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Cover Illustration

Exemplar of a fossilized fungus comb from Fungus-growing termites (Size: around 6cm). It was extracted in the Chad basin from 7 million-year-old continental sandstone in the same sites where the skull of *Sahelanthropus tchadensis*, the earliest hominid, was found. This is the first fossil fungus comb ever found in the world and the first evidence of a symbiotic relationship between termites (Macrotermitinae subfamily) and fungi (*Termitomyces*) in tha past.

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SHORT COMMUNICATION

The first fossil fungus gardens of Isoptera: oldest evidence of symbiotic termite fungiculture (Miocene, Chad basin)

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Abstract Higher termites of the subfamily Macrotermitinae (fungus-growing termites) are known to build fungus gardens where a symbiotic fungus (*Termitomyces* sp.) is cultivated. The fungus grows on a substrate called fungus comb, a structure built with the termites' own faeces. Here we present the first fossil fungus combs ever found in the world. They were extracted from 7-million-year-old continental sandstone (Chad basin). Fossilized fungus combs have an ovoid morphology with a more or less flattened concave base and a characteristic general alveolar aspect. Under lens, they display a typical millimetre-scale pelletal structure. The latter, as well as the general shape and alveolar aspect, are similar to the morphology of fungus combs from extant fungus-growing termites.

Keywords Fossil fungus · Termite fungiculture · Symbiotic · Isoptera · Fungus gardens

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Introduction

Fungus combs of fungus-growing termites (Macrotermitinae, Termitidae) (Figs. 2f and 3a) are distributed throughout the soil to depths of several metres (Grassé 1982; Wood and Thomas 1989). Most of them are characterized by three main features: (1) an alveolar or cellular aspect sometimes brain-shaped (Figs. 2f and 3a); (2) they often have a convex top combined with a concave or more or less flat base (Figs. 2f and 3a); and (3) a pelletal structure formed by the stacking up of millimetre-sized balls (faeces) (Figs. 2f and 3a,b) (Sands 1969; Grassé 1978, 1982; Josens 1971; Darlington 1997; Rouland-Lefèvre 2000).

The convex top combined with the concave base of many fungus combs and the occurrence of millimetre-sized faecal balls result from the building behaviour of Macrotermitinae. Plant material ingested after a brief mastication goes rapidly through the gut without significant digestion. Material accumulated in the rectum is then expelled in one piece, forming the faecal pellet referred to as a mylosphere (Grassé 1978, 1982; Josens 1971). Termites use these faeces in the form of small balls to construct the sponge-like fungus comb on which symbiotic fungi grow (*Termitomyces* spp., *Basidiomycota*) (Sands 1960; Grassé 1982; Wood and Thomas 1989; Rouland-Lefèvre 2000).

The fungus combs are more than just an accumulation of mylospheres: as new faecal material is added (often on the

