GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Coprophilous fungi of Daloa city: New species for the fungal flora of Côte d'Ivoire

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GSC Biological and Pharmaceutical Sciences, 2022, 20(03), 251–260

Publication history: Received on 10 August 2022; revised on 19 September 2022; accepted on 21 September 2022

Article DOI: https://doi.org/10.30574/gscbps.2022.20.3.0362

Abstract

Objective: The objective of this work is to assess the diversity of coprophilous species growing on cow dung in Daloa locality.

Methodologies and results: The study was conducted in Daloa city. This locality is located in a forest area, with a tropical climate favorable to the development of fungi. Several dried samples of cow dung were collected during field trips in the city. Coprophilous species growing on cow dung were collected in the field. In the laboratory, the dung samples were moistened and placed in a humid chamber, in the dark and at a temperature of 28ºC. Microscopic and macroscopic observations were made on the fruiting bodies obtained for species identification.

Conclusion and application of results: At the end of this study, six (7) coprophilic fungi were identified. Among these species are three (3) Ascomycetes: *Ascobolus immersus*, *Fimetariella microsperma*, *Coprotus aurora*. Three (3) Basidiomycetes were observed: *Coprinopsis nivea*, *Coprinopsis lagopus*, *Psilocybe coprophila*. One (1) Zygomycetes was reported, it is *Pilobolus crystallinus*. All these species are new to the fungal flora of Côte d'Ivoire.

Keywords: Coprophilic fungi; Cow dung; Excrement; Fimicole

1. Introduction

Coprophilic fungi are species that feed on animal feces [1]. They are widely distributed in the world. Their presence depends on the animal species present in the regions considered, the climate, the type of vegetation and according to the environmental latitude gradient [2].

Coprophilic fungi are advantageous organisms to study [3]. They can appear on a fresh dung pile within a few weeks and regardless of the season [4]. Herbivore feces provide a habitat for coprophilic fungi [2]. They are small in size and are not edible. They can accumulate heavy metals and some species are endophytic, toxic, cytotoxic, hallucinogenic [5]. Some species can be facultative mycoparasites [6]. They constitute an interesting ecological niche [1].

In nature, coprophilous species participate with insects, earthworms and microorganisms in the degradation and recycling of herbivore excreta as a source of nutrients for plant growth and development [7]. The excreta produced by herbivores are an important source of nitrogen for soil fertilization provided they are degraded by coprophilic

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organisms [1]. Coprophilous fungal species, belong to the Myxomycetes, Zygomycetes, Ascomycetes and Basidiomycetes [8].

Coprophilous fungi are very diversified as almost all taxonomic groups are represented except for lichens and those with their own niche [1]. They do not receive special attention in most management plans of protected areas, dedicated to the conservation of biodiversity [9]. Coprophilous species are little known in Côte d'Ivoire and no scientific study of this scale has yet been conducted in Daloa region of the country. The objective of this work is to evaluate the diversity of coprophilous species growing on cow dung in Daloa.

2. Material and methods

2.1. Study area

This study took place in Daloa city, located in central-west Côte d'Ivoire between 6°53 north latitude and 6°27 west longitude [10]. It has a humid tropical climate with dense forest vegetation that is regressively evolving due to the practice of extensive and shifting agriculture coupled with the uncontrolled exploitation of forest species [10]. Its rainfall is distributed over four seasons: a long rainy season from April to mid- July, a short dry season from mid-July to mid-September, a short rainy season from mid- September to November and the long dry season from December to March [11]. The two dry seasons are separated by one or two months of varying rainfall [12]. The dry and wet seasons alternate temperatures ranging from 24.65°C to 27.75°C on average [11]. Rainfall has decreased from 1868.5 mm in 1968 to 1120.4 mm of rainfall on average in 2005, the region is experiencing a decline in rainfall of about 40 % [13].

2.2. Biological materials

The material used in this study was dried cow dung (Figure 1) which served as a culture medium for the growth of the fungi. Petri dishes were used as culture media. A cardboard box was used to store the petri dishes to avoid sunlight. An oven was used to preserve the culture and to maintain a controlled environment. Tap water was used to regularly moisten the medium. A microscope and a camera were used for the observation of small species and the measurement of organs.

Figure 1 Dried cow dung on petri dish

2.3. Harvesting cow dung

Field trips to Daloa city allowed for the collection of dried cow dung samples on the ground. For the collection of dung samples, plastic bags are the easiest and cleanest to use. They are easy to invert with very little effort. Each bag is used as a glove and hands can be inserted and removed without effort. Samples taken can be stored for several days. It is best to collect sun-dried material, which is material that has been in the wild for a long time.

