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PHENOLOGY, MORPHOLOGICAL, AND ANATOMICAL CHARACTERISTICS OF A STINKHORN MUSHROOM IN GHANA

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ABSTRACT

The genus *Phallus* is a member of the group commonly known as stinkhorn fungi belonging to the family: Phallaceae and Order: Phallales. They are distributed in tropical areas of Africa, Asia, Australia, and the Americas but are rarely reported in Ghana. During the recent minor rainy season (September-December 2022) an undocumented member of the Phallales was found in a rock garden located in the Greater Accra Region, Ghana. The subterranean basidioma developed from an egg-like structure, single or gregarious with a thick gelatinous peridium. The primordium was oval/spherical, or ellipsoidal in shape; initially hypogeous becoming epigeous in the habit by maturity; is 4.0-5.0 cm in diameter, creamy to grayish cream with age. Within 48-72 h, the egg broke to expose the pink-colored stalk, sponge, or reticulate with rounded greyish-dark green cap carrying smooth-walled slimy ellipsoidal basidiospores (2-5µm) with a fetid rotten smell. Within 2- 4 h of the emergence of cap, it was depleted of the ellipsoidal spores by foraging bluish-green bottle flies. The transverse section of the columnar stipe (5.0-8.0 cm long) showed a hollow orifice serving as the passage of basidiospores in the glebal mass. Occasionally, there was a division of the stipe at the cap to show two distinct stipes and caps from the same volva of the basidioma. The pileus was campanulate (bell-shaped), 2-3cm high dark green in color with vertical granular markings, and rounded to hemispheric in shape. Dark-green basidiospores besmear the cap with slimy glebal mass. These descriptions agree with no known penis-shaped morphology of the *Phallus* species with a prominent basal volva 2.0 -3.5 cm high.

Keywords: Stinkhorn; *Phallus* sp., phenology; penis-shaped, phallales; basidiospore; basidioma.

Introduction

The genus *Phallus* has a cosmopolitan distribution in tropical areas and is found in Africa, southern Asia, Australia, and the Americas where it grows in woodlands and gardens in rich humus soil as well as rotten woody material (Borde *et al*., 2021).

Phallus species are commonly known as stinkhorn, with penis-like morphology, and are classified in the family: Phyllaceae; Order; Phallales; subclass; Phallomycetidae belonging to the gasteromycetes group. They give, at maturity, a smelly fetid odour (Kibby, 2015) which attracts insects and plays a role in basidiospore dispersal through these insects (Ingold,1971; Malloch & Blackwell, 1992). Recently *Itajahya rosea* (a phalloid stinkhorn) was found frequently visited by *Drosophila* spp. feeding on the gleba which was found to produce some compounds for attraction and

feeding by *Drosophila* (Borde *et al*., 2021). Nineteen compounds were identified from the gleba while nine compounds were recovered from the pseudo-stipe including hexadecans, pentadecane which were responsible for the attraction of *Drosophila* toward the gleba. Three fatty acids, 9,12-octadecanoic acid, hexadecanoic acid, and benzoic acid ethylester produced served as an appetitive signal through the olfactory response of *Drosophila*, so the flies were fed on the gleba. Two pheromones compounds, heneicosane and (+)-5s,9s)-5,9 dimethylpentadecane, were also reported in the pseudo-stipe and gleba respectively which play a role in *Drosophila* for breeding (Borde *et al*., 2021). *Itajahya* is now treated as a subgenus of *Phallus* which includes four species (Malencon,1984; Kriesel, 1996). Fungal odours have been shown to attract insects living on fungi or substrates decayed by fungi not excepting stinkhorn mushrooms and some other insects (Tuno,1998; Pacioni *et al*., 1991; Bengtsson *et al*., 1991; Jonsell &Nordlander, 1995; Honda *et al.*, 1988; Phelan & Lin 1991; Pierce *et al*., 1991; Pfeil & Mumma, 1993). Currently, *Itajahya* is a subgenus of *Phallus* which includes four species (Malencon, 1984; Kriesel, 1996).

In Africa, *Phallus* (=*Itajahya*) *roseus* was originally regarded in Egypt and is also found in Pakistan, France, Israel, South America, and South Africa (Borde *et al*., 2021). *P. hadrani* (duene stinkhorn or the saw stinkhorn) is widely distributed and native to Asia, Europe, North America, and Australia (https://en.m.wikipedia.org). *P. ravenelii* is found in eastern North America grouped in clusters; *P. rugulosus*, *P. granulosodenticulatum*, and *P. impudicus* are found in Brazil (Stinkhorn.jpg).

