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CYTOLOGICAL STUDIES ON THE AGARIC *Termitomycetes striatus (Heim)* FOUND GROWING WILD IN AHMADU BELLO UNIVERSITY, ZARIA, AND ITS ENVIRONS

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ABSTRACT

Cytological behavior in *Termitomyces striatus* was studied. Samples of the mushrooms were collected and were identified using a mushroom field guide. The samples were preserved in FAA for future used. The samples were ran through anatomical processes in the department of Vetenary medicine, Ahmadu Bello University, Zaria and microscopic analysis was done. The photomicrographs of the results showed that *Termitomycete striatus* is an aseptate mushroom without a cross wall structure in the hyphae. The two taxa of basidiomycetes; Ustomycetes and the true basidiomycetes were based on the differences in their septal ultra structure and life cycle. Formal classification of the fungi into Heterobasidiomycetes and Homobasidiomycetes is shown in Plate 3. In modern taxonomy, homobasidiomycetes roughly corresponds to the monophylectic class Agaricomyetes. *T. striatus* is placed in the family Tricholomataceae due to complete absence of clamp connections as indicated in Plates 1- 2, & 4.

Keywords: Anatomical studies; basidiomycetes; clamp connections; microscopic; *Termitomycetes.*

1. INTRODUCTION

The Agaricales or Eugarics clade is a monophyletic group of approximately 8500 mushrooms species. It forms the largest clade of Homobasidiomycetes [1] Binder et al. [2] and together with the Boletales and Atheliales, it forms the Agaricomycetidae. Species of Agaricales are widespread and diverse on land ranging from desert, grassland, forests, tundra, and shorelines in tropical, temperate, and arctic-alpine habitats. While many Agaricales acquire sustenance by decomposing dead organic matter (as saprotrophs), others parasitize plants or other fungi, and a few capture or parasitize vertebrates or invertebrates. Many groups of Agaricales engage in mutualistic symbioses with the roots of vascular plants where they form ectomycorrhizas (EM), or more rarely associate with unicellular green algae or cyanobacteria as lichens. A few colonize bryophytes. Orchids and mycotrophic plants depend on some Agaricales (*Armillaria*, *Tricholoma*), in addition to other groups of Agaricomycetes, for their nutrition. Several clades of Agaricales include ant or termite symbionts or even nematode predators. Species of *Armillaria*, *Athelia pro parte, Moniliophthora*, and *Mycena* are economically devastating plant

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pathogens, whereas others in the genera *Agaricus*, *Lentinula, Termitomyces, Tricholoma*, and *Volvariella* are highly valuable commercial or collected foods. Psychotropic or hallucinogenic mushrooms of *Psilocybe* and other genera also occur in the Agaricales, and others, as in some species of Amanita, produce toxins lethal to humans. Molecular systematic studies of the Agaricales have radically transformed our interpretations of the evolution and classification of gilled mushrooms and their relatives [3-6]. The overwhelming majority of species produces fruit bodies with gills (lamellae), but the evolution of gills has arisen numerous times in the Agaricomycetes [3]. Likewise, multiple lineages of "*gasteromycetes*" (puffballs, bird's nest fungi, false truffles), species that produce spores in an enclosed fruiting structure, have evolved independently among the Agaricales [7]. Studies by Bodensteiner et al. [8-10] have shown that some non-gilled fungi, including reduced or cup-like (cyphelloid) forms and crustose or resupinate forms, share their evolutionary histories with numerous lineages of Agaricales, including some lineages that evolved in aquatic or marine environments [11-13]. In short, the gross morphology of mushroom fruit bodies is highly plastic and often a poor phylogenetic indicator. These and other studies demonstrated that a broad concept of Agaricales [14], including *Boletes*, some *Polypores*, and the genera *Russula*, *Lactarius* and their allies, does not form a monophyletic group. Thus, the clade containing predominantly genera and families from the suborder Agaricineae [14] was labeled the euagarics clade and represents what we currently regard as the Agaricales [15]. Most family-level relationships based on morphological characters are artificial, but progress is being made to delimit higher-level monophyletic groups.

