



New records on the genus *Tomophagus* and *Battarrea* for mycobiota of Egypt

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Abstract

During an extensive survey of macrobasidiomycota and the effects of climate changes on their distribution supported by Alexandria Research Center for Adaptation (ARCA) in Egypt and Mohamed bin Zayed Species Conservation Fund (MBZ), several specimens collected, examined and preserved. As a result, two species of *Tomophagus colossus* (Fr.) Murrill (Basidiomycota, Ganodermataceae) and *Battarrea phalloides* (Dicks.) Pers. (Basidiomycota, Agaricaceae) were identified and recorded as new records. Both taxa were identified phenotypically and were subjected to sequencing for confirmation. The internal transcribed spacer (ITS) 1–5.8 s – ITS2 rDNA sequences obtained were compared with those deposited in the GenBank Database and registered with accession number MH796120 and MH796121 in the NCBI Database respectively. We provide an updated full description and illustration of both species.

Key words – Agaricaceae – ARCA – Basidiomycota – Ganodermataceae – Ismailia – MBZ – Nile delta

Introduction

Ganodermataceae Donk (Basidiomycota) was described in 1948 on the basis of double walled basidiospores, with an outer (exosporium) layer relatively thin and hyaline, and the inner (endosporium) usually pigmented, thick and often ornamented, rarely smooth (Cannon & Kirk 2007). Taxonomically the family was almost exclusively based on morphological criteria, such as appearance of pilear surface (i.e., dull or laccate), disposition of the hyphae in the pilear surface (i.e., anamixoderm, characoderm, cortex, hymeniderm, trichoderm) and basidiospore characters (shape and texture including some ultrastructural approaches). Despite extensive studies at generic and infrageneric levels (Ryvarden & Johansen 1980, Corner 1983, Gottlieb & Wright 1999a, b, Ryvarden 2004, Torres-Torres & Guzmán-Dávalos 2012), only five genera are currently widely accepted namely: *Amauroderma*, *Ganoderma*, *Haddowia*, *Humphreya* and *Tomophagus* (Ryvarden 2004, Kirk et al. 2008, Le et al. 2012).

Tomophagus has basidiospores with a truncate apex; however, it is characterized by a pale and soft floccose context where chlamydospores are produced. *Tomophagus colossus* was described by Fries (1851) from Costa Rica as *Polyporus colossus* and later transferred to *Ganoderma* by Baker (1918). It is a rare species but it has been reported throughout the tropics, except from East

Africa (Ryvarden & Johansen 1980, Ofodile et al. 2005).

Battarrea phalloides (Dicks.) Pers. (sandy stiltball, tall stiltball, mallee drumstick, sandy stilt puffball) is a distinctive saprotrophic fungus. It is easily recognizable with a stem up to 40 cm long and spores on the upper surface of the cap, and has been described as resembling a puffball on a tall shaggy stick (Hubregtse 2017). *B. phalloides* has a widespread distribution and has been reported from 64 countries and all continents except Antarctica (Ivančević et al. 2016). However, it is typically uncommon, occurring in small, scattered populations sometimes consisting of only one or two individuals. At least 12 species have been described in the genus *Battarrea* Pers. since 1801 (Index Fungorum Database), although 5 taxa are now considered synonyms of *B. phalloides* (Martín & Johannesson 2000, Jeffries & McLain 2004, Ivančević et al. 2016). Previous phylogenetic analyses of *Battarrea* found three well-supported lineages (Martín & Johannesson 2000, Martín et al. 2013, Garrido-Benavent 2014), which differ in their spore ornamentation patterns. These lineages represent cryptic species within the *B. phalloides* group (Garrido-Benavent 2014).

By scanning of available sources of information concerning genera of *Tomophagus* and *Battarrea* in Egypt no record of both taxa have been recorded until now (Abdel-Azeem 2018). Here we report *Tomophagus colossus* and *Battarrea phalloides* which constitutes the first record of these species in Egypt.

