



## ***Lysurus habungianus* sp. nov. (Phallaceae) – A new stinkhorn fungus from India**

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### **Abstract**

This paper presents a description and an illustration of one hitherto undescribed species of stinkhorn under family Phallaceae, genus *Lysurus* Fr., named as *Lysurus habungianus* sp. nov. collected from short grassland under Bamboo plantation of Habungia village, Jorhat District, Assam, India.

**Key words** – Dendrogram – macrofungi – Phallaceae – phylogeny – stinkhorn – undescribed

### **Introduction**

*Lysurus* Fr. is a genus of fungi belongs to the family Phallaceae commonly known as stinkhorn fungi. The species of *Lysurus* have a widespread distribution, but are especially prevalent in tropical areas (Kirk et al. 2008). There are records of 30 spp., 7 varieties and 2 forms of *Lysurus* in the index fungorum (Index Fungorum 2015). In India, Narasimhan (1932), reported *Lysurus gardneri* Berk. from ground of Mysore, Karnataka. Ahmad (1940), reported *Simblum phaeocephalum* Schlecht from Rohtak, Punjab and Tewaria & Khare (1968), reported *Simblum periphragmoides* Klotzsch from Varanasi, Uttar Pradesh. Later on these two fungi taxonomically shifted to the synonyms of *Lysurus periphragmoides*. Patil (1978), reported another species of *Lysurus* named *Lysurus sulcatus* (Cooke & Masee) G. Cunn. from well manured garden soil, Kolhapur, Maharashtra. Agarwal et al. (1984), reported *Lysurus borealis* (Burt) Henn. from Palampur, Himachal Pradesh. Acharya et al. (2010), reported two species of *Lysurus* namely *Lysurus periphragmoides* (Klotzsch) Dring (1980) and a *Lysurus* sp. from Howrah and East Midnapur district of West Bengal. Mohanan (2011) described a new species of *Lysurus* named *Lysurus brahmagiri* C. Mohanan from Kerala. Some of the allied species of *L. habungianus* sp. nov. like *L. mokusin* (L.) Fr. described as a new record from the Island of Madeira (Portugal) by Calonge & Goncalves-Silva (2006). This species is also found in Australasia the Canary Islands, Korea, Japan, China and the Bonin Islands. A new distribution of *L. cruciatus* (Lepr. & Mont.) Henn. in Bulgaria and southeastern Europe was reported by Assyov & Gashtarov (2007). *Lysurus gardneri* (Berk.) new distribution from Czechoslovakia was reported by Kluzak (1974). Recently, Cortez et al. (2011) reported two species of *Lysurus* namely *L. cruciatus* and *L. periphragmoides* (Klotzsch) Dring and one variety of *L. cruciatus* namely *L. cruciatus* var. *nanus* from Rio Grande do Sul State, in southern Brazil. The species is also reported from Africa, Asia, China, Sri Lanka, India, Pakistan, Thailand, Indonesia, Australasia, North America and South America.

Being a biodiversity hotspot hardly a few species of macrofungi have been newly described from North East India. Gogoi & Parkash (2014) reported seven species of stinkhorns excluding *Lysurus* spp. from Hollongapar Gibbon Wildlife Sanctuary, Jorhat, Assam. As per literature review there was no any record of new description as well as distribution of any *Lysurus* spp. from North East India. There is a great possibility of exploration and descriptions of new species of macrofungi from Assam. In this paper, we are describing a new species of *Lysurus* species after thorough macro as well as micro morphological studies followed by literature survey.

