

MACROFUNGI FROM SIDDABABA SACRED GROVE: WEST, NEPAL

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ABSTRACT

During the survey of wild edible mushrooms in between tropical and temperate belt of Nepal in rainy season 2010 -2012, many macro fungal species were collected and identified. The paper highlights on new record of *Volvorella taylorii* (Berk and Broome) Sing. The species was reported and re-described for the first time from Nepal. Along with, taxonomic description of the two species of *Volvorella* found in the same studied area, have also been provided. Phytogeographically, the sample collected area lies within a narrow limit of 9.75 hectare at the altitude between 335 and 400 masl in tropical deciduous riverine forest. The dried specimens are housed in the Tribhuvan University Central Herbarium (TUCH), Kirtipur Kathmandu, Nepal.

Kew words: Basidiomycetes, Taxonomy, *Volvorella taylorii*

INTRODUCTION

Macro-fungal studies have long been of interest to scientists in the world for their significant roles in forest ecosystem and human life. Macrofungi are used in the pharmaceutical industry, the mass production of cultivated fungi is used in the food industry, and they play vital role in biodegradation (De Boer et al. 2005).

Fruiting body of edible species of macro-fungi are mushrooms. Nepal is considered as the homeland for the mushroom floral diversity (Aryal and Budhathoki, 2012a). So far, 816 mushroom species have been identified (Adhikari, 2009; Aryal and Budhathoki, 2013abc and Aryal et al. 2014), which include 228 edible (Christensen et al. 2008), 66 poisonous (Pandey, 2008 and Adhikari, 2009) and 75 medicinal species (Adhikari, 2009; Aryal and Budhathoki, 2013; Aryal et al. 2014). The investigation and study on mushrooms of Nepal started since 19th century (Llyod, 1808). Since then several papers have been published and several botanical investigations have been done. Among these, very few reveal the studies and investigation on wild mushrooms from western Nepal. The area has not been previously investigated so far. The present works explore on the systematics of *Volvorella bombaycina* (Schaeff. ex Fr.) Sing and *Volvorella volvacea* (Fr.) Sing, with the addition of new species *Volvorella taylorii* (Berk and Broome) Sing. Presently three taxa of the genus *Volvorella* have been reported from Siddababa religious forest, a region of tropical climate and west-south region of Nepal. Among the three, one taxon is new to Nepal, and has not been reported previously.

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STUDY AREA

The study area lies in Lumbini zone, Palpa district, Dovan VDC, WN-7, Siddababa temple territory in the west Nepal and towards the north of Butwal Municipality (Figure 1). Phytogeographically, it embraces tropical riverine belt along with loamy sand to sandy loam soil. The forest vegetation is dominated by members of the Dipterocarpaceae, Combretaceae, Leguminosae and Myrtaceae families. This study area included 9.75 hectare of forest (DFO, 2007) and lies between 27.73399°N-27.73400°N latitude and 83.41398°E-83.41400° E longitudes. The altitudinal range varies between 335 and 400 masl. The average annual rainfall is 1391mm (GoN, 2010).

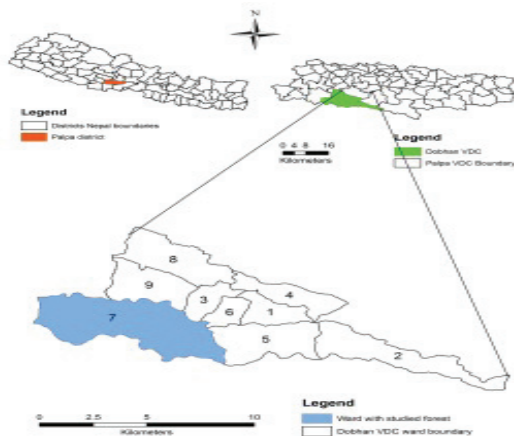


Figure 1. Map of Sample collection site

METHODOLOGY

Survey was conducted from first week of May in 2010 and specimens were collected from first week of June 2010 to last week of October, 2012. Mushroom samples were photographed in their natural habitat and their morphological characters were noted. Spore print was taken. The samples were well dried and packed in wax paper bags. They were wrapped with aluminum foil bags to prevent from the external infection. As well as prevent from the intermixing of the spores, proper tag numbers were used. 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 preserved in a liquid preservative (25:5:70 ml rectified alcohol + formalin + distilled water), (Hawksworth et. al. 1995) and