Materials & Methods

Sampling

Elephant foot (*Tomophagus colossus*) fungus were collected from Faculty of Agriculture Ornamental farm in Suez Canal University (N 30° 37' 27.0", E 32° 15' 58.1"). Basidiomata of *B. phalloides* collected many times from different *Citrus* and *Psidium guajava* fields in El-Sanania area, Damietta Governorate, North East of Egypt (N 31° 26' 18.6", E 31° 46' 32.3") during the period of April 2016 to November 2018. Mature basidiomata of *B. phalloides* were collected during different developmental stages. All basidiomata of both taxa were dried and deposited at the Suez Canal University Fungarium (http://www.wfcc.info/ccinfo/index.php/collection/by_id/1180/).

Phenotypic identification

For phenotypic identification of *T. colossus*, identifications were made with the use of such sources of information as Corner (1983), Gilbertson & Ryvarden (1986) and Wu & Zhang (2003). For identification of *B. phalloides* relevant references were consulted as Singer (1962), Moser (1978), Arora (1986), Castellano et al. (1989), Turner & Szczawinski (1991), Jordan & Wheeler (1995) and Martín & Johannesson (2000).

Molecular identification and phylogenetic analysis

DNA was extracted from fungal dried tissue using an adapted chloroform procedure (Arenz & Blanchette 2011). The internal transcribed spacer (ITS) region of ribosomal DNA was targeted for PCR amplification with the primers ITS1 and ITS4 for large subunit amplification (White et al. 1990). PCR amplifications were done using Amplitaq Gold PCR Master-mix (Applied Biosystems, Foster City, CA) and 1 ml of template DNA using the following parameters: 94 °C for 5 min, 35 cycles of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min, and a final extension step of 5 min at 72 °C. PCR amplicons were visualized on a 1 % agarose gel using SYBR green 1 (Life Technologies, Grand Island, NY, US) prestain and a Dark Reader DR45 transilluminator (Clare Chemical Research, Denver, CO, US). Primers used for PCR were used for sequencing reactions on automated DNA sequencer (Model 3100; Perkin-Elmer Inc/Applied Biosystems – Bioneer, South Korea), according to the manufacturer's protocol. Consensus sequences were assembled using Geneious 9.0 (Kearse et al. 2012) and compared to those in GenBank using BLASTn for identification. The sequences of both taxa were compared with reference ITS sequences from the GenBank database at the National Center for Biotechnology Information (NCBI), using the basic local alignment search tool (BLAST). Evolutionary distance matrices based on the neighbor-joining

algorithm (with max sequence difference of 0.75) were calculated using Kimura's two-parameter model (Kimura 1980). Tree topology was inferred by the neighbor-joining method in the program MEGA7 (Kumar et al. 2016), with bootstrap values based on 1,000 replications.

Light and scanning electron microscopy examination

The microscopic characteristics were observed with the Leitz Research microscope (Laborlux S) and microscopic photos were taken with HD USB electronic digital eyepiece camera. Scanning electron microscopy (SEM) studies were conducted at Unit of Electron Microscope at Assiut University. Sections were removed from dried basidiomata and mounted directly on aluminum stubs using carbon adhesive tabs. The fragments were coated with 8–13 nm of gold using a Baltec SCD050 sputter coater and examined with a JEOL JSM-5900 scanning electron microscope.

Results & Discussion

During more than 2 years of survey two species of *Battarrea* specimens were collected and they are stored at the SCU Fungarium with the numbers SCUF-23M, 97M, 98M, 117M and 212M. Only three samples of *Tomophagus colossus* was collected and stored with the number of SCUF-21M, 555M and 601M. This record of *Battarrea phalloides* and *Tomophagus colossus* is the first reported from Egypt.