## Materials & Methods

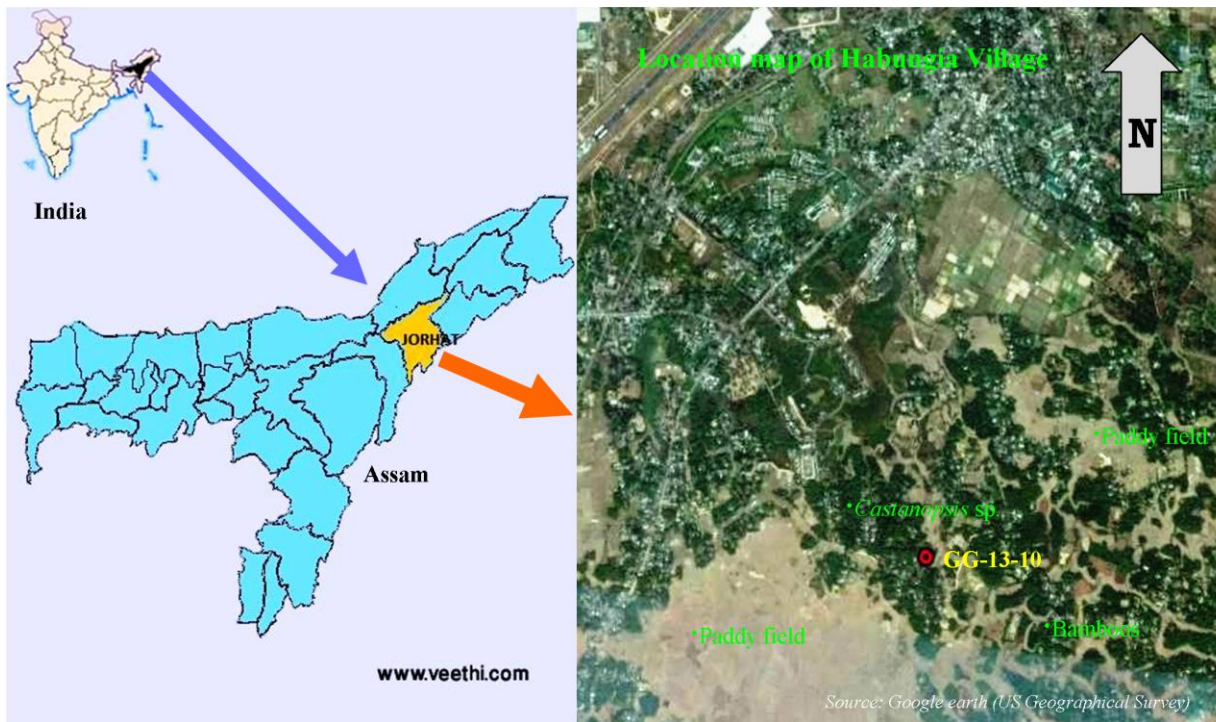
**Study site (Fig. 1)** – The study site is a small village called Habungia under Jorhat district of Assam, India. The village is located at a distance of 8 km from Jorhat district Head Quarter. There are 68 number of households and almost each household has an own bamboo plantation in the village. The main source of income is found to be cultivation.

**Collection and observation** – Collection of the specimens were done during June to October 2013 to 2014 from short grassland and Bamboo plantation of Habungia village, Jorhat, Assam (Fig. 1). The specimens were photographed with SONY DSC-H9 camera in their habitat during the survey period. Different identifying characters viz. colour, odour, texture, substratum, size of whole fruit body or its parts, were noted down in the field. Colour codes and terms followed Methuen Handbook of Colour (Kornerup & Wanscher, 1978). The same specimens were brought to the Laboratory, RFRI, Jorhat for macroscopic and microscopic study and preserved for further study. Micromorphological features were studied from the collected samples mounted in a mixture of 5% KOH, 1% phloxin, Congo red and 30% glycerol, and Melzer's reagent. Spore dimensions were measured under Olympus microscope CX41 and photographed the same spores. Twenty numbers of basidiospores were selected in the slide under the microscope to measure dimensions of the spores (Das & Zhao 2013). The minimum, the maximum and mean value for length and width were measured among the twenty basidiospores. The quotients (length – width ratio) of spore the minimum, the maximum and mean quotient value amongst the measured spores are determined (Das & Zhao 2013). The specimen was allotted accession number as GG-13-10 (Holmgren et al.1990) and deposited to the Rain Forest Research Institute, Jorhat. Authors' names were abbreviated as per Index Fungorum (2015). Spore measurements are recorded based on those of twenty basidiospores. Spore measurement and quotient indicating length–width ratio ( $Q = L/W$ ) are presented as minimum–mean–maximum. A distribution map was created with the aid of Google Earth ([www. google.com/earth](http://www.google.com/earth)). Phylogeny analysis was done by construction of a phylogeny tree or a dendrogram according to UPGMA method (Michener & Sokal, 1957) using the online dendrogram construction utility, DendroUPGMA (<http://genomes.urv.cat/UPGMA>) (Garcia-Vallvé et al.1999).

