

THE GENUS *AMANITA* (Pers.) IN LUMBINI ZONE, NEPAL

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Abstract: During the survey of wild edible mushrooms in tropical to temperate belt of Nepal during rainy seasons in 2010 and 2011, many macro fungal species were collected and identified. The paper highlights on the five species of genus *Amanita* Pers. including new species *Amanita volvata* (Peck) Lloyd with their identification and documentation. The new species was identified and this is being reported and re-described for the first time from Nepal. *Amanita* characterized by stipe with volva or rudiments of volva, annulus present, in some may be absent, clamp connection present or absent, spores amyloid or inamyloid. The study area occupied 0.55 sq. km among the 9.288 sq. Km land and lies within an altitude between 90 and 2500 masl in tropical deciduous riverine forest to lower temperate mixed broad-leaved forest. The dried specimens are housed in the Tribhuvan University Central Herbarium (TUCH), Kirtipur Kathmandu, Nepal. The area embraces many mycophagous ethnic communities. The mycoelements prevailing in this area need sustainable conservation and utilization.

Keywords: Tasonomy; Basidiomycetes; *Amanita volvata*; Macrofungi.

INTRODUCTION

The investigation and study on mushrooms of Nepal started since 19th century (Lloyd, 1808; Berkeley, 1838). The genus *Amanita* contains over 500 named species and varieties (Adhikari *et al.*, 2010). *Amanita* from Nepal has been reported since 20th century (Singh, 1996ab), since then several papers have been published. 42 species and 2 subspecies of *Amanita* are reported from Nepal (Adhikari *et al.*, 2010), since then several botanical investigations have been done. Among these, very few reveal the studies and investigation on wild mushrooms from western Nepal. This area has not been previously investigated so far. This is a preliminary report on highlight on; presently five taxa of the genus *Amanita* Pers. with the addition of new species from Lumbini zone a region of tropical to temperate monsoon climate and west-south region of Nepal. *Amanita volvata* (Peck) Lloyd was identified and this is being reported and re-described for the first time from Nepal have not reported previously been recorded. The new findings have published here.

STUDY AREA

The study area (fig.1) lies in Lumbini Zone, Partial parts of Paklihawa-Rupandehi (90m), Tikkar-Kapilwastu (222m), Ramnagar-Nawalparasi (256m), Thada-Argkhakhanchi (500m), Simaldanda-Palpa (900m) and

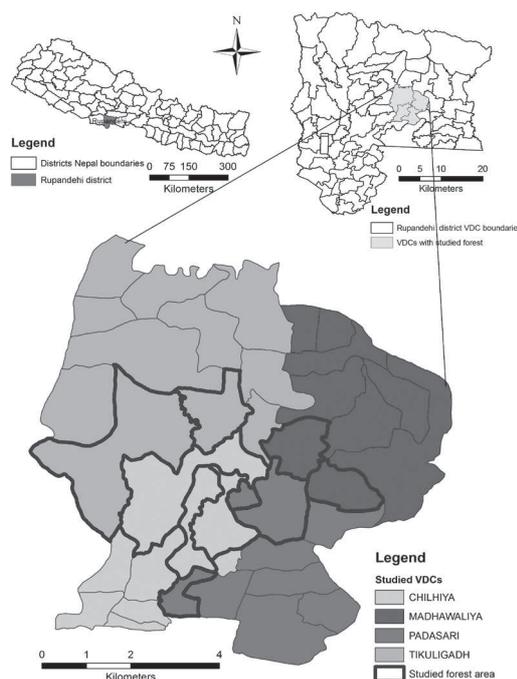


Figure 1: Sample Collection sites

Rsunga-Gulmi (2350m) of mid-west region of Nepal. It lies between 27.4813°- 28.13765° N latitude and 83.09029°- 84.05827° E longitude. The vegetation in forest is dominated by members of the Combretaceae, Dipterocarpaceae, Fagaceae and Leguminosae. The area occupies 6432.8 ha land between 233 and 262 masl. The

average annual rainfall is 1391mm.

