Identification and anatomo-morphological description of three species of boletes collected from western Burkina Faso

[*]Benovana Bakiono[1], Andjièrèyir K. Somda[1], Elise Sanon[1], Kounbo Dabire[2], Marie L. Guissou[3], Samson Nankone[3] and Ragnaguénewindé S. Nana[1]

[1]Team of Phytopathology and Tropical Mycology, Laboratory Bioscience, Department of Plant Biology and Physiology, Training and Research Unit in Life and Earth Science (UFR/SVT), Joseph KI-ZERBO University, 03 BP 7021, Ouagadougou, Burkina Faso
[2]Tenkodogo University Centre, Thomas Sankara University, 12 BP 417 Ouagadougou 12, Burkina Faso
[3]Life and Earth Sciences Laboratory, Norbert Zongo University, BP 376 Koudougou, Burkina Faso
[*]Corresponding author's email: bbenovana@gmail.com

Abstract

Boletes in the broadest sense belong to many genera and even families. They are mushrooms with soft, putrescible flesh, sometimes turning blue on contact with air. They generally grow on the ground, close to trees, where they develop mycorrhizae. In Burkina Faso, these mushrooms contribute significantly to the food supply of some populations, as well as to the regeneration of forest ecosystems through their ectomycorrhizal relationships. The present study was carried out in the gallery forests of Dan, Toussianbandougou and Kotoudeni in Burkina Faso. The objective of this study was to identify different species of boletes in this area by describing their microscopic and macroscopic characteristics. Macroscopic, microscopic and anatomic studies led to identification of three species of boletes namely *Boletellus lindéri*, *Tylopilus balloui* and *Strobilomyces strobilaceus*. Analysis of spore measurements showed a significant difference among the three species. Of these species, *Strobilomyces strobilaceus* was identified as endemic to Burkina Faso.
Keywords: Anatomo-morphological, *Boletellus lindéri*, Burkina Faso, Ectomycorrhiza.

Introduction

Fungi in general, and macromycetes in particular, have an important and irreplaceable role to play in the ecosystem. Ectomycorrhizal fungi are involved in the regeneration of natural and secondary forests, as well as in environmental balance (Bâ *et al*., 2012). They also play a major role in the diet of certain populations, providing energy, protein, lipid, carbohydrate and ash-rich food for those in rural areas (Ekissi *et al*., 2021, Mondo, 2024). Some species such as *Podaxis pistillaris* and *Ganoderma lucidum* are also used in traditional pharmacopoeia in Bukina Faso (Guissou, 2005; Guissou *et al*., 2014). Knowledge of the diversity of higher fungi in tropical Africa remains fragmentary (Kamou *et al*., 2017).

Boletaceaes are multicellular or unicellular fungi. The Boletaceae family we are interested in is well diversified, with around 50 families and over 800 genera (Kirk *et al.*, 2008, Wu *et al*., 2014). However, in Africa, this diversity is so great that it is difficult to make reliable estimates of their numbers (Castaño *et al*., 2017). The fungi of this family are generally characterized by a fleshy appearance and a tubular hymenophore, rarely lamellate or localized. These complex characters give boletes diversity, and this diversity can be observed from one continent to another or from one country to another (Wu *et al.*, 2016; Badou *et al.*, 2018). The forests of western Burkina Faso are rich in bolete diversity (Bakiono *et al.*, 2024), but there is no specific study on species description. The local climate is South Sudanian, with average annual rainfall ranging from 900 to 1100 mm. Forest formation is mainly linked to the presence of watercourses, and vegetation develops in gallery forests. The Kotoudeni gallery forest is located in the village of Kotoudeni, whose land was once covered with natural plant formations and food crops, but has gradually been planted with orchards, providing a good example of the upheavals taking place throughout the Sudanian savannahs (Fig. 1). Its terroir (10° 55'-11° 00'N and 5° 00'-5° 05'W) is located in the department of Orodara, in a tropical South Sudanian climate (800 to 1200 mm annual rainfall), with mostly sandy-clay to clay or silty-clay soils (Fontès *et al.*, 1995). The spontaneous vegetation consists mainly of more or less dense Sudanese shrub savannas (15–60% woody cover), with 152 woody species belonging to 40 families (Fournier, 2016). Four main types of land use have been recognized by ground observation: fields and young fallow land, orchards, "bushes" and forest

galleries (Bene, 2011). The Kotoudéni terroir has seen a considerable reduction in closed forest formations (bushes and forest galleries) in favor of fields and orchards, which have become dominant. This development has taken place against a backdrop of strong demographic growth and technological change (harnessed and motorized cultivation) combined with climatic hazards such as low quantity and poor distribution of rainfall (Bene, 2012). The objective of this study was to identify the different species of boletes forests of western Burkina Faso by describing their microscopic and macroscopic characteristics.

