Ghana J. Sci. **63** (2), 2022, 21 - 30 https://dx.doi.org/10.4314/gjs.v63i2.3

DEVELOPMENTAL MORPHOLOGY, ANATOMICAL FEATURES AND FAIRY RING FORMATION BY TWO GASTEROID BASIDIOMYCETOUS MUSHROOMS, GROWING ON GRASS LAWNS IN GHANA

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ABSTRACT

Calvatia and *Lycoperdon* belong to the Gasteromycetes commonly called gasteroid fungi or puffballs. Their basidiospores are kept in a stomach-like glebal and spores are not discharged forcibly. An account of diagnostic characters, developmental morphology, anatomical features, and fairy ring formation of *Calvatia cyathiformis* and *Lycoperdon* sp. are presented during three (3) years (2017 – 2020) study. *C. cyathiformis* has epigeous habit on the grass lawn appearing after 2 – 3 weeks as a small whitish button and changed colour to cream and finally pinkish to purple at maturity. The basidiocarp was ball-shaped becoming two chambered with basal portion distinct from the upper. This fungus formed a complete circle fairy ring with a striking greening of the grass along the periphery of the circle where the basidiomata emerged. *Lycoperdon* sp. produced basidiocarp which was also epigeous and matured within 2-3 weeks. The basidiocarp was initially greyish white maturing into brownish colour. *Lycoperdon* sp. formed a semi-circle fairy ring without change in the grass colour for three consecutive years. Although fairy rings have been formed by other *Lycoperdon* sp. these present findings of semi-circle fairy ring formation is being recorded for the first time in Ghana.

Keywords: *Calvatia cyathiformis*, *Lycoperdon* sp. puffballs, morphological development, Anatomical characteristics; fairy rings

Introduction

The Phylum/Division Basidiomycota has 32 Orders, 140 families and 473 genera. The Family Holobasidiomycetidae (Homobasidiomycetes) has a basidium which is not divided by primary septa but may sometimes become adventitiously septate (Hawkworth *et al*., 1996). There are 21 Orders and 1,200 species including Gasteromycetes to which the genera *Calvatia* and *Lycoperdon* belong. This group of fungi comprise of what is referred to as the gasteroid fungi. Its etymology in Greek meaning gaster = "stomach" (literally stomach fungi) group of polyphyletic assemblage of basidiomycetous fungi characterized by the fact that their basidiospores mature inside the basidiocarps and are not discharged forcibly from the basidia (Alexopoulos *et al*., 1996; Miller *et al*., 2001). The Gasteromycetes, therefore, have a morphologically closed basidiomata but are phylogenetically distantly related (Pipenbring, 2015; Miller *et al*., 2001). The more or less globose basidiomata containing mass of basidiospores called gleba (an inner fertile portion). This fertile gleba of the basidiocarp is enclosed by a peridium. Depending on the species, the gleba tissue may consist of only one thin-walled generative hypha (monomitic); or consists of both generative and skeletal hyphae (dimitic).

The peridium (an outer sterile layer) of the gasteroids are quite variable but typically consists of 1 to 3 layers. If single layer is present, it is called Peridium; if two (2) layers are present, the outer is called exoperidium and the inner called endoperidium. If three layers exist, then they are identified as exoperidium, the mesoperidium and the endoperidium (Alexopoulos *et al*., 1996; Pipenbring, 2015). The "Gasteromycetes" are variable including different families of species or groups of genera with gasteroid fruiting bodies, belonging to different orders based on morphological and molecular data (Pipenbring, 2015; Miller et *al*., 2001).

Ecologically, gasteroid fungi are usually found as saprotrophs in places rich in decaying organic material with high humidity which is necessary for their development. Such areas include the forests, lawns, courtyards, and parks. They are also found on excrement of animals where their globose basidiomata must protect the basidiospores against drought. *Calvatia* belonging to the family Agaricaceae (Agaricales) is a mushroom. The name *Calvatia* derives from the word calvus (Latin) meaning "bald". The genus is soft and is characterized by stalked- sessile, globose- subglobose, pyriform, subpyriform sporophore. Fruiting body dehisces by irregular fragmentation of the peridium (Hedawoo, 2020).

