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A Russula (*Basidiomycota, Russulales*) with an unprecedented hymenophore configuration from northwest Himalaya (India)

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Abstract – The authors describe a *Russula* in section *Cyanoxanthinae* with a stipe surface that is entirely covered by a hymenial layer in the form of a reticulate-veined extension of the gills all the way down to the stipe base, a hitherto unknown phenomenon in the family *Russulaceae*. The significance of this finding is discussed.

Russula* subsect. *Cyanoxanthinae* / *Bondarzewiaceae* / gills / pores / *Russulales

INTRODUCTION

Russula Pers. is a worldwide very common genus of obligatory root-symbiotic, ectomycorrhizal basidiomycetes (Singer, 1986). Together with *Cortinarius*, it is probably also one of the most diverse genera of hymenomycetes with so far close to 3000 published names worldwide for a total of a little less than 900 accepted taxa (http://www.mtsn.tn.it/russulales-news/welcome.asp). With the exception of Europe where the genus has received a lot of attention from both professional and amateur mycologists in the past, many ill-known species remain to be re-examined (Adamcík & Buyck, 2010; 2011; Adamcik et al., 2010; Buyck & Adamcik, 2011) and many new species still to be described from almost any other continent (Li et al., 2011; Das et al., 2011) and this should probably raise the worldwide diversity of *Russula* to several thousands of species.

In the course of the 20th century, the Friesian concept of the agaricoid, lamellate family *Russulaceae*, including the genera *Lactarius* Pers. (milkcaps) and *Russula,* has been expanded to include also secotioid, hypogeous (Singer & Smith 1960; Albee-scott, 2007) and pleurotoid genera (Redhead & Norvell 1993; Henkel *et al*., 2000), all of which are now presumed to represent synonyms of either *Lactarius* or *Russula* species (Lebel & Tonkin, 2007; Buyck & Horak, 1999). More recently, one new, supposedly ancient agaricoid genus, *Multifurca* Buyck & V. Hofst., was described for a handful of tropical to subtropical *Russula* and one *Lactarius*, all sharing pale colors, regularly forked gills and deep orange spore print, occurring on different continents (Wang & Liu., 2010) including India (Saini & Atri, 1982). *Multifurca*, comprising typical representatives of both *Lactarius* and

Russula in the traditional sense, made a split of the genus *Lactarius* unavoidable (the only other alternative would have been to lump all agaricoid *Russulaceae* in one supergenus - see Buyck *et al.*, 2008; Buyck *et al.*, 2010a,b).