Materials and Methods

Study site

The study site is located in the province of Kénédougou in the extreme west of Burkina Faso, in the administrative region of Hauts-Bassins, between 4°30' and 5°30' west longitude and 10°10' and 12°05' north latitude, covering an area of approximately 8265 km^2. It is bordered to the north and west by the Republic of Mali, to the south by the provinces of Léraba and Comoé, and to the east by the province of Houet. The Dan gallery forest is located some 15 km from the town of Orodara, while the Toussianbandougou gallery forest is situated between latitudes 10° 58' 50" N and 04° 50' 36" W, west of the village of Toussianbandougou, 5 km from Orodara on the Orodara-Bérégadougou road. Natural plant formations, mainly composed of *Berlinia grandiflora*, *Uapaca* sp., *Afzelia africana* and *Malacantha alnifolia*, and fields of annual crops such as cereals, legumes and manioc, along the banks of the villages of Dan and Toussianbandougou. There are also perennial crops such as citrus plantations (*Citrus* sp.) and a palm plantation (*Elaïs guineensis*).

Data collection

The collections were made in 2018-2020 in two steps in the gallery forests of Toussianbandougou, Dan and Monhondon dominated by *Berlinia grandiflora* and *Afzelia africana* but also *Elaesis guinensis.* The first collection was made during the last week of July and the second during the second half of August. Following the method of Bâ *et al.* (2012), boletes porophores were harvested under the host plant's crown, sometimes on the trunk and at some distance (30 m or more) from it, but with the utmost care. All carpophores of the Boletaceae family were collected, photographed with a Canon camera (EOS 100D) and wrapped in aluminium foil for the base camp for macroscopic description. Geographical coordinates were recorded using a Garmin Dakota 20 Taiwan GPS. Samples were dried using a Stockli electric dryer for 12–18 h at 70 °C, depending on consistency. Dried specimens were placed in minigrip plastic bags and transported to the mycotheque of the phytopathology and tropical mycology team of the BIOSCIENCES laboratory at Joseph KI-ZERBO University.

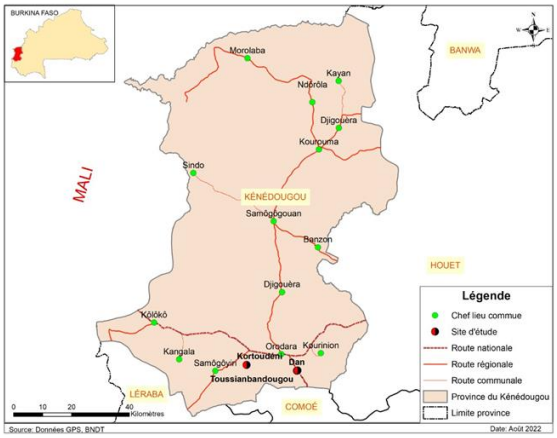

Fig. 1: Geographical map of collection area.

Data processing

The morphological characteristics of each sample were recorded using the methodology proposed by earlier scientists (De Kessel *et al*., 2002; Onguene and Kuyer, 2019). The identification of the fungi required the observation of morphological characters using terms specific to mycology. Macroscopic description was carried out on fresh carpophores. The microscopic study consisted of observing the fungal fragments to determine the cap and stipe coatings. The hymenium elements (tubes) were cut longitudinally and placed in an observation medium between slides. Preparations were made with 5% and 10% potassium hydroxide and Congo Red (1% solution in diluted ammonia to stain walls and contents of structures) for good observation of cystids and basidia. For spores, we used Melzer, a reagent composed of iodine 0.5 g + potassium iodide 1.5 g + 20 mL water + chloral hydrate 20 g, used in the amyloidity test for better observation of spore shapes and ornamentation. Observation of the different parts of the sporophores: color, shape, aspect and dimensions (diameter and thickness), the characteristics of the hymenium: at this level the color of the face of the tubes, their shape, color, density, insertion and color changes of the tubes were noted and the stipe (length, width and consistency). All drawings were made at ×10 ×100 before measuring the various structures (basidia, basidiospores, cystids, etc.) in micrometers. We took 70 spores, 30 basidia and 30 pleurocydtids from each species studied. For elements other than spores, we measure the total length (in micrometers) of the element, and as width (in µm), we measure the most swollen part, as indicated by Buyck (1994). Only the extreme values (maximum and minimum lengths of the largest elements, as well as widths) of the elements measured are used in the description and

