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**Study of some new species of the *Pluteus* genera for the fungal flora of Morocco**

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**ABSTRACT**

Seven species of the *Pluteus* genera were encountered in the forest of Mamora (North Western Morocco), including four new species to the fungal flora of Morocco: *Pluteus luctuosus*, *P. depaupreratus*, *P. podospileus*, and *P. griseoluridus*. Two species, reported by Malençon and Bertault in 1970, have been described for the first time: *Pluteus pellitus* and *Pluteus satur*. *Pluteus romellii* (= *Pluteus lutescens*), a common species, was described by Malençon and Bertault (1970) and Outcounit (2011).

**Keywords:** Morocco, Mamora, *Pluteus*, fungal flora.

**INTRODUCTION**

The family *Pluteaceae* Kotl. & Pouzar (Agaricales, Basidiomycota) includes three genera: the *Pluteus* Fr. without both annulus and volva, the *Chamaeota* (W.G.Sm.) with annulus but without volva and *Volvariella* (Speg.) with volva but without annulus<sup>1,16,21</sup>. Some *Chamaeota* species have been transferred into *Pluteus*<sup>16</sup>.

The *Pluteus* genera, created by Fries in 1836<sup>20</sup>, belongs to the order of *Pluteales*, characterized by free and pinkish lamellae with an inverse bilateral trama, stipe without volva and mostly exannulate, smooth and pinkish spores<sup>3,8,15,17,21</sup>. It comprises about 300 species<sup>11</sup> and is distributed in all continents except Antarctica<sup>21</sup>. Species of *Pluteus* are fleshy and can meet throughout the year if conditions are favorable, on rotting stumps, dead branches and old pile of sawdust<sup>14,17,21,22</sup>. These genera are not saprotrophic and presumably never mycorrhizal<sup>17</sup>. A number of *Pluteus* species can with practice be identified in the field. There is however a group of small brown-capped species that can only be reliably separated by reference to their microscopic features<sup>12</sup>.

In Morocco, Malençon and Bertault<sup>13</sup> are reported sixteen species, of which nine are a description (*Pluteus chrysophaeus*, *P. cinereofuscus*, *P. cinereus*, *P. cyanopus*, *P. hiatulus*, *P. lutescens*, *P. minutissimus*, *P. and patricius* *P. salicinus*) and are mentioned only six (*P. cervinus*, *P. leoninus*, *P. marginatus*, *P. pellitus*, *P. saturated*, *P. semibulbosus*). El-Assfoury *et al.* are reported for the first time, in the forest of Mamora, *Pluteus petasatus* in 2003 and *Pluteus cervinus* in 2006.

In this study seven species of the *Pluteus* genera encountered in the forest of Mamora (northwestern Morocco), were studied.

**MATERIALS AND METHODS**

The surveys carried out in the cork oak forest of Mamora (North western Morocco) from winter to spring in 2009, 2010, 2011 and 2012, enabled us to encounter the *Pluteus* genera. The specimens of these species were collected and brought to the laboratory. The macroscopic descriptions of carpophores 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 spores and cuts at the level of the hymenium, cuticula, flesh and stipe. The dimensions of the basidiospores, cystidia, basidia and sometimes sterigmata are measured via a micrometric eyepiece large field 10 × (18 mm) to 10 mm divided scale graduations 100 (0.1 mm). The microscopic observations have been

realized using an optical microscope (magnification  $\times 400$ ). The mounting liquid was the rainy water. The identification of species was based on work of Courtecuisse & Duhem<sup>6</sup>, Malençon & Bertault<sup>13</sup> and Roger<sup>19</sup>.

## RESULTS

The seven species encountered in the forest of Mamora, have been integrated into three sections. The species of section *Pluteus* characterized by pleurocystidia typically divided into hooks, while those sections *Celluloderma* and *Hispidoderma* have thin-walled pleurocystidia generally smooth and undivided in hooks<sup>5</sup>.