Phytogeographically the area lies in tropical and temperate zones embracing interesting different types of forests and soil composition. The tropical riverine belt composed of *Acacia catechu*, *Adina cordifolia*, *Anogeissus latifolius*, *Bombax ceiba*, *Dalbergia latifolia*, *Dalbergia sissoo*, *Eugenia jambolana*, *Mallotus philippensis*, *Phoenix sylvestris*, *Shorea robusta*, *Schleihera oleosa*, *Syzygium cumini*, *Tectona grandis*, *Terminalia alata*, *T. tomentosa* and *T. belerica* etc. with an increasing altitude of *Castanopsis indica*, *Ehretia acuminata*, *Leucosceptrum canum*, *Lithocarpus elegans*, *mallotus nepalensis*, *Quercus lamellosa*, *Quercus semecarpifolia*, *Rhododendron arboreum* and *Schima wallichii*. The northern belt of this area has loamy sand, while the southern belt consists of sandy loam to loamy soil.

MATERIALS AND METHODS

Surveyed was done from 15th to 31th May and specimens were collected from 1st June to 31st Oct in 2011 and 2012. Mushroom samples were photographed in their natural habitat and their morphological characters were noted. Spore prints were taken. The samples were well dried and packed in wax paper bags, and wrapping with aluminum foil to prevent from the external infection as well as prevent from the intermixing of the spores, with proper tag numbers. The habitat including ecological parameters viz. altitude (by altimeter), vegetation composition, soil type, soil pH (by digital pH meter), soil moisture (by nail pH meter), humidity, temperatures and time (by means of thermo-Hygrometer) were recorded. The paper bags were brought to central Department of botany, Tribhuvan University, for further microscopic examination.

Some of the collected specimens were also preserved in a liquid preservative (25:5:70 ml rectified alcohol + formalin + distilled water), (Hawksworth *et. al.*, 1995). The specimens were studied based on the macroscopic, microscopic and habitat characteristics. Xanthochoric reaction (Tulloss, 1994) was determined by placing a small piece of material in 5% KOH solution, followed to the NH_3OH and FeSO_4 (Solution & Crystal) and its colour compared with the commercial colour chart leaflet. For microscopic study the specimens were sectioned by using a sharp razor blade. The cyanophilia was observed in cotton blue mounted in lactophenol reagent. The photographs were taken with a SONY DSC S980 Camera, macroscopic and microscopic characters were studied under the compound microscope, Olympus No. 575096, Tokyo (Japan), and latitude and longitude were taken by means of GPS compass.

The voucher specimens were identified with the help of relevant taxonomic literature viz.

(Singer, 1986; Kumar *et al.*, 1990 and Watkinson *et al.*, 2000), monograph (Gerrit, 1998) and Website (Jstor.org, Index fungorum, tropicos.org, Mycobank.org, biodiversity library.org). The voucher specimens are deposited in TUCH, Nepal.

RESULTS:

1. *Amanita volvata* (Peck) Lloyd

Amanita volvata f. *avellaneosquamosa* (S. Imai) E.J Gilbert, in Bresadola. *Iconogr. Mycol.* 27 (Suppl. 1): 304 (1941)

Local name: Besare chya.

Taxonomic position: Basidiomycetes, Agaricales, Amanitaceae (Figure 1.1, 1.2 and 1.3)