## Results

**Ecological study** – The fungus is found to be saprobic, where it grows solitarily on short grassland and on mulch of bamboo plantation.

The mature fruit body is originated from white (8A1) to brown (8E6) roughly round, partially buried in the soil or bamboo leaf litter, volva or Egg (Figs 7–8, 12). The volva is attached to the soil with a white (8A1) and thick rhizomorph consists of mass of fungal hyphae. The egg is soft and the peridium is white (8A1) to brown (8E6) colored on the external surface and has gelatinous layer inside (Fig 8). The diameter of a full grown egg was found 2–2.5cm (Figs 7–8, 12). It was observed that an egg took 5–7 days to be full grown and after that it was ruptured as the stipe expanded and broke through in the upper side giving rise the main fruit body of the fungus and the outer layer of the egg was still attached at the base of the matured fruit body, creating a volva at the base of the stipe. The matured fruit body which was 8.5 to 11cm high and 2.5–3cm width (Figs 5, 6), differentiated in to a brownish latticed cap, pileus or head (a receptaculum) and a spongy, hollow, white (8A1) to cream coloured (3C) stalk or stipe. The stipe 7–8cm high and 2.5 to 3cm width, walls composed of 1 layered chamber, wall 0.3 to 0.4 cm. The size of the head was found to be 2.5–3 cm tall and 3–3.5 cm width (Fig. 4). The cap formed brown lattice or mesh. There are 52 to 58 small pentagonal to hexagonal meshes on the head.



**Fig.1** – Map of study site. • (indicates the location of Habungia village on the globe)

The size of the meshes were found to be 3–4mm × 4–6mm. Sharp ridges were found on the outer surface of the arms of the mesh, without corrugations on the sides and the inner surface were found to be flat. Some spiny structures were found on the sharp ridges of the outer surface of the arms. The lengths of these spiny structures were found to be 0.5cm to 1.5 cm and white (8A1) in colour. A dark olive-green spore mass, the gleba, fills the inner surface of the lattice and extended outwards between the arms (Figs 2–6). Like other members of the Phallaceae family, the gleba has a foetid odour that attracts flies and other insects which help to disperse its spores. The stipe has found to be spongy, hollow, cylindrical, white to cream in colour, attained a length 7–8cm high and 2.5– 3cm width (Fig. 6).

Microscopic study – Dimension of basidiospores (Figs 9, 11) were measured in side view from 20 numbers of basidiospores. Basidiospores were elongated or elliptical in shape, hyaline thin walled smooth and minutely apiculate. The minimum, maximum and mean value for length of the basidiospores is found to be 4.5 µm, 4.9 µm and 4.7 µm respectively. Accordingly, the minimum, the maximum and the mean value for width of the basidiospores are found to be 2.4 µm, 2.8 µm and 2.6 µm respectively. The minimum, the maximum and the mean quotient value amongst the measured spores were found to be 1.71, 1.88, and 1.81 respectively. Stipe cells were found ellipsoidal to polygonal (5-6 sided), cell size 5–8µm x 3–4µm, egg cells were filamentous, septate and hyaline, 14 to 16 µm broad.

*Lysurus habungianus* G. Gogoi & V. Parkash, sp. nov.