It was recently stated that cytology, perhaps more than in any other science, progress depends upon manipulative skill and that type of low cunning which is needed to apply old methods to new uses. This truth is beautifully illustrated in the case of the fungi where, in cytological work, we deal with structures and artifacts lying close to the limits of visibility, and only the most cunning of cytologists succeed in devising significant new methods. However, within the last two decades a sufficient number of ingenious new techniques have been conceived to further progress to the point where it can be said with confidence that the general topography of the fungal cell has been comprehensively mapped. Nonetheless, our knowledge of the fine structure of the thallophytic body is still rudimentary, and we are decidedly ignorant of the formation and chemical nature of its organelles. Interest in the present is shifting from the descriptive to the experimental phases of cytology; recent developments in quantitative optical microscopy and particularly in histo-chemistry insure that cytological investigation of the dynamic features of cellular development become an increasingly important subdivision of Biology. The cytological studies on mushrooms is very important as it aids one to place them better into their various taxonomic group in the cytology level, that is more satisfying than just using the morphological features. Also, most information covers only a limited taxonomic role of this organism in the ecosystem. The aim of the research is

- to serve as a base study for mushroom taxonomy and
- to view and appreciate these organisms at their cellular level

1.1 Morphology of *Termitomyces striatus (*Heim)

The genus is distinguished from all other genera of agarics by the distinctive pseudorhiza and its obligate association with termite nests. The cap of the fruit body has a prominent umbo; the cuticle consists of repent, filamentous and hyaline hyphae; the gills are usually free to sub-adnate, either having a notch at the point of attachment to the stipe, or have a decurrent tooth; hymenophoraltrama initially bilateral, then becoming regular – sub intermixed; cystidia present; basidia normal; spores smooth, ellipsoid, hyaline, with continuous homogenous, rather thin-walled; spore-print pink; stipe with a long tapering end pseudorhiza reaching the termite nest some distance below the soil surface. The genus obtains its nourishment from the material of the termite nest which is constructed of woody material masticated by the termite workers. Convex compact, fleshy, or somewhat tough in the stipe; hyphae without clamp connections. The primordial usually develop in the holes of the termite nests and can be observed by removing the soil from where the carpophores is growing and exposing the nest. They are cosmopolitan in nature, several species of *Termitomyces* are now well-known developmentally and biochemically. Commonly occur in the tropical regions of Asia, Africa, and south pacific. Nuclei and chromosomes of Basidiomycotina are generally smaller than those of Ascomycotina. The number of nuclei in the mycelium, hyphae of carpophores, cystidia, basidia and spores differs according to races, species or genera. The small size of nuclei as well as chromosomes makes their study difficult. In most cases, the descriptions with respect to somatic nuclear division in Basidiomycotina have been subordinated to those in Ascomycotina and Deuteromycotina [16,15] has expressed the view that aberrations from

the normal pattern of life cycle in the Agaricales, if constant, can be taxonomically useful. An important use of cytological characters is derived from the fact that not all cells of the carpophores of the dikaryotype are binucleate. It has been shown by Kuhner [17] that many hyphae of the interior of stipe and cystidia of species such as *Coprinus disseminatus* often contain more than two nuclei.

1.2 *Termitomyces* spp

There are more than 60 *Termitomyces* taxon described, but reliably only 18 species, collected mainly from west Africa, are reasonably well known, the rest are either synonyms or badly described and difficult to identify. A genus, *Sinotermitomyces* has been reported from China, but species are difficult to classify. Characteristically, Termitomyces has pinkish spores, a cap and stipe at the top of a long 'pseudorrhiza' that arises from the comb of the termites' caste and the cap has a 'perforatorium' that assists the mushroom to penetrate the hard ground and there is atermite association. Recent molecular studies supports the placement of *Termitomyces* in the Tricholomataceae [18].