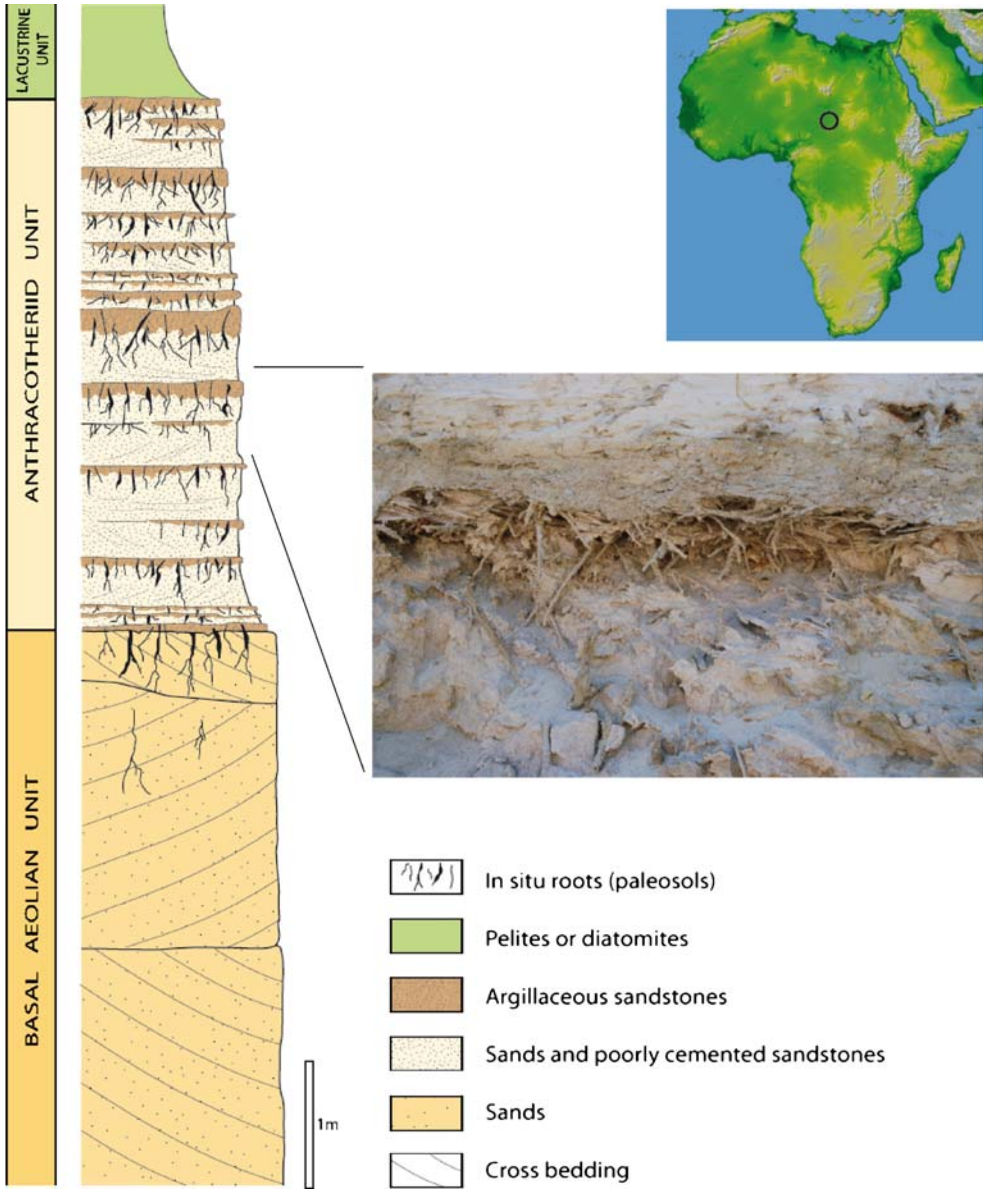

Fig. 1 Synthetic stratigraphic column. The fossilized fungus combs were collected in the palaeosol levels inside the anthracotheriid unit (the section is a synthesis of several smaller profiles in the same area)

top of the comb), the old part of the comb is eaten (often at its base) (Sands 1960; Alibert 1964; Josens 1971). The full significance of the fungus in termite nutrition is still debated (Bignell 2000), but it may have some benefits: the fungal symbiont degrades complex substances (such as lignin or cellulose) into substances that can be used by termites (Wood and Thomas 1989; Hyodo et al. 2000).

Fungus combs have been known and described since the 18th century (Grassé 1982). Whereas fossilized termite nests are well known and described (Coaton 1981; Bown 1982; Sands 1987; Genise and Bown 1994; Bown and Laza 1990; Schuster et al. 2000; Genise 2004; Darlington 2005), fossilized fungus combs have never been reported until now. The aim of this paper is to present the first examples of fossil fungus combs and to compare them with the fungus combs of extant species.

Materials and methods

The rock exposures are located 600 km NNE from N'Djamena (Chad). The deposits belong to the geodynamic setting of the Chad basin (Schneider 1968, 1989; Schuster et al. 2005). These Upper Miocene continental formations have attracted attention since the discovery there of the earliest known hominid (*Sahelanthropus tchadensis*) (Brunet et al. 2002). The fossiliferous sites, estimated to be around 7 million years old (Vignaud et al. 2002), are dated biochronologically by the evolutive degree of their faunas.