Figs 2–12

Mycobank No. MB 812277

Facesoffungi number: FoF 00645

TreeBASE Submissions ID: 17469

Etymology – Named after Habungia village, type locality.

Material examined – India, Assam, Jorhat District, Habungia village, short grassland of bamboo plantation, 09 August 2013, G. Gogoi, GG-13-10(Holotype), RFRI-10 (ex-holotype culture), 26.7049°N Latitude and 94.18648°E Longitude, Elevation 102 m.



Fig. 2



Fig. 3



Fig. 4



Fig. 5



Fig. 6



Fig. 7

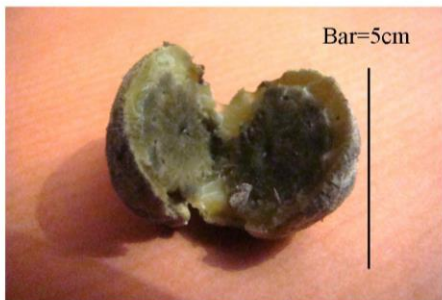


Fig. 8

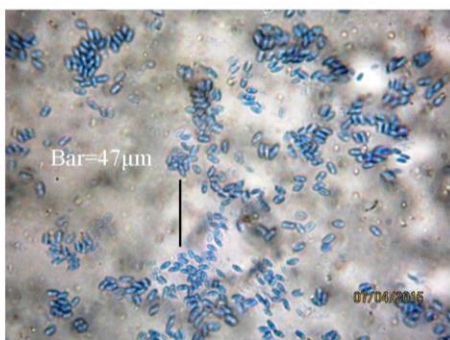


Fig. 9



Fig. 10

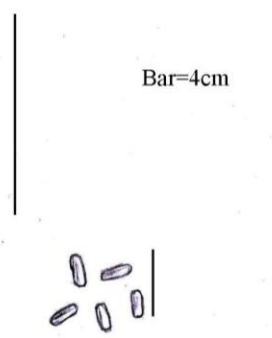


Fig. 11 Bar=12µm

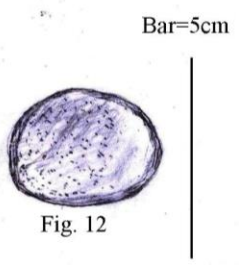
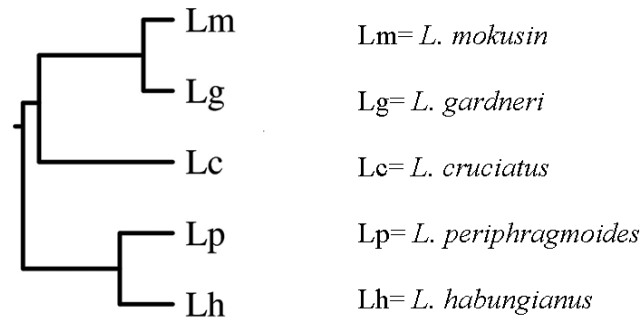


Fig. 12

**Figs 2–6** – Fruit bodies of *Lysurus habungianus* sp. nov. **Figs 7–8** – Eggs, **Fig. 9** – Basidiospores, **Fig. 10** – Line diagram of *Lysurus habungianus* sp. nov. Fruit body, **Fig. 11** – Line diagram of Basidiospores, **Fig. 12** – Line diagram of an Egg



**Fig.13** – A classical Phylogenetic tree or dendrogram of five *Lysurus* spp. using the online dendrogram construction utility, DendroUPGMA

