Botanica
ISSN 2538-8657
https://doi.org/10.35513/Botlit.2023.2.2
2023, 29(2): 50–58

Original research

New and confirmed species of operculate cup fungi (Ascomycota, Pezizomycotina) in Tunisia

Ridha El Mokni[1, 2*]

[1] University of Monastir, Faculty of Pharmacy, Department of Pharmaceutical Sciences "A", Avenue Avicenna, 5000 Monastir, Tunisia
[2] National Research Institute of Rural Engineering, Water and Forests, Department of Forestry, 2080 Ariana, Tunisia
[*] Corresponding author. E-mail: ridha.elmokni@fphm.rnu.tn

Abstract

El Mokni R., 2023: New and confirmed species of operculate cup fungi (Ascomycota, Pezizomycotina) in Tunisia. – Botanica, 29(2): 50–58. https://doi.org/10.35513/Botlit.2023.2.2

Three taxa of macromycetes, *Otidea alutacea*, *Otidea bufonia* and *Phylloscypha phyllogena*, were first recorded in Tunisia. Occurrences of *Humaria hemisphaerica* and *Peziza vesiculosa* were confirmed from the northwestern part of Tunisia more than 100 years after their first records in the northeast and the southwestern parts of the country, respectively. The species reported belong to three families of the Pezizales order (Otideaceae, Pezizaceae and Pyronemataceae). Short descriptions of their distinctive macroscopic and microscopic features, ecology and geographical distribution in Tunisia are given. The locality, the data of collection and field photographs for each species are also presented.

Keywords: Humaria, North Africa, macrofungi, Otidea, Pezizales, Phylloscypha.

INTRODUCTION

Ascomycota remains the largest and most diverse group in the kingdom of fungi, comprising ca. 59% of all known fungal taxa and including almost 3.255 genera with more than 64.000 named species (Schoch et al., 2009; Vega et al., 2012; Money, 2016). The Pezizales order is the richest in this group, including mainly saprophytic fungi with disk- or cup-shaped operculate above or belowground ascocarps. The order includes genera such as *Aleuria*, *Geopora*, *Gyromitra*, *Helvella*, *Morchella*, *Otidea*, *Peziza*, *Sarcoscypha*, *Sarcosphaera*, *Scutellinia*, *Tarzetta*, *Terfezia*, *Tirmania*, *Tuber* and *Verpa*.

In Tunisia, studies and first inventory data relating to fungi belonging to the Ascomycetes group, including Pezizales, come back to Patouillard (1897), Maire (1906, 1909), Le Gal (1952), Malençon (1952) and Dennis (1984). After that, few researchers have been interested in Tunisian Ascomycetes and the Pezizales group. Mycological research during the last two decades (Slama et al., 2006, 2009; El Mokni et al., 2013; El Mokni & El Aouni, 2019; Ben Hassine Ben Ali & Aschi-Smiti, 2014; Ben Hassine Ben Ali & Stephenson, 2016; Ouali et al., 2016, 2020) has led to few novelties and increased the number of taxa in the Tunisian Ascomycota to 80 species, where the richest orders are the Pezizales with 43 species, the Helotiales (16) and the Xylariales (9).

As a part of these investigations and studies focusing on improving and updating knowledge (inventory and taxonomy) on the taxa of the Ascomyc-

etes in Tunisia, field explorations have allowed us to discover or rediscover several infrequent species of Ascomycetes.

The present study aimed to provide information about three new species and two rediscovered species for the Tunisian macromycota of the Otideaceae, Pezizaceae and Pyronemataceae families (Pezizales order) and to provide new data regarding their occurrence in the northwestern Kroumirian oak forests.

MATERIALS AND METHODS

Data presented in this study are based on specimens collected through fungal surveys and sampling performed between 2001 and 2023 in various localities and areas of Tunisia's northwestern Kroumirian oak forests. Kroumiria is defined geographically in Tunisia as a series of mountainous chains, southwestern to northeastern, which rise to 1203 m a.s.l. at Djebel El-Ghorra on the Tunisian–Algerian border. The area corresponds geologically to the largest unit of the Numidian *flysh* field (a detrital sedimentary deposit with turbiditic affinities), where the substrate is acidic with a clay sandstone ground including a series of argillite with intercalation of sandstone Benches and a marly base. Bioclimatically, it is Tunisia's most privileged region, having a lower-humid bioclimate and a temperate winter (annual pluviometry is up to 2200 mm). Vegetation is mainly dominated by oak species (mainly *Quercus canariensis* Wild, *Quercus suber* L., *Quercus coccifera* L. and with very restricted areas *Quercus afares* Pomel, *Quercus ilex* L. and *Quercus ×numidica* Trab.) with sporadically planted conifers and eucalyptus at little extends accompanied by remarkably diverse undergrowth composed by shrubs and bushes (El Mokni & El Aouni, 2019).