1.3 Developmental Plasticity-A Challenge to Taxonomists

Developmental plasticity is an inborn property of an organism to adapt to the fluctuating environment and is enabled by the differential expression of the genome according to the immediate needs. Spontaneous fruitbody plasticity is widely observed during mushroom cultivation although mushroom farmers usually regard the variant form as abnormal. Unlike abnormal forms caused by infections [19], the variant form just reflect the response toward a certain stimulus for example, the uprolling of the *Pleurotus* cap exposing the gills or the premature exposure of the fruit body proper (hymenophore) from a basal bulb of *Volvariella* to the stagnant air, and the turmor outgrowth on the caps of *Agaricus bisporus*. Once the environmental conditions change or become better, normal fruitbodies appear again. In *Volvariella bombycina*, spontaneous variants in the forms of exposed cap, morchelloid forms, supernumerary gills, inverted caps, enclosed form (gasteromycetoid form) and even the Agaric form without basal volva which is a characteristic feature of the genus *Volvariella* were found together with the normal Agaric form [20]. All of these normal and variant fruitbodies except the morchelloid form matured with spore formation. Yet there were some irregularities; gills were heavily covered with mucilage, asynchronous maturation of spores borne on the same basidium, the number of spores/sterigmata borne on a basidium ranged from 2-4, and the basidiospore shape which is also of taxonomic value was not egg-shaped but heart-shaped [20]. By invitro and invivo studies using the gills of *Coprinus cinereus*, basidial maturation can be disturbed by exogenous factors leading to arrestment of development without spore formation, a basidium with fewer than 4 spores / sterigmata, a mosaic basidium bearing both spores and hyphal tips and giant spherical spores [21,22]. By carrying out a developmental study with *V. bombycina*, some of the variants were found to be actually the embroyonic form which under favourable conditions will transit into the normal agaric form [23]. Therefore, care is needed to differentiate this developmental plasticity with the disease symptoms caused by pathogens. The latter requires an environmental study isolating the causative agent. In addition, it is common to find the different morphological forms when a fungus is subcultured onto different media, the same old phenomenon named plasticity. Therefore, when variant fruitbodies are encountered, the critical thing to do is to check for any unfavorable environmental conditions and contaminants present.

Taxonomists in the old days stressed on morphological features (phenotype in genetic term) for classification purpose, but a developmental Biologist regards the phenotype which is the product of interaction between environment and genotype. No wonder why, taxonomists are collecting information at the DNA level in addition to the morphological and physio-chemical features of organisms. This information collected in addition to the strains preserved in a culture collection center would surely reflect and conserve the fungal biodiversity on earth.

1.4 Karyotype Analysis

Fungal chromosomes are minuite and at the limit of resolution by conventional light microscope. With the development of Pulse Field Gel Electrophoresis (PFGE) [24], the karyotypes of various mushroom species showing molecular size and numbers of chromosomes have been worked out. Successfully devised was a freeze-grinding method to prepare the intact chromosomal DNAs of the yeast *Saccharomyces cerevisiae* which is commonly employed as the size marker [25] but such method is found causing too much mechanical damage to most mushroom chromosomal DNAs of size greater than 2Mb (million base pairs).

The importance of karyotype for identification of species is not justified as chromosomal polymorphism among strains is now known to be a widespread phenomenon. Yet electrophoretic karyotype is a tool to reveal chromosomal aberration during growth and

development if any in such fungi. Strain instability and sectoring are commonly encountered especially for old cultures, but no one has looked at this phenomenon from this aspect, perhaps simply because of the lack of funding for basic research.