**Table 1** Morphological comparison of *Lysurus habungianus* sp. nov. with four allied species.

Sl. No	1	2	3	4	5
	<i>L. mokusin</i> (L.) Fr., <i>Syst. mycol.</i> (Lundae) 2(2): 288 (1823)	<i>L. cruciatus</i> (Lepr. & Mont.) Henn., <i>Hedwigia</i> 41(Beibl.): (172) (1902)	<i>L. gardneri</i> Berk., <i>London J. Bot.</i> 5: 535 (1846)	<i>L. periphragmoides</i> (Klotzsch) Dring, <i>Kew Bull.</i> 35(1): 70 (1980)	<i>L. habungianus</i> sp. nov.
<i>Lysurus</i> spp.					
<b>Fruit body size</b>	11–18cmx1.5–3 cm	6-15 x1-2 cm	17cmx2cm	7- 15 cmx3.5	8.5-11cmx3-3.5cm
<b>Egg Size</b>	1–3 cm	5 cm	3 cm	5 cm	2.5cm
<b>Egg colour</b>	White	White	White	white or buff	White or brown
<b>Stipe shape</b>	Angular(4–6 arms fused)	Cylindrical, hollow, single	Cylindrical, hollow, single	Cylindrical, hollow, single	Cylindrical, hollow, single
<b>Stipe colour</b>	White to pink to red	White or yellow	White	Orange to reddish	White or cream
<b>Stipe size</b>	10–15 cmx1.5–2.5 cm	5-12.5cm x1-2.5	15 cm x 2 cm	6-11.5 cm x1-3 cm	7-8cmx 2.5 -3cm
<b>Cap shape</b>	Angular (4–6 arms fused)	4-7 arms (free)	4-6 arms united at tip	Latticed spherical	Latticed spherical
<b>Cap size</b>	0.8 to 3 cm long	1-2.5 cm long	1.5-2cm long	1.5–3.5 cm	3-3.5 cm
<b>Spore shape</b>	Cylindrical, smooth, hyaline	Elliptical to oblong, smooth	Elliptical, smooth. hyaline	Elliptical to oblong, smooth.	Elliptical to oblong, smooth
<b>Spore size</b>	4-6x 2-2.5 μm	3–4 x1.5–2 μm	4-5 x 1.5 μm	3.5-4.5 x 1.5-2 μm	4.7 x2.6 μm

Notes – The matured fruit body which was found 8.5 to 11cm high and 2.5–3cm width. The stipe was found spongy, hollow, white (8A1) to cream colored (3C) with 7-8cm high and 2.5 to 3cm width. The cap formed brown (8E6) lattice or mesh. There were 52 to 58 small pentagonal to hexagonal or irregular meshes on the head. The size of the meshes was found to be 3–4mm × 4–6mm. Sharp ridges were found on the outer surface of the arms of the mesh, without corrugations on the sides and the inner surfaces were found to be flat. Some sterile, white (8A1) and spiny structures were seen on the sharp ridges of the outer surface of the arms originating from the adjoining area of arms of the mesh (Figs 2-6). The length of these spiny structures was found to be 0.5 to 1.5cm. The minimum, the maximum and the mean value for length of the basidiospores were found to be 4.5 µm, 4.9 µm and 4.7 µm respectively. Accordingly, the minimum, the maximum and the mean value for width of the basidiospores were found to be 2.4 µm, 2.8 µm and 2.6 µm respectively. The minimum, the maximum and the mean quotient value amongst the measured spores were found to be 1.71, 1.88, and 1.81 respectively.

### Phylogeny analysis

Morphological features of four allied species of *Lysurus habungianus* sp. nov. were collected from the existing literatures and tabulated (Table 1) as follows for phylogeny analysis by using UPGMA (Unweighted pair group method with arithmetic mean). A phylogenetic tree or dendrogram (Fig.13) was constructed from the tabulated morphological features of the five *Lysurus* spp.

Phylogenetic tree (Fig.13) showed that all the five fungal species were originated from same ancestral gene; *Lysurus habungianus* and *L. periphragmoides* belong to the same clade which was parallel to each other in their evolutionary line and both were more primitive than the rest three species. *Lysurus cruciatus* formed an independent branch which was slightly advanced in comparison to the previous two species. *L. gardneri* and *L. mokusin* formed a separate branch parallel to *L. cruciatus*. *L. mokusin* was found most advance species in comparison to the other four species. *L. gardneri* and *L. mokusin* were belonging to the same clade but parallel to each other in their evolutionary line.