Figure 1.1: *Amanita volvata*

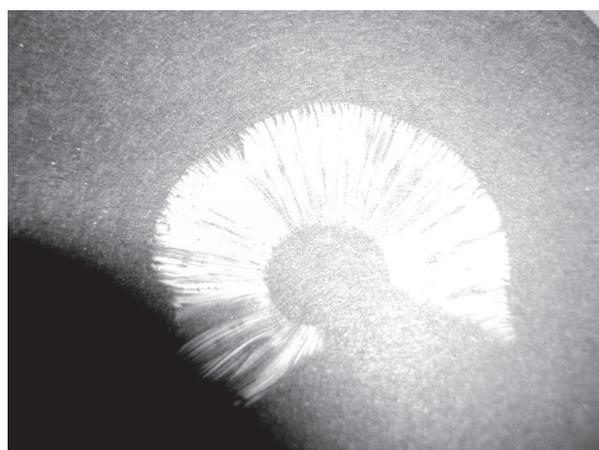


Figure 1.2: Spores print

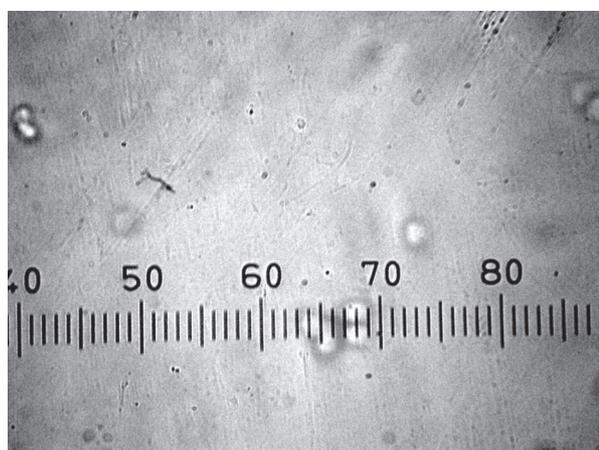


Figure 1.3: Spores (1div=3.75µm)

Identifying characters: The species is quite rare. Absence of annulus and the presence of the large somewhat cup-shaped persistent volva. The pileus is not smooth as is usually the case in the species with a persistent membranous volva, but it is more or less scaly with minute tufts of fibrils or tomentose hairs. Sometimes the margin is not very distinctly striate. The colour varies from white to brownish. The lamellae, which are white in the fresh, in dried specimens assume a dull yellowish-brown colour, except on the edge which remains white and is more or less floccose.

Justification: Another species of *A. volvaceus*, Bull., has similar volva, but its spore and lamellae are pinkish or flesh-coloured and it belongs, therefore, to the subgenus *Volvaria*. Hence this new species *A. volvata* distinctly differed from the *A. volvaceus*.

Taxonomic description:

Pileus: 5-10 cm convex to flat, margin with radial striations, hairy or floccose-scaly, white to cream, pale yellow; surface often with numerous patches of thin veil remnants. **Gills:** free, broad, crowded; white. **Stipe:** 5-10 x 1-1.5 cm, white; surface floccose-scaly to powdery, also discoloring; slightly tapering upward, base emerging from a thick, membranous, fleshy, saccate volva, persistent; no ring is left on the stipe, bulbous base. **Flesh:** white, discolour when cut, bruised or damaged. **Odor:** not distinctive. **Taste:** not distinctive. **Spores:** elliptic, smooth ornamentation, cell wall thick, colour hyaline, amyloid, deposit white, cream or yellowish measurement: 9-11 x 6-7µm in size. **Spore print:** white.

Habitat: in open field, often sandy soils in mixed woods and roadsides. Rare, not common. Found in eastern North America. **Ecology:** Mycorrhizal. **Season:** July-October. **Edibility:** Poisonous. Not edible.

Chemical reaction (Tulloss, R. E. 1994):

With NH_3 :negative on all the parts.
 With KOH : yellowish-grey on cap surface. .
 With $FeSO_4$ solution: Stipe: outer: negative & inner: Beacon 3109, Cap: negative. Gills: Raw Silk 0351.
 With $FeSO_4$ crystal: Stipe outer: negative, inner: Beacon 3109, Cap: negative, Gills:Sandalwood 0485.

Specimen examined: Nepal, Rupandehi, Dudrachhya WN 7, 245 m, long. 27° 29.3.45'N, lat. 083° 26.4.36'E, Coll No. 1007123, on humus rich liter soil in Sal forest, Aryal, HP. 21.08. 2011. New to Nepal. Common

Distribution: North America and Nepal.

2.Amanita caesarea (Scop.) Pers.

Syn. meth.fung. (Gottingen) 2: 252 (1801)
 Caesar's mushroom (Eng.)

Local name: Suntale chyau, Salle chyau, Phul chyau, Dhar shyamo (Tamang).

Taxonomic position: Basidiomycetes, Agaricales, Amanitaceae. (Figure 2.1, 2.2 and 2.3).