identification. Finally, for basidiospores, we measure the length and width of each spore (in µm), and calculate the Q ratio (length/width) of the spore. The ratio of spores measured enables us to determine the shape of the spores, using the key proposed by Buyck (1993). Measurements were entered into an Excel 2016 spreadsheet, and the parameters measured were Q ration, maximum and minimum length, and maximum and minimum width. Scanning Electron Microscopy (SEM) was used to study only spore characteristics such as shape and size, which are clearer and more precise than with light microscopy. The method is fairly simple and can be divided into 3 short stages. The first step is to cut out a fragment of the sporophore lamella and place it on an adhesive glue covering the surface of the silver spots. The second step is metallization, which consists in placing each stud with the piece of lamella in a metallizer fitted with a glass bell covered by a lid with a valve. In the third step, the metallized studs are removed and placed in the electron microscope connected to a computer for image visualization.

Results

Identification of boletes species

There was an anatomical and morphological diversity of boletes collected from western Burkina Faso. Three bolete basidiomes were described and observation of macroscopic characteristics allowed the three boletes to be classified in the genera *Boletellus, Tylopilus* and *Strobilomyces*. The description enabled us to identify the following species: *Strobilomyces strobilaceus*, *Boletellus lindéri* and *Tylopilus balloui*. These three species belong to the Boletacaes family. Results of the statistical analysis of basidiospore width and length for the three species showed a significant difference (Fig. 2). Basidiospores measurements showed a significant difference between *S. strobilaceus* and the other two species, namely *B. lindéri* and *T. balloui.*

Description of *T. balloui* (Peck) Singer

The species was collected on August 9, 2019 at Mondon, Kénédougou (Orodara) Coordinates: 10° 52' 16,3 " N; 4° 50' 04,96" W. Alt. 467 m. It is characterized by a whitish cap, plano-convex in shape, hemispherical when young, and a uniform coating with a mucilaginous, adhesive appearance when wet (Fig. 3A & B). The fungus has a gregarious and often cestipitous growth habit. The diameter of the cap varies from 1.5 to 4 cm, and the thickness of the flesh from 0.1 to 0.3 cm. The hymenophore is milky white, the pores are angular and very fine (20 to 30 cm), the tubes were short (20 to 30 cm) and the flesh is bright white and fleshy. The stem is cylindrical, central, small and concolored at the cap. The *T. baloui* species identified has a pustular coating and a solid internal structure, with length and thickness ranging from 1 to 4 cm and 0.5 to 1 cm, respectively. Pileics cells are giant, elongated, smooth and septate (Fig. 4A). Pleurocystidia are cylindrical, with rounded apices and clear contents 22–36 × 7–10 µm (Fig. 4D); basidia are tetrasporic, cylindrical and elongate: 25–38 × 8–10 µm (Fig. 4H). Spores are numerous, hyaline to melzer, apiculate, smooth ellipsoid to elongate: 6–12 × 4–5 µm (Qm =2.1, N = 70) (Fig. 3D & E).

Description of *S. strobilaceus* (Scop.: Fr.) Berk

The sample was collected on August 26, 2020 in the Koutoudeni gallery forest, bank dominated by *Berlinia grandiflora*, coordinates: 10° 56' 97.6" N and 5° 01' 23.3" W alt.483 m. It has a blackish cap, a hairy coating (Fig. 5A & B), fungal odor, with a diameter of: 2–12 cm, flesh thickness varying from: 0.4- 2.5 cm, flesh is flesh and whitish, blackening on contact with oxygen (Fig. 5C). The hymenium is tubular, whitish and blackens to the touch, covered by a veil. The pores are thick, angular in the center and thin at the margin, tubular: 0.3–2.1 cm, with a brownish spore. The stipe is concolorous with the cap, and is: 4.5–18.5 cm and 0.3–1.6 cm wide, with a cylindrical shape of hardened, fibrous consistency and a honeycombed coating.

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Microscopic observations showed giant hyphae on the cap lining, with contents that were sometimes smooth and partitioned (Fig. 6A). The stem cells are largely clavate, with cross-shaped contents (Fig. 6B & C). Pleurocystidia cylindrical and subventral (Fig. 7E): 34–65 × 10–18 µm, basidia cylindrical, giant, tetrasporic: 42–67 × 11–18 µm with pronounced, elongated sterigmata (Fig. 6F), spores are subglobose to elongate with apicles and are peculiarly striated: 12–17 × 5—11 µm (Qm = 1, 57, N =70) (Fig. 6E & F).