2.4. Cultivation

The culture of the dung samples was carried out according to the "wet chamber" method. In the laboratory, the samples were moistened and placed in petri dishes to stimulate the development of the fungi. The humidified samples were placed in incubation at 28°C, in the dark. A few days later, the fungi start to grow.

2.5. Observation and Identification

Two types of observations were made:

- Macroscopic observations were made on the young and adult appearance of fruiting bodies (sporocysts, basidiomas and ascomata);
- Microscopic observations were made on the mycelium, reproductive organs and spores of asexual and sexual reproduction.

Determination and identification were carried out through the works and keys proposed by Bell [14][15], Richardson & Bell [16], Richardson [17][18][19], N'Douba [20][3], Courtecuisse & Duheim [21] and Cheype [22].

3. Results

Several coprophilous species were cited in this study.

3.1. Ascomycota-Pezizomycotina

3.1.1. Family: Ascobolaceae

Ascobolus immersus Pers. : Fr.

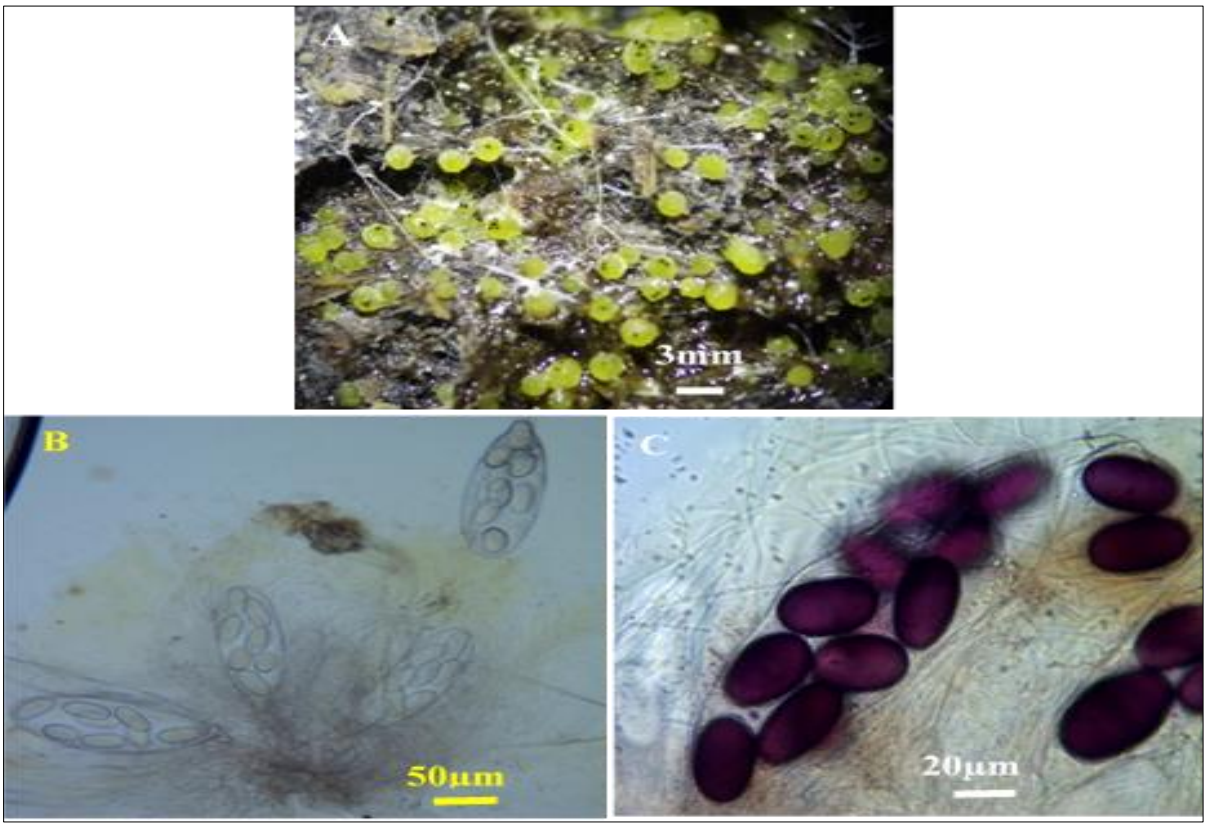

Figure 2 *Ascobolus immersus*; A: Apothecia on cow dung; loupe view; B: Immature asci and spores; C: Mature asci and spores

