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First Report on *Choiromyces meandriformis* Vittadini. from Tunisia

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Abstract:

Ascomycete fungi are mainly represented by desert truffles. Truffles fructification of these mushrooms depend on climatic conditions, above all on a well-adequate and well-distributed rainfall. These mushrooms are economically important and widely distributed in the arid and semi-arid regions. Ascomycetes made a mycorrhizal association with various vascular plants species. Survey trips were realised in spring period from 2010 to 2019. During survey about Ascomycete fungi in arid and desertic lands in Tunisia, morphological characterization of harvested truffles and soils analysis were carried out. Results proved a first identification of *Choiromycesmeandriformis*Vittadini species, host plant, locality and soil physicochemical characterization in Tunisia.

Keywords **—Ascomycota, Tuberaceae, morphological description, *Acacia sp.***

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I. INTRODUCTION

Truffles are of the most searched and appreciate edible fungi in the world. The genus *Choiromyces* was initially proposed by [1]. From this date, nomenclature of *Choiromyces* genus has strongly changed. *Mylitta venosa* described from Sweden by [2] was later nominated *Tuber niveum* Desf. and *Terfezialeonis* Tul. & C. Tul. [3]. Then[4] named it *Choiromyces venosus*. The species was considered conspecific with *C. meandriformis* (Fr.) Th. Fr. [5].

C. gangliiformis, which has only lighter spore color, was considered as a developmental stage of *C. meandriformis*[6-7].

Moreno

[8]Moreno et al. (2012) confirmed that the genus *Choiromyces* include at least three species: *C. venosus- meandriformis*, *C. magnusii* and *C. alveolatus*. According them, C. *venosus* and *C. meandriformis* from central and Mediterranean Europe are strongly attached and related to the same taxon while *C. magnusii* was considered a separate species, only associated to the Mediterranean genus *Cistus.*

Choiromyces venosus (= C. meandriformis) is recognized with its large irregularly lobulated whitish ascomata and with sinuous veins which fille all gleba attributed to 'meander-like' description (which is the origin of the epithet *C. meandriformis* [1. Specific spore ornamentation of C. venosus is spiny. The best way to tell the species apart is its very strong and nauseating at maturity odour[6], or "spirituous to aromatic that turns unpleasant after drying" [9]. It is known by numerous common names: 'white truffle', 'truffle of Mallabia' 'Hungarian truffle', 'pig's truffle' 'truffle of the Caucasus' and 'white truffle of Transylvania'.

Choiromyces sp. Geographical distribution is relatively large; in Europe, America and Australia [8], China, Iran. In Africa, only the species *Choiromycesechinulatus* was reported in South Africa [10]. In North Africa there is no confirmation of *Choiromyces* sp. presence.

Bonito et al. 2013, reflection about dispersal events of ancestral truffle species that could have been correlated with host plant migration or host switching probability may originated the diversification of *Choiromyces* species around all continents.

Choiromycesvenosus (Fr.) Th. Fr. (synonym: *Choiromycesmeandriformis*Vittad.), is an ectomycorrhizal [11]species generally associated to oak plant, *Eucalypus*sp.and*Carpinionbetuli* and *Melampyrobihariensi-Carpinetum*[12]and *Cistus*[8]. *Choiromyces* species of the Northern hemisphere could be linked to angiosperms or *Pinaceae* roots.

In Tunisia, there is few studies deal with these fungi. [13]had described the presence of *Terfeziaboudieri* Chat., *Terfezialeonis*Tul. (= *Terfezia arenaria*Moris.), *T. metaxasi* Chat., *T. Hafizi* Chat., *Tirmaniaovalispora*Pat. (= *T. nivea* Desf.), *Phaeeangiumlefebvrei* Pat. (*Phaeangium Pat.* = *Picoa*Vitt.), *Terfeziaclaveryi* Chat. and *Terfeziaboudieri*var. pedunculata. [14] had confirmed the presence around Tunis of *Terfeziaclaveryi*Tul. species. Six other desert truffles species were next identified in Tunisia [15]. The appearance of these fungi requires appropriate rainfall during the winter season[16]; Tuberaceae truffles have never been described nor identified in Tunisia.

This research aims to identify new inventory in truffles species from last studies conducted in 2006 (using morphological description of ascocarps, asci profile and ascospores size and shape), to localize and describe fungi species and their natural soil sites (pH, organic matter, phosphorus content and texture) and to enumerate their host plant species.