Description of *Boletellus linderi* Singer

Sample collected in Burkina Faso, Kénédougou province, coordinates 10° 53' 35,6" N and 4° 50' 23,3" W. Alt. 448 m. The cap is plano-convex in the adult state and brownish-yellow in color with a rough appearance, squamulose coating, diameter (Fig. 7A & B): 4–17 cm, thickness 0.3–1 cm. Tubular hymenophore is yellowish and rapidly

changing to blue after cutting with angular and thick pores (8–12 cm), easily detachable from the cap. Flesh is yellowish and flesh, turning greenish after cutting, with a fruity odor. The stipe is cylindrical with a central insertion (Fig. 7A), concolorous with a cylindrical cap, length 5–12 cm, diameter 0.5–2 cm, solid consistency. Microscopically, giant, elongated hyphae with heterogeneous terminal cells, hyphae in the cap are septate with some contents and others with smooth contents (Fig. 8A). The hyphae on the cap are longer and not septate. Basidia are tetrasporic, subclavate to elongate (Fig. 8D), pleurocystidia are few and variable in size: 25–60 × 8–15 µm; Basidia: 25–35 × 8–12 µm; basidiospores are abundant (Fig. 7C), smooth, elongate: 10–13 × 5–7 µm (Qm =2.1, N = 70).

Discussion

The present study was based on the anatomo-morphological description of boletes collected in western Burkina Faso. A total of three bolete basidiomas were described, and observation of macroscopic characters enabled the three boletes to be classified within the Boletellus genus, Tylopilus and Strobilomyces. Thanks to morphological and anatomical description analyses and scientific documentation (Bâ *et al*., 2012, Wu *et al*., 2014), we have identified three species namely *B. linderi*, *T. balloui* and *S. strobilaceus*. These three species all belong to the Boletacaes family. Among the three, the presence of *B. linderi* has been reported in Côte d'Ivoire (Vanié-Léabo, 2016). Boletetacae family presents some very interesting species but is less diversified in terms of specificity, covering few species (Raspé *et al.*, 2016). It has several characteristics that differ from those of *S. strobilaceus*. However, there is a slight resemblance in spore ornamentation. The *Strobilomyces* genus comprises species whose differentiation is very complex (Han *et al.*, 2019). *S. strobilaceus* is a rare and curious species that resembles no other with its scaly, woolly cap and stipe. It was collected only in the Kotoudeni forest gallery and is an endemic species in Burkina Faso that should be protected. The spores are elongated, the pleurocystidia are very large (34–65 × 10–18 µm) and the cells are pillate with cross-shaped membranes. This ornamentation is specific to the *S. strobilaceus*. The work of Han *et al.* (2019) shows genetic diversity within the Strobilocyces genus. *Boletus linderi* has been reported in countries such as Côte d'Ivoire, particularly in forests dominated by species such as *Bernilia grandiflora*, *Afzélia africana* (Vanié-Léabo *et al.*, 2017).

The boletes were collected in forest gallery sites in areas with annual rainfall of between 900 and 1200 mm^3, at an altitude of 470 m. Their hosts are ectomycorrhizal species such as *Bernilia grandiflora*, *Afzelia africana* and *Uappaca*, with which they maintain a symbiotic relationship. Plant formations containing these different species are conducive to the fruiting of ectomycorrhizal fungi such as boletes (Leabo, 2016). Previous studies by Sanon (2015) revealed an interesting richness of russula in the Dan gallery forest. This diversity is thought to be linked to several factors, namely rainfall, the physical (permeability, structure, texture) and chemical (nitrogen, phosphorus, hydrogen) components of the soil: and the distribution of ectomycorrhizal species such as *Berlinia grandiflora*, *Uappaca* sp., *Afzelia africana* and *Malacantha alnifolia* ((Vanié-Léabo, 2016). The distribution of *S. strobilaceus* in Burkina Faso is specific because the species has only been collected in the Kotoudeni forest, and its presence is thought to be linked to certain ectomycorrhizal species such as *Pinus* and *Querzus* (Sato *et al.*, 2007). However, the species has been collected in several localities in Bulgaria (Assyov *et al.*, 2011). In West Africa, boletes are generally found in Sudano-Guinean type forests dominated by mushroom partner trees (Castaño *et al.*, 2017). These areas are also characterized by abundant rainfall frequencies. The three genera identified have been by several authors as complex genera within the Boletaceaes; for finer data it is necessary to go to the molecular level (Sato and Hattoro, 2015; Sato *et al.*, 2007).