The fungal karyotype can also be revealed by chromosome spread at the meiotic prophase stage. Up to now, such technique first developed in plant system has only been applied to the mushroom *Coprinus cinereus* [26]. This protocol requires good preparation of chromosome spread and the equipment scanning electron microscope for best resolution. In comparison, electrophoretic karyotype requires high yield of protoplasts which are then embedded in agarose, in-situelysis of protoplasts to release the chromosomal DNAs which are protected from mechanical shear by the agarose and an equipment to do PFGE. PFGE is an important tool for gene mapping and for gene cloning especially when using vectors such as yeast artificial chromosome which can harbor DNA up to 1Mb. Therefore, the latter technique should have wider uses. All these in future will build up into a transformation system involving a particular gene or chromosome for strain improvement

2. MATERIALS AND METHODS

2.1 Study Area

This work was carried out in the Faculty of Veterinary Medicine, and the Department of Biological Sciences, Faculty of Science Ahmadu Bello University Zaria.

3. COLLECTION AND IDENTIFICATION OF T. STRIATUS

3.1 Collection of Mushrooms

A field guide was used for identification and some paper bags to put the mushrooms. On hunting for fungi (*T. striatus*), they are found in habitat that is suitable for germination of their spores. They are found growing on termites mount. Some species of such kind may be poisonous and some are edible but do not collect and mix them up in the same bag because even the spores of poisonous fungi can make you sick. Also it is a good idea if you are new to the hobby of mushroom hunting to make sure you have made an Identification that is accurate by using competent persons. Collected mushrooms were preserved in glass bottles with Formalin acetic acid (FAA) at room temperature in the laboratory for further use.

3.2 Used of Spore Print

Identifying mushrooms require a basic understanding of their macroscopic structure. Most are Basidiomycetes and gilled. Their spores, called basidiospores, are produced on the gills and fall in a fine rain of powder from the caps as a result. At the microscopic level the basidiospores are shot off -the basidium and then fall between the gills in the dead air space. As a result, for most mushrooms, if the cap is cut off and placed gill-side-down overnight, a powdery impression reflecting the shape of the gills (or pores or spines) is formed (when the fruit body is sporulating). The colour of the powdery print, called a spore print is used to help classify mushrooms and can help to identify them. Spore print colors include white (most common), brown, black, purple-brown, pink, yellow, and cream, but almost never blue, green, or red.

3.3 Used of Macroscopic Features

Stipe and stipe length: The stipe of *T. striatus* is cylindrical, pale grey without annulus and can reach up to 14 cm in length and 1.5 cm in width.

- Pileus length: This was in the range of 12-14 cm.
- Margin of fruit body: Produces fruit bodies with incurved margin and smooth type of pileus.
- Color of fruit body: Produces whitish to creamy color
- Gills: Produces crowded, free whitish gills
- Flesh: Produces a soft and inflated flesh.
- Spore print: The spore print shows a creamy coloration
- Annulus: They usually lack both an annulus and other velar remnants.

I. Pseudorrhiza: They have a pale grey, smooth, solid and fibrous pseudorrhiza growing up to 14 cm long.

3.4 Used of Microscopic Features

A hymenium is a layer of microscopic spore-bearing cells that covers the surface of the gills. In the non-gilled mushrooms, the hymenium lines the inner surfaces of the tubes of Boletes and polypore, or covers the teeth of the spine fungi and the branches of corals. In Basidiomycetes, usually four spores develop on the tips of thin finger-like projections called stigmata, which extend from a club-shaped cell called a basidium.

The most important microscopic feature for identification of mushrooms is the spores themselves. Their color, shape, size, attachment, ornamentation, and reaction to chemical tests often can be the crux of identification. Spores often have a protrusion at one end, called an apiculus, which is the point of attachment to the basidium, termed the apical germ pore, from which the hypha emerges when spore germinates [22]**.** This aspect of experiment requires a careful and stepwise procedure in order to get a concise result. This includes**;** fixating, cutting, embedding and sectioning, clearing, staining, mounting and viewing.

3.5 Histological Processes

3.5.1 Fixating

This serves to 'fix' or preserve cell or tissue morphology through the preparation process.