The ascomata are subglobose to turbinate, with an indistinct margin, yellowish brown, ocracy yellow, olive green, becoming brownish with age (Figure 2 A). They are punctuated with black papillae. Asci (200 µm x 40-46.42 µm) are

amyloid and hyaline. Paraphyses (1.66- 2 µm) are cylindrical, septate sometimes branched towards the base and yellowish in color. Ascospores (40-46.65 µm x 26.64-30 µm) are 8 per asci. They have an ellipsoidal shape and a thick wall. They are smooth, hyaline when young (Figure 2 B) and purplish purple when mature (Figure 2 C).

3.1.2. Family: Lasiosphaeriaceae

Fimetariella microsperma J. C. Krug

The perithecia is pyriform, membranous and brown. It is densely covered with hypoid hairs in particular under the neck, coriaceous and blackish. The perithecia is membranous, the interior of the perithecia presents octospores asci. The asci are uni-tunicate, non-amyloid, cylindrical, long-stalked, without obvious apical apparatus. Spores are unicellular, uniseriate obliquely to almost vertically. Each spore is surrounded by a thick, ephemeral envelope, sometimes invaginating away from the largest germinal pore. The spores are hyaline in the young state becoming yellowish then dark brown with maturity. They are ellipsoidal to narrowly ovoid, smooth, slightly narrowed at one or both ends (Figure 3). The spores have two germinative pores.

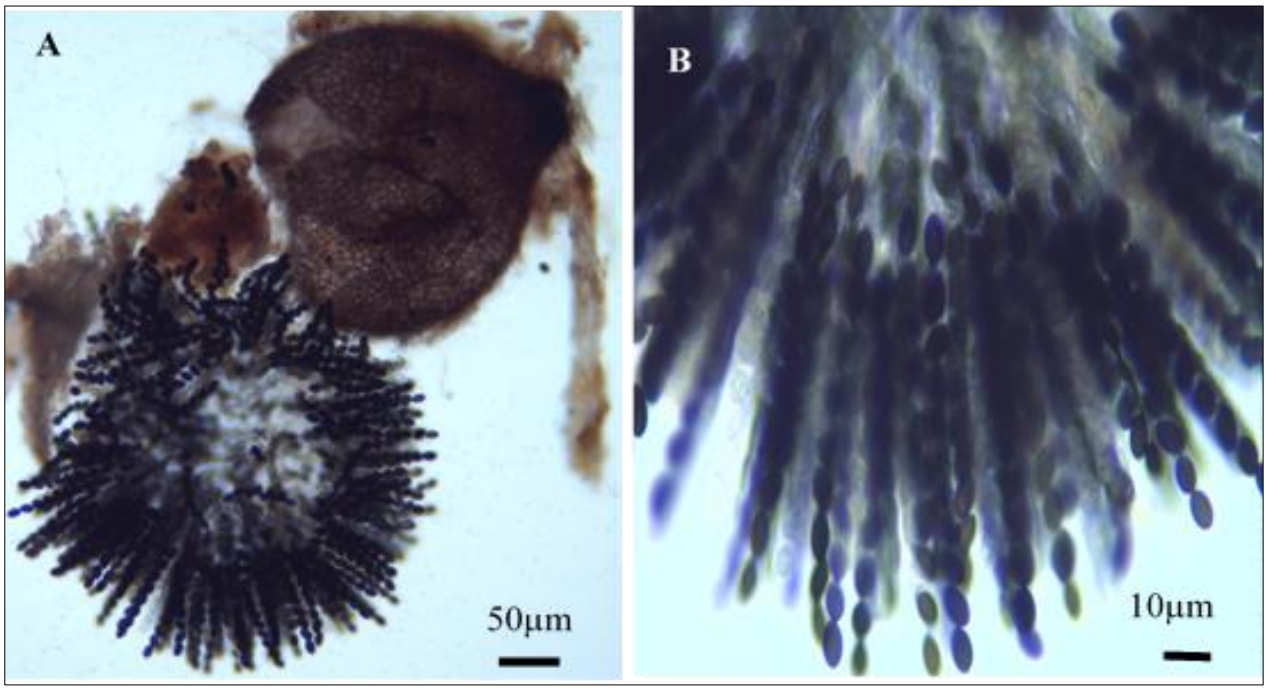

Figure 3 *Fimetariella sp*.; A: Open perithecia with asci and spores; B: Asci and spores

The sporocysts of this fungus grow vertically on the moistened dung. They are unbranched and produce towards the top a vesicle crowned by a black lenticular sporocyst.