Members of this family are commonly known as "puffballs". There are 140 records of *Calvatia* species on website, out of which 58 are valid names under the genus (http:www. indexfungorum.org as cited in Hedawoo, 2020). Verma *et al*. (2018) recently gave account of mushrooms belonging to the genus *Calvatia* including *C. cyathiformis*. Most species are edible when young, e.g., *Calvatia gigantean* (Batsch) Lloyd (giant puffball) and *Calvatia utriformis* (*Bovistella utriformis* (Bull.) Demoulin and Rebriev) were reported as edible (William & Arora, 2008) while others have pungent odour and are inedible e.g., *Calvatia fumosa*. *Calvatia* is flask-shaped and has both exosporium and endosporium but are quite thin and fragile and no special provision is made for the liberation of the basidiospores. The exosporium breaks into flakes and falls away revealing the endosporium that gradually breaks to expose the basidiospore. In the case of *C. cyathiformis* the basidiocarp is pear-shaped pinkish to purple in colour producing copious spores (estimated at 6 – 7 trillion spores) which besmear the basidiocarp and later the spores turn brownish in colour (Alexopoulos *et al*., 1996; Aneja & Mehrotra, 2011; Verma *et al*., 2018).

The genus *Lycoperdon* has 18 genera and 160 species and also includes the puffballs, so called because of the method of spore dispersal. In *Lycoperdon*, both the endo- and exoperidium are distinct. This gasteroid basidiomycetes is epigeous, cylindrical to ovoid in shape with apical dehiscence.

Some members of the Basidiomycota form what is known as the "fairy rings" during their developmental history. Fairy rings have been found during the development of mushrooms (Agaricales, Gasteromycetes, Agaricaceae, etc.) such as *Clitocybe maxima* (*Infundibulicybe geotropa), Clitocybe dealbata* (fool's funnel mushroom) *Calocybe cambosa* (St. George's mushroom), *Agaricus campestris, Lycoperdon depressum* (=*C. rivulosa), Scleroderma verrucosum, Lepiota raevum, L. procera* (parasol mushroom), *L. rhabcodes, Leucopaxillus gigantea, Marasmous oreades* (Encyclopedia Britannica, 2020).

A fairy ring also known as a fairy circle, elf circle, elf ring or pixie ring is a naturally occurring ring made of mushrooms (https:// www.woodlandtrust.org.uk, 2019). The fungus sprouts lots of small thread of the mycelium in a circular shape. Later, the mushrooms pop out of the ground at the edge of the circles, and some

can grow up 600 metres in diameter (Vickers, 2019). These perfect to near perfect circles have been in the past attributed to witches or fairies, representing the path of dancing fairies (Alexopoulos *et al*., 1996; Pipenbring 2015; Encyclopedia Britannica, 2020). The rings have also been known to signify fairy villages, where they use mushrooms as their dining tables (https://fantasticfungi.com) and would dance and celebrate within the mushroom ring limited space. It was a taboo in some cultures to step into the circle as you will become invisible and trapped forever. In other cultures, they believe that the fairy rings are portals to another world.

There is, however, a scientific explanation for the magical phenomenon of fairy rings. As the subterranean mycelium grow and divide along a circle and at maturity, fertile basidiomata appear along the periphery of the circle (Encyclopedia Britannica, 2020). It is known that fairy rings are of three types (Aneja & Mehrotra, 2011). First, the development of the basidiocarps has no effect on the vegetation e.g., *Lepiota morgani* and *L. procera* (Odamtten et al., 2022). Second, there is increased growth of the vegetation where the fungus is growing e.g., *Chlorophyllum molybdites* and *Lepiota personatum* (Alexopoulos *et al*., 1996; Aneja & Mehrotra, 2011). In this instance inside the circle of mushrooms, there is a distinct zone of the grass which is noticeably greener than the grass elsewhere in the vicinity. The greener colour is attributed to the nitrogenous substances which became available to the grass as the older hyphae of the fungus die and disintegrate (Alexopoulos *et al.,* 1996; Aneja & Mehrotra, 2011; Encyclopedia Britannia, 2020). In the third type of fairy ring, the vegetation is damaged, sometimes so badly as to influence the aesthetic value of the grass, or the vegetation is nonexistent.