3.5.2 Cutting

This involves cutting out fixed tissues into appropriate portions of transverse, longitudinal and cross sections and placing in embedding cassettes for further processing.

3.5.3 Embedding and sectioning

Biological samples often need to be solidified to allow fine sectioning. Thin slices improve the access of dyes, probes, and antibodies and reduce the overlay of different cells layers. For light microscopy, paraffin wax is the most frequently used hard matrix for cutting. This was followed by a clearing agent, usually xylene, to remove the ethanol. Finally, molten paraffin wax infiltrates the sample and replaces the xylene. The cut out tissues was dehydrated for paraffin embedding using ethanol of various concentrations from 70% to100% within two changes.

- Xylene was used as the clearing agent and paraffin wax (56-58 degree Celsius) was used in two changes forone and a half hour each. Embed tissues into paraffin blocks
- Trim paraffin blocks to an optimal cutting surface including the sample with a small paraffin frame
- cut 3-10 µm slices (5 µm is commonly used); use a brush to draw the section onto the knife holder
- place paraffin ribbon or slice in 40-45ºC water bath with a 2nd wet brush (it will expand and wrinkles will vanish)
- fish out swimming paraffin section using glass slides and a brush to position the section
- dry sections at 37ºC (lower baking temperatures are better for subsequent antibody detection)

3.5.4 Clearing

This involves de waxing the paraffin with the tissues in it. The following steps were involved.

- 2 changes of xylene, 3-10 min each (3+ changes for sections >25 µm) [deparaffinise]
- 2 changes of 100% ethanol, 3 min each [re-hydrate]
- 2 changes of 95% ethanol, 3 min each [re-hydrate]
- rinse in distilled water

3.5.5 Staining

This involves the use of a stain to a sample to color the cells, tissue components or metabolic processes. This process may involve immersing the sample (usually before mounting) in a dye solution and then rinsing and observing the sample under a microscope. Two staining methods was employed to the tissues of the mushroom after clearing; crystal violet staining method and hematoxylin and eosin (H and E) staining method.

3.6 Photographic Analysis

Having mounted and viewed, to enhance further microscopic analysis, the digital camera was used to take pictures of the viewed cells for further interpretation

4. RESULTS AND DISCUSSION

Within the Basidiomycetes, three main lineages can be discerned; two leading to the Ustomycetes and one leading to the true Basidiomycetes, these two taxa created by Moore (1972) was on the basis of differences in their septal ultrastructure and life cycle. As seen from the plates above, the *Termitomyces striatus* is thus classified as an aseptate mushroom lacking a crosswall structure in their hyphae. Formal classification of fungi into Heterobasidiomycetes (those with septatebasidia) such as the jelly fungi, smuts and rust and Homobasidiomycetes (those with aseptatebasidia) which includes most mushrooms place the species (*Termitomyces striatus)* under the group of the Homobasidiomycetes.

In modern taxonomy, homobasidiomycetes roughly corresponds to the monophyletic class Agaricomycetes whereas heterobasidiomycetes are

paraphyletic and as such corresponds to various taxa from different taxon.

Heim in 1942 [29] originally placed the genus in Amanitaceae, Julich [30] erected the monophyletic family Termitomycetceae and later Pegler [20] placed the genus in plutaceae. According to Singer [15], the cyanophilous spores, the bilateral lamella trame of young carpophores, the siderophilous staining of the basidia and the spore hilum of the open pore type, places the genus in Tricholomataceae next to Lyophyllae in its own tribus, Termitomyceteae, due to complete absence of clamp connection (parts of the this information indicated in Plates 1-4 above).