3.1.3. Family: Curculionidae

Coprotus aurora (P. Crouan & H. Crouan) K.S. Thind & Waraitch.

The apothecia (1-3 mm) is slightly concave at the beginning then discoid, sesile, of greasy texture to the touch. The hymenophore is smooth, orange-yellow, the external surface is slightly granular. Asci (155 x 10 µm) are cylindrical, octospores, uniseriate vertical and sometimes irregular, with hooks at the base, not amyloid (Figure 4). The paraphyses are filiform, septate, with sparse carotenoid pigments. They are enlarged at the apex. Spores are ellipsoid, smooth, hyaline, enveloped in a mucilaginous mass. They are uni-nucleated. The excipulum is globose, formed by spherical cells of irregular sizes, with cylindrical hyphae.

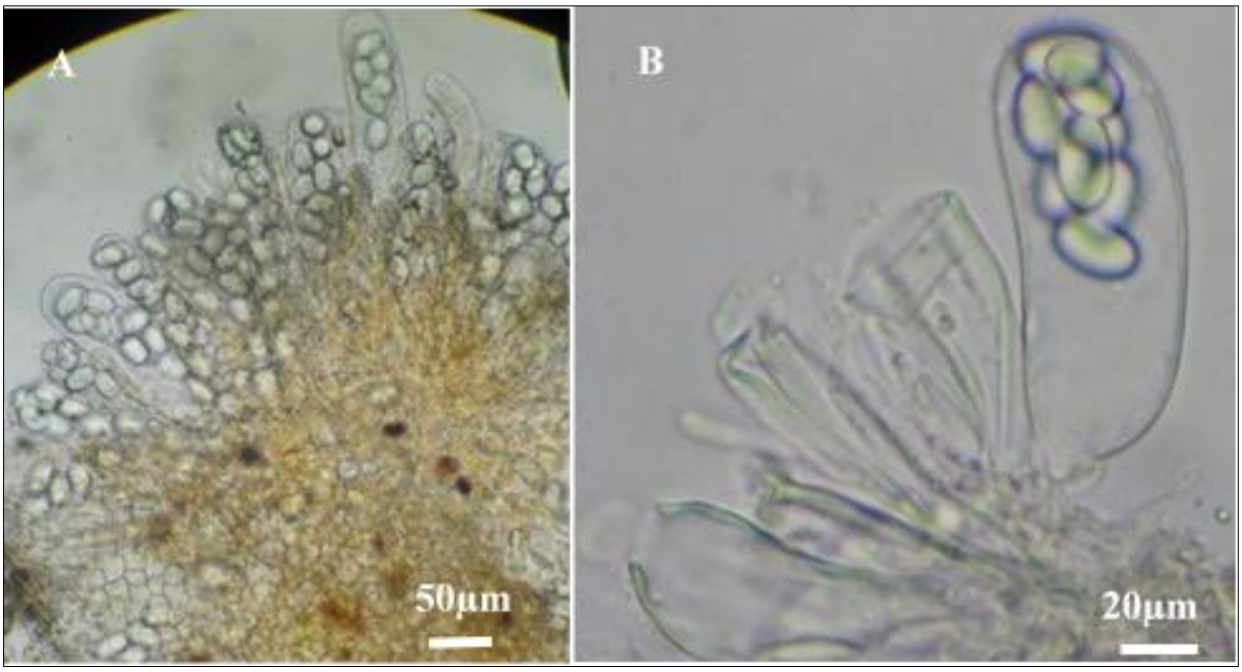

Figure 4 *Coprotus aurora*; A: Asci and spores; B: Asci without spores

Pilobolus crystallinus (Wiggers) Tode

Trophocysts (350-400×250-300 µm) are ovoid to globose, with rhizoidal extensions. They are slightly pigmented with orange color. Sporangiophores (1.5-5.0 mm×115-150 µm) are cylindrical, long and topped with black sporangia (Figure 5A). Sporangia (250-350×240-340 µm) are hemispherical to ovoid, tough and smooth-walled. Columella are smooth, mammiform, 180-200×160-175 µm, subsporangial vesicles (500-700×400-600 µm) smooth- walled, without pigmentation, ovoid to globose. Spores (6-12 x 4-7 µm) are cylindrical, yellow with homogeneous contents, smooth and short-cylindrical (Figure 3B).