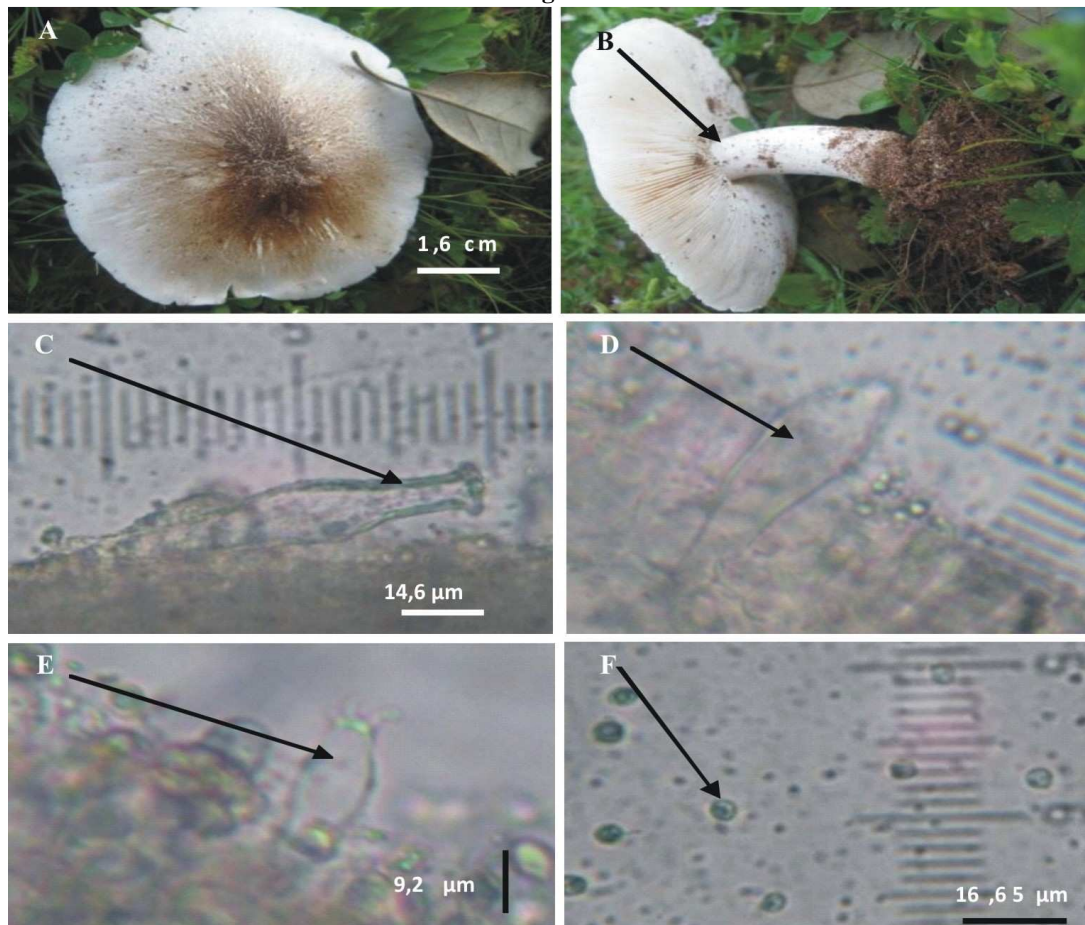
### Pluteus Section

*Pluteus pellitus* (Pers.) P. Kumm, 1871. Harvested on 03/15/2009 in the forest of Mamora on land and dead trunks of *Quercus suber*.

The pileus (cap) (7-8 cm) convex to plano-convex, finely silky brown in the center and dark greyish-white. The flesh is white, soft, thick in the center and tapered toward the margin. The margin is straight and smooth. The pileipellis is filamentous. The stipe (4.5-5 x 0.6 cm) is white to the base, central, equal, arched, and presents full rhizoids at the base. The lamellae are tight, slim, distant, unequal, and white become pink with age. The lamellar edge is simple and white (Figure 1A and B).

Basidia (36 x 6  $\mu\text{m}$ ) are ovoid and fusiform, narrow tetrastrigmate (Figure 1 E). Basidiospores (6-8 x 4.5-5  $\mu\text{m}$ ) are ellipsoid ( $1.3 < Q < 1.6$ ) and subhyaline to very pale yellow (Figure 1, F). The pleurocystidia (73 x 13  $\mu\text{m}$ ) are fusiform, thickened and crowned with hooks, some are thin-walled and acute apex without brackets (Figure 1, C). The cheilocystidia (56.6 x 10  $\mu\text{m}$ ) are pyriform to sphaeropedunculate (Figure 1, D). The pileipellis is filamentous filled with intracellular pigment. The clamp connection is present.

Fig. 1



Pileus (A), lamellae insertion and stipe (B), pleurocystidia(C), cheilocystidia (D), basidia (E) and basidiospores (F) of *Pluteus pellitus*

## Section Celluloderma Fayod

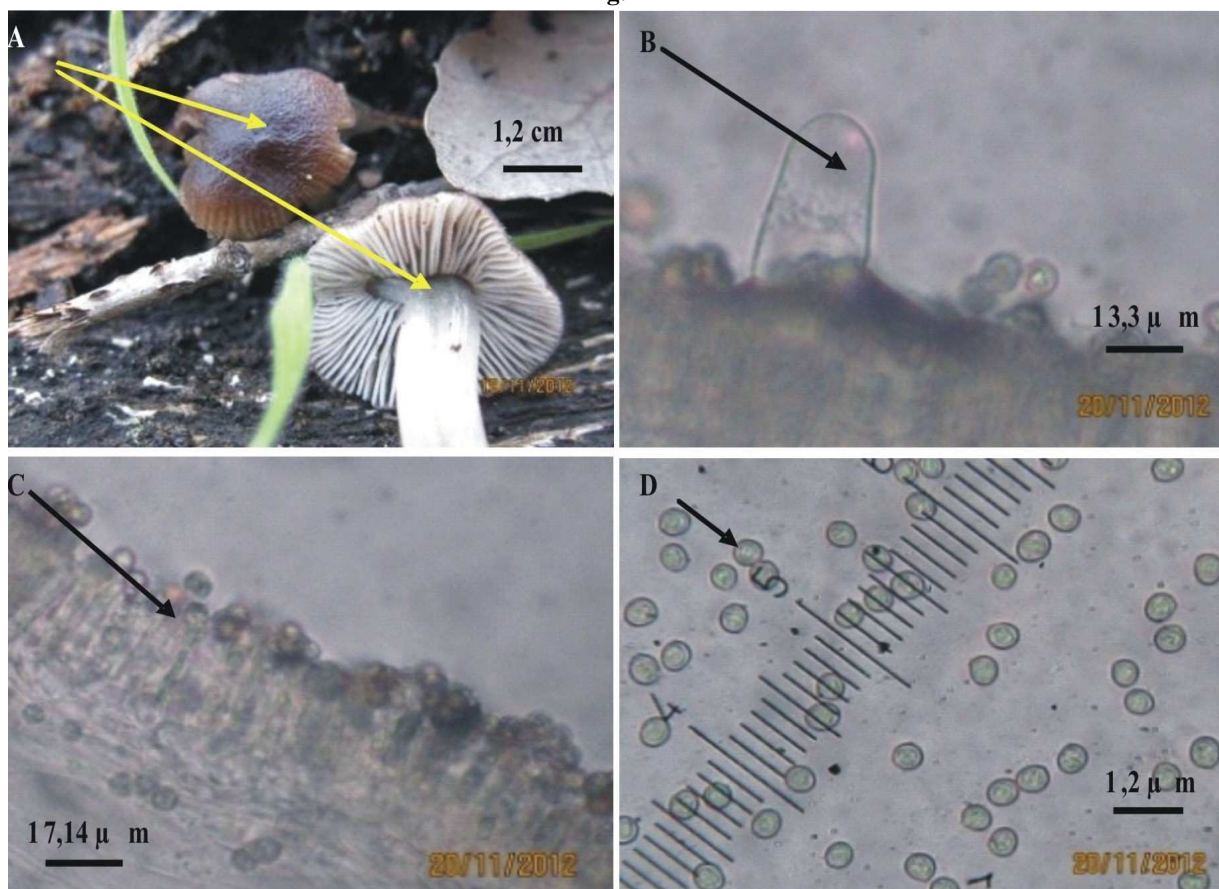
## Subsection Eucellulodermi Singer ex Singer

