



ISSN: 0976-3031

Available Online at <http://www.recentscientific.com>

International Journal of Recent Scientific Research
Vol. 5, Issue, 7, pp.1244-1247, July, 2014

**International Journal
of Recent Scientific
Research**

RESEARCH ARTICLE

TWO NEW SPECIES OF ASCOMYCETES FOR THE MYCOFLORA OF MOROCCO

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ARTICLE INFO

Article History:

Received 15th, June, 2014

Received in revised form 27rd, June, 2014

Accepted 14th, July, 2014

Published online 28th, July, 2014

Key word:

Morocco, Jerada, Ascomycetes, Pezizales.

ABSTRACT

Surveys were conducted in the Jerada mine site (Northeastern Morocco) have revealed the existence of two new Ascomycetes species. These species have been harvested in *Pinus halepensis*, belong to the Pezizales Order: *Geopora arenosa* (Fuckel) S. Ahmad 1978 and *Helvella leucomelaena* (Pers.) Nannf. 1941. The obtained results are part of the contribution to the determination of the fungal diversity in Morocco, which remains incomplete till no.

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INTRODUCTION

Ascomycota is the largest Phylum of the Fungi Kingdom with about 65 000 described species (Kirk *et al.*, 2001). Members of this phylum reproduce sexually or meiotically via production of ascospores formed inside a sac-like structure called an ascus (Carris *et al.*, 2012). Ascomycota includes both unicellular and multicellular forms; it is divided into three monophyletic sub-phylum: Taphrinomycotina, Saccharomycotina and Pezizomycotina (Hansen and Pfister, 2006).

According to the classification of Kirk *et al.*, (2008), the Pezizales are an order of the Pezizomycotina subphylum within the Ascomycota phylum; the order contains 16 families, 199 genera, and 1683 species. According to the molecular phylogeny of the Pezizales order belongs to the Class of Pezizomycetes, Subdivision of Pezizomycotina, Division of Ascomycota, it features the following Families: Ascobolaceae, Ascodesmidaceae, Carbomycetaceae, Discinaceae, Glaziellaceae, Helvellaceae, Karstenellaceae, Morchellaceae, Pezizaceae, Pyronemataceae, Rhizinaceae, Sarcoscyphaceae, Sarcosomataceae and Tubercaceae (Hibbett *et al.*, 2007). Species in this order are saprophytic, mycorrhizal, or parasitic on plants (Egger and Paden, 1986). They grow on wood, leaves, soil and dung (Egger and Paden, 1986; Kirk *et al.*, 2008).

Most Pezizales form cup-shaped fruit bodies (apothecia) on soil, dung or plant debris. Apothecia vary in size from less than a millimeter to approximately 15 cm, and may be stalked or sessile (Eriksson *et al.*, 2001). The ascospores are single-celled, bipolar symmetrical, and usually bilaterally symmetrical, varying from roughly spherical to ellipsoidal to occasionally fusoid (Hansen and Pfister, 2006). The ascospores of some species develop surface ornamentations such as warts, ridges, or spines. The tissues of the ascomata are fleshy and

often fragile. Although the majority of species are known only in the teleomorphic state, the anamorphs of some species are known (Hansen and Pfister, 2006). In this study, two species of the order Pezizales have been described.

MATERIALS AND METHODS

The study materials were collected from Jerada mine site (northeastern Morocco) in 2009 and 2012. The site contains the forest of BeniYaala, which is an artificial plantation of Aleppo pine (*Pinus halepensis*) (Azzedine, 2004) (Fig.1). Necessary morphological and ecological characteristics of the samples were recorded and they were photographed in their natural habitats. Then, the samples were taken to the laboratory for further investigations. Two types of observations were made. Macroscopic observations are focused on youthful appearance and adult fruiting bodies (ascomata). Microscopic observations are concerned the ascospores, ascus and paraphyses. Spores measurements are made in water and in lactic acid with cotton blue (CB). The obtained information was compared to (Breitenbach and Kraenzlin, 1984; Kempton and Wells, 1970; Weber, 1972; Weber, 1975; Yao and Spooner, 1996; Abbott and Currah, 1997; Dougoud, 2001, 2003; Dupuy, 2008; Tanchaud, 2013; Darlene and Jonathan, 2011).

RESULTS

Two species have been described in this study:

Geopora arenosa (fuckel) s. Ahmad, monogr. Biol. Soc. Pakistan 7: 176 (1978).

Synonyms:

Humaria arenosafuckel, jb. Nassau. Ver. Naturk. 23-24: 321 (1870) [1869-70].

lachnea arenosa (fuckel) sacc., syll. Fung. (abellini) 8: 167 (1889).