II. MATERIAL AND METHODS

Many prospecting trips were conducted in spring at various sites. During trips, local markets visits and an oral survey with local habitants were accomplished to localize truffle sites in each region.

A. Truffle identification

Truffles species identification was based on macromorphological characterization of peridium and gleba of each harvested ascocarp from fresh tissues under a binocular microscope (Leica MS 5). Microscopic studies of gleba were conducted on rehydrated sections cut from specimens. The measurements of dimensions of the ascus and ascospores were performed in distilled water using a Leica DMLS microscope. Ascospore dimensions were measured mounts on at least 100 randomly selected spores

B. Truffle site characterization

1) Location of truffle site

Global positioning coordinates were recorded using a portable global positioning system (Meridian) in truffle emplacement. The map of the harvested truffle species site was produced using Arc View GIS 3.2 software. This card referred to truffle species which has been morphologically identified in the laboratory.

2) Host plant identification

Plant host determination was based on the identification of all plant species found in proximity of ascocarps location in a cercle of 0.5 m of radius according to [17]. The centre of the cercle was the truffle position.

3) Soil analysis

Soils samples were taken beneath harvested ascocarp at 0–10 cm of depth using an auger, in three replicates. Soil samples were passed through a 2 mm mesh sieve and subjected to various analyses. Soil pH was determined by the saturated paw method [18]. Organic matter was determined by the partial oxidation method [19].Texture class was analysed accordingISO 11277 and available phosphor content is determined by vanadomolybdophosphoric Yellow Color method.

III. RESULT AND DISCUSSION

From last study carried out in 2006 [15], results confirmed the presence of a new truffle species: *Choiromycesmeandriformis*[1]. This species has

never been mentioned in North Africa. Common name of this truffle species is "tansylvanian big white truffle".

Choiromycesmeandriformis[1]

This is the first report on *C. meandriformis* presence in Tunisia. Harvested ascocarps were generally spotted reddish brown. The mean characteristic of *C. meandriformis*is its typical odor specially when mature this aroma was overpowering and nauseous. The glebe was composed of numerous whitish-ferruginous fertile veins. The nearness plant species to *C. meandriformis*ascocarpes location was only *Acacia cyanophylla* (Fig. 1), some ascocarps were found attached to roots of this plant species. The soil analysis of *C. meandriformis* site, showed aclaysubstrate with low alkaline pH (7.94), relatively rich in organic matter (3.61%) and totally deprived of available phosphorus (0 ppm).

Fig.1 *Choiromycesmeandriformis*ascocarp next to*Acacia cyanophylla* plant

C. meandriformis ascocarps have irregular form, with ochre to gray smooth surface (Fig. 2). Globose spores are yellow-brown with truncate spines ornamentation. There were always eight spores in each ascus of *C. meandriformis* (Figs 3)**.** This Ascomycete belongs to Tuberaceae family.

Fig. 2 *Choiromycesmeandriformis*fruiting bodies.

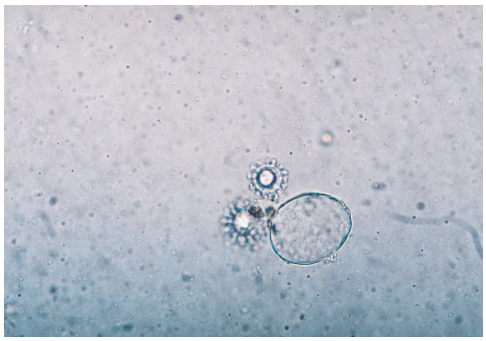

Fig.3 *Choiromycesmeandriformis*ascospores

This truffle was localised only in a natural land belongs to the coast of Mahdia (Fig. 4), located in semi-arid bioclimatic floor in lower-level variant mild winter with an annual rainfall between 300 and 400mm/year.