During a three-year period (2017 – 2020), two gasteroid basidiomycetous mushroom belong to the genera *Calvatia* and *Lycoperdon* were observed growing on grass lawns on the campus of the University of Ghana, in the Greater Accra Region, Ghana. The two species from separate genera formed distinct fairy rings in the successive 3 years. This paper reports some phenological, morphological and anatomical studies carried out simultaneously on the fungi with the view to documenting their development, anatomy and fairy ring formation observed during the stated period.

Experimental

The two gasteroid mushroom *Calvatia cyathiformis* and *Lycoperdon sp*. were observed during the rainy season of 2017 – 2020 growing on the grass (*Chrysopogon aciculatus*) lawns within the University of Ghana, Legon (5.6506°N, 0.1962°W Digital Address GA - 490 - 3845) in the Accra Metropolitan District, Greater Accra. They were identified using the Encyclopaedia of mushrooms (Dickinson & Lucas, 1979; Davis et al., 2012; Piepenbring 2015; Verma *et al*., 2018). The morphological, anatomical and other characteristics were also studied to augment the data.

Phenology, morphological and anatomical studies of Calvatia and Lycoperdon species

Calvatia cyathiformis (Bosc) Morgan (= Lycoperdon cyathiformis Bosc. Morgan)

The initial stages of the fruiting body were subterranean, and the demarcation of the circle was clearly marked as darker greener complete circular path prior to the appearance of the first button of the developing fruiting body (Fig. 1). Thereafter, photographs were taken of the development of the fruiting body till maturity in 2 – 3 weeks. A Samsung Galaxy A51 Camera Phone was used. Photographs were taken every year for 3 years (2017 – 2020).

A diagrammatic representation of the fairy ring was also drawn (Fig. 2) to show the aesthetic of the circle and the arrangement of the basidiocarps as well as the architecture of the exterior of the basidiocarps (Fig. 3).

A longitudinal section through the cap was made using a sharp dissecting kitchen knife (Fig. 3) to show the gleba, stipe, the ecto- and endoperidium. The thick basal portion that is slightly narrower than the upper section, the globose to turbinate tapering into large and well-developed stout rooting base for anchoring the mushroom. The gleba region bearing numerous basidiospores interspersed with hyphae-like capillitia were drawn to illustrate the glebal anatomy (Fig. 4).

Lycoperdon sp.

Same procedure was followed as spelt out for *Calvatia*. A Samsung Galaxy A51 Camera\Phone was used. The developmental stages took 2 weeks to be completed (Fig 5). A longitudinal section through the various stage of development was made and recorded (Fig. 7) as well as the external morphology of the fruiting body of *Lycoperdon* (Fig. 8). The Semi-circle fairy ring formed was also noted and photographed (Fig 6).

