$ is cylindrical, central, fibrous, sinus, bulbous at the base and light brown. The lamellae are notched adnate, crowded, unequal, broad and brown-olive color. The lamellar edge is smooth and whitish (Figure 5 A and B).

The basidia (33.3 $\times 8.3~\mu m$) are hyaline, clavate and tetrasporic. The sterigmata measures 4 μm (Figure 5 D). The pleurocystidia (76.6 \times 11.6-16.6 μm) are closely fusoïdes, subcylindrical, hyaline crystals in the top and crown thickwalled (Figure 5, C). The basidiospores (6-10 \times 5-6.6 μm) are sub-gibbous, ellipsoids and present four and six bumps (Figure 5 E).

DISCUSSION

The classification of Inocybe genus is primarily based on the morphology of spores, the shape and distribution of cystidia and morphology of the stipe. The spores can be ellipsoids, wavy. The pleurocystidia amygdaliformes or cheilocystidia have a thick wall. The stipe may be uniform or have a significantly bulbous base (Heim 1931; Kuhner & Romagnesi, 1953; Kuhner, 1980; Kuyper, 1986; Stangl, 1989; Kobayashi, 2002). Ecological, anatomical, and molecular evidence suggest that Inocybe is ectomycorrhizal and symbiotic with numerous families of angiosperms and gymnosperms such as the Betulaceae, Casuarinaceae, Cistaceae, Dipterocarpaceae, Fabaceae, Fagaceae, Myrtaceae, Nothofagaceae, Pinaceae, Salicaceae and Uapacaceae among other families (Agerer, 1987-1998; Glen et al., 2001. Horak, 1977, 1980; Kuyper, 1986; Matheny and Watling, 2004; Matheny et al., 2003. Singer, 1986). The Inocybe genus is often divided into three sub genera (Inocybe (Fr.) Fr. 1863, Inosperma Kuhner and Mallocybe Kuyper) (Kuyper 1986; Stangl, 1989), sometimes unofficially treated as genera (for example Mallocybe in GenBank taxonomy, Benson et al., 2009) (Ryberg, 2009). The Mallocybe and Inosperma are recognized by smooth spores and lack of metuloides yet the Inocybe is recognized by smooth or gibbous spores and cystidia metuloides (Courtecuisse & Duhem, 2000).

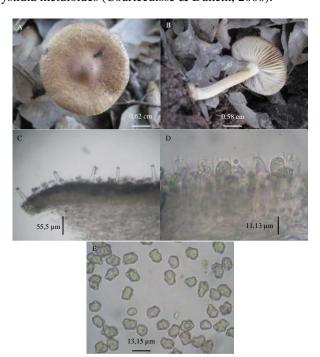


Figure 5: Pileus (A), stipe and lamellae insertion (B), pleurocystidia (C) basidia (D), basidiospores (E) of *Inocybe margaritispora*. (× 400)

CONCLUSION

In Morocco the Inocybe genus is fairly well represented (Malençon and Bertault, 1970). The Species of Inocybe tigrina, I. rhodella I. pallida and I. umbrina are newly described for the fungal flora of Morocco. While Inocybe margaritispora has been described and encountered in Tangier and Larache under Quercus suber by Malençon and Bertault in 1970.

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