Rock exposures commonly present large, flat-to-gently-undulating sandstone surfaces. Geological sections rarely exceed a few metres in height. The palaeontological assemblage comprises a rich fauna that includes mammals, birds, reptiles and fish (Vignaud et al. 2002). They are associated with rhizoliths in high density (Fig. 1) and insect trace fossils, including many termite nests (Schuster et al. 2000). Among this new Upper Miocene termite material, a dozen alveolar fossils have been extracted from the anthracotheriid unit (Vignaud et al. 2002) situated between a well-developed aeolian unit at the base (Schuster et al. 2006) and a lacustrine one on the top of the section (Fig. 1) (section of Fig. 1 is a synthesis of several profiles in the same area). The anthracotheriid unit is composed mainly of sand and argillaceous sandstone alternations (Fig. 1). The fossils were found in argillaceous sandstones characterized by dense networks of roots (palaeosols) and termite nests. Argillaceous sandstones were deposited during wet periods that enabled the development of dense vegetation accompanied by ephemeral pools and discontinuous lake expansions. Sands and poorly cemented sandstone beds are interpreted as a Sahelian environment which developed during dry periods. Termite nests and root trace fossils are often truncated by others palaeosols or by aeolian deposits, which indicates that termite colonisation was contemporaneous with the bearing palaeosol (Tessier 1959; Cloud et al. 1980).

Results

The fossils are roughly ovoid in shape, often with a flattened concave base (Figs. 2d and 3c). The best-preserved specimen (Fig. 3c) is 6.5 cm wide and 2.5 cm in height. It is composed of irregular subhorizontal to undulating plates linked together by vertical walls (Fig. 3c). The external aspect is clearly alveolar, composed of millimetre-scale small, flattened-to-oval cells.