*Pluteus satur* Kuhner & Romagnesi 1956, harvested on 11/19/2012 in the forest of Mamora on dead trunks of *Quercus suber*.

The pileus (1-2 cm) is dark brown, circular, plano-convex and has labyrinthine. Flesh is grayish white, thinner in the center and tapered toward the margin. The margin is straight and serrated. The pileipellis is smooth. The stipe (2 x 0.2 cm) is sub-cylindrical, central, fistula and greyish white. The lamellae are uneven, tight, distant, stubby and pink beige. The lamellar edge is smooth and whitish (Figure 2, A).

The basidia (30 x 8.3  $\mu$ m) are clavate, cylindrical, tetrasterigmate (Figure 2, C). The basidiospores (5-6.6 x 4.3-5  $\mu$ m) are hyaline, smooth thick walled, subglobose ( $1.05 < Q < 1.15$ ) and have lipid droplets (Figure 2, D). The pleurocystidia are clavate, obese (60 x 23.3  $\mu$ m) or cylindrical (50 x 18.3  $\mu$ m), and hyaline thinner wall (Figure 2 B).

Fig.2



Pileus (A), lamellae insertion and stipe (B), pleurocystidia (C), basidia (D), and basidiospores (E) of *Pluteus satur*

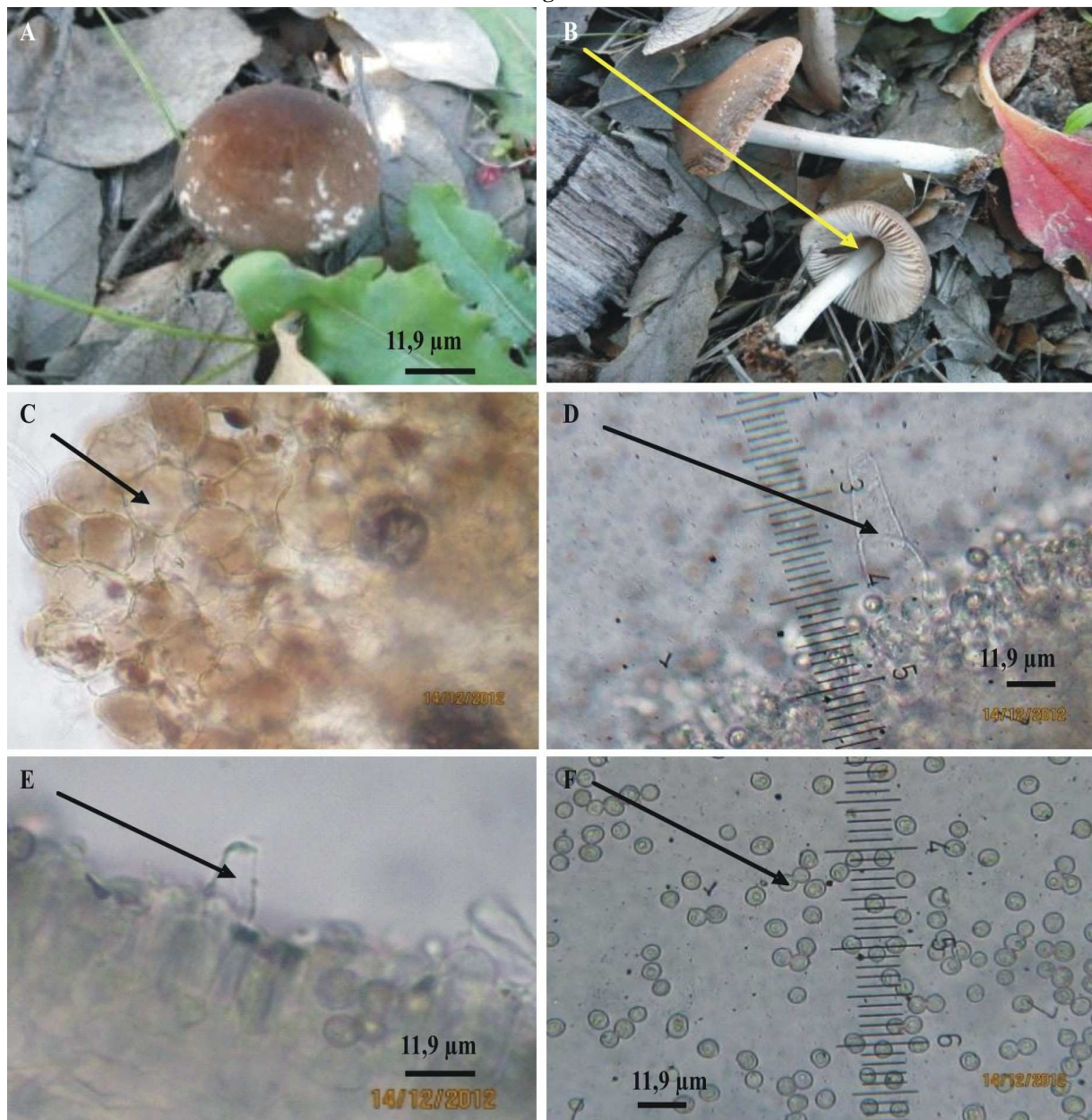
*Pluteus griseoluridus* P.D Orton 1984 harvested on 12/13/2012 in forest of Mamora on dead trunks of *Quercus suber*.