Taxonomy

Tomophagus colossus (Fr.) Murrill, Torrey 5: 197, 1905. MH796120

Fig. 1 a–d

Specimens Examined – Elephant foot (*Tomophagus colossus*) fungus were collected from Faculty of Agriculture Ornamental farm in Suez Canal University (N 30° 62' 33.30", E 32° 26' 67.46"), Ismailia, Egypt, on the soil probably attached with decaying wood/root, 25 December 2016, 13 November 2017, 9 January 2018, leg. Ahmed M. Abdel-Azeem, det. Ahmed M. Abdel-Azeem (SCUF-21M, 555 and 601).

T. colossus has a pantropical overall distribution (Fungal Red list Initiative 2018). Basidiomata annual, laterally stipitate to sessile, unguulate, dimidiate, large, distinctly light weight, broadly attached. Pileus 16–24 × 10–12 cm, up to 7.5–8 cm thick near base; surface deeply zonate, weakly sulcate near base while gradually deeply sulcate towards margin, glabrous, slightly laccate, that can be easily peeled off, irregularly swollen, light yellow to reddish yellow, mostly dark orange at the base, becoming greyish orange in older specimens; with light cream colour patches due to separation of cuticle surface (Fig. 1a). Hymenial surface showing angular to rounded pores (Fig. 1b).

Hyphal system dimitic. Generative hyphae 2.5–3.4 µm wide, thin- to thick-walled (wall up to 0.5 µm thick), clamped, branched, rare, hyaline. Skeletal hyphae 4–5.8 µm wide, thick walled (wall up to 3 µm thick), mostly aciculiform but also little branched arboriform, smooth (Fig. 1c). Basidia not found. Basidiospores 14–16.8–20 × 9–11.3–13 µm, length/width ratios Q=1.25–1.63–1.80, broadly ellipsoid to ovoid, truncate or rounded at the apex; exospore smooth and hyaline; endospore ornamented partially reticulate, pale yellow (Fig. 1d). Chlamydospores 15–18 × 12–14 µm, spherical, verrucose, thick-walled.

Sample SCUF21M was subjected to molecular identification based on sequence analysis of the ITS regions. A search of the GenBank database via BLAST revealed that the ITS region sequence of MH796121 exhibited high similarity (100%) with *T. colossus*. This relationship was also evident from the phylogenetic tree constructed using ITS region sequences. The isolate MH796120 clustered together with *T. colossus* isolates, thus confirming their closest relationship at the species level (Fig. 2).