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Lachnea arenosa (fuckel) sacc., syll. Fung. (abellini) 8: 167 (1889) var. *Arenosa*.
Peziza arenosa (fuckel) cooke, mycogr., vol. 1. Discom. (london): fig. 117 (1876)
Peziza arenosa (fuckel) cooke, mycogr., vol. 1. Discom. (london): fig. 117 (1876) var. *Arenosa*.
Peziza arenosa var. *Bloxamii* cooke, mycogr., vol. 1. Discom. (london) (4): 68 (1875).
Sepultaria arenosa (fuckel) boud., hist. Class. Discom. Eur. (paris): 59 (1907).
Sepultaria arenosa (fuckel) boud., hist. Class. Discom. Eur. (paris): 59 (1907) var. *Arenosa*.
Sepultaria arenosa var. *Dodgeirehm*, trans. Wis. Acad. Sci. Arts lett.17 (1914) [20].

Specimens recovered from the pine forest of the Jerada mine site (harvesting in the 1st of December, 2012), are solitary or in groups and dispersed among the mosses, on peaty soils and needles and small pieces of wood under *Pinus halepensis* (Fig. 2A).

Apothecia, 0.5-3 (4) cm in diameter, spherical and slightly buried at first, then cup-shaped and spreading, sessile, margin crenate to deeply incised (Fig. 2A). Hymenium surfaces is smooth to slightly rough, greyish brown (Fig. 2A). Flesh thin, brittle, ± 1 mm thick, whitish. Smell and taste odor and taste: indistinct. Asci 240 x 14-20 µm 8 spores uniseriate, cylindrical (Fig. 2C). Paraphyses: cylindrical, narrow, straight or slightly flexuous branched at the base, slightly septate, 6-9 µm up slightly extended at the apex (Fig. 2C). Ascospores, 19.5-30 x 10-13.5 µm, ellipsoid-fusoid, smooth, with 1-2 large guttules, hyaline (Fig. 2B). External hair, 1000 x 6-10 µm, wavy, branched one or many times, thin septa, thick-walled, brownish. Ectal excipulum terminals to hyphae clavate subglobose, sometimes cylindrical (Fig. 2D). Growth mode: solitary or gregarious. Ecology: solitary or in groups and dispersed among the mosses, on peaty soils and needles and small pieces of wood under *Pinus halepensis*. Frequency: rare. Edibility: inedible.



Figure1 Overview of the forest of Beni Yaala in Jerada (A), waste of craft coal wells transformed into isohumic soils and on which fungi grow (B).

Helvella leucomelaena (Pers.) Nannf., in Lundell & Nannfeldt, Fungi exsicc. upsal. 21: 952 (1941).

Synonyms:

Acetabula leucomelaena (Pers.) Sacc., Syll. fung. (Abellini) 8: 61 (1889).

Acetabula leucomelaena var. *helenae* L. Remy, Bull. trimest. Soc. mycol. Fr. 80(4): 585 (1965) [1964].

Acetabula leucomelaena (Pers.) Sacc., Syll. fung. (Abellini) 8: 61 (1889) var. *leucomelaena*.
Acetabula leucomelaena var. *percevalii* (Berk. & Cooke) Boud., Hist. Class. Discom. Eur. (Paris): 40 (1907).
Acetabula leucomelaena var. *semi hypogaea* Wichanský, eskáMykol. 13(1): 24 (1959).
Acetabula percevalii (Berk.&Cooke) Masee.
Geopyxis cookei var. *percevalii* (Berk. & Cooke) W. Phillips.
Geopyxis percevalii (Berk. & Cooke) Sacc. [as 'percevali'], Syll. fung. (Abellini) 8: 69 (1889).
Paxina leucomelaena (Pers.) Kuntze [as *leucomelas*], Revis.gen. pl. (Leipzig) 2: 864 (1891).
Peziza leucomelaena Pers. [as '*leucomelas*'], Mycol. eur. (Erlanga) 1: 219 (1822).
Peziza percevalii Berk. & Cooke [as '*percevali*'], in Cooke, Mycogr., Vol. 1. Discom. (London): fig. 192 (1875).
Pseudotisra diculata var. *percevalii* (Berk. & Cooke) Cooke [20].

Helvella leucomelaena (Pers.) Nannf., was encountered on February 14th, 2009, on clayey soil, on sandy soil with needles and on small dead branches of *Pinus halepensis* group with copies packed against each other.