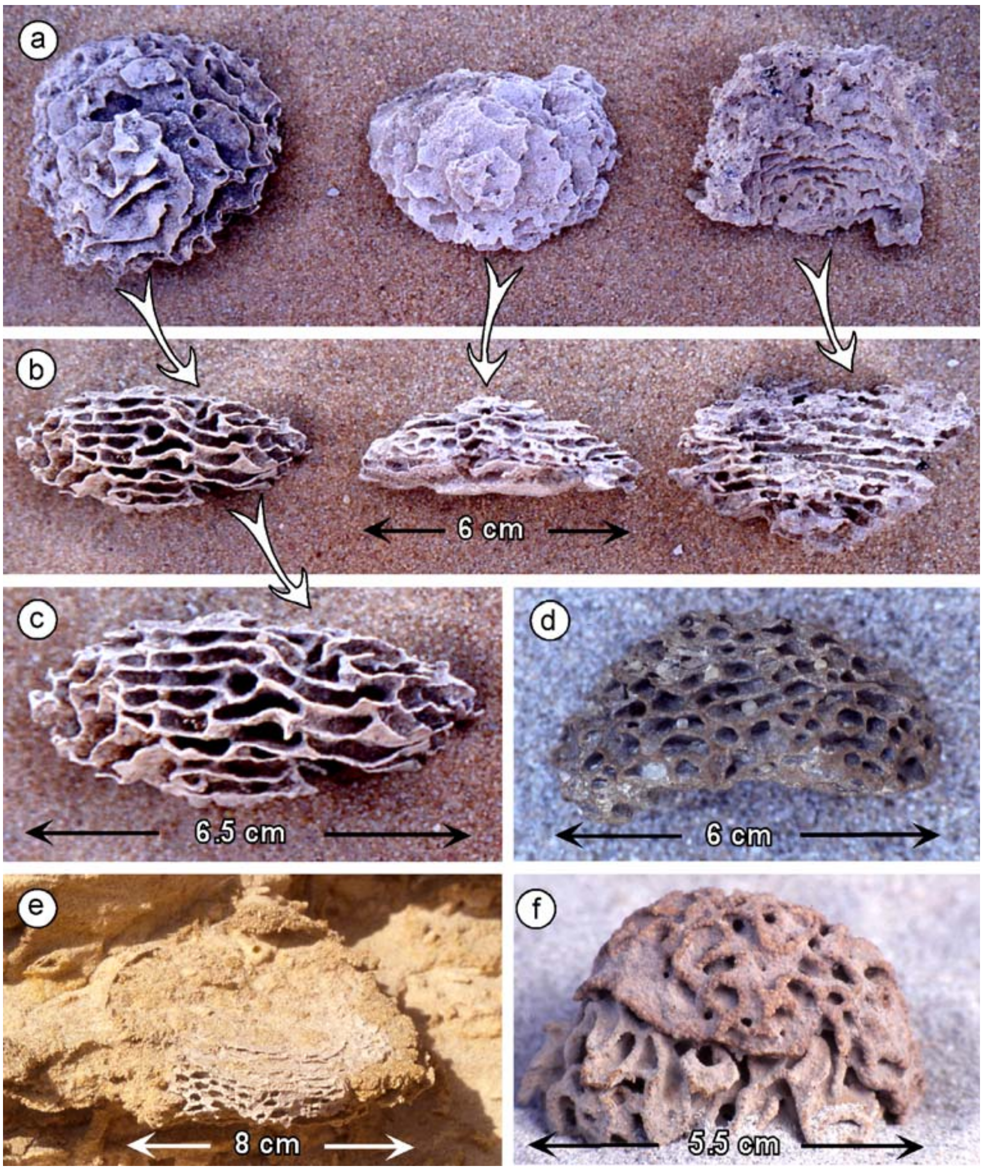

Fig. 2 Fossilized fungus combs in plane (**a**) and side view (**b**). Close view of “laminar” (**c**) and “sponge” (**d**) type. **e** In situ fungus comb in poorly cemented sandstone. **f** Fungus combs of the extant species *Odontotermes* sp. (material courtesy of the University of Dijon, France)

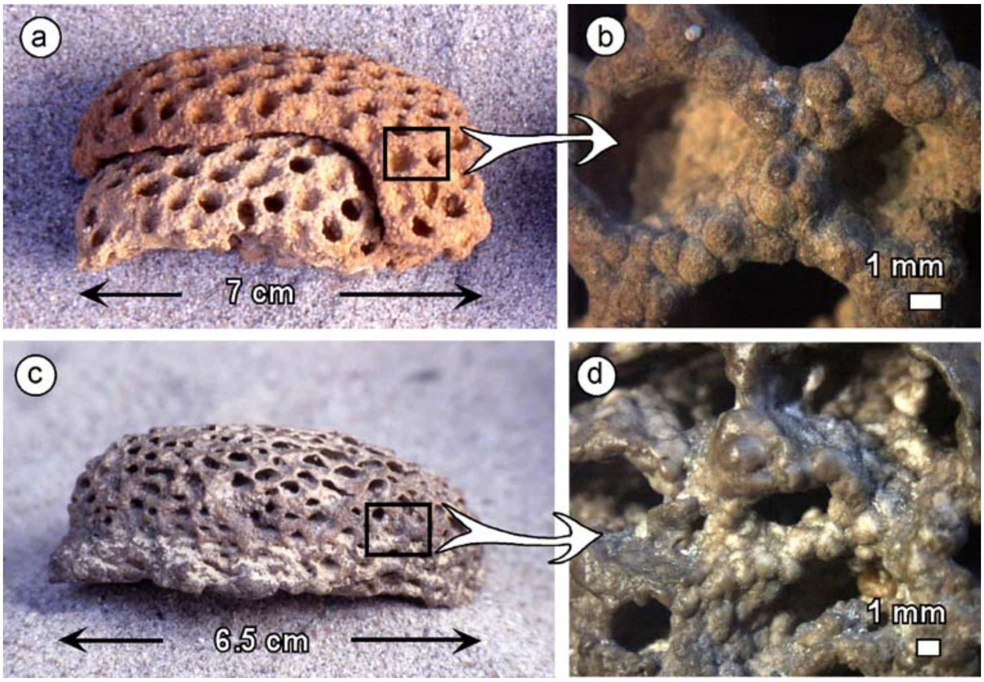

Fig. 3 Structure comparison between the fungus combs (**a, b**) of extant fungus-growing termites (*Odontotermes* sp.) (material courtesy of the University of Dijon, Dijon, France; photograph by A. Peppuy in the Tam Dao National Park in North Vietnam) and fossil fungus combs (**c**, **d**)