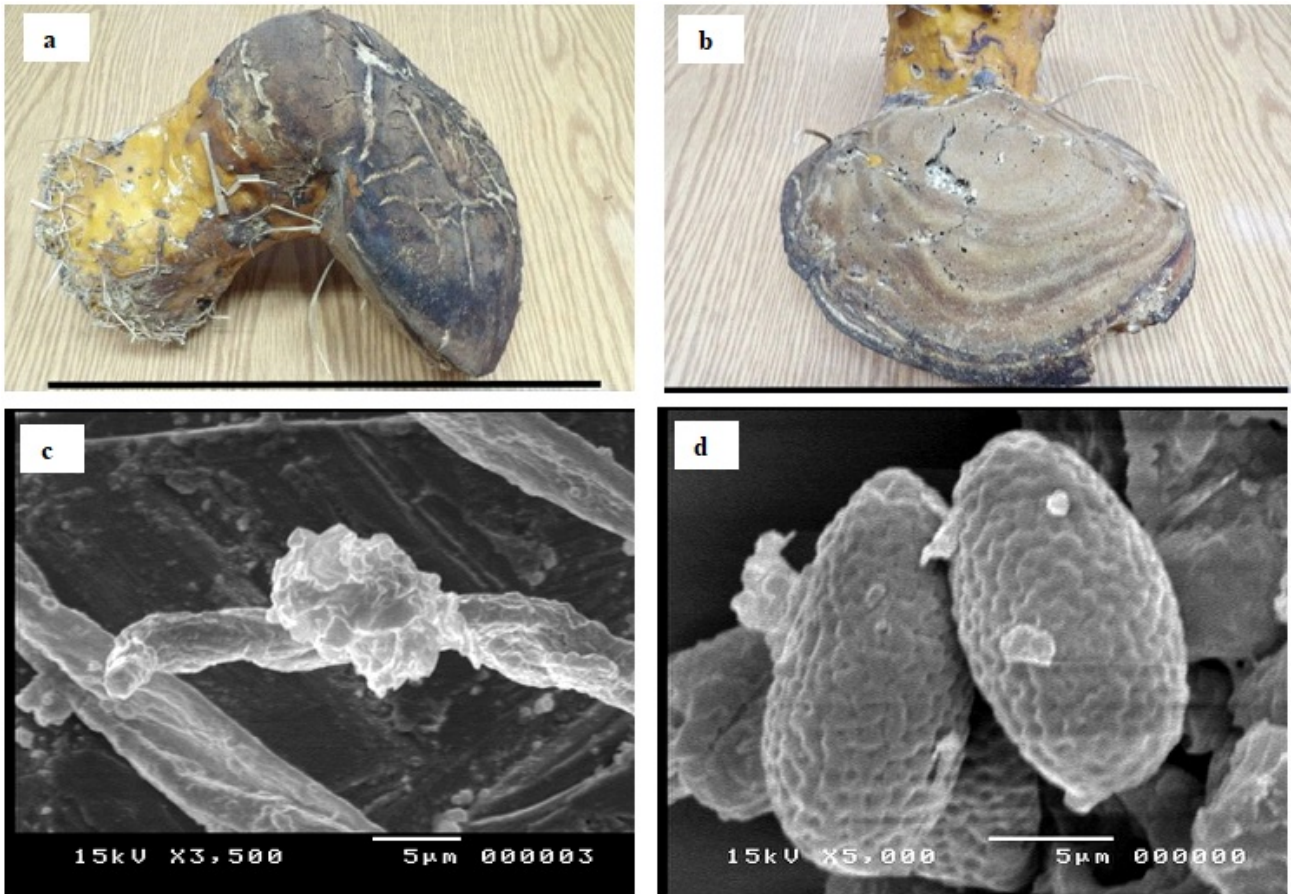


Fig. 1 – a-d *Tomophagus colossus* ((Fr.) Murrill, Torrey 1905). a Basidiomata (lateral view, Scale bar: 20 cm). b Hymenial surface of basidiomata showing angular to rounded pores (lower view, Scale bar: 20 cm). c Skeletal hyphae by SEM. d Mature basidiospores by SEM.