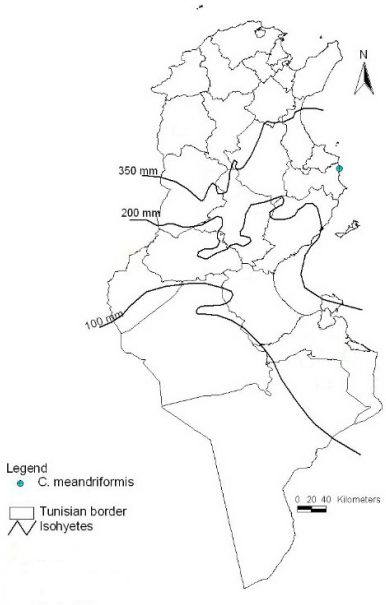

Fig. 4 Localisation of *Choiromycesmeandriformis*species in Tunisia

Taxonomically, our results showed, for the first time, the presence of *Choiromycesmeandriformis*Vittadini in Tunisia. *C. meandriformis* collected from Mahdia region is a common species in Europe and United States [19].*Choiromycesmeandriformis* has occurs in Great-Britain and in Austria, it is common in the Baltic countries, in Sweden, in Northeastern Poland and in South Germany [5]. *Choiromycesmeandriformis* is widely spread in the Carpathian Mountains of Hungary [12].

This species is considered toxic and causes mild gastrointestinal problems [19]but no intoxicity effect was demonstrated by [5]. Moreover, this truffle is consumed in many countries as in Hungary where it is considered as a sweet flavor[12] besides *C. meandriformis* is used to adulterate Tuber magnatum species [20).

Associated plant to *Choiromycesmeandriformis* was *Acacia cyanophylla*. This fungus improves the plant development. *C. meandriformis* is found in Navarra associated with *Quercus robur* and it is generally associates with deciduous and coniferous trees [21]. *Choiromycesmeandriformis* host were hornbeam (*Carpinus betulus*) and spruce *(Piceaabies*) in forestry lands of Hungary [12]. Difference on plant species specter is related to bioclimatic properties of every ecosystem. Fungi are from microorganism species that tolerate hard condition an modify their living strategy to overrunning the root system of new and numerous vegetation species [22].

By pedological analysis, *Choiromycesmeandriformis* required clay soil with low basic pH.*C. meandriformis*' soil in Hungary was slightly alcalic or neutral with high humus, various phosphorous and medium or high potassium contents with traces of lime. However, according [21] *Choiromycesmeandriformis* grows in acid soils with high rainfall and prefers clayey soils.

Soil characterisation is an important tool to characterize requirements of these fungi. [14] elucidated that texture and soil type affect the growth of mycelia and the shape of ascocarps. Most truffle species develop in high pH calcareous soils[23-24], but some are founded in soils with neutral pH values [25-26]. Moreover, some species required moderately sandy soils [27].Commonly, the appearance of truffles depends specially on soil type and climatic conditions (rainfall, temperature and moisture) during the current year. The characteristics of desertification in arid and semi-arid regions include[28] (Herrera et al. 1993) loss or disturbance of the vegetation cover, increase in soil erosion, loss of available nutrients and organic matter and/or diminution in microbiota activity, thus affecting suitable nutrient cycling. It is known that the natural equilibrium of a given ecosystem can be disturbed by changes in the activity of natural agents (climatic, ecology, etc.). Consequently, soil difference between natural sites of Tunisian and previous enumerated locality could explained.

[12] declared that the fruiting period of *C. meandriformis* between June to September contrariwise our harvest period in spring season). Difference could be accredited to the humidity and rainfall quantity and repartition discard and difference between humid European countries and arid lands in Tunisia.

In brief, this taxonomic diversity of Truffle species recovered from different Tunisian soils evidenced a novel biodiversity among fungi associated to wild plant species. *Choiromycesmeandriformis*Vittadini was reported for the first time on Tunisian soils. *Acacia cyanophylla* was the host plant found neighbouring *Choiromycesmeandriformis*. This Tuberaceae truffle species grows in clayey, fertile soil with low basic pH. No final conclusion about truffe species spectra in Tunisia must be announced, since other species could be present. In fact, harvesting company was limited in all studied period by climatic conditions last years. Study must be accomplished by other prospecting trips when favourable conditions reign and in forestry regions during all year specially in summer and autumn seasons (the ideal appearance period of *Tuber* species).

IV. CONCLUSIONS

Declaration of *C. meandriformis*existence in Tunisia and in North Africa for the first time can promote the cultivation ability of *C. meandriformis* for gastronomic purposes since this fungus recently came into edible valorization interest. Mycorrhizal relationship with this Tuberaceae species needs investigations both in natural condition and in experimental level that could allow valuable data on reforestation and fight against desertification strategies in arid and desertic lands.

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