Results and discussion

The first striking feature on the grass on which *Calvatia cynthiformis* appeared was the appearance of a clear circle called fairy a ring of markedly darker greener grass (Fig 1) prior to the appearance of the initial button of the developing fruiting body. The mycelium of the Basidiomycota arises as a primary monokaryotic mycelium before, a secondary dikaryotic mycelium is formed. In many of the saprobic dikaryotic basidiomycetes, this dikaryotic mycelium inhabits the subterranean soil, becoming prolific and abundant in the soil (Alexopoulos *et al*., 1996). The dikaryotic mycelium has the tendency to grow in all directions from the central point forming a large invisible circular colony. The greener colour of the grass (*Chryopogon aciculatus*) along the circumference of the fairy ring has been attributed to the availability of nitrogenous substances made available to the grass as the older hyphae of the fungus die and disintegrate (Alexopoulos *et al*., 1996; Aneja & Mehrotra, 2011). This present finding agrees with observations made elsewhere by Alexopoulos *et al.* (1996) and Aneja & Mehrotra, 2011). When the time comes for sporulation, and spore fruit body formation, the tertiary mycelium, which is also dikaryotic, forms complex basidiocarps at the periphery of the circular colony resulting in the formation of a ring called fairy ring (Figs. 1 & 2) Fig. 1 shows the fairy ring formed during the 3 years period (2017 – 2020) of observation. The diameter of the fairy ring after 3 years was wider than the previous years (Fig. 1). It is estimated that in *Agaricus praenimosus* the mean growth of a ring is 12cm in radius every year (Aneja & Mehortra, 2011); the same is reported of *Armillaria bulbosa* which could cover 15 ha of the forest floor in Canada having spread for about 1500 years and achieved a harvest weight of 10,000kg (Carlile *et al.,* 2005). It is conjectured that in all cases of occurrence of fungal fruiting bodies in circles, it is merely a reflection of the way in which fungi grow, given a uniform spread of food material (nutrients) and no physical or biological obstructions. This is the first record of *Calvatia cyathiformis* forming fairy ring under natural conditions in Ghana. The fruiting bodies (basidiocarps) are shown at different development stages in Fig 2 (Labelled 1 – 9) arranged in a circle (Fig 1 & 2).

The puffball, *C. cyathiformis* emerged as a small whitish “egg” or button and changed colour to cream and finally to pinkish epigeous basidioma (Fig 1). The epithet *cyathiformis* means flask- shaped and was growing in a ring formation along the periphery of the fairy ring.

The fruiting body was 6 – 8 cm high and 6 - 6.5 cm wide (depending on the maturity and availability of water in the soil). It was ball-shaped when young but developed a thick basal portion that is slightly narrower than the upper portion, globose to turbinate or sub-pyriform, tapering abruptly into a large well- developed thick stout rooting base and deeply wrinkled (Verma *et al.,* 2018) (Figs. 3A & B). The outer surface was ornamented pinkish to pale brown in colour (Fig 1). The basidioma breaks up into small, mosaic – polygonal – like scales (Fig. 3A) eventually becoming very pale brown besmeared with brown-colored basidiospores (Fig. 1) enclosed in a peridium (Fig. 3A). *C. cyathiformis* as shown in Fig. 3A has both exosporium and endosporium which are quite thin and fragile with no special provisions made for the liberation of the basidiospores. The exosporium breaks into flakes and falls away revealing the endosporium that eventually breaks to expose millions of the brown basidiospores (Alexopoulos *et al.,* 1996; Aneja & Mehrotra 2011; Verma *et al.,* 2018) (Fig. 3B).

According to Verma et al. (2018) the interior of the fruiting body *C. cyathiformis* is white and firm when young becoming two-chambered textually, with the basal portion distinct from the upper portion, becoming yellowish and finally deep purple as it matures and turns into a spore dust of purplish to brownish colour. These observations agree with our findings in Fig. 1 & Fig. 3 showing the distinct upper glebal region and the narrower base forming the stipe. Basidiospores were globose, numerous and tightly packed intermingled with a capillitial thread – like hyaline hyphae. The basidiospores were covered in spines (Fig. 4). Although we did not measure the dimensions of the basidiospores, Verma *et al.* (2018) reported that the basidiospores were 3 – 6 µm in diameter, globose and covered with spines 0.5 – 1.0 µm long.

The diagnostic characters of *C. cyathiformis* in this paper included its habits of occurrence on grass (*Chrysopogon aciculatus* syn. *Andropogon aciculatus*; Phylum: Spermophyta; Class Monocotyledonae). Other common names of this grass are golden beardgrass. Love grass, Sea grass, Spear grass, Needle grass, lesser spear grass etc. (Clayton *et al.,* 2014). *C. cyathiformis* is oval or ball-shaped and white when young but on maturity changes colour to pink and develops a basal portion like an inverted pear fruit. (Fig. 3). The surface of the mushroom was smooth and creamish-white initially but became more pinkish, brown and broken up into mosaic-like scales (Fig. 1; Fig. 3A & B). These characters are mostly in consonance with what was reported in India by Verma *et al*. (2018). There was a striking deeper greening of the grass along with the fairy ring circle where the fruiting body emerged. This is the first documented greening of grass along a fully circled ring attributed to the presence of *Calvatia cyathiformis* in Ghana.