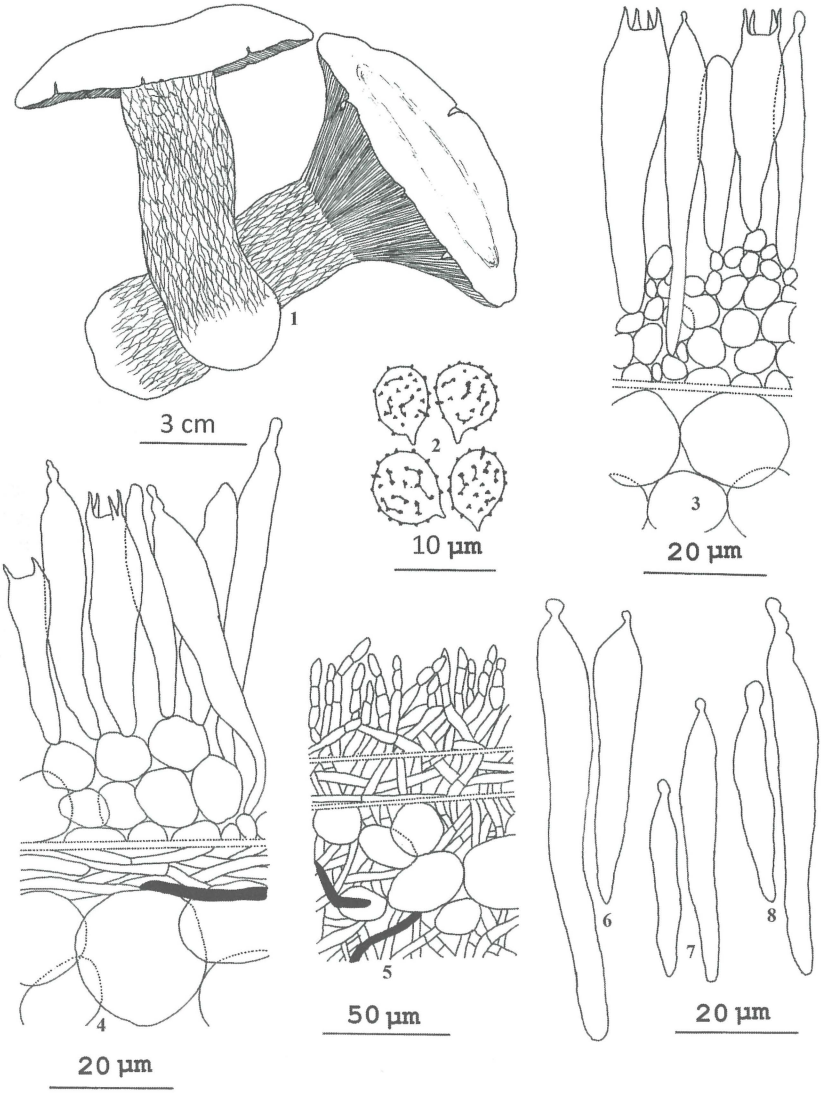
Molecular studies in the past decennium have also shown that resupinate, crust-like fungi are the closest existing relatives to *Russula* and *Lactarius,* as already suggested by some morphological similarities in hymenial features with *Multifurca* species (Buyck, 1995; Buyck *et al.*, 2008). At least a few of these crust-like fungi with smooth hymenophore seem to have their natural place within family *Russulaceae* (Larsson & Larsson, 2003; Larsson 2007; Binder *et al.*, 2005).

The configuration of the hymenophore within family *Russulaceae* has thus changed from a strictly lamellate configuration in the Friesian concept to a much wider variation in a molecularly based definition of the family, where it ranges along a continuum from regularly equal to regularly unequal or regularly forked lamellate in agaricoid forms, over a disorganization of the gills in secotioid forms to an strictly gleba-like configuration in the gasteroid, hypogeous forms, or else, completely smooth in the few resupinate representatives. Corralloid, hydnoid and poroid hymenophore configurations known from other families in the Russulales have not yet been demonstrated in family *Russulaceae*. In all of the above cases, the reduction or complete disappearance of the stipe seems to accompany the change of the strictly agaricoid lamellate hymenophore configuration.

In this paper, the authors describe a typical agaricoid *Russula* that was collected in the northwest Himalaya (India), but is unique in the fact that its hymenophore extends in a reticulate fashion almost up to the base of its normally developed stipe.

MATERIAL AND METHODS

This communication is based on the survey made for the collection and study of Russulaceous fungi from Chakrata (Kailana) in Uttrakhand, which is situated between Tons and Yamuna rivers between 30.67°N latitude and 77.85°E longitude at an elevation of 2209 meters in the north-west Himalaya (India).The macroscopic features pertaining to different parts of the carpophores were noted from the fresh fruiting bodies in the field. Observations for microscopic details were made by cutting free hand sections from the dried material revived in 5% KOH. For staining the sections Cotton blue was used while Lactophenol was used as mounting medium. Melzer's reagent was employed for performing iodine reaction while Cresyl blue was used for performing metachromatic reactions. Microscopic details were drawn with the aid of a camera lucida. Spore ornamentation was examined also with a Scanning Electron Microscope. For the examination of reticulation on the stipe surface stereo-microscope was used. Spore measurements are based on "n" spores and the Quotient (Q=L/W) was calculated from the mean values of length(L) and width(W). The colour terminology used is after Kornerup & Wanscher (1978). The examined material has been deposited in the herbarium of the Botany Department, Punjabi University, Patiala (PUN), with duplicate material at the Field Museum of Natural History (F), Chicago, and the Museum national d'histoire naturelle, Cryptogamie (PC), Paris. Herbarium abbreviations follow Holmgren *et al.* (1990).