The pileus (1-2 cm) is dark brown at the center, plano-convex and cracked gray, circular and brown. The flesh is thin and whitish. The margin is simple and smooth. The pileipellis is smooth. The stipe (2.5 x 0.25 cm) is wide, hail, cylindrical, central, full, fibrillose and grayish white. The lamellae are uneven, tight, free, bulging and pinkish color. The lamellar edge is smooth and white (Figure 3, A, B and C).

The basidia (33.3 x 8.3  $\mu$ m) are clavate, cylindrical, tetrasterigmate (Figure 3 E). The pleurocystidia (80 x 10-16.6  $\mu$ m) are spindle-shaped, the center of obese, hyaline and thinner wall (Figure 3, D). The basidiospores (5-6.6 x 4.3-5  $\mu$ m) are hyaline, smooth, thick walled, and subglobose ( $1.05 < Q < 1.15$ ) (Figure 3, F).



Fig.3



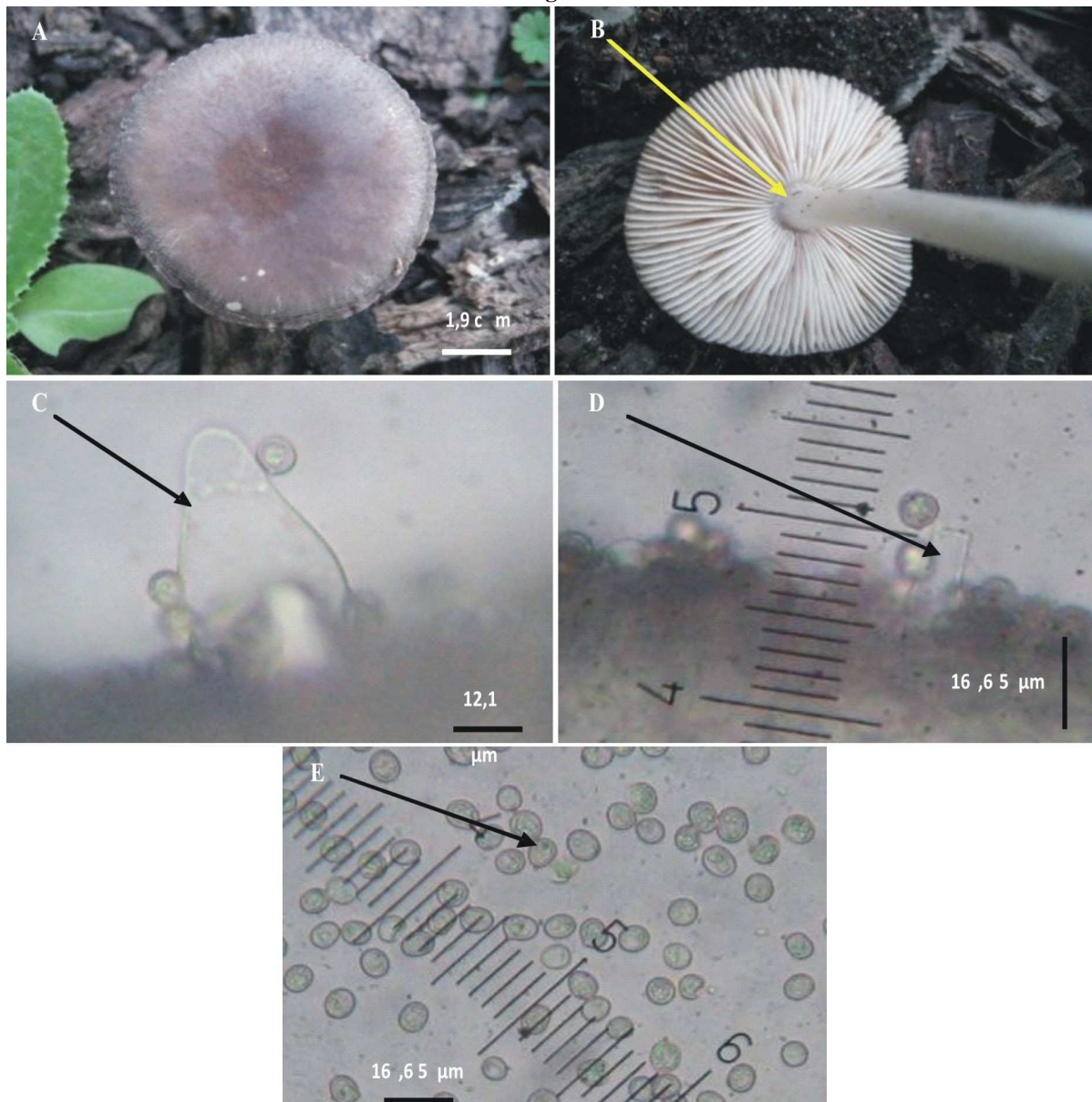
Pileus (A), lamellae insertion and stipe (B), pileipellis cellular (C), pleurocystidia (D), basidia (E) and basidiospores (F) of *Pluteus griseoluridus*