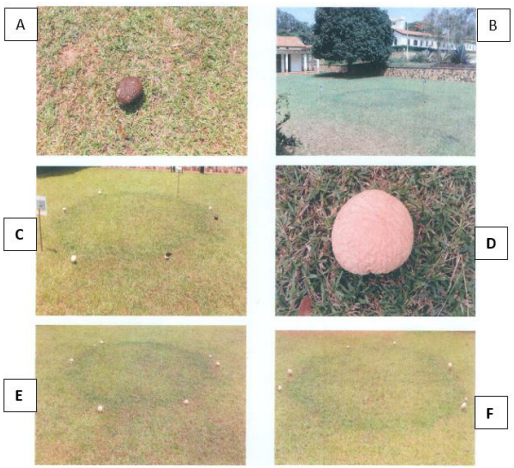

Fig. 1: Gasteroid basidiomycete *Calvatia cyathiformis* growing on grass lawn (*Chrysopogon aciculatus*) in the courtyard of Department of Plant and Environmental

Biology on the University of Ghana Campus.
A: Mature fruiting body of *C. cyathiformis* with a smear of brown released basidiospores on the surface of the basidioma.
B: A deep green colouration of a fairy ring prior to the appearance of fruiting body of the fungus.
C, D, E and F: Photograph of the fairy rings with fruiting bodies formed at the periphery of the circle during the rain seasons of the 1st, 2nd and 3rd years of observation. (Note the fruiting bodies of *C. cyathiformis* varying from 6 – 7 and the extension of the circle diameter during the 3rd year.
D: A mature basidiocarp of *C. cyathiformis* (magnified for better view).

Fig. 2: Diagrammatic representation of the fairy ring of *C. cyathiformis* showing different stages (1-9) of the basidiocarp at the periphery of the fairy ring circle.

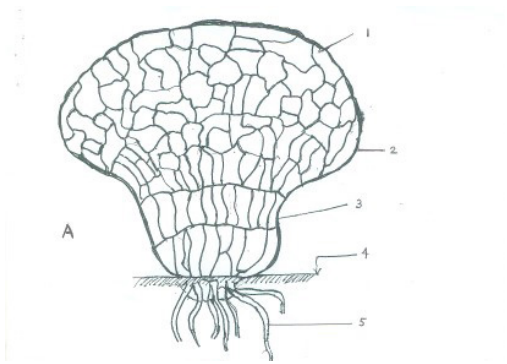

Fig. 3a: Drawing of fruitbody of mature *Calvatia cyathiformis* showing external morphology (parts 1-5).

1. Top of convex hemispherical cap showing polygonal architecture
2. Exosporium
3. Narrower neck showing stipe
4. Substratum
5. Mycelial strands enlarged into rhizomorph-like rhizoids

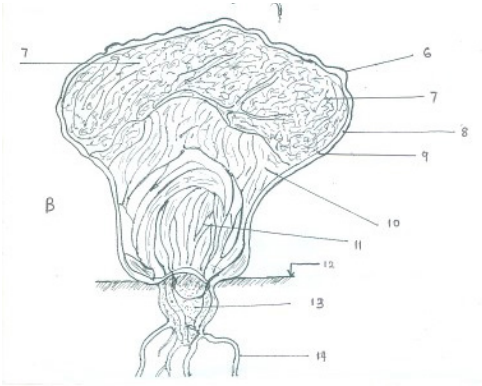

Fig. 3b: Longitudinal section of mature fruiting basidioma of *Calvatia cyathiformis.*
6. Warty, undulating exosporium
7. Gleba region: mass of branched capillitial-like hyphae mingled with basidiospores forming the foamy gill region
8. Exosporium
9. Endosporium
10. Developing glebal mass
11. Central stipe region
12. Substratum
13. Subterranean hidden portion of stipe
14. Hyphal strands covering serving as holdfast to the soil

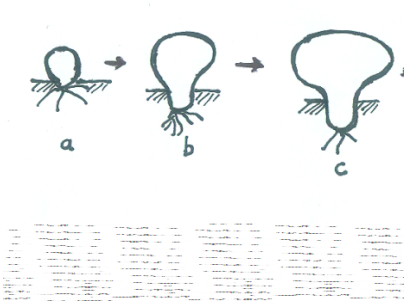

Fig. 4. Drawing of gleba region of *C. cyathiformis* showing magnification of,

a – capillitial-like hyphae; **b** - basidiospores with spiny encrustations on the exterior.