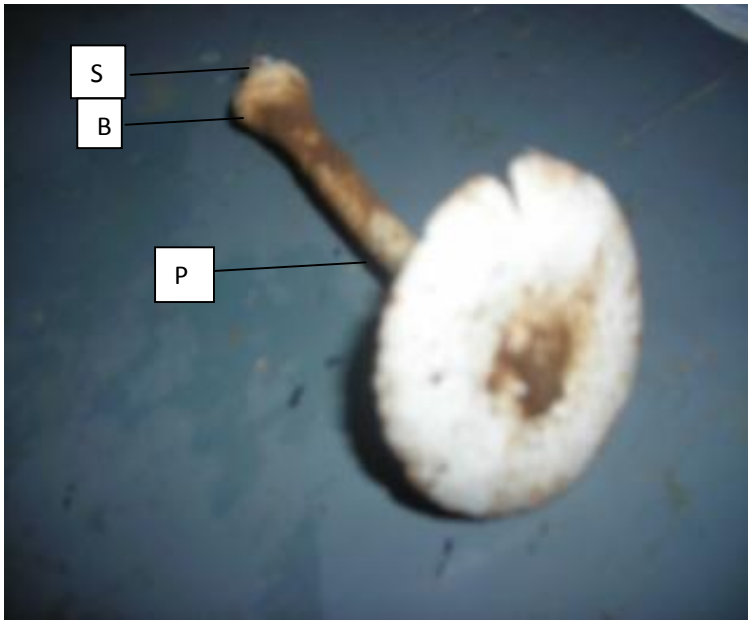


Fig. 1. Mature *Termitomyces striatus* showing pileus and stipe
B = Bulbous base, S = Stipe, P = Pileus

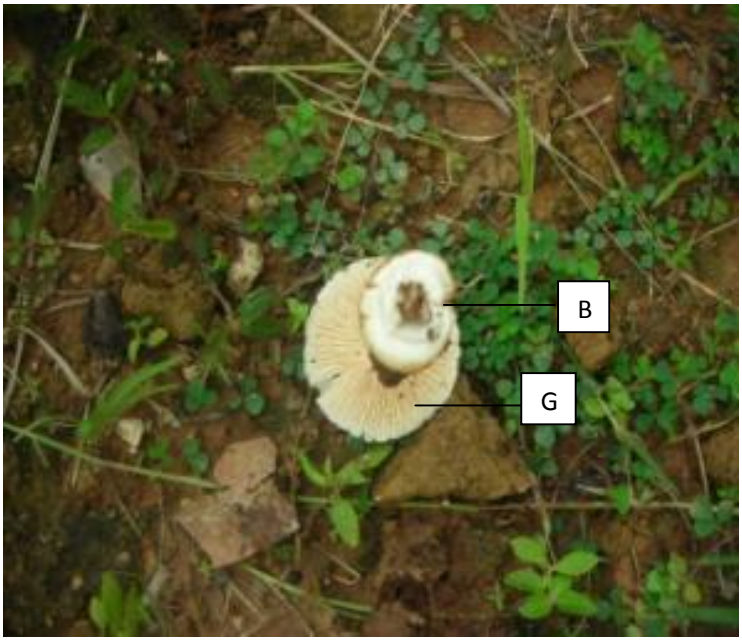


Fig. 2. *Termitomyces striatus* in inverted position showing bulbous base
B = Bulbous base, G = Gills