*Pluteus luctuosus* Boudier 1905 harvested on 11/22/2010 in the forest of Mamora on dead trunks of *Quercus suber*.

The pileus (2.5 cm) is dark brown to ochraceous, circular, smooth, plano-convex, brown and with cracks at the center. The flesh is more or less thin and white. The margin is straight, wavy and slightly ridged. The pileipellis is smooth. The stipe (4.5 x 0.4 cm) is cylindric, central, right in sinus fibrous and whitish. The lamellae are unequal, less crowded, free, bulging and pinkish color. The lamellar edge is whitish (Figure 4, A and B).

The basidia (34.9 x 6.6 µm) are clavate, close to the base, tetrasterigmate (Figure 4 D). The pleurocystidia (72.5 x 14.3 µm) are lageniformia, obese, hyaline to narrow top wall and thinner (Figure 4, C). Basidiospores (6.66-7.6 x 5-5.6 µm) are hyaline, smooth thick walled and broadly elliptical ( $1.15 < Q < 1.3$ ) (Figure 4, E).

Fig.4



Pileus (A), lamellae insertion and stipe (B), pleurocystidia (C), basidia (D) and basidiospores (E) of *Pluteus luctuosus*

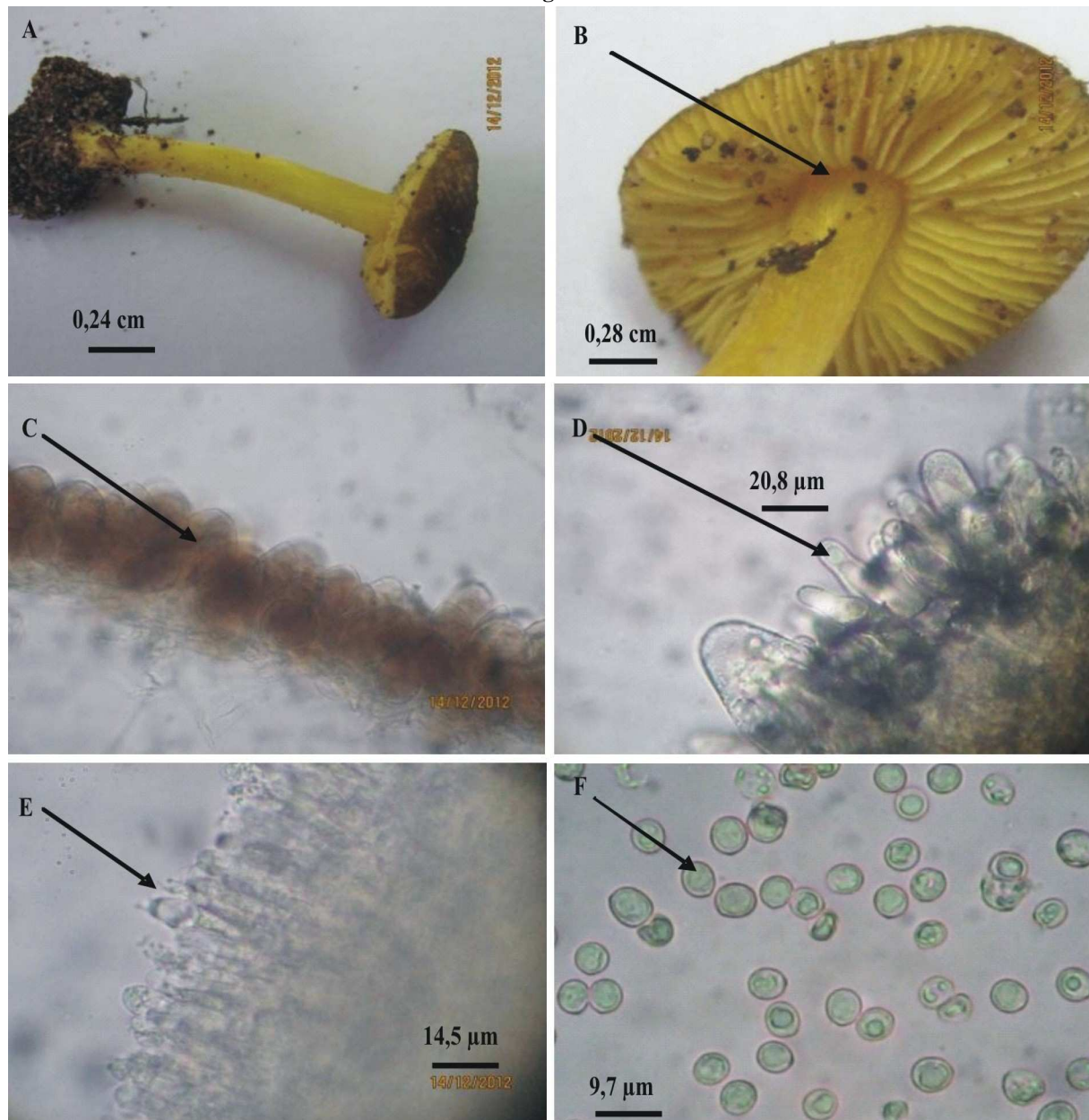
*Pluteus romellii* (Britzelm.) Sacc. 1895. Harvested on 12/13/2012 in the forest of Mamora on dead trunks of *Quercus suber*.