Taxonomic description:

Pileus: 8-20 cm, hemispherical to flat, orange red, washing out to yellow, cuticle separable sometimes with evident membranous remains of white veil,



Figure 2.1: *Amanita caesarea*,

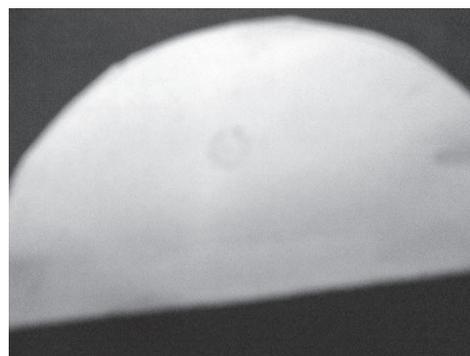


Figure 2.2: Spores print

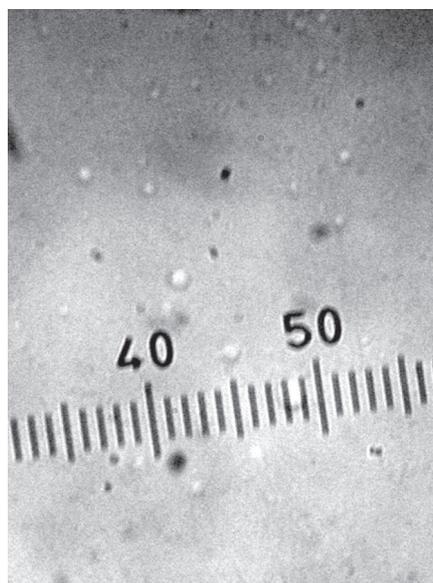


Figure 2.3: Spores (1div=3.75µm)

margin striate. **Gills:** free, crowded, yellow. **Stipe:** cylindrical, 8-15 x 2-3 cm, narrowing at the top, hollow when mature, with yellow falling annulus, slightly swollen at the base, with large white membranous volva. **Flesh:** whitish, yellowish beneath cuticle. **Odor:** without evident. **Taste:** good. **Spores:** elliptical, oval to spherical, ornamentation: smooth, cell wall thick, colour hyaline, white, measurement: 8-14 x 5-8.5 µm in size. **Spore print:** white.

Habitat: Soil on Quercus-Rhododendron forest.

Ecology: mycorrhizal. **Season:** June- August.

Edibility: Edible excellent. Used as vegetable.

Chemical reaction (Tulloss, R. E. 1994):

With NH_3 : reddish on cap surface.

With KOH : yellowish-grey on cap surface.

With FeSO_4 solution : Stipe: outer: negative & inner: Pale Cream 0328, Cap: negative. Gills: Ivory 0315.

With FeSO_4 crystal: Stipe: outer: negative, inner: Antique White 0940, Cap: negative, Gills: Macrame 095.

Specimen examined: Nepal, Rupandehi, Dudrachhya WN 7, 255 m, long. $27^\circ 29.4.45' \text{N}$, lat. $083^\circ 26.4.36' \text{E}$, Coll No 100755, on humus rich liter soil in Sal forest, Aryal, HP. 21.07. 2012. New to area of collection. Edible, Rs 250/Kg, Common.

Previously reported:

Manichur (Adhikari, 1976); Kathmandu market (Adhikari, 1991a); growing on soil. In moist shady place in *Pinus roxburghii* forest, Bajrayogini (1600m, no F. 1.2.1) (Adhikari, 1991b); Kathmandu market, (Adhikari and Adhikari, 1996-97); growing on soil, in moist shady place, in *Pinus roxburghii* forest, Bajrayogini (1600m, no 93077) (Adhikari, 1996b); Tokha (1670m); Kakani (1790m) (Adhikari *et al.*, 1996); Kathmandu (Ason) market (Bhandary, 1984; Singh, 1966ab; Pandey and Budhathoki, 2002); Swayambhu and Chautara (Cotter, 1987); Nagarkot (1620m) and Tokha (1679m) (Joshi and Joshi, 1999); on soil Sundarijal (1400m), and in the Kathmandu (Ason) market. (Pandey, 2008); (Pandey *et al.*, 2006). (Christensen *et al.*, 2008) and Muse (Lukla) (2783m), (Rana and Giri, 2008).

Distribution: N. America, Japan, Europe, Africa, India and Nepal.