Phallus has a wider distribution in the tropics and is made up of about 65 species; *P. drewsii*, *P*. (Calogne *et al*., 2008; Kasuyo, 2008; Desjardin & Perry, 2009). The fruiting body (basidioma) of the species of the Phallales develops starting from an egg-like structure ('witches egg') with a thick gelatinous peridium that protects against mechanical damage and desiccation. The fungus produces abundant subterranean mycelia of different strains (+ or -) which are haploid. During the rainy season, the mycelium ramifies and enmeshes leaf litter and stumps, and eventually the nucleus of the positive strain pairs up with the negative one to form a dikaryotic mycelium. This then undergoes conjugate division and forms the characteristics of a rounded oval or ellipsoidal-shaped egg with a thick gelatinous peridium that protects it against mechanical damage like the egg of a hen.

The basidioma (fruiting body) expands within the egg (by inhibition of water), breaks out, and expands to a height of 10 -15 cm (depending on species) producing or forming a volva, and the stalk stands out with the pileus or cap on top. The pileus carries a slurry or slimy spore-bearing mass called gleba which may drop off the cap because it is deliquescent. The basidiospores usually emit a foul-smelling fetid odour which attracts flies or sludges to greedily eat the basidiospore/slime mix. The development and emergence of the basidioma take a couple of hours 48-72h or more (depending on the species). The cap (pileus) can be stripped of the slime/basidiospore mix of the gleba within 2-4h of the emergence of the stalk-beaning cap (Dickinson and Lucas, 1979). The stalk of the stinkhorns (which are ephemeral mushrooms) appear and last for 12-24h or even for a few days.

The stalk can be white (*Phallus impudicus*, *P. indusiastus, P. hallus* (=*Itajahya rosea*), red (*P. rubicundus; Mitunus elegans*) pink, or shades of orange (Borde *et al*., 2021). Studies have documented a wide range of invertebrates that visit mature stinkhorns for consumption and dispersal of basidiospores, including, blue bottle flies, beetles, bees, slugs,

Drosophila etc. (Oliueirva & Morate, 2000; Chen *et al*., 2014; Borde *et al*., 2021). These are attracted by volatiles emanating from the fetid slime/spore mixture.

The macrofungi collection of Ghana in the Ghana Herbarium, GC at the University of Ghana has data on 206 macro-fungi species belonging to 50 genera (Motey, 2006). Of these, 21 species belonging to 12 genera are of foreign origin. Curiously, there was no record of Phallales in the inventory at that time. However, a subsequent survey carried out in southern Ghana (Mortey, 2006), recorded *Dictyophora indusiata* (Vent; Pers) Desv: from Aburi and Atewa Forest Reserves in the Eastern Region. This phalloid possesses a veil or indusium covering the cap and stipe when found in prime conditions. *Phallus impudicus* was recorded at Legon / Accra, *P. rubicundus* was found in the Eastern Region and Aiyaola Foreest Reserves whiles *Itayahya rosea* was found in the Achimota Forest Reserves in the Greater Accra Region (Mortey, 2006). *I. rosea* is a rare species found not only in semi-desert habitats such as Egypt, and Morocco but in Ghana, Israel, Pakistan, France, South America, and South Africa (Borde *et al*., 2021).

During the minor rain season in September-December 2022. An undocumented member of the Phallales was found growing in a rock garden in the Adenta Municipality of the Greater Accra Region. This paper reports the phenology, morphological and anatomical characteristics of this member of the Phallales and the visit by ramifying flies. The paper also add to the list of macro fungi collection in Ghana.

Experimental

Location of the study area

This member of the Phallales was spotted on 18th October 2022 in a rock garden soil (of the humus type) with sparse vegetation in a residential area at Ashalley Botwe in the Adenta Municipality of the Greater Accra Region (Coordinates N 4^0 31^1 28^0 E O^0 55^1 56 Latitude 4557651; Longitude 0.99322071).

The fruiting body/basidioma

The fruiting body was subterranean dikaryotic in the form of an egg structure, oval, spheroidal, or ellipsoidal in shape. The eggs were either solitary or gregarious and were cream to off-white in colour, becoming greyish with age (Fig. 1A and B). A diagrammatic representation of the developmental stage of the fruiting body up to the final mature stage when the pileus or cap has emerged with the stipe to show the characteristic penis-like morphology is shown in Figure 3A. During the development of the morphological characteristics of the fruiting body, the top of the egg opens presumably by expanding to show a hollow orifice originating from the inner peridium (Fig. 2A and Fig. 4).