rest are in dry preservation (Atri and Saini, 2000). The specimens were studied based on the macroscopic, microscopic and habitat characteristics, slide was prepared and spores were measured with microphotograph of fruiting body from the prepared slide. For determining the groups, chemical reaction (Tulloss, 1994) was carried out using different solution of 5% KOH, NH₃OH, FeSO₄ (crystal and solution) and its colour was compared with the commercial colour chart leaf let. (www.asianpaints.com). For microscopic study, the specimens were sectioned by using a sharp razor blade. The cyanophily was observed in cotton blue mounted in lactophenol reagent. The photographs were taken by Digital Sony DSC S980 Camera. Macroscopic and microscopic characters were studied under an Olympus microscope (Model No. CX 22), and latitude and longitude were taken by means of GPS compass (Garmin, Eterex, Germany).

The samples were identified with the help of relevant taxonomic literature (Shaffer, 1957 and 1962; Singer, 1986; Saccardo, 1887 and Seok et al. 2002) and Website (biodiversity library.org, Index fungorum, Jstor.org, Mycobank.org, Scirus.com and tropicos.org). Basically, the mushroom was identified on the basis of spore print (colour), size of spore, gills and its habitat. The voucher specimens are deposited in TUCH.

RESULTS

KEY TO THE SPECIES

Identifying Characters of the Volvarella

It bears basidia in a well defined hymenium, which becomes exposed while basidiospores are still young and underdeveloped, the basidiocarp in order Pluteales (Agaricales) is fleshy, sub fleshy, leathery, membranaceous or fragile. Basidia are 2, 4 or 8 spored and one called at maturity. The spores are forcibly discharged from the sterigmata at maturity.

Volvorella taylorii (Berk and Broome) Sing.

Monoson, H.L., A.S. Methven and W.J. Sundberg. 1993. Illinois species of *volvariella* (Basidiomycetes, Agaricales, Pluteaceae). *Mycotaxon* 49: 269-278. Shaffer, R.L. 1957. *Volvariella* in North America. *Mycologia* 49(4): 545-549. Kuo, M. 2008. *Volvariella taylotia*. Retrieved from the *Mushroom Expert.Com* Web site: http://www.mushroomexpert.com/volvariella_taylori.html

Local Name: Peeiroutee (Tharu).

Taxonomic Position: Basidiomycotina, Basidiomycetes, Hymenomycetes, Agaricales (Pluteales), Pluteaceae, *Volvariella* (Figure 2.).

Taxonomic description :(Kuo, 2008)

Basidiocarp: Initially grayish white egg-like structure consisting of a basidiocarp covered with volva. On maturing, the basidiocarp consisting of pileus and stipe emerge out of the volva (Figure 2.1-2.2). Annulus and clamp connection are absent. Macro and micro morphological characters with systematic treatment of chemical reaction (Table 1, 2 and 3).

Host: Saw dust. Edibility: Edible. Ecology: Saprobic, growing alone or gregariously on the ground; found in woods, often near brushpiles and woody debris, or in urban

areas in grassy waste places. Habitat: Terrestrial, saprobic in nature (the specimens were collected from the rice straw debris on 12 August, 2011) found growing in this area from the month of June/July to October/November (according to local inhabitants). Season: May to October; with heat, all year.

Specimen examined: Nepal, Palpa district, (Siddababa Religious forest), 380m; long. 83.49875° E; lat. 27.57412° N; Aspect: 220° W; Slope: 40° S/W; Temp.: 20.3-31.9° C; Humidity: 72-87%; Soil pH: 7.6; Soil moisture: 6.5, Time: 17.02 pm; Voucher No. 1009428; date: 09.09.2012. Growing on compost piles (decomposed paddy straw), Palpa. No previous record from the country, hence new to Nepal. Distribution: widely distributed in India and Nepal.