The pileus (1.2 cm) is dark brown in the center on a yellow background, circular, plano-convex brown, with and smooth ridge at the center. The flesh is yellowish, thinner at the center and tapered toward the margin. The margin is straight and serrated. The pileipellis is smooth. The stipe (2.5 x 0.25 cm) is cylindrical, central, hollow, fibrillose and light yellow. The lamellae are uneven, tight, free, bulging and yellowish. The lamellar edge is smooth and white (Figure 5, A, B and C).

The basidia (33.3 x 6.66 μm) is clavate, cylindrical, tetrasterigmate (Figure 5, E). The pleurocystidia are lageniformia, obese (53.3-66.6 x 23.3-28.3 μm) or cylindrical (50 – 60 x 6.6-10 μm), hyaline and thinner wall (Figure 5, D). Basidiospores (5.4 x 5.4 μm) are hyaline, smooth, thick-walled and subglobose or globular ( $1 < Q < 1.05$ ) (Figure 5 F).



Fig.5



Pileus (A), lamellae insertion and stipe (B), pileipellis cellular (C), pleurocystidia (D), basidia (E) and basidiospores (F) of *Pluteus romellii*

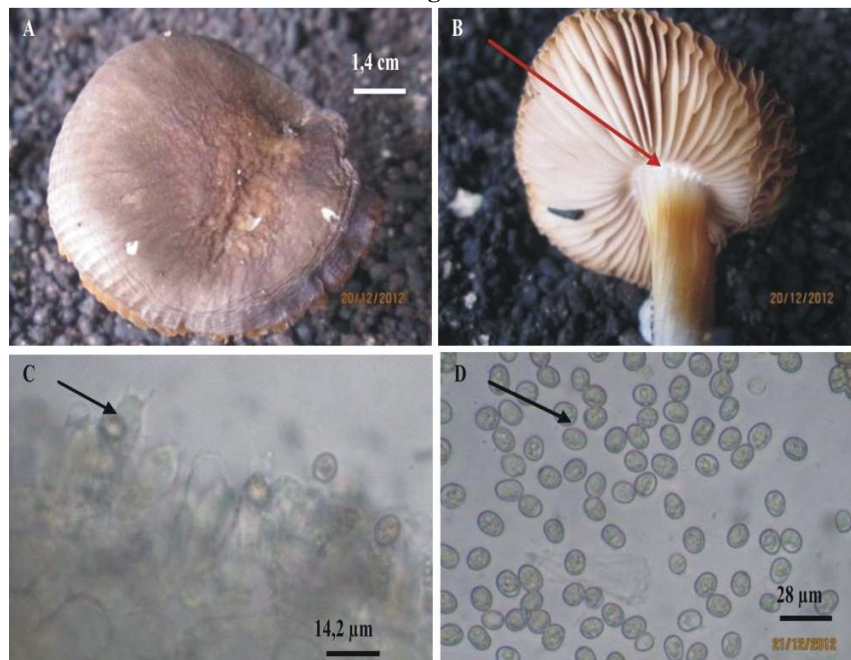
#### Subsection Mixtimi Singer ex Singer

*Pluteus podospileus* Sacc. & Cub. 1887. Harvested on 11/20/2012 in the forest of Mamora on dead trunks of *Quercus suber*.

The pileus (2.5 cm) is dark brown to brown to blond-brown margin, circular; plano - convex and cracked fine line at center. The flesh is thin, whitish and spongy. The margin is straight and striped. The pileipellis is smooth. The stipe (3 x 0.4 cm) is cylindrical, central, fibrillose, and hollow and brown to yellowish brown to the lower part. The lamellae are uneven, little tight, free, bulging and pinkish color. The lamellar edge is wavy and white (Figure 6, A and B).