Anatomical studies

The anatomy of the developing fruiting body (basidioma) was studied by cutting a longitudinal section of the egg by hand using a very sharp Okapi® kitchen knife to show the gleba, stipe, and peridium (Fig. 2B and 4). It also revealed the early stages of the phenology of development. Photographs were taken of the mature fruiting body, stipe, and cap as well as the smear of basidiospores on the pileus coming with the slimy gleba (Fig. 5A&B). Consequently, the basidiospores morphology was depicted by photomicrograph under photomicroscope (Leica Computer Model 1CC50W, Germany) with a software (Leica LAS EZ Version 1.8.0 for the dimension of basidiospores) (Fig. 8). A longitudinal and transverse sections of the stipe was made with the sharp kitchen knife to show the hollow nature of the stipe (Fig. 4 & 7) and the nature of the opening or orifice in the stipe leading to the pileus or cap (Fig. 7 and 8).

Discharge of basidiospores from gleba to the tip of the cap

An absorbent filter paper was placed at the bottom of a plastic transparent bowl (20×12×6cm) and then moistened with 150 – 200 ml of tap water. An unopened egg of the fungus was placed on filter paper to absorb water for 48 -72h. The changes that occurred are recorded in the photograph (Fig. 11).

Dimensions of the morphology of the fungus (mushroom) parts

Measurement was recorded of the egg, fruiting body, pileus, stipe, etc. using a centimeter ruler. Dimensions of the basidiospores were compared with measurements under the photomicroscope using an eyepiece graticule and stage micrometer.

Insect visitation of the cap to consume the basidiospores

The slimy gleba bearing the basidiospores was visited by bottle files during the period of observation (Fig. 5A and B).

Morphology of the stipe and cap region of the fruiting body

There was a peculiar phenomenon with the stipe emanating from a single volva; it emerges as two stipes with two separate caps at the rear tip region but remained attached at the base (Fig. 9). A detailed illustration of the cap (pileus) region of the fruiting body is shown in Fig. 10 (A and B).

Results

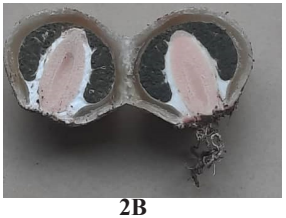
The basidiomata of the mushroom developed from abundant subterranean mycelium. They were initially hypogeous (or nearly so) but became epigeous in habit and visible to the naked eye. The basidiomata were either solitary or gregarious (Fig. 1A and B) and were creamy to off-white in colour which later turned greyish with age agreeing. The eggs were enclosed with a thick gelatinous exoperidium (Fig. 2A) showing a thin endoperidium that surrounds the gleba region with developing basidiospores and a central young receptacle or stalk (Fig. 2B).

A **B**

Fig. 1A: Picture of a solitary egg of a developing basidoma of *Phallus*

Fig. 1B: Picture of a gregarious colony of the egg of developing basidiomata of *Phallus*

2A



2B

Fig. 2A: Photograph of the egg of *Phallus* sp. about to release the pileus & stipe through the hole at the top leaving the volva to cover the base

Fig 2B**:** Longitudinal section of the development of the fruiting body of *Phallus* sp. showing thick external peridium, gleba (carrying basidiospores) & a central initial stipe

The final mature morphology of this fungus was penis-like in shape with a volva, stipe, and cap akin to a typical *Phallus* (Fig. 3B). During the development of the morphological characteristics of the family body, the top of the egg opens presumably by expansion, by

imbibition of water to show a hollow orifice origination from the inner peridium (Fig. 4, Fig. 2A). At maturity, the primordium grows to extend the stipe and cap to the top (Fig. 3B). The gleba is deliquescent and smells like excrement or rotting flesh which attracts insects like the blue bottle fly *Calliphora vomitoria* (family: Calliphoridae) (Fig. 5A and B). Within 2 - 4 hours of the emergence of the stalk (stipe), the cap is stripped of the slimy basidiospores by this visiting insect (Fig. 5). The stalks or stipes collapse after a few days or within 12 - 24 h because they are ephemeral (Fig. 6).