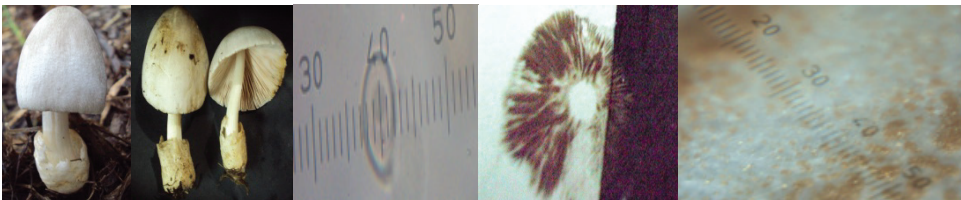


Figure 2.1 Figure 2.2 Figure 2.3 Figure 2.4 Figure 2.5

Figure 2. *Volvorella taylorii*. Figure 2.1 Basidiocarp, Figure 2.2 Dorsal and ventral view of basidiocarps, Figure 2.3 Spore (1div=1.66 μ m), Figure 2.4 Spore print, Figure 2.5 Distance between two lamellae (1div=11.66 μ m).

Volvorella bombaycina (Schaeff. ex Fr.) Sing.

Syn. *Volvaria bombaycina* (Berk. Sec Barb), Phillips, Mushrooms and other Fungi of Great Britain and Europe. 112, (1981). - Orson and Millar, Mushrooms of North America, 170, (1984). - Purukayastha and Chandra, Manual of Indian Edible Mushrooms, 127, (1985). - Saccardo, Sylloge de fungorum vol. 5, 656, (1887). - Konemann, The Encyclopedia of Mushrooms, 121, (1999), -Krieger, The Mushroom Handbook, 453, (1967).-Rea, British Basidiomycetaceae, 94, (1922).-Koneman. Mushrooms of Japan, 122, (1999).

Local Name: Kathchueenya (Tharu)

Taxonomic Position: Basidiomycotina, Basidiomycetes, Hymenomycetes, Agaricales (Pluteales), Pluteaceae, *Volvariella* (Figure 3.)

Taxonomic description:

Basidiocarp: White cap covered with silky fibrils, viscid, sac-like, dirty spotted volva, growing on wood or ground (Table 1, 2 and 3). Annulus and clampconnection absent.

Host: living trees. Edibility: Edible. Ecology: Saprobic, growing alone or gregariously on the ground; found in woods, often near brushpiles and woody debris, or in urban areas in grassy waste places. Habitat: Usually solitary, on rotting broadleaf tree trunks, in holes in living trees, sometimes some yards above ground, and on hollow stumps. Season: May to October.

Specimen examined: Nepal, Palpa district (Siddababa Religious forest), 400m; long. 83.51675° E; lat. 27.61321° N; Aspect: 220° W; Slope: 40° S/W; Temp.: 20.3-31.9° C; Humidity: 72-87%; Soil pH: 7.6; Soil moisture: 6.8, Time: 17.02 pm; Voucher

No. 1107979, Date: 15.09.2012. Growing on living tree (*Shorea robusta*). New to area of collection.

Previously reported: On groove of populus trees, Kirtipur from 1320m (Pandey, 2008).

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

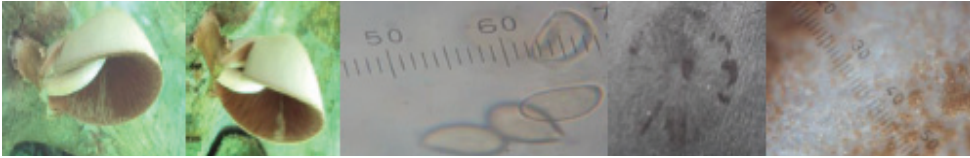


Figure 3.1

Figure 3.2

Figure 3.3

Figure 3.4

Figure 3.5

Figure 3. *Volvorella bombaycina* (Schaeff. ex Fr.) Sing.

Figure 3.1 and 3.2 Basidiocarp, Dorsal and ventral view, Figure 3.3 (1div=1.66µm), Figure 3.4 Spore print, Figure3.5 Distance between two lammelle (1div=11.66 µm).

Volvorella volvacea (Fr.) Sing.

Syn. *Volvorella volvacea* (Bull. ex Fr.) *Volvaria volvacea* (Bull.) Sacc. Sing.

Local name: poor chhtha (Tharu).

Common name: Paddy Straw mushroom, Chinese mushroom or Contonese mushroom.