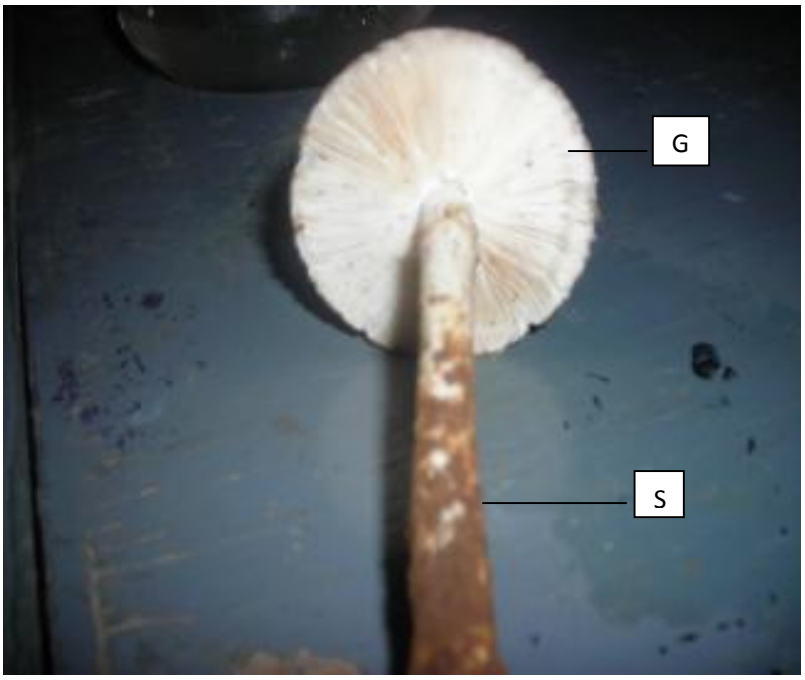


Fig. 3. *Termitomyces striatus* showing the gills
G = Gills, S = Stipe

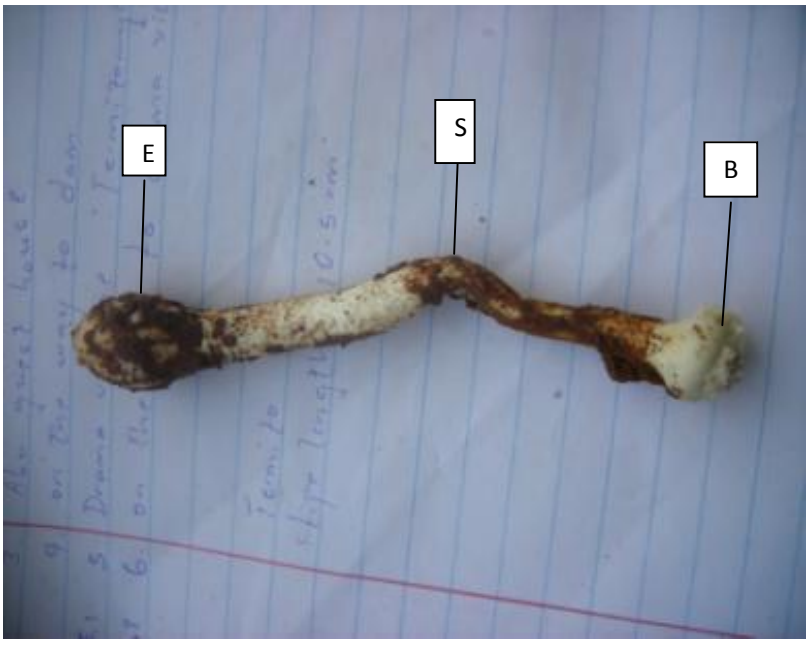


Fig. 4. Young *Termitomyces striatus* button stage
E = Enclosed gills, S = Stipe, B = Bulbous base

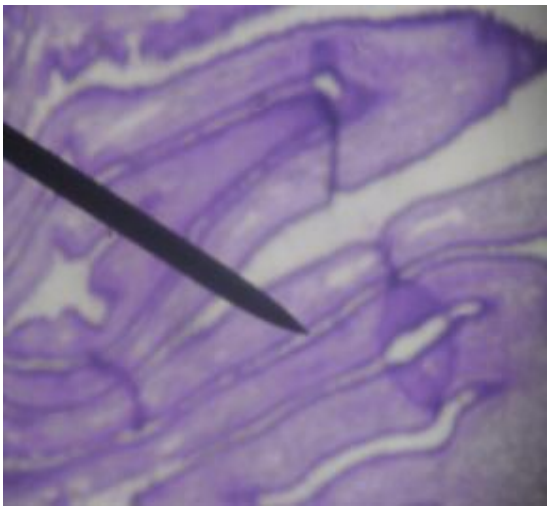


Plate 1. T. S. Pileus *Termitomyces striatus* (hypha) mag.(3x10^{-6} µ)