The primordium of this phalloid mushroom was spotted in the soil on 13th October 2022 after a previous day's rain and in about 48 – 72 h the life cycle was completed and the basidiospores were completely eaten and depleted in 2 – 4 h by the blue bottle fly. The transverse and longitudinal section of the stalk showed a clear hollow orifice (passage-way) through which the gleba and accompanying basidiospores pass to the tip of the cap (Fig. 4 and 7).

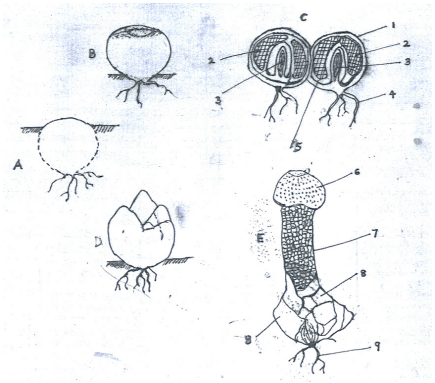


Fig. 3A: Developmental stages of *Phallus* sp.
Legend
A – Buried egg of *Phallus*
B – Fully exposed basidioma
C – Transverse section of fully exposed basidioma
1. Exoperidium
2. Gleba (bearing basdiospores)
3. Initial development of stipe
4. Rhizoid
5. Endoperidium

D – Fruiting body showing ruptured exoperidium and emerging developing stipe (stalk)
E. Final mature stage with penis-like appearance
6. Pileus or Cap
7. Stipe with reticulate covering
8. Volva
9. Rhizoid or mycelia acting as holdfast

Fig. 3B: Mature fruiting body (basidioma) of a *Phallus* sp. showing a penis-like morphology.

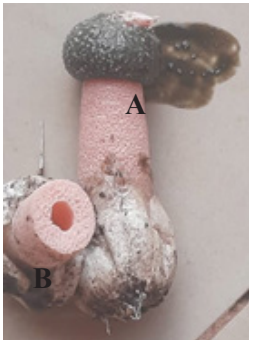


Fig. 4. : Mature fruiting body (basidioma) of *Phallus* sp. showing A= Penis-like morphology
(Note the discharge of basidiospores close to the pileus or cap). B= Transverse section of the stipe with hollow passage.

A **B**

Fig. 5A and B: Collapsed stalk with a bottle fly (*Calliopora vomitoria*) arrowed eating spilled basidiospores

Fig. 6: Whole intact fruiting body; (right): Whole fruiting body with ruptured cap and slimy basidiospores. (**Note** the propagative rhizomorph hypha at the bottom of the volva).

Fig. 7: Longitudinal section of the pinkish & reticulate stalk in pair

Descriptions and dimensions of the developmental stages of the phalloid mushroom
Habit: Eggs or primordia solitary or gregarious in humus soil in a rock garden found on 13th October 2022 and the eggs took 48 - 72 hr. (or more) to complete their life cycle. The eggs were 4.0 - 5.0 cm in diameter: creamy to an off-white color, turning greyish white with age (Fig. 1A and B).
Stipe (stalk): Cylindrical slightly swollen in the middle; transverse and longitudinal sections showed a hollow orifice serving as a passage for glebal mass with basidiospores (Fig. 4, 6, 7, and 8). Stipe columnar ranging in height 5.0 - 8.0 cm, soft, spongy, reticulate and pinkish in color bearing rounded cap (Fig. 6 and 7). Occasionally, there was a division of the stipe towards the cap /pileus region showing two distinct stipes and caps emanating from the same volva (Fig. 9).
Pileus (cap): Pileus was campanulate, 2-3cm high, dark green in color with vertical granular markings, rounded to truncate, and hemispheric in shape (Fig 10A and B). Pileus had an opening emanating from the stipe bearing dark green spores with a fetid smell besmearing the cap. (Fig 5A&B, 10A&B).
Basidiospores: Basidiospores are mainly ellipsoidal to cylindrical or spherical in shape with smooth walls (Fig 9); dimensions, 2.0-2.5µm.

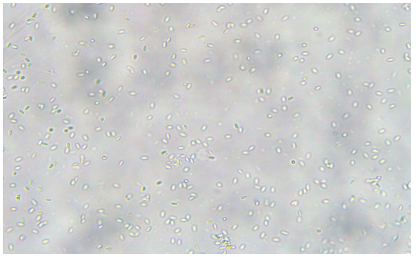


Fig. 8: Smooth & ellipsoidal basidiospores of *Phallus* sp.