Taxonomic Position: Basidiomycotina, Basidiomycetes, Hymenomycetes, Agaricales (Pluteales), Pluteaceae, *Volvariella* (Figure 4.).

Taxonomic description:

Basidiocarp: Sporophores usually growing solitary or gregarious on rotten paddy straw heaps; centrally stipitate (Table 1, 2 and 3). Annulus and clampconnection absent. Host: Rice straw. Edibility: Edible. Ecology: Saprobic, growing alone or gregariously on rotten paddy straw heaps. Habitat: greenhouses or gardens, on sawdust or other vegetable residue, also in areas where field mushrooms are cultivated. Season: May to October; when the temperature remains optimum (22-30°C), they are found throughout the year.

Specimen examined: Nepal, Palpa district (Siddababa Religious forest), 335m; long. 83.41400° E; lat. 27.73400° N; Aspect: 220 ° W; Slope: 40° S/W; Rain fall: 15 mm, Temp.: 20.3-31.9°C; Humidity: 72-87 %; Soil pH: 7.6; Soil moisture: 6.3, Time: 17.02 pm; Voucher No. 1109856, Date: 15.09.2012. Growing on compost piles New to area of collection.

Previously reported: On groove of populus trees, Kirtipur from 1320m,(Pandey, 2008).

Distribution: Europe, North America, India and Nepal

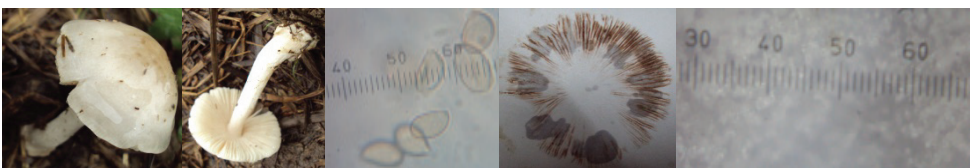


Figure 4.1

Figure 4.2.

Figure 4.3

Figure 4.4

Figure 4.5

Figure 4. *Volvorella volvacea* Figure 4.1 and 4.2 Basidiocarp of dorsal and ventral view, Figure 4.3 Spore (1div=1.66µm), Figure 4.4. Spore print, Figure 4.5 Distance between two lammelle (1div=11.66 µm).

DISCUSSIONS

Volvorella is principally a tropical genus, and some species are also found in temperate region, frequently in forests, cultivated fields, gardens, straw heaps and greenhouse; a few grow on wood and one is parasitic on other mushrooms. These phytodiversity and ecological conditions provide a good homeland for the growth of tremendous amount of parasitic, saprophytic and mycorrhizal fungi in the region (Aryal et al., 2012b). All the members of the genus *Volvariella* play an important role in forest ecology due to saprophytic in nature which occurs in the *Shorea-Schima-Castanopsis-Quercus-Rhododendron* and *Betula utilis* forests ranging between 60 and 3000 m in Nepal. This saprophytic fungi and its prevailing environment has not been well studied.

Species of this genus are indigenous and are well appreciated in the study sites. It has high medicinal importance and high protein content (Shing and Shing, 2005). The increasing depletion of forest is an alarming signal in the appearance, distribution and dominance of these species. The present investigation shows that vascular flora favors the growth of numerous species of mushrooms. The litter debris of these floras favors the regulation and maintenance of temperature and moisture in the soil.

CONCLUSION

The recent investigation on macro-fungal studies of this area is the genus *Volvariella* created an enthusiasm towards its intensive exploration. This genus is widely spread throughout the country in tropical to temperate belt of Nepal. It needs extensive investigation to find out their morphological characters, species richness and distribution pattern and species diversity. It needs special attention to conserve against the threat to avoid their unmanaged and unscientific exploitation by the people. Therefore, methods of harvesting should be done by application of more scientific rather than traditional methods. The mycoelements prevailing in this area need sustainable conservation and utilization. Hence, *Volvariella taylorii* is a new record from Nepal.

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Table 1. Summary of macro-morphological characters of basidiocarp.