At the sampling localities, relevant notes were taken, mainly those related to fungi ecology, morphology, and geographic position, and photographs of fungi using a *Nikon COOLPIX P520* camera. The action of micromorphology features was examined by using water, cotton blue dissolved in water (CB/W) or lactic acid (CB/LA). Taxonomic identification of the collected macrofungi was prepared based on the available literature containing identification keys and descriptions (Hansen & Knudsen, 2000; Spooner, 2001; Davis et al., 2012; Van Vooren, 2012; Olariaga et al., 2015). The nomenclatural databases Index Fungorum (2023) and MycoBank (2023) were used to find the most appropriate names of taxa. Chorology and eventual occurrences in Tunisia were checked in the online international network and databases, Global Biodiversity Information Facility (GBIF, 2023). Collected specimens are deposited at the Herbarium of Monastir University (not listed in the Index Herbariorum).

RESULTS AND DISCUSSION

Pezizales

***Otidea alutacea* (Pers.) Massee** (*Otidea* (Pers.) Bonord., Otideaceae Eckblad)

Description. Apothecia 15–75 mm high, 8–48 mm wide, yellow to light brown, initially ear-shaped, then expand to capulate, sploit, erect, in clusters, subsessile or short stipe, asymmetrical, truncate at apex, margin wavy (Fig. 1, A–B). Spores 14–15.5 × 7–8 µm, hyaline to yellowish green, ellipsoid to elliptical, smooth, guttulated, usually two. Asci 146–178 × 11–12 µm, cylindrical, nonamyloid. Paraphysis 3–4 µm wide, hyaline, slender, hooked, branched below. Excipulum textura globosa, cells compactly arranged, parenchymatous cells, 11–14 µm wide cells, terminal cells larger than medullary excipulum, hyaline, interconnected, septate hyphae (Olariaga et al., 2015: 182–184).

Habitat. In Tunisia, apothecia were found growing on leaves of *Quercus canariensis* Willd. Only three localities are known in rich soils within Kroumirian oak forests (Jendouba, northwestern Tunisia).

Specimen examined: Tunisia, Jendouba, Aîn Draham, on loam-clayey soils within *Quercus canariensis* Willd leaf-litter, 827 m. a.s.l., 36°45'37" N, 008°41'57" E, 15 November 2012, Collector Ridha El Mokni (REM).

Notes. *Otidea alutacea* is common and widespread throughout Britain and Ireland, particularly in broadleaved woodlands and occasionally under conifers in mixed woodlands. In contrast, *Otidea onotica* is distributed throughout most of mainland Europe. It occurs also in many parts of North America. The species has been reported for the first time to the Tunisian macromycota and is the third for the whole African