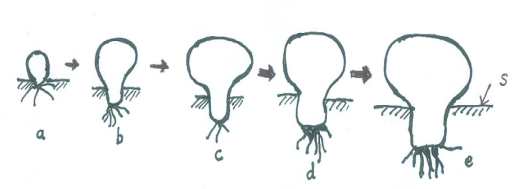

Fig. 5. Diagrammatic drawing of the morphological changes of fruiting body of *Lycoperdon* sp.From the initial egg-like button (**a**) to the mature basidioma (**e**); substratum (**s**) (b) *Lycoperdon sp.*

This fungus is another puff-ball gasteroid. The initial stages of the fruiting body was subterranean like *C. cyathiformis* and the mature basidiosmata made an ominous appearance after 2 – 3 weeks on a clear semi-circle path without any visible change in the vegetation. This is the second type of fairy ring formation (Aneja & Mehrotra, 2011) (Fig. 6). The fruiting body was initially greyish white and with maturity, turned brownish (Fig 8).

The stages in the development of the sporophore are shown in Fig. 5. The longitudinal section of the fruit body was divided into two chambers textually with the basal portion distinct from the upper portion. The upper glebal portion was whitish in colour with the basal narrower portion forming the stipe having thick mycelial strands or rhizomorph securing the fruit body into the soil (Figs 5 - 8). The fruiting body had both exosporium and endosporuim (Fig. 8) and was generally ovoid to pear-shaped at maturity. In *Lycoperdon*, both the endo- and exoperidium are distinct (Pipenbring, 2015) with apical dehiscence of spores. As seen in this paper, the gasteroid basidiomycete is epigeous and either grows on grass lawns or on decaying wood.

The genus *Lycoperdon* includes some of the most common puffballs. The diagnostic characters of the *Lycoperdon* in this paper included its habit of occurrence on grass (Clayton *et al*., 1994; Alexopoulos *et al*., 1996). After its ominous appearance on the grass the fruiting body was a button ball-shaped and white turning greyish white to light brown and finally diving into two chambers; the upper glebal portion was whitish with a basal narrow part forming the stipe with rhizomorph-like mycelial strands at the base (Figs 7 to 8) holding the fruiting body firm to the soil. The *Lycoperdon* is pure white inside at the top and as spores form it begins to turn yellowish brown (Alexopoulos *et al.* 1996). This agrees with the appearance of our specimens (Figs 7 - 8). According to Pipenbring (2015) and Alexopoulos *et al*. (1996) the fruiting bodies of Lycoperdales are enclosed by an exoperidium and endoperidium. This agrees and underscores our finding of the distinct presence of both the endo- and exoperidium in *Lycoperdon* sp. in this paper.

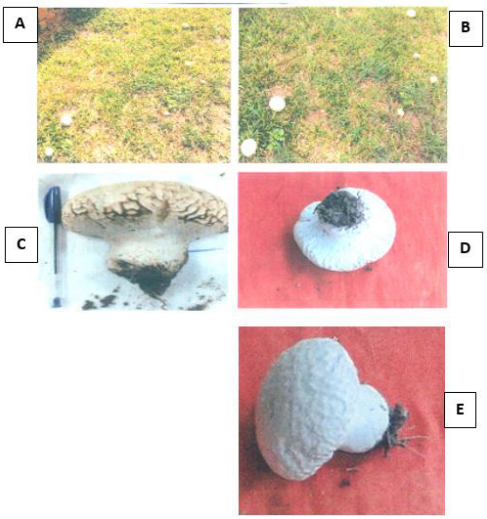

Fig. 6a: Photograph showing mature basidiocarps (fruiting bodies) of *Lycoperdon* sp. Arranged in a semi-circle fairy ring (Note the non-greening of the grass/substrate on which The mushroom is growing).
C, D and F: Harvested basidiocarp of *Lycoperdon* sp. (Note the change in colour From greyish white to creamish brown; middle left and right).