Conclusion

Macroscopic and microscopic descriptions have enabled us to identify three species of boletus: *B. linderi*, *T. balloui* and *S. strobilaceus*. From these studies, we note a morphological and anatomical diversity in the various species described in the Boletaceae. In view of their importance in the environment, boletes constitute a group of mycorrhizal fungi that should be better known, studied and valorized to safeguard forest ecosystems in Burkina Faso.

Novelty statement

Some studies have been conducted on the studies of the anatomical and morphological characters of macroscopic mushrooms such as Amanitas, Russulas and polypores in West Africa and particularly in Burkina Faso. However, there is no study on the characterization of boletes in Burkina. This study provides the scientific community with information on the diversity and scientific knowledge of boletes in Burkina Faso.

Acknowledgements

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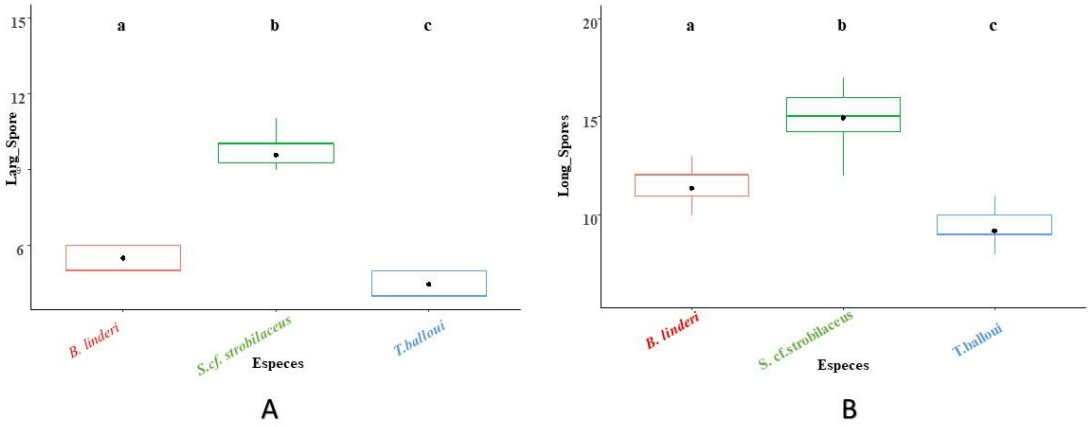
Contribution of authors

BB and ES planned the collections, analysis and interpretation of the results, and also contributed to the description and identification of the species. RSN and AKS participated in the collection of samples. MLG, SN and KD contributed to the correction and improvement of the manuscript.

Conflict of interests

Authors declare no conflict of interest.



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Shapiro-Wilk normality test
data: data$Long_Spores
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Fig. 2: Representation of the medians of the widths (A) and lengths (B) of the basidiospores of the three species of boletes.