The basidia (33.4 x 10 μm) are clavate, cylindrical, tetrasterigmate (Figure 6, C). The pleurocystidia (30 x 13 μm) are cylindrical, hyaline and thinner wall. Basidiospores (7.34 -8.4 x 5.99-6.66 μm) are hyaline, smooth thick walled and subglobose or broadly elliptical (1.15 < Q < 1.3) (Figure 6 D).

Fig.6



Pileus (A), lamellae insertion and stipe (B), basidia (C) and basidiospores (D) of *Pluteus podospileus*

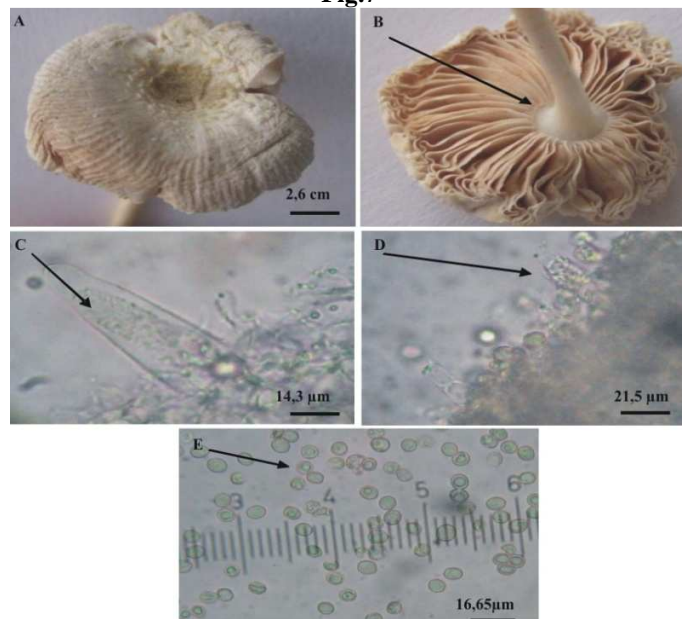
**Section Hispidoderma Fayod**

*Pluteus depauperatus* Romagnesi, harvested on 04/21/2011 at house in Kenitra on white wood paneling decoration.

The pileus (1.5 cm) is creamy white to pink reflection, circular, scaly to ridged center to the margin and plano-convex. The flesh is thin and white. The margin is ridged and flared. The pileipellis is smooth. The stipe (4.5 x 0.2 cm) is cylindrical, central, hail, swollen open bottom, white and sometimes tinged with pink spores. The lamellae are uneven, tight, free, bulging and pinkish color. The lamellar edge is simple and whitish (Figure 7, A and B).

The basidia (31.6-36.6 x 6.66-8.32 μm) is clavate, obese and tetrasterigmate (Figure 7 D). The pleurocystidia (76.6 x 8.32 μm at the top and 13.32 μm at the center) are lageniformia, obese center and hyaline thinner (Figure 7 C). Basidiospores (5.9-8.32 x 5-6.6 μm) are hyaline, smooth thick walled and subglobose (1.05 < Q < 1.15) (Figure 7, E).

Fig.7



Pileus (A), lamellae insertion and stipe (B), pleurocystidia (C), basidia (D) and basidiospores (E) of *Pluteus depauperatus*

### DISCUSSION AND CONCLUSION

The *Pluteaceae* are currently separated of *Amanitaceae* by evidence molecular<sup>4</sup>. In the case of *Pluteus*, the practical application of a biological species recognition is even more challenging than other groups of *Agaricales* due to the hardship of growing members of the genus in culture<sup>2</sup>. The traditional subdivision of *Pluteus* into three sections (*Pluteus*, *Celluloderma* Fayod and *Hispidoderma* Fayod) is essentially supported by molecular data<sup>9</sup>. The section *Pluteus* typically has a pileipellis with a cutis and thick-walled pleurocystidia while section *Hispidoderma* has some forms of filamentous pileipellis and thin-walled pleurocystidia<sup>21,24</sup>. The section *Celluloderma* is defined by a cystoderm (cellular) pileipellis composed of ellipsoid to saccate-pyriform to vesiculose cells with or without cystidioid elements<sup>10,21</sup>.

The seven species have been encountered in the forest of Mamora (North western Morocco) haven't neither annulus nor volva and are characterized by a pileipellis filamentous, fibrillo-combed, squamulose and fluffy to hispide as *Pluteus pellitus* or by a pileipellis glabrous, velvety micaceous and sometimes cracked or punctuated (*Pluteus luctuosus*; *P. depaupreratus*; *P. satur*; *P. podospileus*; *P. griseoluridus* et *P. romellii*)<sup>6</sup>. Yet our identification based on keys of Citérin and Eyssartier<sup>5</sup> and Justo & Castro<sup>8</sup> have helped to integrate *Pluteus pellitus* in section *Pluteus*, while *Pluteus satur*, *P. griseoluridus*, *P. luctuosus* et *Pluteus romellii* in section *Celluloderma* (Fayod) subsection *Eucelluloderma* (Singer ex Singer) and *Pluteus podospileus* in subsection *Mixtimi* (Singer ex Singer) and finally *Pluteus depaupreratus* in section *Hispidoderma*.

Among the studied species, four are new to the fungal flora of Morocco (*Pluteus luctuosus*, *P. depaupreratus*, *P. podospileus* and *P. griseoluridus*). *Pluteus pellitus* and *Pluteus satur*, are described for the first time in this study, have been already reported by Malençon & Bertault<sup>13</sup>. *Pluteus romellii* reported in the Rif, Middle Atlas, Tangier and in forest of Mamora is described by Malençon & Bertault<sup>13</sup> (1970) and Outcoumit<sup>18</sup> in Tangier.