3. *Amanita chepangiana* Tulloss and Bhandary

In *Mycotaxon*. XLLIII (1): 235-242, 1992; Chepang slender Caesar (Eng.).

Local name Gobre musa (Che.) Salleo (Magar), Seto Kukhura phooley chyau (Nep.)

Taxonomic position: Basidiomycetes, Agaricales, Amanitaceae. (Figure 3.1, 3.2 and 3.3).

Taxonomic description:

Pileus: 13 – 19 cm wide, pure white, sometimes with a slight grayish to brownish or Yellowish tint over the center, convex at first later umbrella like. **Gills:** free, white in color close to sub crowded, white, truncate. **Stipe:** cylindrical, fibrillose, white, 15 – 18 cm X 2 cm, skirt like white annulus, just 1cm below the cap, at the base



Figure 3.1: *Amanita chepangiana*,



Figure 3.2: Spores print,

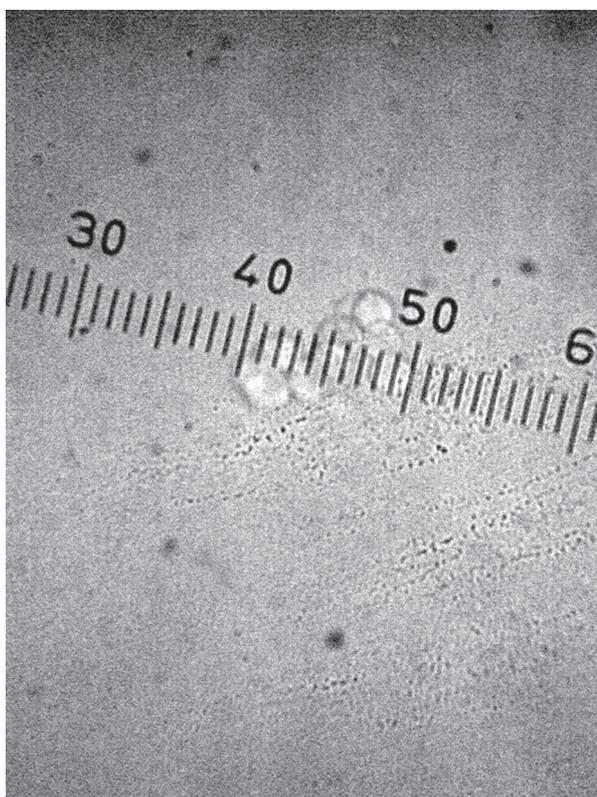


Figure 3.3: Spores (1div=3.75µm)

large membranous volva, white, saccate. **Flesh:** white. **Odor:** none. **Taste:** mild. **Spores:** oval to subglobose, ornamentation smooth, cell wall thin, colour hyaline, inamyloid, white, measurement: 9.2 - 12.5 X 8 - 10 μ m in size. **Spore print:** white.

Habitat: Litter (debris) on the Dipterocarpaceae and Fagaceae forest. **Ecology:** mycorrhizal. **Season:** July-August. **Edibility:** Edible preferred by Chepang excellent. Used as vegetable.

Chemical reaction (Tulloss, R. E. 1994):

With NH_3 : negative on all the parts.

With KOH : yellow in gills.

With FeSO_4 solution: Stipe: outer pale yellow & inner Beacon 3109, Cap: yellowish. Gills:

Antique white 0940, Pileus: negative.

With FeSO_4 crystal: Stipe outer pale yellow, inner sugarcane 0332, Cap: Daffodil 0308, Gills:

Suede 0N01, Pileus: negative.

Specimen examined: Nepal, Rupandehi, Parroha VDC, 233 m, long. 27°29.2.45'N, lat. 083° 26.2.36'E, Coll No 100772, on humus rich liter soil in Sal forest. Aryal, HP. 21.07. 2011. New to area of collection. Edible. (Rs 350/- to 400/- per Kg). Common.

Previously reported: Jugedi, Chitwan (Tulloss and Bhandary, 1992); listed in Adhikari & Durrieu (1996); Adhikari (2000b); on soil, Amrit Dharapani Samudayak Ban Chitwan (200m), (Pandey, 2008); listed in Christensen *et al.*, (2008b).