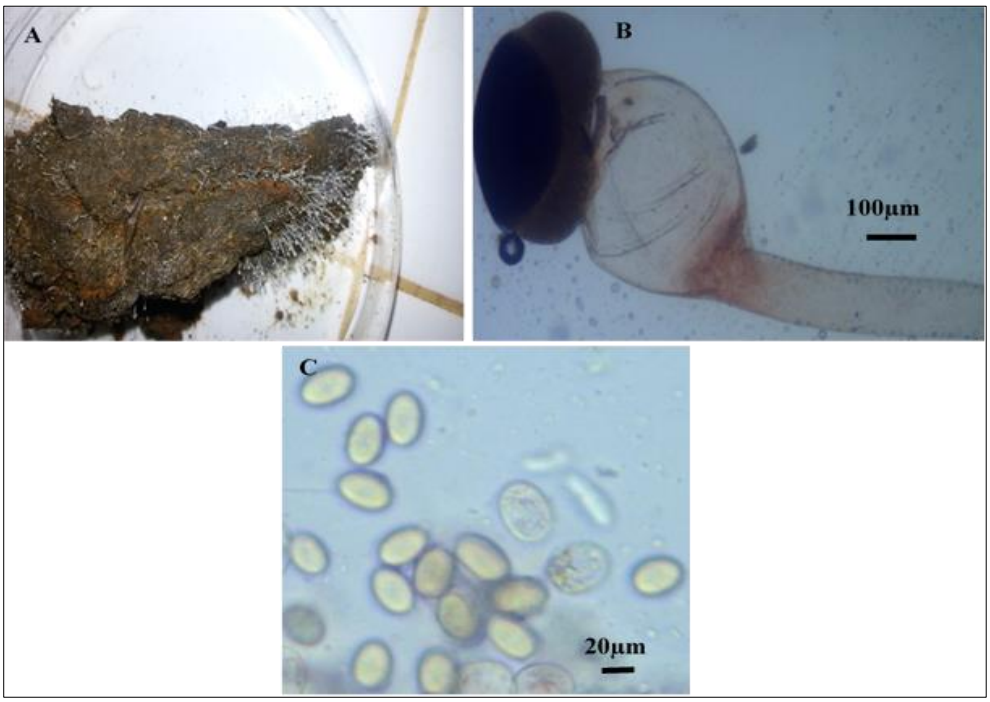

Figure 5 *Pilobolus crystallinus;* A and B: Sporocystophore and sporocyst; C: spores

3.2. *Basidiomycota* - Homobasidiomycetes - Agaricales

3.2.1. Family: Coprinaceae

Coprinopsis lagopus var. vacillans (Uljé) P. Roux & Guy Garcia 2006 ; Syn. Coprinus lagopus var. vacillans Uljé 2000.

The cap (3-6 cm), has a conical form then conico-convex or campanulate while spreading. The margin is striated then rolled up before deliquescence. It is long striated, the lining is formed by a fibrillated to squamulose veil. The cap is bristly in the center, labile, whitish, silvery gray, on a whitish then brownish gray cuticle (Figure 6 A) and finally blackish gray. The blades are tight, adnate to free, brownish gray and finally black with deliquescence. The stipe (4-10 x 0.6-1 cm) is white, stubby (at first), clavate, pruinose to flaky especially at the base. The pulp is whitish then pale brownish gray. The spore is black, the spores (6-9 x 5.5-7.5 x 4.5-6 µm) are ovoid to subglobose, amygdaliform, with a central germinal pore. Cheilocystidia (40-100 x 20-30 µm) variable, cylindrical, clavate, vesicular, oblong, pleurocystidia (80-140 x 25-50 µm) lageniform somewhat vesiculated (Figure 6 B).