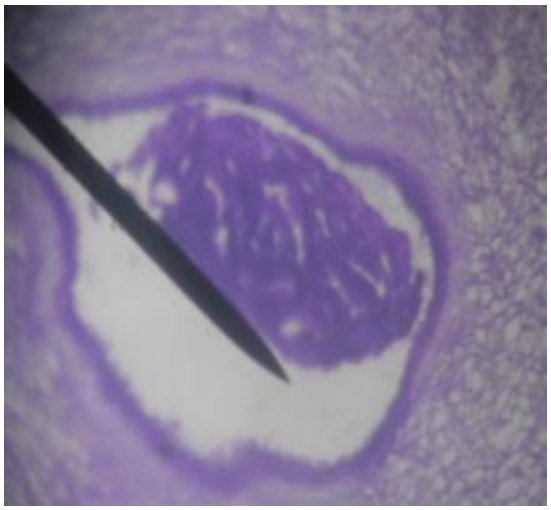


Plate 2. T. S. pileus *Termitomyces striatus* (condensed nucleus) mag. (3x 10^{-6})

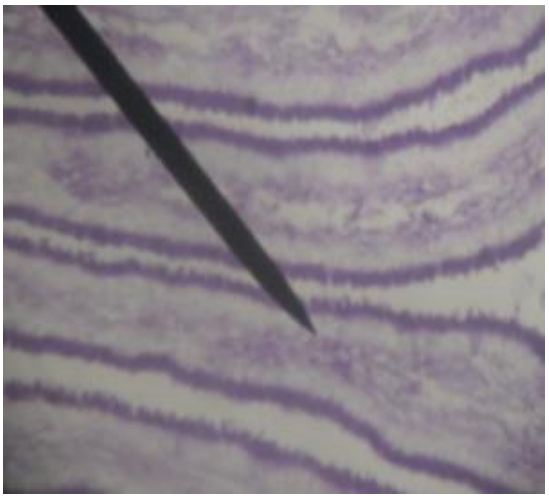


Plate 3. L. S. Pileus *Termitomyces striatus* aseptate hypha mag. (3x 10^{-6})

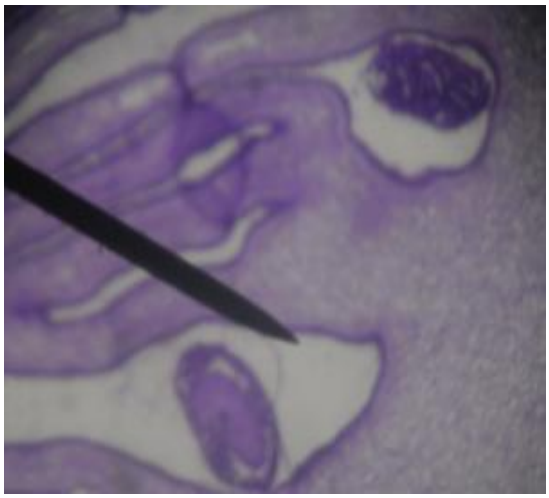


Plate 4. L. S. Pileus *Termitomyces striatus* cellular conformation Mag. (3x 10^{-6})

5. CONCLUSION

As the world of science and technology is increasing daily in advancement, the world of the mushrooms is also receiving more interest from scientist in the world. The study of mushrooms is very important as more and more research is being made on these fascinating organisms to harness any possibility of providing the populace with sustainable food source, serving as reliable source of medicines and most importantly as long as the Biological world is concerned, placing them accurately into their various taxonomic groups. Cytological study alone is not the best method for analyzing, understanding, and placing mushrooms into their various taxonomic groups. Therefore, biotechnology of this mushroom should be done to have a precise taxonomy of their genera.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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