Distribution: Mostly in *Shorea robusta* forest. Occurs in Fagaceous forest also China, South East Asia and Nepal.

4. *Amanita fulva* Fr.

Observ. Mycol. (Havniae) 1: 2 (1815)

Tawny grisette (Eng.)

Local name: Tahar shyamo (Nep.)

Taxonomic position: Basidiomycetes, Agaricales, Amanitaceae. (Figure 4.1, 4.2 and 4.3).



Figure 4.1: *Amanita fulva*,

Taxonomic description:

Pileus: grey in colour, glabrous, when mature orange-brown, paler towards the margin and darker in the center, 4 - 10 cm in diameter. It develops an umbo

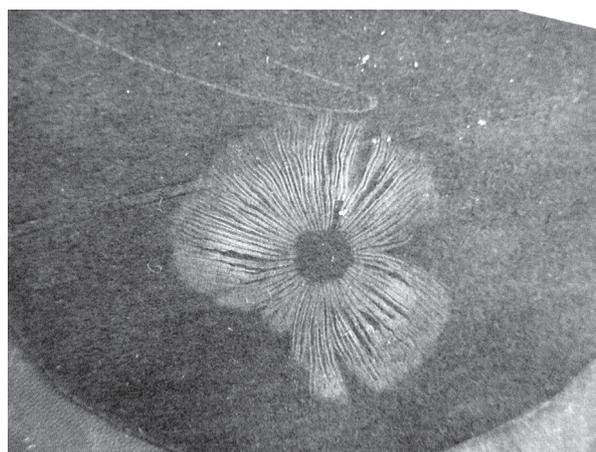


Figure 4.2: Spores print,

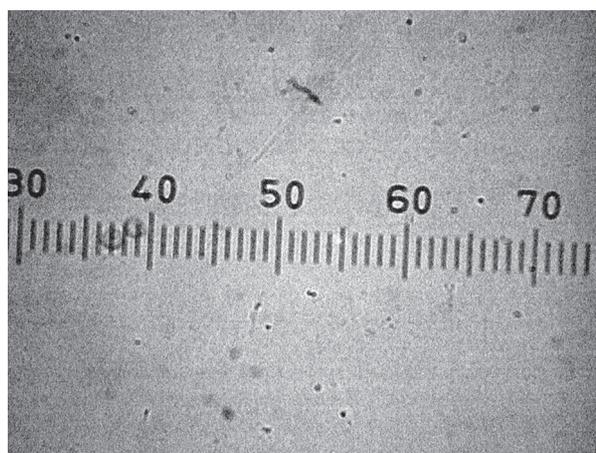


Figure 4.3: Spores (1div=3.75 μ m)

when expanded, and has a strongly striated margin, surface is smooth, slightly sticky and slippery when moist and glistens; later it may dry, usually free of volva remnants. Infrequently, roughly polygonal pieces of the veil may remain on the surface. **Gills:** white, free, long, crowded. **Stipe:** 8-12 X 0.5-1.5 cm, white and smooth, sometimes tinged with orange-brown and with very fine hairs, slender, annulus absent, hollow and quite fragile, tapering towards the top, universal veil which initially encapsulates the fruiting body is torn and develops into a white, sack-like volva with characteristic rusty-brown blemishes. **Flesh:** white to cream, soft. **Odor:** not distinctive. **Taste:** not distinctive. **Spores:** oval to spherical, ornamentation smooth somewhat hairy cell wall thick hairy appendages, colour hyaline, inamyloid, white, measurement: 8-9 x 12 - 12.5 x 9.3 - 12.0 μ m in size. **Spore Print:** white.

Habitat: On soil, growing along or scattered, near broad-leaved and mixed forest. **Ecology:** Mycorrhizal. **Season:** June-August. **Edibility:** Edible, but not popularly used (Commonly used in Gurung, Kumhal and Lodth mycophagous ethnic casts during my studies).

Chemical reaction (Tulloss, R. E. 1994):

With NH_3 :negative on cap surface.

With KOH : negative to slightly pale yellow, on cap surface.