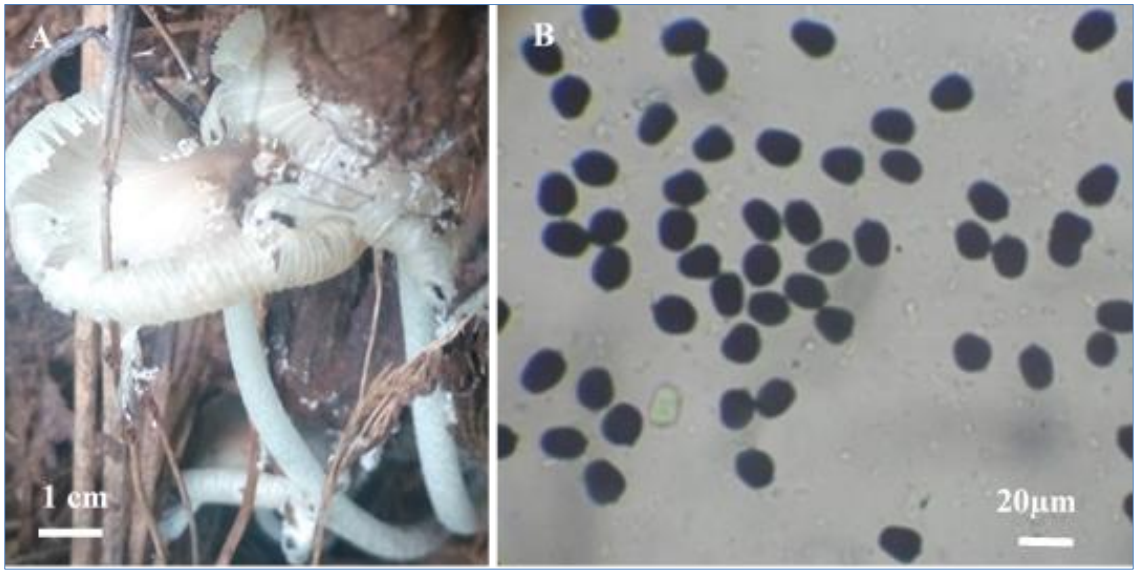

Figure 6 *Coprinopsis lagopus*; A: carpophore; B: spores

Coprinopsis nivea (Pers.) Redhead, Vilgalys and Moncalvo = *Coprinus niveus* (Pers. : Fr) Fr.

The cap (1.5 to 4 cm) is ovoid to conical when young, whitish in color (Figure 7). The surface of the cap is completely covered with a white, flaky, mealy veil. In the adult state (1 cm), the cap may curl back and its color changes from white to gray-black. The stipe (1-12 x 0.3-0.5 cm) is hollow and white. It is fragile, flaky then glabrous with age. It bears a tuft of whitish mycelium at the base (Figure 7). The blades are free, white then black. Basidia are sometimes bisporate. Basidiospores (13.30 x 10 µm) are smooth, with a central germinal pore, sometimes slightly oblique. Cheilocystids (34-58 x 14-45 µm) are usually globose. Pleurocystids (65-135 x 30-40 µm) are cylindrical to fusiform.