Figs 1-8. *Russula* sp. PUN 322 (*Cyanoxanthinae*) **1.** Mature carpophores. **2.** Basidiospores. **3.** Cross section through hymenophore. **4.** Cross section through hymenial layer on stipe surface showing basidia and cystidia. **5.** Cross section through pileipellis. **6.** Pleurocystidia. **7.** Cheilocystidia. **8.** Hymenial cystidia on stipe surface (all drawings N.S. Atri).

DESCRIPTION

***Russula* sp. (subsect. *Cyanoxanthinae* Singer)** Figs 1-10

Fructifications up to 10 cm in height. **Pileus** up to 9 cm broad, first convex then expanded, finally infundibuliform; margin irregular, splitting at maturity; surface dry, pruinose, light yellow ($4A_4$) in the center, white along the margin;

cuticle not peeling. **Lamellae** adnate, but some extending down to the stipe in a network like pattern, unequal but not in series, highly forked, close to crowded, moderately broad, white, unchanging, with edges fimbriate. **Stipe** up to 9 cm long, 3.5 cm broad, stout, tough, surface reticulately netted because of the extending lamellae from its tip right up to the base, pruinose, white with yellowish tinge near the base, unchanging, solid, breaking up into shreds on applying slight pressure. **Flesh** white, unchanging, up to 1.2 cm thick in the cap. **Taste** initially mild then sour to tardily acrid after some time. **Odour** distinct, not bad. **Spore deposit** white. **Chemical Color Reactions:** Pileus surface and flesh turn coffee brown in conc. sulphuric acid, unchanging in KOH, NH_4OH and formalin; stipe surface turns reddish in Guaicol, unchanging in $FeSO_4$.

Spores ellipsoid, measuring 6.5-8.2 × 5.5-7 µm excluding ornamentation, Q = 1.17; ornamentation of small, blunt, amyloid warts, locally fused or connected by fine lines in an incomplete reticulum, suprahilar spot not amyloid, apiculus up to 2.2 µm long. **Basidia** 30-46 × 5.5-11 µm, clavate, 4-spored with sterigmata up to 5.5 µm long. **Hymenial cystidia** abundant, 36-78 × 4.5-8 µm, versiform, ventricose to clavate, usually with a small appendage, originating deep in the subhymenium, similar but smaller near the gill edge, 24-48 × 3.5-6.7 µm, with distinct, sulfovanilin positive contents. **Hymenophoral trama** solely made up of sphaerocysts; subhymenium up to 26 µm thick, multilayred. **Pileipellis** two-layered, showing strong metachromatic reactions in all parts when observed in Cresyl blue; suprapellis in the form of a definite turf of highly septate, upright hyphal extremities, 7.5-22 × 3.7-5.5 µm, with the terminal cell rounded to attenuated; subcutis gelatinized, formed of slender, irregularily tangled, septate hyphae mixed with scattered oleiferous fragments and embedded pileocystidia; context heteromerous. Cystidial elements on pileus small and scattered in the tufts of the suprapellis, also present in subpellis and underlying context becoming gradually longer as they are deeper inbedded in the tissue. **Stipe** surface fertile throughout, covered by a hymeniform layer of similar aspect as on the gills (fusoid to ventricose cystidia measuring 34.5-68 × 4.5-8 µm; clavate 2 and 4-spored basidia measuring 19-38.5 × 5-9.5 µm; hymenial cells originating from a subhymenial layer). **Clamp connections** absent.

Examined material: Uttarakhand: Chakrata, Kailana (2209m), growing scattered on humicolous soil under *Quercus leucotrichophora,* N.S.Atri, PUN 322, 24 August 1980 (Duplicates F, PC as *Russula* sp.).