### REFERENCES

1. Ammirati, J.F. Traquair, J.A. & Horgen P.A. Poisonous Mushrooms of the Northern United States and Canada, University of Minnesota Press, United States of America, 396 (1985)
2. Banerjee, P. & Sundberg, W.J. Preliminary observations on germination of *Pluteus* basidiospores. *Mycologia*, **85**: 811–813 (1993a)
3. Bouillard B., Dictionnaire: plantes et champignons. Éditions scientifiques, techniques et médicales, Paris, 875p (1997)
4. Cannon, P.F & Kirk, P.M. Fungal Families of the World, CABI Books, United States of America, 294p (2007)
5. Citérin, M. & Eyssartier, G. Clé analytique du genre *Pluteus* Fr. Documents Mycologiques Tome XXVIII, fascicule n°111 pp 47-67 (1998)
6. Courtecuisse, R. & Duhem, B. Guide des champignons de France et d'Europe. Ed. Delachaux et Niestlé, S. A. Lausanne (Switzerland), Paris, 476p (2000)
7. El Assfour A., 2006. Biodiversité fongique du Maroc: Inventaires et étude des Basidiomycètes de la forêt de la Mamora. Thèse de Doctorat, Université Ibn Tofaïl, Faculté des Sciences, Kénitra, Maroc, 294p.
8. Justo, A. & Castro, M.L. An annotated checklist of *Pluteus* in the Iberian Peninsula and Balearic Islands. *Mycotaxon* **102**: 231–234 (2007)
9. Justo, A. Vizzini, A. Minnis, A.M. Menolli, Jr. N. Capelari, M. Rodríguez, O. Malysheva, E. Contu, M. Ghignone, S. & Hibbett, D.S. Phylogeny of the *Pluteaceae* (Agaricales, Basidiomycota): Taxonomy and character evolution. *Fungal Biology Mycologia*, **85**: 811–813 (2010)
10. Homola, R.L. Section *Celluloderma* of the genus *Pluteus* in North America. *Mycologia* **64**: 937–1210 (1972)
11. Kirk, P. Ainsworth and Bisby's Dictionary of the Fungi, 9th Edition. CABI Bioscience, UK Centre, Egham, UK; P.F. Cannon, CABI Bioscience, UK Centre, Egham, UK; J C David, CABI Bioscience, UK Centre, Egham, UK; J A Stalpers, Central bureau voor Schimmelcultures, Utrecht, The Netherlands, 624p (2005)



12. Liffé, R. Getting to grips with *Pluteus*. Field Mycology, **11(3)**: 78-92 (2010)
13. Malençon, G. & Bertault, R. Flore des champignons supérieurs du Maroc, Tome I. Faculté des Sciences de Rabat, Maroc, 601p (1970)
14. Marshall, N.L. The Mushroom Book. A popular guide to the identification and study of our commoner fungi, Garden-City New York Doubleday, Page and Company, 173p (1923)
15. Mehrotra, R. S. & Aneja, K. R. An Introduction to Mycology. New Age International (P) Limited, Publishers, New Delhi, 539p (1990)
16. Minnis, A.M. Sundberg, W.J. Methven, A.S. Sipes, S.D. & Nickrent, D.L. Annulate *Pluteus* species, a study of the genus *Chamaeota* in the United States. Mycotaxon, **96**: 31-39 (2006)
17. Orton, P.D. Pluteaceae: *Pluteus* & *Volvariella*, in Henderson, P.M. Orton, P.D. and Watling, R. (eds), British Fungus Flora: *Agaricus* and *Boleti.*, Royal Botanic Garden Edinburgh **4**: 1-98 (1986)
18. Outcoumit, A. Contribution à l'étude de la diversité fongique du Maroc et mise en évidence de quelques espèces fongicoles et de l'importance des espèces sécotoïdes dans la systématique des Basidiomycètes. Thèse de Doctorat, Université Ibn Tofail, Faculté des Sciences, Kénitra, Maroc. Volume II, 497p (2011)
19. Roger ,P. Les champignons. Edition du Club Français Loisirs, Paris, avec l'autorisation des Editions Solar, 288p (1981)
20. Si-Si Y. Tolgor, B. & Tai-Hui, L. New Chinese records of *Pluteus* collected from Jilin Province, China, Mycosystema, **30(5)**: 794-798 (2011)
21. Singer, R. The Agaricales in Modern Taxonomy (4th Ed.). Königstein im Taunus, Germany: Koeltz Scientific Books, 981 (1986)
22. Slézac, A-M. Les Champignons, Editions Liber SA, Genève, Suisse, 268p (1995)
23. Taylor J.W., Jacobson D.J., Kroken S., Kasuga T., Geiser D.M., Hibbett D.S. & Fisher M.C., 2000. Phylogenetic species recognition and species concepts in fungi. Fungal Genet. Biol. 31: 21–32.
24. Vellinga, E.C. *Pluteus*. In: Bas C, Kuyper ThW, Noordeloos ME, Vellinga EC (eds) Flora Agaricina Neerlandica. A.A. Balkema, Rotterdam, **2**: 31–55 (1990)