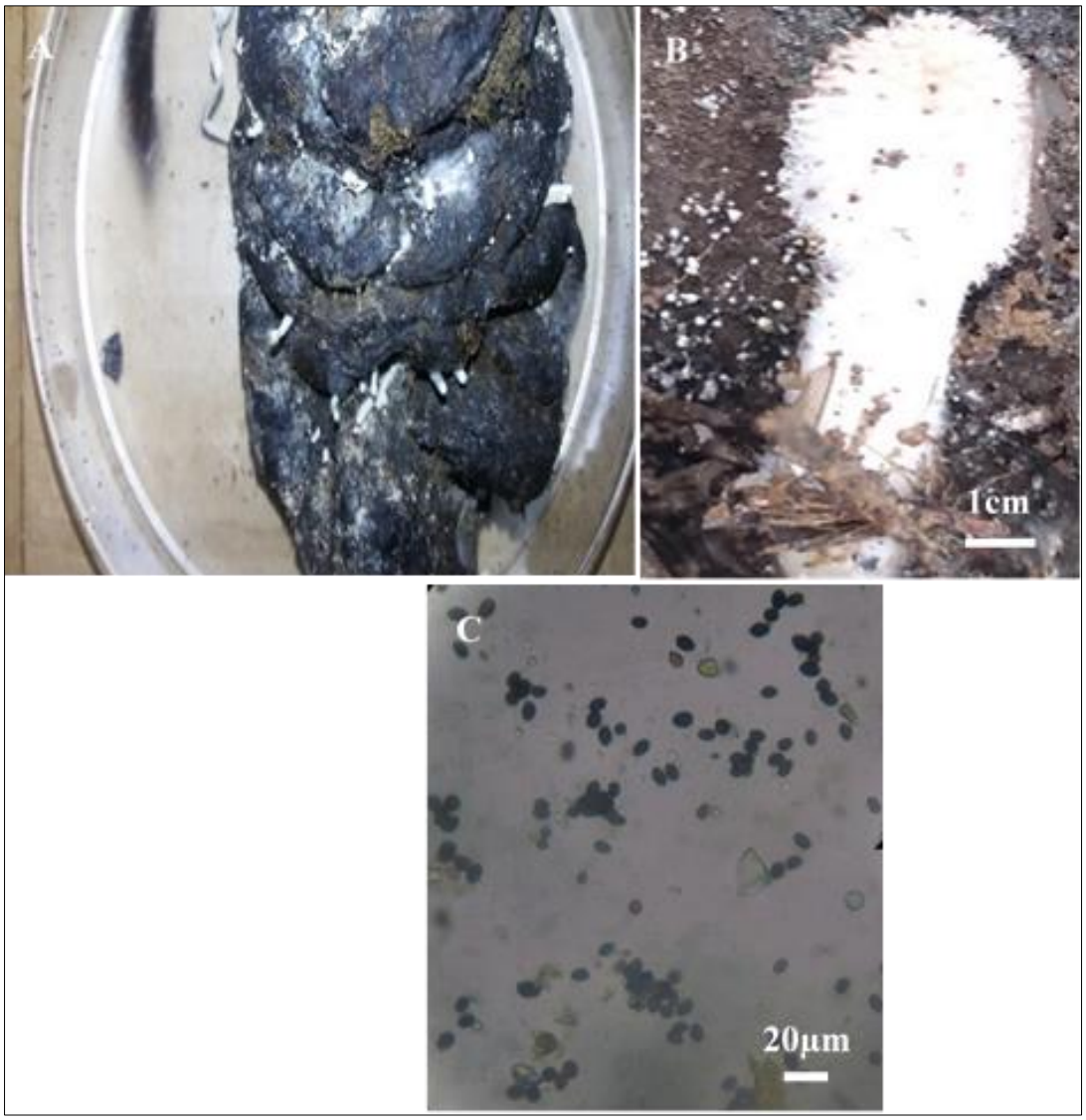

Figure 7 *Coprinopsis nivea*; A: young carpopBhore on cow dung; B: carpophore; C: spores

3.2.2. Family: Strophariaceae

Psilocybe coprophila (Bull. : Fr.) Quélet

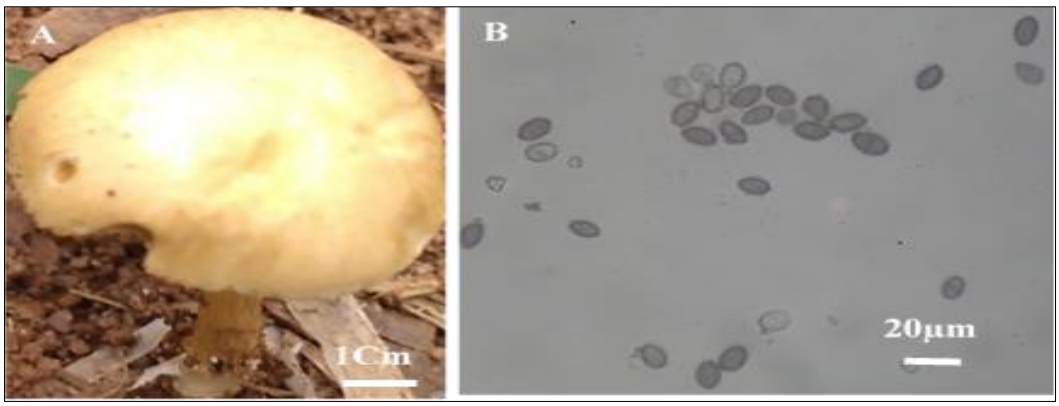

Figure 8 *Psilocybe coprophila*; A: Carpophore; B: Spore

The cap (0.6-0.8 cm) is yellow brown to dark reddish brown, hemispherical to convex (Figure 8). It is sometimes slightly crenate at the margin and translucent from the margin to mid-radius. The blades are adnate to slightly decurrent. The stipe (1.5-3 x 0.3-0.4 cm) is hollow, whitish when young (whitish veil) then light to dark brown after loss of the veil. The flesh is ochracate. Spores (12-14 x 7 µm) are purplish brown, more or less hexagonal according to the direction of observation. Basidia (33 x 10 µm) have 4 basidiospores. The cheilocystids are about 26-34 x 7-10 µm.