Fig. 9: Photograph of a pair of stalks of *Phallus* sp. emerging from one volva; joined at the base but split into two distinct caps.

A **B**

Fig. 10A: Photograph showing rounded or oval-shaped pileus/cap of *Phallus* sp.

10B: Magnification of the pileus to show the architectural markings on the pileus of *Phallus* sp.

Volva of the fruiting body after the emergence of stipe: About 2.0 -3.0 cm high, creamish white in color attached to the rhizomorph at the base of the fruiting body (Fig. 4 and 6). The volva was prominent and sheathed to cover the base of the unbranched stipe or receptacle.

Discharge of basidiospores from gleba to the tip of cap: The phalloid egg placed in water in a bowl showed interesting findings. Firstly, the eggs opened in 48 -72 h producing the characteristics basidiomata with volva, stipe, and cap at the tip. There was a breaking of a pink 'hymen' covering the tip of the cap releasing copious basidiospores (Fig. 11). The 'hymen' is seen lying near the cap in Fig 11 colored pink.

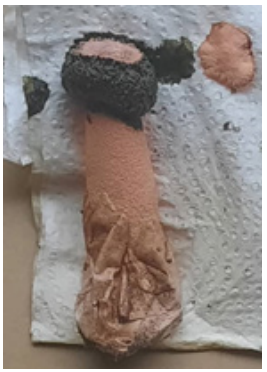


Fig. 11: Photograph of the mature fruiting body (basidioma) of *Phallus* sp. initially placed in a bowl of water as an egg & going through its phenology in 48-72h to mature. Bottom portion (volva), Middle (stalk or stipe), Tip (grayish black pileus with a clear opening). Juxtapositioned to the tip of the pileus is the pinkish ruptured hymenium which covered the exit of the basidiospores.

Discussion

The genus *Phallus* is a well-known and widespread gasteroid fungal genus from tropical and temperate zones characterized by an ovoid to spherical basidioma before ripening (maturity) to form cylindrical hollow pale yellowish to pinkish as well as white pseudo-stipe also called receptacle (Index Fungorum, 2022).

In this present study, the fungus was spotted at an early stage of development forming globose or ovoid basidiomata, which superficially resemble an egg beneath the surface of the soil. This agrees with the onset of the development of the gasteriods in the genus *Phallus.* The diameter of the egg ranged between 4.0 - 5.0 cm in agreement with the data of Moore-Landecker (1996) and Index Fungorum (2022) (Fig. 1A and B). The young basidioma was covered with a peridium and underneath the peridium was a well-defined gelatinous layer (Fig. 2B). The gleba carrying

the basidiospore was attached to the peridium at the base only (Fig. 2B). The basidiospores were mainly ellipsoidal to cylindrical or spherical in shape with a smooth wall (Fig. 8) ranging in dimension (2.0 - 2.5 µm). This is in the agreement with the report of Li *et al*. (2014), Piepenbring (2015), and Philips *et al*. (2018) for the genus *Phallus*. The fetid smelling basidiospores attracted flies like the *Calliphora vomitoria* (bottlefly) Family: Calliphoridae (Fig 5). These flies are attracted to garbage, rotting animal carcasses, and fetid odour (https:en.m.wikipedia.org>wk). They are common in Europe, Americas, and Africa, Greenland, and Mexico. Fungal odours including stinkhorns in the genus *Phallus* (Honda *et al.,* 1988; Pacioni *et al.,* 1991; Bengtsson *et al*., 1991; Phelan & Lin 1992; Tuno, 1998). The blue bottle flies depleted the cap of the basidiospores in 2 - 4h. This phenomenon has been reported in the genus *Phallus* (Moore-Landecker, 1996; Alexopoulus *et al.* 1996; Liu *et al.,* 2005; Liu *et al.,* 2014).