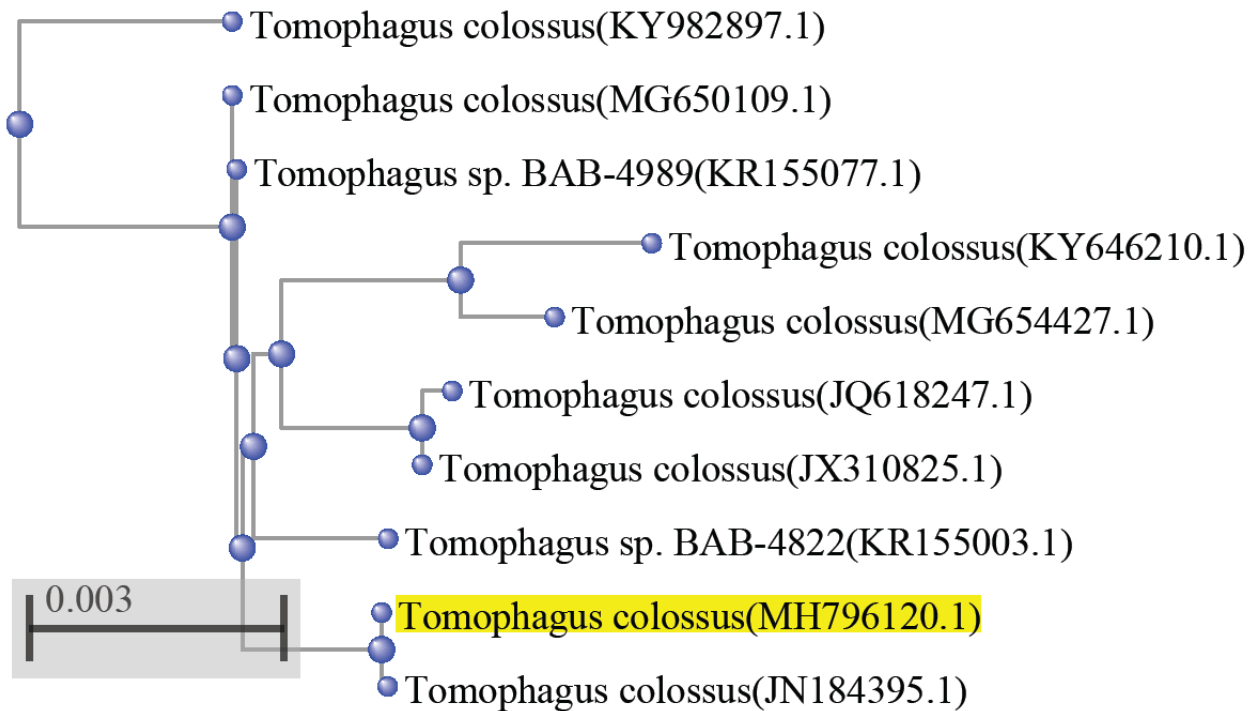


Fig. 2 – ITS sequence tree of *Tomophagus colossus* (SCUF21M-MH796120) made through NCBI blast based on neighbour joining method with max sequence difference of 0.75.

Battarrea phalloides (Dicks.) Pers., Syn. Meth. Fung.: 129, 1801. MH796121

Fig. 3 a–d

Specimens Examined – Nile Delta of Egypt, collected from *Citrus* and *Psidium guajava* fields in El-Sanania area, Damietta Governorate, north east of Egypt (N 31° 26' 18.6", E 31° 46' 32.3"), 25 April 2016, 23 May 2016, 17 May 2017, 30 June 2017, 27 May 2018, leg. Ahmed M. Abdel-Azeem, det. Ahmed .M. Abdel-Azeem (SCUF-23M, 97M, 98M, 117M and 212M).

Battarrea phalloides is a terricolous saprobic taxon, decomposing rotten leaves or other organic matter, occurring in warm-temperate, Mediterranean to tropical climate, on sandy and clay soils, in deserts, semideserts, steppes, or ruderal sites, in dry clear forests, often under trees in shade, sometimes on woody debris or sawdust, fruiting in all seasons (Gibson 2013). Old basidiomata may last for several months after they have dried up (Ellis & Ellis 1990).

Battarrea phalloides is rare everywhere but distributed worldwide (Howladar et al. 2013). Basidiomata developed rapidly, rust in color, stipe expanding up to 35 cm in length and 1.4–1.8 cm in width and bearing spore sac (Fig. 3a). Exoperidium basally remaining as a volva and apically as scales on the endoperidium. Endoperidium and spore sac is hemispherical to conical, 2.8 cm to 7.2 cm in diam. (Figs. 3b, c), later splitting circular along the equator and deteriorating. Upper surface velutinous to densely fibrillose and dry, margin crenate to eroded.



Fig. 3 – a–e *Battarrea phalloides* ((Dicks.) Pers. 1801). a Basidiomata in the field. b Basidiomata (lateral view, Scale bar: 30 cm). c Basidiomata (top view, Scale bar: 5cm). d Elaters by SEM. e Mature basidiospores by SEM.