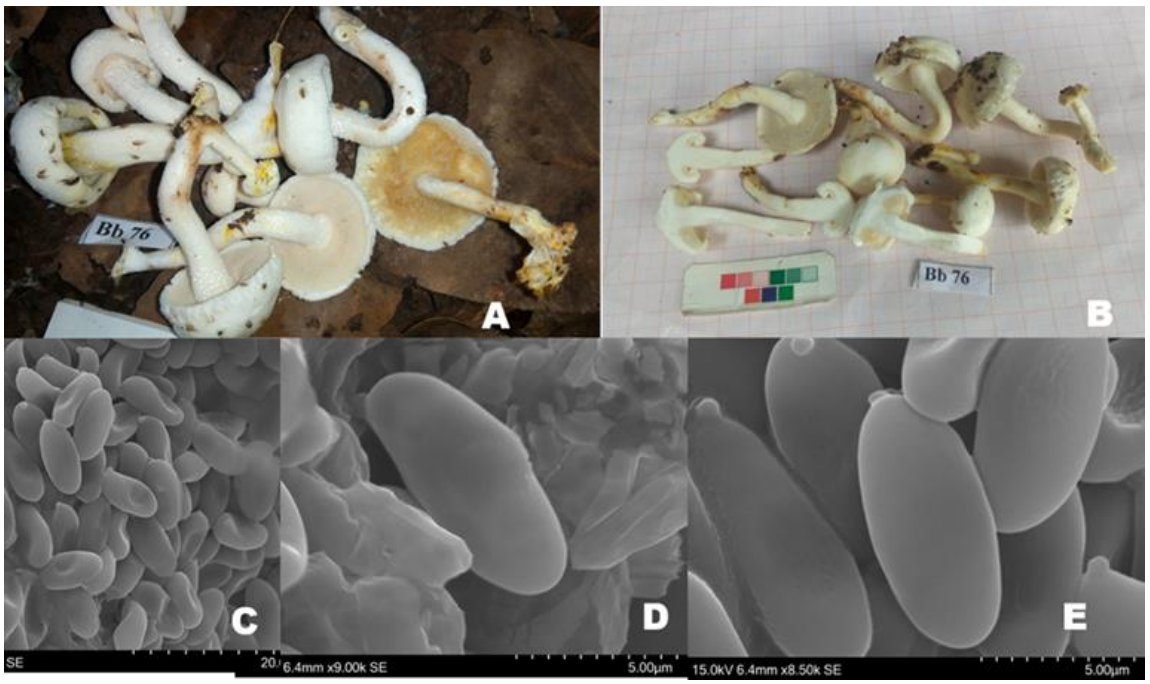

Fig. 3: *Tylopilus balloui*. *In situ* carpophores of *T. balloui* (A), ex-situ carpophores of *T. balloui* (B), immature basidiospores (C), mature basidiospores (D, E).

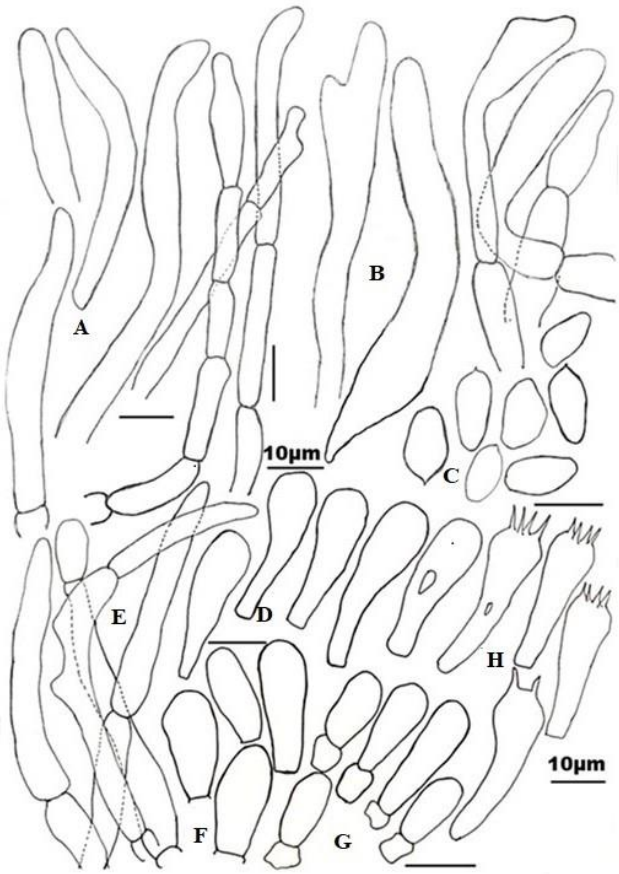

Fig. 4: Microscopic elements of *Tylopillus balloui*. **A:** cap hyphae, **B:** stipe hyphae, **C:** basidiospores, **D:** pleurocystids, **E:** hymenium cystids, **F:** basidia, **G:** basidioles, **H:** basidia, (scale =10 µm).