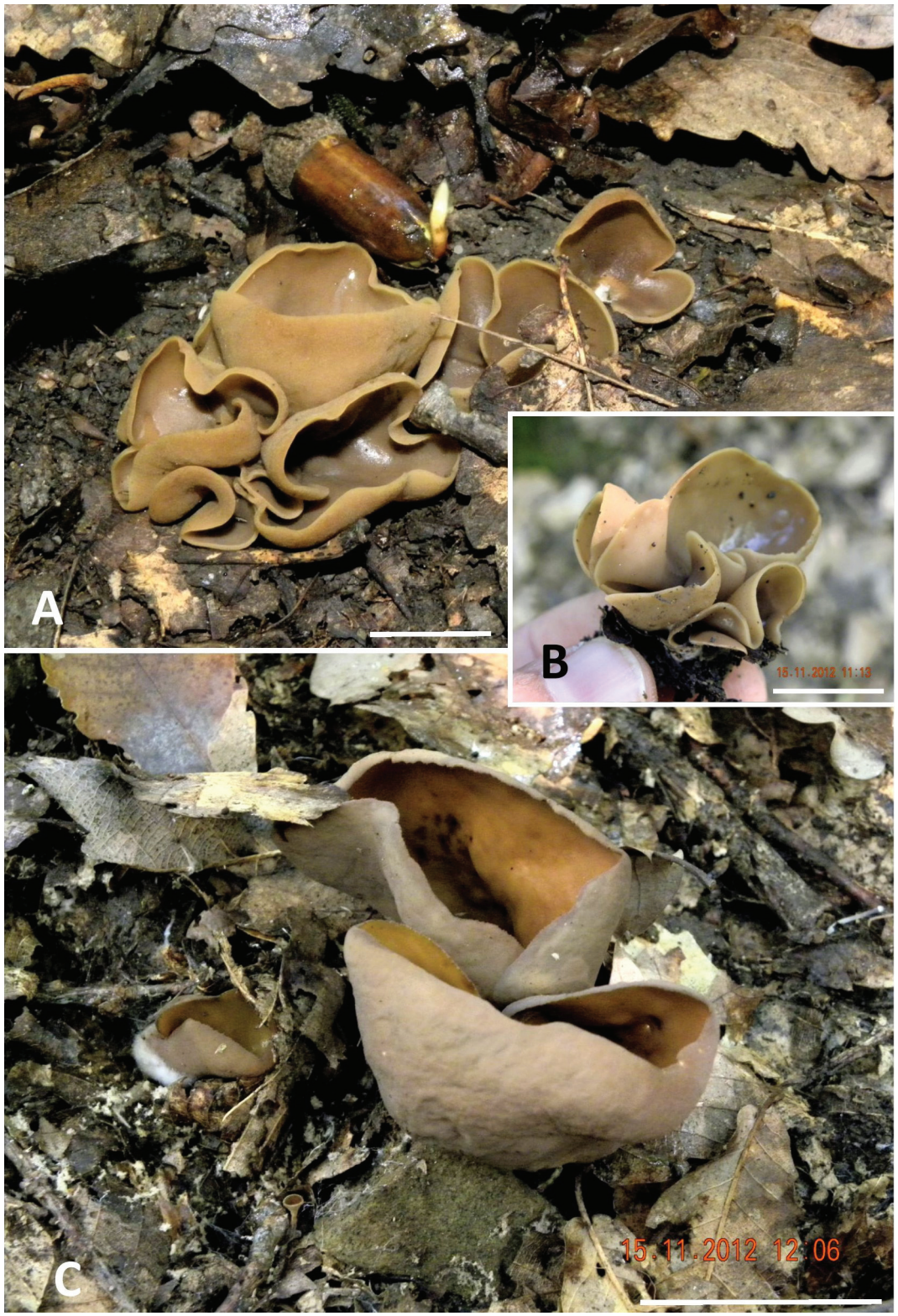

Fig. 1. Apothecia of *Otidea alutacea* on leaf-litter of *Quercus canariensis* in Tunisia (A and B). Apothecia of *Otidea bufonia* on leaf-litter of *Quercus canariensis* in Tunisia (C). (Béni Mtir, northwestern Tunisia, 15 November 2012). Photographs by R. El Mokni. Scale bars: 20 mm

continent (Dennis, 1984; Olariaga et al., 2015; GBIF, 2023). The species is characterised by its medium brown receptacle and light yellowish-brown basal mycelium that lacks brown resinous exudates. Morphologically, it is similar to *Otidea cochleata* due to a identical truncate apothecial shape. However, *Otidea alutacea* can be differentiated by the woody brown apothecia, whereas fruitbodies of *Otidea cochleata* are dark-coloured (Naseer et al., 2019).

***Otidea bufonia* (Pers.) Boud.** (*Otidea* (Pers.) Bonord., Otideaceae Eckblad)

Description. Apothecia 15–45 mm high, 17–32 mm wide, gregarious, rarely caespitose, initially ear-shaped, then soon expanding and becoming deeply cup-shaped, split, stipitate or sessile (Fig. 1, C). Hymenium is initially orange-brown, sometimes olivaceous brown, then dark orange-brown, when dried, greyish brown, slightly purple. Receptacle surface dark brown, occasionally pale rusty brown or purplish brown or with olivaceous tones, slightly hygrophanous, in drying slightly paler, when dried dark orange-brown, warty, seldom slightly wrinkled at the base. Basal tomentum and mycelium abundant, brownish white to light brown. Spores narrowly fused, rarely ovoid, inequilaterally, with two large guttules, very rarely with a third small guttule, smooth, hyaline to pale yellowish, (12–)13–16.5(–18) × 6–7.5(–8) µm (12.4–16.1 × 6.3–7.3 µm); Paraphyses hooked, a few curved, of the same width or slightly enlarged at apices, 3.5–5(–7) µm wide, without notches or rarely with a notch on the underside, when fresh containing small, refractive, light brownish yellow guttules; when dried light brownish yellow. Asci 143–172 × 10–12 µm (Olariaga et al., 2015: 206–209).

Habitat. Apothecia grow gregariously in deciduous forests, saprotrophic on the leaf-litter of *Quercus canariensis* on compacted heavy, rich soils, and more rarely on burnt soils within Kroumirian oak forests of Aîn Draham and Béni Mtir (Jendouba, northwestern Tunisia), very frequent.