Gleba pulverulent consisting of capillitia and elaters. Volva membranous, presenting similar color and surface as the stipe, partially adhered to the stipe base. Capillitium hyphae thin-walled,

mostly septate, smooth, little branched, hyaline to pale yellow, with clamp-connections up to 5 μm in diam. Elaters 32–80 μm long and 3.5–7.0 μm wide, pale yellow, walls smooth, tapering and bearing spiral thickenings (Fig. 3d). Basidiospores thick walled, rusty brown, finely warted, 5.0–6.5 μm in diam (Fig. 3e). Under SEM, the spore ornamentation of all Egyptian specimens appears with anastomosing truncate ridges.

Sample SCUF-23M was subjected to molecular identification based on sequence analysis of the ITS regions. A search of the GenBank database via BLAST, where ITS sequences (AF215648, AF215650, AF215656 and AF215658) from Martín & Johannesson (2000) and unpublished sequences from GenBank database (DQ184684 and DQ184689) were used, revealed that the ITS region sequence of SCUF-23M exhibited high similarity (99%) with *Battarrea phalloides* and deposited under the number MH796121. This relationship was also evident from the phylogenetic tree constructed using ITS region sequences. The isolate SCUF-23M (MH796121) clustered together with *Battarrea phalloides* isolates, thus confirming their closest relationship at the species level (Fig. 4).

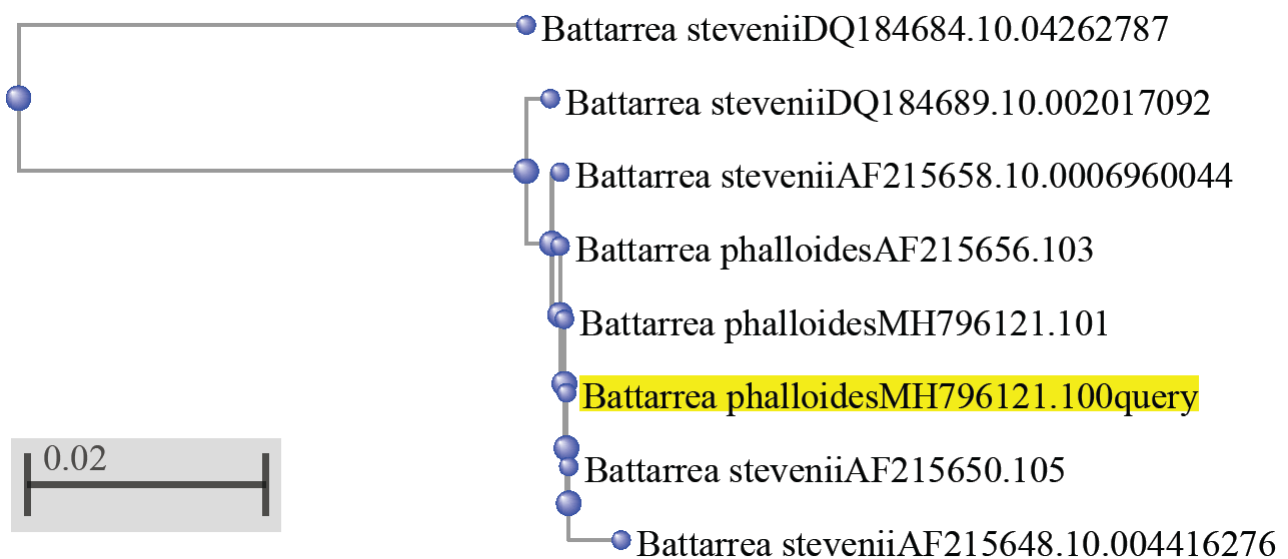


Fig. 4 – ITS sequence tree of *B. phalloides* (SCUF-23M- MH796121) made through NCBI blast based on neighbor joining method with max sequence difference of 0.75.

Conservation status

Conservation of fungi in Egypt and worldwide remains very low, and more education on fungal conservation is urgently needed (Abdel-Azeem et al. 2018). Although these species distribution is rare in Egypt and the conservation status of this fungal species according to the IUCN criteria (2015) needs more studies.

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