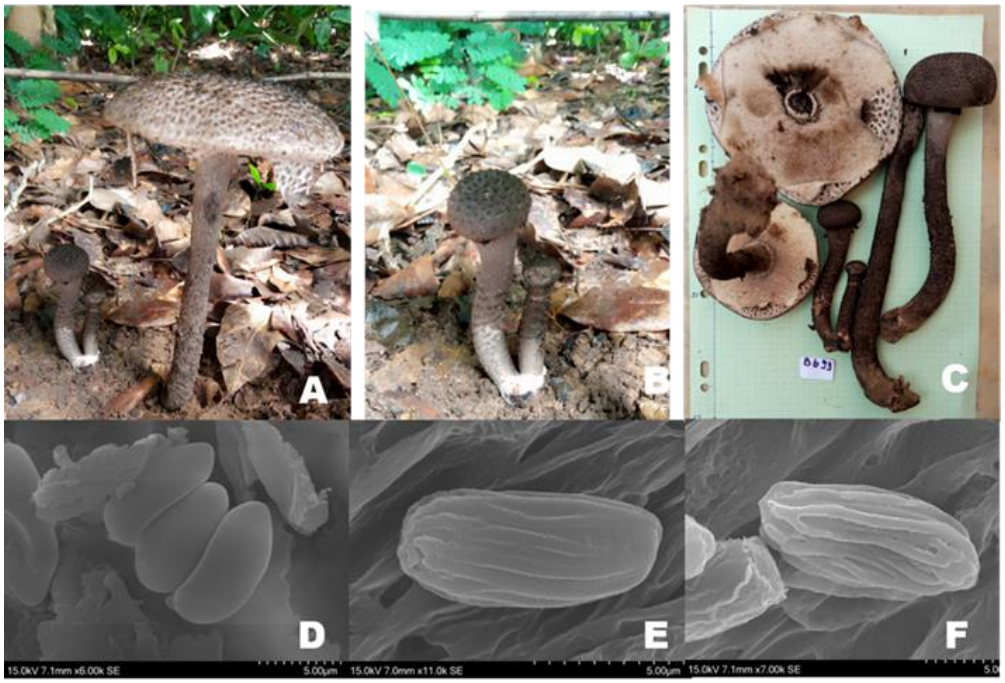

Fig. 5: Carpophores of *Strobilomyces strobilaceus*. **A:** *in situ* adult carpophore; **B:** *in situ* young carpophore;

C: *ex situ* carpophores; **D:** immature basidiospores; **E & F:** mature basidiospores.

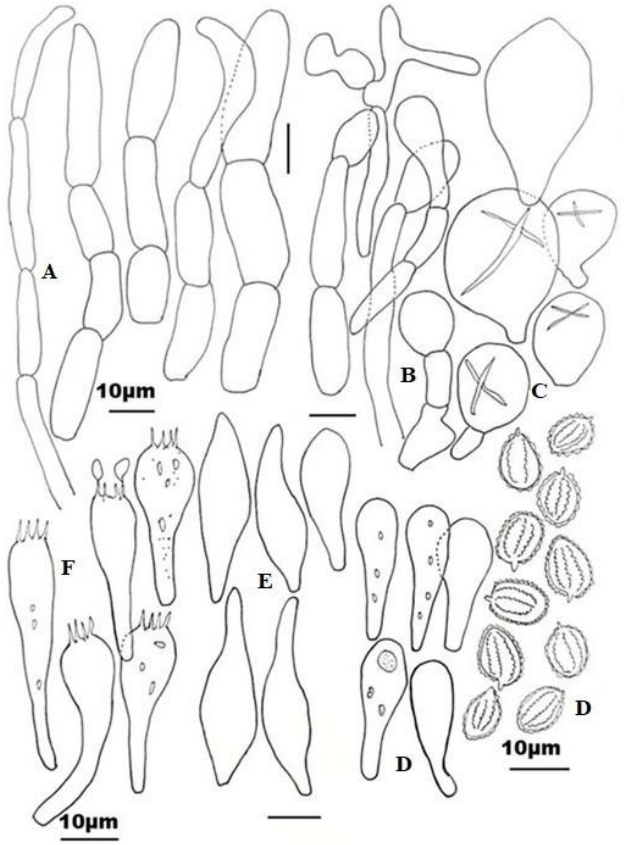

Fig. 6: Microscopic cells of *Strobilomyces* cf. *strobilaceus*. **A:** cap hyphae, **B:** stipe hyphae, **C:** cross cells, **D:** basidioles, **E:** pleurocystidia, **F:** basidia, **G:** basidiospores, (scale = 10 µm).