They are commonly wider than they are high, resulting in a rectangular shape (Figs. 2b–d and 3c). The height of each cell is relatively constant at around 2 mm on average, whereas the width commonly ranges from 2 to 8 mm. Many cells on the upper part of the structure (Figs. 2d and 3c) have a more or less arched top with a concavity oriented downwards. In all specimens studied, the cells were empty, resulting in the typical sponge-like structure. The walls of many fossils display a pelletal texture composed of juxtaposed small balls of about 1 mm on average in diameter (Fig. 3d). All specimens, as well as the majority of other fossils (bones, termite nests and roots), are partly to entirely silicified.

Discussion

The fossils look like termite fungus combs of extant Macrotermitinae (Heim 1977; Grassé 1978, 1982; Wood and Thomas 1989; Darlington 1997; Rouland-Lefèvre 2000; Mueller and Gerardo 2002) (Figs. 2f and 3a,b). Both extant and fossil examples of fungus combs share similar structures, in particular, the general alveolar, cellular or sponge-like aspect or the pelletal texture formed by the stacking up of millimetre-sized balls of faecal matter (mylospheres) (Figs. 2 and 3). The general shape of fungus combs of extant termites and the alveolar aspect can be highly variable depending on the termite species examined (Rouland-Lefèvre 2000). However, all extant examples share two important features: the occurrence of mylospheres and the sponge-laminated-like aspect (Figs. 2 and 3). These two main features are present in the fossils described in this article (Figs. 2b–e and 3c,d). Based on these morphological characteristics, the Chadian fossils are interpreted as fossilized termite fungus combs.

It is difficult to identify the genera of termites based on the shape of the combs because the species of about 12 genera build a large diversity of types of fungus combs (alveolar, globular and oolitic) or formed in vertical units (Heim 1977; Grassé 1982; Darlington 1997; review in Rouland-Lefèvre 2000). Lepage and Noirot (personal communication) believe that the general shelved aspect of the fossilized fungus combs illustrated in Fig. 3c is typical for combs of *Odontotermes* sp. After Darlington (1997), Fig. 2a–c belongs to the "laminar" type of *Odontotermes* fungus combs, whereas Figs. 2d and 3c,d belong to the "sponge" type. Fossil fungus combs presented in Figs. 2e and 3c are particularly similar in aspect and structure to the hemispherical fungus combs of *Odontotermes pauperans* (Grassé 1982) (same shape, convex upwards, flat to concave base and similar size). Nevertheless, the comparison remains difficult because the morphology and the size of the comb differ among species of this genus (Darlington 1997).

The ecological distribution of Macrotermitinae is broad. They are found from the fringes of tropical rain forests out to the edges of semi-arid steppes (Wood and Sands 1978; Bignell and Eggleton 2000; Aanen and Eggleton 2005). They are generally detritivores, feeding mainly on dead wood, dead grass, dung and roots of dead or living plants, depending on the different species. Many species of *Microtermes* sp. and *Odontotermes* sp., whose fungus combs are comparable with the fossilized ones described herein, locate their nests in savanna to Sahelian environments. *Odontotermes pauperans* are also common in Sahelian environments of the southern Sahara (Grassé 1984).

An important question is how such damp fragile structures can be preserved. If the nest is abandoned by the termites, the comb soon decomposes. Only rapidly desiccated fungus combs result in hard preservable structures. The occurrence of numerous interbedded aeolian sands can support this process of fossilization. However, it is obvious that a rapid silicification following the desiccation is necessary for the preservation of such fragile structures.

All extant species of fungus-growing termites have a single origin and all extant species of symbiotic termite fungi as well (Aanen et al. 2002). The symbiosis is probably far more than 7 million years old. The significance of the present discovery is to show for the first time that recognisable fungus combs can be preserved as fossils.

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