Fig. 7. Longitudinal Section of *Lycoperdon* sp.

Fig. 8: Photograph showing different sizes of harvested mature fruit bodies of *Lycoperdon* sp.

In this study, *Lycoperdon sp*. formed a semi-circle fairy ring in three consecutive years without the greening of the vegetation akin to the second type fairy ring formation reported by Aneja & Mehrotra (2011). Fairy rings have been formed by other *Lycoperdon* species (*L. depressum* = *L. rivulosa*) (Encyclopaedia Britannica, 2022). However, these present findings of semi-circle fairy ring formation by *Lycoperdon* in Ghana are being recorded for the first time.

Species of *Calvatia* and *Lycoperdon* have been recorded from different types of soils and substrates, humicolous soil, grassy soil, sandy soil, on manure, on soils of moist and shady places (Verma et al., 2018). This agrees with grassy humicolous soil that the macro-fungi reported in this paper was found. Some *Calvatia* species as well as *Lycoperdon* were reported to be edible if the inside is fresh and pure white and should be consumed within 24 hours of harvest. All *Calvatia* species are edible (Morris, 1987), but only in the immature state before the commencement of spores maturation and while the gleba is still firm and white (Hedawoo, 2020). Edibility of *Calvatia cyathiformis*, *Calvatia fragilis, Lycoperdon perlatum* is supported by many researchers e.g., (Coetzee & Wyk, 2009; Chakraborty et al., 2012; Verma et al., 2018; Hedawoo, 2020). These species are referred to as the "poor man's sweetbread" due to their texture and flavour. An account on the ethnomycological use of *C. cyathiformis* and *C. gigantea* use as medicine have been recorded (Rai et al., 1993; Buyantogtokh et al., 2020). Two steroids including calvasterol A (14α-hydroxyergosta-4, 7, 9, 22-tetraen-3-6-dione) and calvasterol B (9α, 14α-dihydroxyergosta-4, 7, 22-trien-3-6-dione) and a novel dimeric steroid and calvasterone were detected from *Calvatia cyathiformis* (Kawahara et al., 1993; 1994; 1995).

On nutritional status, Aletor (1995) estimated 13.2% - 46% crude protein and had a gross energy value of 3.07 kcal/g. for *Calvatia cyathiformis*. Vetter (1990) compared the potassium, copper, manganese, zinc and phosphorus contents of *Calvatia* - complex with a large number of other edible macrofungi and found higher values of these elements in *Calvatia* - complex than other edible macrofungi.

Calvacin, a new antitumor agent was detected in basidiocarps by some researchers (Roland et al., 1960; Beneke, 1963; Lam et al., 2001). The discovery of the oncostatic properties of *Calvatia* species directly inspired an investigation into their possible antiviral activity (Cochran, 1978). Viterbo et al. (1975), reported that calvatic acid exhibited antibacterial, antifungal as well as antitumor effect.

Ethnomycology and biotechnological potential of *C. cyathiformis* and other *Calvatia* (*C. craniformis, C. excipuliformis, C. gigantea* and *C. utriformis*) are a source of food and traditional medicine has been reviewed by Coetzee and Van Wyke (2009). The record of *C. cyathiformis* and *Lycoperdon* sp. in Ghana extends the list of the edible and possible ethnomycological value of these macrofungi indigenous in Ghana. Wild mushrooms like *Calvatia* and *Lycoperdon* represent a major and untapped source of potent new pharmaceutical products, hence, the need for further research into these species.

Acknowledgements

We thank Mr. Anthony Adu-Gyamfi of the Ghana Herbarium, University of Ghana for the identification of the grass in the field in this paper, Miss. Afi Nunana for assisting in putting the document in a word format.

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Received 14 Jul 21; revised 19 Oct 22.