With FeSO_4 solution: Stipe: outer negative & inner Bliss 0171, cap: negative. Gills: Tinge of Rose 8084.
 With FeSO_4 crystal: Stipe: outer negative, inner: Pale Rose 0421, cap: negative, gills: Sugarcane 0332.

Specimen examined:

Nepal, Rupandehi, Gajedi WN 2, 262 m, long. $27^\circ 29.545'N$, lat. $083^\circ 26.536'E$, Coll No 1010524, on humus rich liter soil in Sal forest, Aryal, HP. 21.06. 2011. New to area of collection. Common.

Previously reported:

Kathmandu valley (Bhandary, 1984); growing on soil, in the most shady places, in the *Pinus roxburghii* forest, Godawari (1515m no. 93002, 93005), Bajrayogini (1600m, no. 93075, 93085), Gokarna (no. 93097, Kakani (1800m, no. 93115), Nagarkot (1700m, no. 93044) (Adhikari, 1996a); Matatirtha (1650m), Lele (1500m), Tokha (1670m) (Adhikari *et al.*, 1996); in Adhikari & Parajuli (1996); listed in Adhikari (2000b); growing on soil, in the moist shady places, in the pine forests, Phulchowki (no.9800P22) and Daman (no. 9800D52) (Adhikari and Manandhar, 2004); (Pandey *et al.*, (2006) and Christensen *et al.*, (2008b).

Distribution: Europe, Japan, North America and Nepal.

5. Amanita pantherina (DC.) Kromb.

Naturgetr. Abbild. Beschr. Schwamme (Prague): 29 (1846)

Panther mushroom, Panther cap (Eng.),

Local name: Mritu chhatrika, Manga shyamo, Bhut chyau, (Nep.).

Taxonomic position: Basidiomycetes, Agaricales, Amanitaceae. (Figure 5.1, 5.2 and 5.3).



Figure 5.1: *Amanita Pantherina*

Taxonomic description:

Pileus: globose, then convex to expanded, 6-10 cm diameter, cuticle brown, covered with small white pyramidal warts, ochraceous to dark brown, striated margin. **Gills:** crowded, free, white. **Stipe:** 8-12 X 1-1.5 cm, narrowing towards top, striated, white, with frayed, pendulous not striated white annulus, with ring-like floccose zone at the base and the strongly swollen base

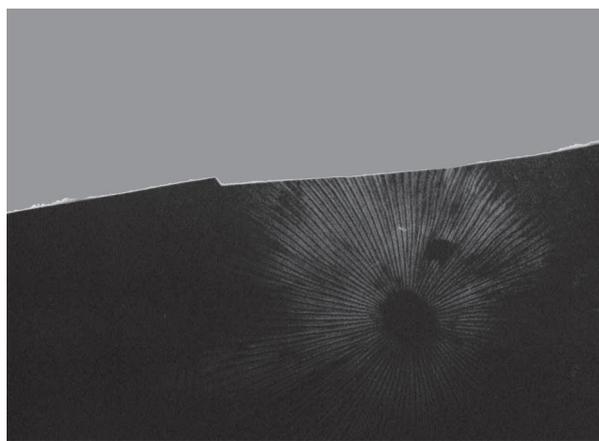


Figure 5.2: Spores print

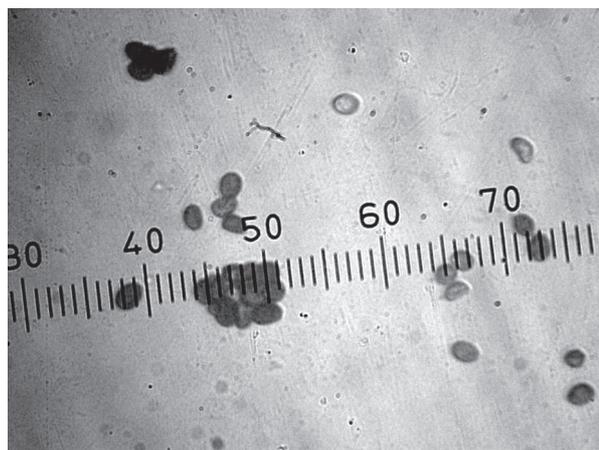


Figure 5.3: Spores (1div=3.75µm)

encased in a white volva. **Flesh:** white, unchanging. **Odor:** faint, pleasant. **Taste:** acrid. **Spores:** oval to ovoid, ornamentation smooth, cell wall thick, colour hyaline, inamyloid, white, measurement: 10-12 X 7-8 µm in size. **Spore print:** white.