Specimen examined: Tunisia, Jendouba, Aîn Draham and Fernana, on compacted loam-clayey soils within abundant leaf litter of *Quercus canariensis* Willd. and *Quercus suber* L., 857 m. a.s.l., 36°45'53" N, 008°42'17" E, 10 January 2012, Collector REM; 15 November 2012, Collector REM.

Notes. *Otidea bufonia* was reported for the first time to Tunisian macromycota and for the second time to the whole African continent (GBIF, 2023). Compared to *Otidea alutacea*, *Otidea bufonia* has a dark brown fruit body on its outside (versus pale brown) and concolorous or slightly paler hymenium (somewhat dark, yellowish or medium brown, mainly when dry in *Otidea alutacea*). In addition, basal mycelium is brownish in *Otidea bufonia*; however, it is pure white in *Otidea alutacea* (Olariaga et al., 2015; Naseer et al., 2019). Moreover, *Otidea bufonia* is ecologically distinguished from the remarkably similar *Otidea mirabilis* Bolognini & Jamoni, a species usually occurring in coniferous forests on calcareous substrates.

***Peziza vesiculosa* Bull.** (*Peziza* Dill. ex Fr., Pezizaceae Dumort.)

Description. Apothecia 10–50 mm high, 20–50(–100) mm wide, sessile, globose, becoming urn-shaped, often contorted when clustered (Fig. 2). Margin incurved, remaining so in age, at times eroded or cracked in age. Hymenium (inner surface) light-brown, pale yellow-brown to medium brown, frequently convoluted or wrinkled. Outer surface granulose to furfuraceous, sometimes indistinctly so when weathered, tan, pale-buff, to nearly white. Flesh thin, pale yellow-brown, fragile; odour and taste mild; *spores* 20–23(–24) × (9–)11–12(–13) µm, elliptical, smooth, lacking oil droplets; *asci* 296–345 × 19.6–23.5 µm. Paraphyses with yellow granules, conspicuously clavate, 5.9–8.8(–9.8) µm broad at the tips (Hansen & Knudsen, 2000: 65).

Habitat. On the leaf litter of introduced *Eucalyptus camaldulensis* Dehnh. in Kroumirian forests, within only two localities.

Specimen examined: Tunisia, Jendouba, Fernana, on sandy clayey soils within leaf litter of introduced *Eucalyptus camaldulensis* and therophytes, 434 m. a.s.l., 36°41'40" N, 008°42'59" E, 17 February 2014, Collector REM.

Notes. *Peziza vesiculosa* was first reported from Oasis d'El-Guettar in the Gafsa region (southwestern Tunisia) as *Aleuria vesiculosa* Boudier (Patouillard, 1897). Its occurrence in Tunisia was confirmed after more than 100 years of its first observation (GBIF, 2023). New localities were recorded in Kroumirian oak forests in the Jendouba region (northwestern

Tunisia). The species could be confused with *Peziza fimeti* (Fuckel) E.C. Hansen (it was recorded only in Morocco within North Africa; GBIF, 2023), from which it could be easily distinguished by its large (2–5(–8) cm versus 2 cm) and exterior pale tan (versus light brown) with minute wart-like pustules (versus granulose) fruit body (Gibson, 2017).

***Phylloscypha phyllogena* (Cooke) Van Vooren** (*Phylloscypha* Van Vooren, Pezizaceae Dumort.)

Description. Apothecia 25–50 mm high, 30–80 mm wide, growing solitarily or in dense clusters, and cup-shaped (Fig. 3). Flesh is thin and fragile, and cup-sides are often tight. Cups are sessile, attached to the substrate at a narrow central point on the bottom. Hymenium is dark purplish brown to a dark reddish grey, while the outer surface is similar to the inner surface or may have more purplish tones. The cup margin is thin, with a sharp edge, and turns black as it dries. Spore print hyaline (translucent) to pale cream. Ascospores ellipsoid, covered with warts, measure 17–23 × 8–13 µm. Asci operculate, 8-spored, and cylindrical, 215–285 × 11.5–13.5 µm (McKnight & McKnight, 1987; Elliott & Kaufert, 1974, as *Peziza badio-confusa* Korf).

Habitat. On soil more rarely on well-decayed logs. Fruit bodies usually appear in early spring (March) in mixed oak forests of Kroumiria. Currently, eight localities were recorded.

Specimen examined: Tunisia, Jendouba, Aîn Draham, Oued Zéen, on loam-clayey soils within leaf litter and mosses, 375 m. a.s.l., 36°49'05" N, 008°50'09" E, 19 March 2018, Collector REM; 21 March 2022, Collector REM.