DISCUSSION

Although one might think to be confronted with an unknown mushroom genus because of the unfamiliar aspect of the fungus, microscopic features allow to place it without much difficulty. Sequencing was not attempted because of the heavily molded nature of all tissues (microscopic examination confirmed presence of several fungal contaminants in surface tissues and underlying context) and because of the straightforward morphological features. The strong metachromatic reactions to Cresyl blue in most parts of the fruit body (especially easy to observe at the pileal surface), the typically cylindrical to attenuated, capitate pileocystidia that are also present in subpellis and underlying context, the small spores with non-amyloid supraphilar plage and low ornamentation, form and size of basidia, hymenial cystidia and, finally, the strongly septate hyphal extremities at the pileal surface are all typical features of subsection *Cyanoxanthinae* Singer in subgenus

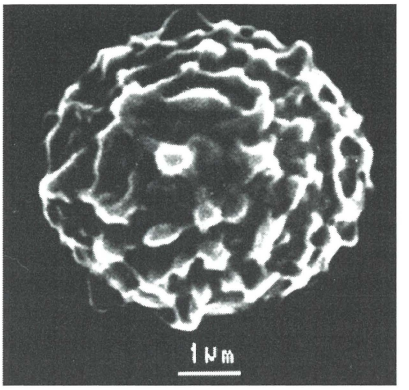


Fig. 9. *Russula* sp. PUN 322 (*Cyanoxanthinae*). Detail of the spore ornamentation as seen with a Scanning electron microscope.

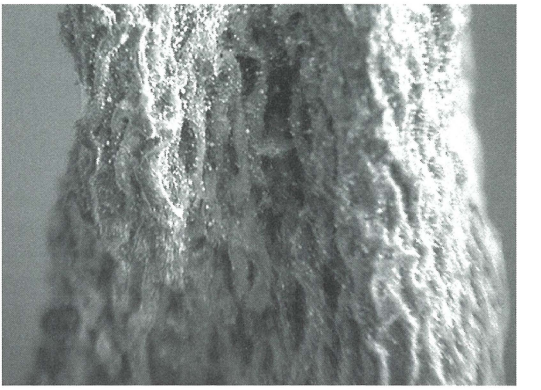


Fig. 10. *Russula* sp. PUN 322 (*Cyanoxanthinae*). Detail of the stipe surface as seen under a stereoscope.

Heterophyllidia (Sarnari, 1998). This placement is also supported by several field characters: no color reaction to ferric salts, a white spore print, unchanging context, mild to slightly acrid taste, and finally, the strongly forking, crowded gills that are characteristic for several other species in the subsection (*R. variata* Bann. for ex.). Pale yellowish *Cyanoxanthinae* with very similar features have been described from other continents (Buyck, 1994; Romagnesi, 1985). Although we refrain here from a more precise identification or eventual description of a new taxon due to the very bad condition of the specimen, there can be no doubt in our opinion on its placement in *Cyanoxanthinae*.

Regardless of whether or not this unique reticulate extension of the gills right down to the stipe base reflects a one-time artifact or a taxon-related, constant feature, one cannot deny the fact that this unique and hitherto unknown morphology bridges part of the enormous gap that existed between agaricoid, lamellate forms in *Russulaceae* and the few known poroid taxa in other families of the *Russulales*, e.g. *Bondarzewia*.

One argument that would favor the hypothesis of an artifact lies in the systematic placement of the species: subsect. *Cyanoxanthinae* is not one of the oldest groups in *Russulaceae* although it occupies an almost basal position the *Heterophyllidia* clade which may explain the presence of several *Cyanoxanthinae* with highly forked gills (Buyck & Hofstetter, 2010). The strongly reticulate, almost poroid configuration of the stipe hymenium should not be mistaken for an indication of a close relationship with *Bondarzewiaceae*. It should merely be seen as an indication that a potential convergence of the hymenophore configuration between these two families seems possible. It is yet another example in hymenomycetes that shows us how easy it can be to change hymenophore configurations.

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