Habitat: Usually grow on near broad-leaved trees (oak, beech, birch), occasionally near coniferous trees, in broad-leaved and mixed forest. **Ecology:** mycorrhizal. **Season:** July-August. **Edibility:** Toxic and produce dramatic symptoms similar to those of *A. muscaria* (L.) Lam.

Chemical reaction (Tulloss, R. E. 1994):

With NH_3 :negative on cap surface.

With KOH : negative to slightly pinkish, on cap surface. With FeSO_4 solution: Stipe: outer white & inner cool Grey 6163, Cap: Marco Polo 8607, Gills:Lavender Dew 0941.

With FeSO_4 crystal: Stipe outer white, inner Pale Cream 0328, Cap: Brick tone 8639, Gills: Lavender Dew 0941.

Specimen examined:

Nepal, Rupandehi, Parroha WN 9, 257 m, long. $27^\circ 29.59'N$, lat. $083^\circ 26.23'E$, Coll No 100773, on humus rich liter soil in Sal forest, Aryal, HP. 12.08. 2011. New to area of collection. Common

Previously reported:

On ground in forest, Phulchowki (no. 1101) Kathmandu valley (Singh and Nisha, 1976); listed in Bhandary, (1984) and Adhikari (1995); in *Quercus-Rhododendron* forest of Phulchowki (1780m), along with the mosses, Kakani (1700m), in mixed forest, Sundarijal (1780m) (Adhikari *et al.*, 1996); listed in Adhikari (1996a, 2000b, 2008, 2009); in status and conservation of fungi (Adhikari, 2000a); on ground in *pinus roxburghii* forest, Nagarkot (1670m, no.9800111, 14.8.1998) (Adhikari and Manandhar, 2004) and place, date and collection number not mentioned, in temperate region. (Nepalese Fungi, 2004).

Distribution: North and South America, Japan, Europe, South Africa, North Asia and Nepal.

DISCUSSION

Members of this family characterized by free gills, presence of annulus and a volva; although annulus of some species disappear early (*A. volvata* and *A. fulva*) and volva may sometimes buried and difficult to see or may slough off. They bear white spore prints. All are mycorrhizal and their spores are hyaline and inamyloid.

Shape of the Pileus of the *A. volvata* is convex to flat and surface covered by numerous patches of thin veil remnants, whereas hemispherical to flat shape in *A. caesarea* and surface is covered by membranous remains of white veil. Similarly *A. chepangiana* has convex shape and surface is pure white and smooth and *A. fulva* has umbo and rough polygonal pieces of the veil on the surface. Likewise *A. pantherina* has globose and surface covered with small white pyramidal warts. Stipe is slightly tapering upwards in *A. volvata*, cylindrical in *A. caesarea* and *A. chepangiana* whereas slender in *A. fulva* and narrowing towards top in *A. pantherina*.

Out of five, three species viz. *A. caesarea*, *A. chepangiana* and *A. fulva* are edible. All the taxa of the genus *Amanita* Pers. plays an important role in forest ecology due to mycorrhizal association with vascular flora. They occur in the *Shorea-Schima-Castanopsis-Quercus-Rhododendron* and *Betula utilis* forests ranging from 90m to 4000m in Nepal. This ectomycorrhizal fungi and its prevailing environment have not been well studied (Adhikari, 1990; Adhikari and Parajuli, 1994; Cotter, 1987).

CONCLUSION

The recent investigation created an enthusiasm towards its intensive exploration. The *Amanita volvata* (Peck) Lloyd is new record for Nepal. The all reported mushrooms are widely spread throughout the country in tropical to temperate belts. It needs extensive investigation to find out their morphological details, species richness, distribution pattern and

species diversity index. It needs special attention to be conserved against the threat to avoid their unmanaged and unscientific exploitation. Harvesting should be done more scientifically rather than traditional methods. The mycoelements prevailing in this area need sustainable conservation and utilization.

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