Ascocarp, 4-7 cm in diameter, broad, sessile to sub-stipitate, deeply cupulate, serrated regular margin crenate, sometimes open and extended to irregular lobes, thin brownish to blackish inside, blackish-gray outside, clearing towards the base to become almost white in the lower third (Fig. 3A and B). Stipe: when present, very short, consisting of whitish folds or blunt ribs (Fig. 3A). Hymenium smooth, dark brown to blackish, often crumpled to the folded center, even outside, pubescent, upper whitish to dark greyish brown glabrous (Fig. 3A and B). Flesh thin, elastic, whitish to light smell of mushrooms. Asci, 280-320 x 12-16 µm, thin walled, cylindrical to 8 spores uniseriate slightly swollen at the ends, aporhynchus (Fig. 3C). Ascospores, 23-27 x 12-14 µm, elliptic to very rounded corners containing a guettule, smooth, thin-walled (Fig.3D). Paraphyses, 320-380 x 10-12 µm, wide, straight, sometimes discreetly septate, broad at apex, the walls are pale brownish (Fig. 3C). Ectal-excipulum terminals to hyphae clavate subglobose, sometimes cylindrical.

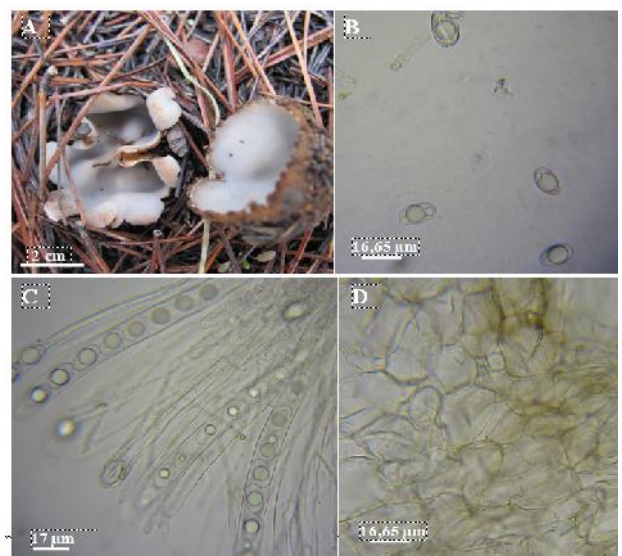


Figure 2 *Geopora arenosa* (Fuckel) S. Ahmad 1978: *In situ* Ascocarp and the other derived from its substrate (A); Ascospores (B); Asci-cylindrical, hyaline, contains 8 ascospores (C); Ectal excipulum: angularis subglobose textura

with brown broad cells, 24–70 µm (D). The microscopic observations are made in water at the magnifications of x 400.



Figure 3. *Helvella leucomelaena* (Pers.) Nannf. 1941: Apothecia, with hymenium smooth dark brown to black (A and B); Ascus (33.3 µm) (C); Ascospores (30 µm) (D). The microscopic observations are made in water at the magnifications of x 400.

DISCUSSION AND CONCLUSION

Geopora species are difficult to differentiate basing only on their morphology. They have been classified in other genera, for example *Humaria*, *Lachnea*, *Scutellinia* and *Sepultaria* (Burdsall, 1968; Zhuang, 2005). Specimens of *Geopora* were identified by morphology as being conspecific have been placed in different clades by analysis of ITS sequences, indicating incorrect species identification (Tamm *et al.*, 2000). *Geopora arenosa* belongs to the Family of Pyrenomataceae, Order of Pezizales, Sub-Class of Pezizomycetidae, Class of Pezizomycetes, Sub-Phylum of Pezizomycotina, Phylum of Ascomycota, kingdom of Fungi (Kirk *et al.*, 2008).

In Morocco *G. arenosa* was collected on sandy-clay soil, covered with needles and twigs of *Pinus halepensis* and among mosses in winter lonely or in groups. Young specimens are barely hypogean, mature their open top flush right at ground level or slightly epigeic, crenate margin, then tearing into irregular lobes. Ascospores, 18.2- 24.6 x 11.8-14.5 µm, are smooth. The anatomical descriptions and ecological needs cited by the authors are largely consistent with mine by consequent *G. arenosa* is a new species for the mycoflora of Morocco. *G. sumneriana* differs to *G. arenosa* by its larger apothecia diameter (3-7 cm), its pale yellowish to brownish hymenophore, thick flesh, its largest spores, 24-30 x 14-16 µm, and it grows in the spring, often in groups with copies packed against each other. Certain authors reported the differences between *G. arenosa* and *G. arenicola* seem tenuous (Breitenbach and Kraenzlin, 1984; Yao and Spooner, 1996). On others propose to compare it with a closely related species, *G. foliacea*, often larger, which is hymenium yellowish, thicker flesh and slightly larger spores (Dougoud, 2003). In Switzerland the species is found in the litter on sandy, wet soil or limestone forests and open areas in the summer, it is rare and inedible (Breitenbach and Kraenzlin, 1984). In France, *G. arenosa* is encountered on sandy or gravelly soil, also in the dunes, Apothecia 0.5-3 cm in diameter. Hymenium greyish cream. Ascospores ellipsoidal,