### Discussion

*Lysurus habungianus* sp. nov. is morphologically distinct, and unlikely to be confused with any other species, like *Lysurus mokusin* (L.) Fr. which has a distinct angular form of stipe (Tejera et al. 1998) and this character differentiated the species from other *Lysurus* spp. *L. mokusin* is the type species of the genus *Lysurus* (*Lysurus* Fr. Mycobank retrieved 2015). The "eggs" of *L. mokusin* are white, gelatinous measuring 1–3 cm in diameter, and are attached to the ground by thickened strands of mycellium called rhizomorphs. The stipe of the hollow, spongy mature fruiting body has dimensions of 10–15 cm by 1.5–2.5 cm and ranges in color from white to pink to red, with 4–6 distinct deeply grooved sides divided lengthwise by ribs. The spores are cylindrical in shape, smooth, thin-walled, and hyaline, with dimensions of 4–6 by 2–2.5µm (Smith 2005).

*Lysurus cruciatus* (Lepr. & Mont.) Henn. initiate from an white colour "egg" up to 5cm across and matured fruit body is 6-15 cm high, consisting of a stem with a head composed of 4-7 (but usually 5) arms. The arms are 1-2.5 cm long, short and thick, hollow, and three-sided. The stem is 1-2 cm thick, and is usually tapered downward. It is hollow and white, or tinged yellowish above and white below. Basidiospores are Spores 3-4 x 1-2 µm; elliptical to oblong and smooth (Miller & Miller 2006). In *Lysurus gardneri* Berk. the "egg" is whitish up to 3 cm across and the stipe is more or less cylindrical, hollow, up to 15 cm long and 2 cm thick, white, lumpy and wrinkled. Head composed of 4-6 short arms that are usually united at their tips. Arms white, lumpy, each with a sterile basal portion that is not covered with spore slime, on the upper, inner surfaces finely velvety, markedly lumpy, and covered with dark brown spore slime (Kuo 2013). Basidiospores have dimension of 4-5 x 1.5 µm.

*Lysurus periphragmoides* (Klotzsch) Dring which is commonly called as the stalked lattice stinkhorn or chambered stinkhorn (McKnight & McKnight 1987) and it was originally described as *Simblum periphragmoides* in 1831. The basidiomata of this species, extend up to 15 cm tall, consists of a reddish latticed head (a receptaculum) placed on top of a long, hollow, cylindrical, reddish stalk. The white to buff colored "egg" grows up to 5 cm in diameter and the matured basidiomata are up to 15 cm

tall. The cap or receptaculum is typically 1.5–3.5 cm in diameter and forms a red or orange lattice, or mesh. The stipe is 5–15 cm by 0.8–3 cm thick. Basidiospores are elliptical or oblong in shape, smooth and have dimensions of 3.5–4.5 by 1.5–2.5  $\mu\text{m}$ .

The species *Lysurus habungianus* is similar to *Lysurus periphragoides* (Klotzsch) Dring which is commonly called as stalked lattice stinkhorn or chambered stinkhorn. Although, *L. periphragoides* is a morphologically variable species, as a result, it has acquired an 18 number of synonyms (Kirk et al. 2008). Still, the present species can be differentiated on the basis of white, sterile, spiny structures found on the upper side of the chambered head of the species which were not ever been observed and reported earlier. Moreover, in *L. periphragoides* the stipe and head are generally yellow or red in colour (Guzman 1984) but in *L. habungianus* the stipe is always cream or white and the cap is always brown in colour. The classical phylogenetic tree (Fig. 13) also showed that the *L. habungianus* is the most primitive in comparison to the other four allied fungal species and it has very much similarity with *L. periphragmoides* but both were parallel to each other in their evolutionary line.

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