The stipe (stalk receptacle) which comes above ground is diagnostic of the genus and species of the phallales. The stipe may occur in various forms such as an unbranched column with a veil on top (*Phallus indusiastus*) or with a receptacle extended into arms (*Aseroe* sp.) or stipe (receptacle) terminating as a pileus-like hollow network (*Simblum* sp.) or with receptacle as a sessile hollow network e.g., (*Clathus* sp.). This phalloid is reported for the first time in Ghana and has a prominent volva, unbranched hollow receptacle (Fig. 3, 4, 5, and 6) with delicate pink to deep pink colour. The stalk or stipe was spongy, reticulate bearing a rounded pileus. The stipe colour varies in color depending on the *Phallus* species. The colour may be white, delicate to deep pink, lilac, orange, or orange-red. In this *Phallus* species reported in this paper, the stipe was hollow, delicate to deep pink with a rounded hemispheric cap (Fig. 3, 6, 7, & 10) characteristic of this species of *Phallus*. The appropriate epithet of this phalloid can be established in future phylogenetic analysis using ITS, LSU, and DNA sequences (Agana *et al*., 2017) using appropriate Genebank data, beyond the scope of this paper.

The bright colours of the basidiomata of Phyllales are due to the presence of carotenoids (Piepenbring, 2015) which also occurs in many other groups of fungi in the Agaricales (*Amanita, Cantharellus*), Dacrymycetales, Ascomyctes, Deuteromyctes and Pezizales (Eschavarri-*Erasun et al.*, 2002; Anon, 2021; Katra, Conlan, & Guel 2020; Poorniammal *et al*., 2021).

In this paper, there was a division of the stipe (receptacle) showing two distinct stipes and caps only at the top but joined in the middle (Fig. 9). This may be partly attributed to a genetic aberration, but present data cannot confirm this occurrence.

There was another interesting observation. The fresh eggs of the phalloid placed in water repeated the phenology of the formation of the stipe, and cap at the tip in 48 – 72 h. There was the breaking of the hymenium covering of the cap releasing copious basidiospores for the first time (Fig. 11) and ‘‘severed hymenium ’cover recorded for the first time.

Conclusion and recommendations

There is great diversity in body structure among the various genera and species in the Phallaceae. However, they invariably begin their development as an oval or rounded structure in the soil called ‘witches egg’. The diagnostic characteristics of the *Phallus* species in this paper included its habit of ominous appearance in the soil first hypogeal habit and epigeous at maturity with creamy to whitish grey colour at maturity. The eggs were either solitary or gregarious (Fig. 1A and B) in

uniformity with the habit of members of the genus *Phallus*. A longitudinal section of the egg stage (Fig. 2B) showed the characteristic arrangement of a gelatinous peridium (Piepenbring, 2015) and a developing stipe. It took 48 – 72h for the stipe (receptacle and cap) to appear to expose the ellipsoidal basidiospores in the gleba at the tip of the cap (Fig. 12). The slimy gleba deliquescenced and smelled like rotten fish or meat to give a fetid odour which attracted the green/blue butterfly *Calliphora vomitoria* (Calliphoridae). In the 16th century, John Gerald called the Phallales as ''pricke mushroom'' or ''fungus virilis penis effigie''with morphology that looks like a penis and smells like rotten meat (Dickinson & Lucas, 1979). This was confirmed by subsequent researchers (Anon, 2017; Anon, 2020; Borde *et al*. 2021; Anon 2022). This description agrees with the penis-like gross morphology of the *Phallus* sp. in this paper (Fig. 3B, Fig. 8, Fig. 13).

The *Phallus* sp. reported in this paper is different from *Phallus indusiatus*, *P. impudicus, Itajahya rosea, P. rubicundus* recorded in Ghana (Motey, 2006; Borde *et al*., 2021). The specific epithet can only be ascertained using molecular and phylogenetic methods available for phyllales to show their evolutionary diversity (Hosaka *et al*., 2006; Borde *et al*., 2021).

Members of the Phallales in Ghana have been reported in four locations and there is no documented occurrence in the over 7 parks and 6 Ramsar sites spread over the entire country's forest reserves, national parks, Ghana (https://en.m.wikipeida.org). Future studies should be extended to countrywide to update the occurrence of the Phallales in their ecological niches. Members of the *Phallales* are used in enthomedicine. For example, *Phallus rubiscundus* is used in the curing of sores and subcutaneous ulcers, scabies, carbuncle, and fistula (Ying *et al.,* 1987). It is also used in dispelling poison, causing detumescence of swelling and promotion of granulation; it can be applied as powder on wounds for healing (Ying *et al.* 1987). *P. Phallus indusiatus* is edible when the slimy spores are removed (Piepenbring, 2015). Gregory *et al*. (1966) showed that *P. indusiatus* contains antitumor compounds. The ethnomedicinal and pharmaceutical uses of the Phallales have not been exhaustively investigated in Africa and require urgent attention in the interest of public health delivery but will require a study on their domestication and commercial production.

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