18 - 26.5 x 12-15 µm, containing one or two guttules each year between October and January in its preferred settings on sandy soil along the coast and on stony ground in land. In Britain *G. arenosa* has large ascospores ellipsoid-fusoides 27-30.5 x 13.5-15-12 µm, it should not be considered as a synonym of *G. arenicola*, but it may be synonymized with *G. sumneriana* (Lantieri *et al.*, 2009). *G. arenosa* is also found in Spain (Catalonia in littorals sandy pine), Australia, Belgium, Germany, Luxembourg, Italy, United States of America, Panama (in inland dunes consolidated by *J. macrocarpa* plant, on mosses, very common (Yao and Spooner, 1996).

Helvella leucomelaena belongs to the Helvellaceae Family, Order of Pezizales, Sub-Class of Pezizomycetidae, Class of Pezizomycetes, Sub-Phylum of Pezizomycotina, Phylum of Ascomycete, and Kingdom of Fungi (Kirk *et al.*, 2008).

The *Helvella* genus includes approximately 52 species (Kirk *et al.*, 2008). Its distribute on is basically north temperate, with only one recognized tropical species. Traditional taxonomy of the genus has been mainly based on ascoma shape and color and the presence or absence of projecting hyphae on the sterile surface (ectal excipulum) of the apothecium (Dissing 1966; Abbott and Currah, 1997; Tanchaud, 2013). Characters such as ascus development (aporphynchous or pleurorhynchous), ascospore shape and size, paraphysis color, and the presence of pigment in the apothecium ectal excipulum cells have proven to be of taxonomic value in some species (Weber, 1972; Harmaja, 1979; Abbott and Currah, 1997).

The Helvellaceae family includes ascocarps stipitate receptacle which have very specific characteristics: The rarely regular cap (*H. leucomelaena* ...) is usually formed of two or three lobes and reflective sometimes fused (*H. lacunosa*). The stipe often furrowed (*H. crispa*) can be smooth (*H. spadicea*). In terms of taste, for a long time *Helvella* species were considered edible after cooking, the results of recent studies have been dismantling that particular *H. crispa*, one of the most consumed, contained non labile toxins responsible for many poisonings. Therefore all *Helvella* are considered now as toxic (Dupuy, 2008). *H. leucomelaena* is characterized by elliptical spores tops very rounded and paraphyses exceeding the asci level of 20 to 30 µm barely swollen at the ends (Dupuy, 2008), which concorde perfectly with the features of our taxon. *H. leucomelaena* is probably closely related to *H. solitaria* because the two species are often found in the same locality (Dupuy, 2008). *H. leucomelaena* grows solitary to gregarious in soil and has a widespread distribution in North and South America, Europe, North Africa, Australia and Asia (Dissing, 1966; Abbott and Currah, 1997). This species was reported in Norway, Sweden, Denmark, Great Britain, Germany, Holland, France, Switzerland, Austria, Czechoslovakia, Yugoslavia, Italy, North Africa (Algeria: Oran, Bois de Pins, Tunisia under Conifers) and Argentina (Dissing, 1966).

H. leucomelaena is often the first mushroom encountered in the first sunny days of February, under the pine forests on sandy Boyard or Saint-Trojan (Oéron) with an apothecia, often small but up to 10 cm in diameter, blackish in the inside, blackish-gray in the outside, clearing towards the base to become almost white in the lower third. Apothecia is marked by a virtually nonexistent pseudostipe, often semi hypogeous in youth, it has then a margin which may be straight but more often irregularly eroded (Dupuy, 2008). In France and in

particular in Finistère, *H. leucomelaena* occurs in conifers in winter and spring, semi hypogeous then emerging, deeply cup, hymenium gray brown to blackish, outer surface white base fruiting, spores 18-23 x 10-14 µm. Species moderately frequent but still visible every year, between February and April, sometimes as early as December or January, under the pine trees on the coast or inland (Tanchaud, 2013).

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