SN	Taxa	Cap				Gills			Stipe		Flesh-colour	hyphae		
		colour	surface	margin	shape	size (lxw)/cm	colour	ornamentation	colour	surface			shape	size (lxw)/cm
1	<i>Volvariella taylorii</i>	grayish topinkish gray or brownish gray, evenly coloured (not markedly darker over the center portion)	dry, hairy	not lined	convex or broadly conic, becoming broadly convex to nearly flat	2.4-3.1 x 5.8-6.1	whitish becoming pink	free from the stem, closed or almost distant	whitish or grayish to brownish	dry, smooth or at the apex, finally hairy	tapering gradually to apex, with a slightly swollen base, the base encased in a thick sac-like gray volvas at the stem base	2.6-6.5 x 1	white	thin walled, inamyloid
2	<i>V. bombycina</i>	ochre-white towards disc, silvery white towards outside, whitish or tinged yellowish, darker,	cuticle adnate, shiny, with fibrils or fibrous scales, dry covered with silky hair, darker,	not lined, rounded and curved, then straight and regular	at first parabolic then convex or almost flat, sometimes slightly umbonate, oval becoming bell shaped to broadly convex to nearly plane,	20-May	whitish becoming pink or flash colour	free from the stem, crowded, with numerous lamellae, very ventricose, becoming toothed	white	dry, smooth, easily detachable from cap, outer cuticle often with yellowish brown or brownish markings	more or less equal but usually tapering somewhat to apex, slender, bulbous base, encased in a thick white to yellowish sac-like volva, persistent, free, wide, large and membranous	6-10x0.3-0.5	pure white	thin in cap, gills and stipe, inamyloid
3	<i>V. volvacea</i>	grayish	soft and fatty touch	sometimes split, velvety, free	Complanate at first, later becoming umbonate, presence of distinct radial sepia coloured streaks up to the middle of the pileus	10-May	rosy buff when young, flesh coloured with reddish tinge at maturity	long, crowded, distinctly formed, free, thin	petiole milky white, below it dull white, usuallu buff	hairy, membranous	Central, cylindrical, attenuated upward, solid, hollow when mature, presence of basal bulb, having well developed volva. Persistent, sepia above bilobed,	5-12x1	white	soft, slightly fibrous in stipe, inamyloid

Table 2. Summary of micro-morphological characters of basidiocarp.

SN	Taxa	Basidiospores	Basidia	Spores	Spore print	Cystidia	Pleuro-and Cheilocystidia	Hymenophoral trama
1	<i>Volvariella taylorii</i>	4.5-9 × 4.6.5 µm, ellipticle, smooth	8.5-12.5 × 3.5-6.5 µm, tetra sterigmata bearing tetrabasidiospore	5.5-9 × 4-6 µm, more or less ellipticle, or somewhatovoid, smooth	Salmon pink (cherry brandy x126)	85-95 x 35 µm	mostly fused-ventricose	inverse
2	<i>V.bombycina</i>	6.5-10.5 × 4.5-6.5 µm, ellipticle, smooth	Basidium with 4-spored	7-9 × 5-6 µm, pale pink, ellipticle, smooth, inamyloid	Salmon pink (cherry brandy x126)	well over 100 µm	Abundant, clavate	inverse
3	<i>V. volvacea</i>	6.8-9.3 × 4.6-6.3 µm, oval to ovoid, sometimes obovoid with hilar appendage, smooth, thin-walled	18.7-28.9 × 6.8-11.9 µm, clavate, tetrastrigimatic	7-9 × 5-6 µm, pale pink, ellipticle, smooth, inamyloid	Salmon pink (cherry brandy x126)	75-90 x 45 µm	clavate to ventricose	inverse

Table 3. Chemical test.

SN	Voucher Number	Taxa	NH ₃ OH	KOH	FeSO ₄ solution	FeSO ₄ crystal
1	1009428	<i>Volvariella taylorii</i>	Negative on all the parts	Yellowish-grey in colour	Bliss 0170, on cap surface	Romance 0p10, on cap surface
2	1107979	<i>V. bombycina</i>	Negative on all the parts	Pale-yellow in colour on cap	Bliss 0170, on cap surface	Romance 0p10, on cap surface
3	1109856	<i>V. volvacea</i>	Negative on all the parts	Yellowish-grey in colour	Lavender Dew 0941, on cap surface	Ting of Rose 8084, on cap surface

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