4. Discussion

Most ethnomycological investigations have focused on edible fungi [23]. According to Lecomte (2008), coprophilic fungi have been relatively neglected and not given attention in study projects. Indeed, ethnomycological data related to the coprophilic fungal flora prior to this study are non-existent. A total of six (7) new coprophilic fungal species for the fungal flora of the Ivory Coast were cited and identified in Daloa city. They are: *Ascobolus immersus, Fimetariella microsperma*, *Coprotus aurora*, *Coprinopsis nivea, Coprinopsis lagopus*, *Psilocybe coprophila* and *Pilobolus crystallinus*. In this work on the inventory of coprophilous fungi of Morocco [3], listed 116 coprophilous species for the Moroccan fungal flora. Richardson [19] in his work on "coprophilous fungi of France" cited 169 coprophilous species. [24] have identified 97 species in the South Atlantic precisely on the Falkland Islands.

The present work was done only on cow dung and identified seven (7) coprophilous species. Richardson [25] in a study on "diversity and occurrence of coprophilic fungi", cited nineteen (19) coprophilic species. This species diversity is due to the variety of animal dung used: sheep, cattle, deer, rabbit, hare and grouse dung. The cow dung collected in Daloa was moistened and placed in a humid chamber for cultivation. A few days later the fungal species appeared. In his work on "Contribution to the knowledge of the fungi of the upper Arve valley (Haute-Savoie), Cheype [22] did not proceed by culture in a humid chamber. He collected in the field the fruiting bodies of mushrooms present on cow dung.

In this study, three (3) coprophilous Ascomycetes were discovered, they are: *Ascobolus immersus, Fimetariella microsperma, Coprotus aurora*. Doveri [26] recorded eight (8) coprophilous Ascomycetes which are: *Cercophora gossypina, Delitschia vilgaris, Fimetariella microsperma, Hypocopra lojkaeana, Melanospora zamiae, Pressia isomera, Sporormiella minipascua* and *Trichodelitschia munkii.* N'Douba [20] in their work on coprophilous ascomycetes of Morocco cited ten (10) species. These species are the following: *Podospora curvula, Podospora pauciseta, Podospora minuta, Sporormia fimetaria, Sporormiella australis, Sporormiella grandispora, Sporormiella intermedia, Sporormiella minima, Saccobolus, glaber* and *Podospora dagobertii.*

Coprophilic fungi are easy and beneficial to study because the study can be done throughout the year regardless of the season. They do not depend on rainfall. All that is required is to moisten the collected dung, incubate it in the humidity chamber and follow its evolution. According to [1], coprophilous fungi constitute a very diverse ecological niche because all taxonomic groups are represented. Also he mentions that they can be studied all year round because their culture is easy to realize.

From 2005 to 2006, a study on coprophilic fungal species present on wild rabbit droppings was carried out in the park of a regional hospital (Region V, Chile). At the end of this study, 85 species were recorded, of which 46 species in warm periods and 39 in cold periods [2]. The excrements produced by the herbivores are a contribution for the fertilization of the soils. The degradation of these excrements by the coprophilic community (fungi, bacteria, arthropods) is often the only source of nitrogen [1]. Davaine & Varney [27], cited and described four (4) coprins in their study entitled: Four coprophilous coprins encountered in 2009. These species came from the excrements of several herbivores.

For these authors, excrement is a food source for many insects, bacteria and fungi. Fungi have an essentially lignicolous diet. Herbivores have a very rich bacterial flora that can degrade cellulose into carbohydrates that can be assimilated by the animal. The lignins are thus rejected in greater proportion. The coprophilous fungi use them for their food.

5. Conclusion

This first study carried out on coprophilic fungi has allowed to know the diversity of coprophilic fungi of Daloa city. A total of seven (7) new coprophilous fungal species for the fungal flora of the Ivory Coast were cited and identified. Among these species are three (3) Ascomycetes: *Ascobolus immersus*, *Fimetariella microsperma* and *Coprotus aurora*. Three (3) Basidiomycetes were observed: *Coprinopsis nivea, Coprinopsis lagopus* and *Psilocybe coprophila*. One (1) Zygomycetes was reported, it is *Pilobolus crystallinus*.

Compliance with ethical standards

Acknowledgments

I would like to thank Professor Douira Allal and the Laboratory of Plant, Animal and Agro-industry Productions. Faculty of Sciences Kenitra, University Ibn Tofail Kenitra for his contribution to the development of the document. We would also like to thank Dr Kouyaté Amadou for his help with the translations.

closure of conflict of interest.

Authors have declared that no competing interests exist.

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