Notes. *Phylloscypha phyllogena* was reported for the first time in Tunisian macromycota and is the third record for the whole inland African continent (GBIF, 2023). The species could be confused with *Phylloscypha boltonii* (Quél.) Van Vooren & Hairaud (a European species not reported from Africa; GBIF, 2023) from which it could be mainly distinguished by its olive-coloured (versus pale lilac, purple to purple-brown coloured) hymenium, which could sometimes be reddish brown, but with olive highlights (Van Vooren et al., 2021: 114).

Humaria hemisphaerica (F.H. Wigg.) Fuckel (*Humaria* Fuckel, Pyronemataceae Corda)

Description. Apothecia 5–15(–20) mm high, 10–30 mm wide, solitary cup- or saucer-shaped, attached

Fig. 2. Apothecia of *Peziza vesiculosa* on the leaf litter of introduced *Eucalyptus camaldulensis* within Kroumirian forests of Fernana and Béni Mtir (Jendouba, northwestern Tunisia, 17 February 2014). Photograph by R. El Mokni. Scale bar: 20 mm

Fig. 3. Apothecia of *Phylloscypha phyllogena* on leaf litter of *Quercus canariensis* (A) and inside view of the apothecia (B) (Aîn Draham, Oued Zéen, northwestern Tunisia, 21 March 2022). Photographs by R. El Mokni. Scale bars: 30 mm

to the ground, sessile (Fig. 4). Outer surface fringed with stiff, brownish hairs, thick-walled, multiply septate and covered with pointed dark brown hairs, distinctly hair on the margin. Inner surface whitish or greyish. Asci 8-spored. Paraphyses with clavate thickenings. Spores 22–27 × 12–15 µm, broadly elliptical, hyaline, with two drops (Hansen & Knudsen, 2000: 99).

Habitat. Recorded on leaf litter within Kroumirian mixed oak forests, with only two localities.

Specimen examined. Tunisia, Jendouba, Aîn Draham on leaf litter within loam-clayey soils, 669 m. a.s.l., 36°45'29" N, 008°42'21" E, 15 November 2012, Collector REM.

Notes. *Humaria hemisphaerica* is very similar to *Humaria setimarginata* Sánchez-Flores, Raymundo, Van Vooren & García-Jiménez (a Mexican native species), from which it could be distinguished mainly by its large apothecia (up to 30 mm versus up to 15 mm wide), whitish to pale grey coloured hymenium (versus greyish white) and long paraphyses (up to 9 µm versus up to 7 µm in *Humaria setimarginata*) (Sánchez-Flores et al., 2023). The species was first reported in the Tunis region (northeastern Tunisia) as *Lachnea hemisphaerica* Weberb. (Patouillard, 1897). Its occurrence in Tunisia and the whole inland African continent (GBIF, 2023) is confirmed here, after more than 100 years of its first observation. New localities were recorded in Kroumirian oak forests in the Jendouba region (northwestern Tunisia).

CONCLUSIONS

As a result of the current study, the number of Tunisian Ascomycota increased by three species belonging to the order Pezizales, and currently, it includes 46 species. *Helvella* (Helvellaceae), with seven species, remains the richest genus. Two genera, *Otidea* and *Peziza*, currently contain four species, whereas the genera *Scutellinia* (Cooke) Lambotte (Pyronemataceae) and *Tuber* P. Micheli ex F.H. Wigg. (Tuberaceae) include three species each. Records of other species of Ascomycota in the whole of North Africa as well as Tunisia are highly probable, but further exhaustive and continuous studies should be performed, mainly in the oak forests.

Fig. 4. Apothecia of *Humaria hemisphaerica* on leaf litter of *Quercus canariensis* (Béni Mtir, northwestern Tunisia, 15 November 2012). Photograph by R. El Mokni. Scale bar: 20 mm

ACKNOWLEDGEMENTS

Thanks are due to all colleagues and friends of the Regional Agricultural Development Commissions (RADC) of Aîn Draham and Jendouba, forest services of Ghar Dimaou (El Feidja, Aîn Sultan, Âadissa and El Ghorra), Fernana and Aîn Draham (Aîn Draham, Aîn Sellam, Aîn Zana, Tabarka and Babouch) for their technical support and their help during my surveys within Kroumiria region since 2001. I am so grateful to all Italian 'Passione Natura e Micologia' and French 'Ascofrance' members for essential discussions on the subject. I also thank anonymous reviewers for their helpful comments and suggestions to improve the first version of the manuscript.

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