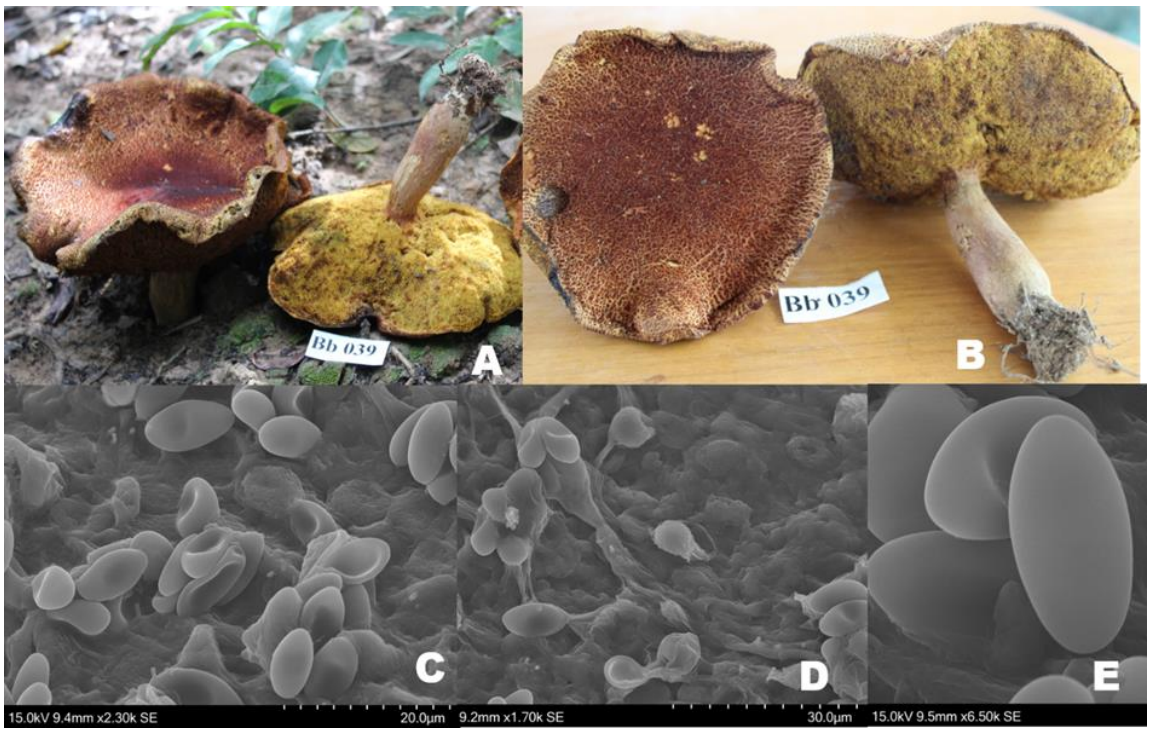

Fig. 7: Basidioma of *Boletellus linderi in situ* photo of *B. linderi* (A); *ex situ* photo of *B. linderi* (B); Basidiospores (C–E).

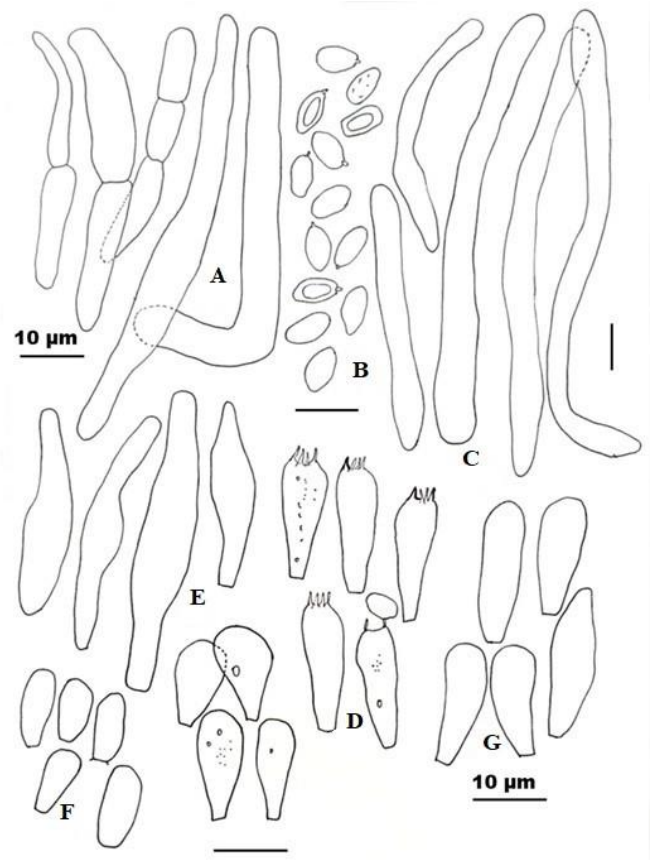

Fig. 8: Microscopic cells *Boletellus lindri*. **A:** cap hyphae, **B:** basidiospores, **C:** stipe hyphae, **D:** basidia, **E:** pleurocystidia, **F:** cheilocystidia, **G:** young pleurocystidia (scale = 10 µm).

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