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Full Length Research Paper

Morphology and taxonomy of *Sarcoscypha ololosokwaniensis* sp. nov.: A new Ascomycota species from Serengeti National Park-Tanzania

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Traditional taxonomy emphasizes the morphological features to characterize a taxon. *Sarcoscypha* is a genus in Sarcoscyphaceae family which display wide array of morphological variations. The genus is widespread in northern hemisphere and boreal regions, but also occurs in sub tropical areas and in the southern hemisphere. Both macro and micromorphological features including (ascocarp size, colour, shape, exterior surface of the fruit body, asci size, shape, as well as ascospore size, ends and lipid bodies) were used in a conventional taxonomic analysis of fresh *Sarcoscypha* material collected from southern hemisphere in Tanzania. Results showed that compared with similar species from northern hemisphere, Tanzanian materials were relatively smaller, smooth, vivid sharp red inside a saucers-shaped ascocarp, sessile to substipitate, microscopically unsheathed ascospores with two lipid bodies, distinctive geographical distribution, and unique season of fructification. Furthermore, a dichotomous identification key constructed for the six close similar species proved that Tanzanian material differed from other close species compared. Therefore based on conventional morphological taxonomy Tanzanian material from Serengeti National Park is described for the first time as a new Ascomycota; *Sarcoscypha ololosokwaniensis* sp.nov.

Key words: Ascocarp, *Ascomycota*, *Sarcoscypha*, Serengeti, Tanzania.

INTRODUCTION

Classification of fungi is constantly anguished by contradictions. This is due to the lack of complete knowledge about all the fungal organisms. Ascomycota, also known as sac fungi, is a recently discovered class. In fact, it was once classified within Deuteromycota. Ascomycota is a sister group to basidiomycota. *Sarcoscypha* represents one of the numerous examples of fungal genera in which a sound taxonomy is only achieved on the basis of vital macro- and especially microscopical characters gained from the study of fresh collections ("vital taxonomy"). Harrington (1990) reported the importance of fresh material for species diagnosis, especially for noting ascospore guttulation, which is vital in recognition providing the distinction of species from different bioclimates.

Sarcoscypha Boud. is a genus in a family Sarcoscyphaceae of the ascomycete fungi. It was described in 1885 by Jean Louis Émile Boudier based on Peziza tribe by Fries in 1822 in *Systema mycologicum* (Fries, 1821-1832). Species of *Sarcoscypha* are characterised by a cup-shaped apothecium which is often brightly colored, saprophytic species growing on decaying woody material from various plants such as plants of the rose family, beech, hazel, willow, elm, and in the oak trees (Baral, 2004). According to the fungal dictionary 10th edition of 2008, the genus comprises about 28 species which tentatively are accepted world-wide, about 6 of which occur within Europe and North America. Phylogenetic relationships study in the genus by Harrington (1998) hypothesized that the most recent common ancestor of the genus originated in Europe. Many appear to be endemic to volcanic islands in the

Table 1. Ten species of mushroom described for the first time from Zanzibar-Tanzania by Berkeley in 1885 original described name, and current name.

S/No	Original described name	Present name
1	*Agaricus missioni*s Berk.	*Lepiota missioni*s (Berk.) Sacc.
2	*Agaricus rhodofephalus* Berk.	*Lepiota rhodofephal*a (Berk.) Sacc.
3	*Agaricus vagu*s Berk.	*Clitocybe vag*a (Berk.) Sacc.
4	*Hiatula benzoni*i (Fr.) Mont.; Berkeley	*Agaricus benzonii* Fr.
5	*Agaricus arethus*a (Berk.) Sacc.	*Omphalina arethus*a (Berk.)
6	*Agaricus obfuscescens* (Berk.) Sacc.	*Pleurotus obfuscescen*s (Berk.) Sacc.
7	*Agaricus mediu*s Fr., Berkeley	*Volvariella medi*a (Schum.ex Fr.) Singer
8	*Agaricus nicotianu*s Berk,	*Agaricus nicotianu*s Berk & M.A Curt.
9	*Agaricus alboquadratu*s Berk.	*Psilocybe alboquadrat*a (Berk.) Sacc.
10	*Agaricus trisulphuratus* Berk.	*Cystoagaricus trisulphuratu*s (Berk.) Singer

subtropics. A high number of species (about 60) have ever been combined in the genus *Sarcoscypha*, many of these were often only collected a single time and especially the old descriptions are very inadequate, often re-descriptions of the type material, if any exists, are lacking thus they remain virtually unknown. Many of the taxa have later been found to belong in other genera of the Sarcoscyphaceae (Harrington, 1996; Harrington and Potter, 1997; Spooner, 2002).

Sarcoscypha coccinea (Scop.) Lambotte is known to be used as a medicinal plant by the Oneida Indians (Seaver, 1928), as a table decoration in Scarborough, England by arranging their fruit bodies with moss and leaves (Dickinson and Lucas 1982). The species is also known to be edible (Arora 1986) and a good source of food for rodents in the winter, and slugs in the summer (Brown 1980). Some *Sarcoscypha* species have been also found to posses some bioactive compounds which might be potential in bioremediation (Tortella et al., 2008).

In Tanzania, mushroom forming fungi are poorly collected, sparingly studied and relatively underutilized. The inventory of mushroom in Tanzania was done by Berkeley (1885) who described 10 new species from Zanzibar (summarized in Table1),followed by Hennings (1893) who described several species of Agaricales from western Tanzania.

Pegler (1977) in his Agaric Flora of East Africa book described also exclusively several *Agaricales* from the country. Recently a comprehensive study of edible and poisonous mushrooms of Tanzania was done by Härkönen et al. (1995, 2003) who reported more than one hundred taxa and described several new. More taxonomic studies on specific genus of the basidiomycete include Buyck et al. (2000); Tibuhwa et al. (2008) who all worked on a genus *Cantharellus* Fr. from miombo-woodland of Tanzania and Magingo et al. (2004) who studied *Odumensiela* sp. Apart from Härkönen et al. (1995, 2003), who studied a few Ascomycete, the rest of the studies never mentioned the Ascomycete nor presented any mushrooms in the Serengeti National Park, one among the protected areas of Tanzania with unique complex ecosystem. Serengeti National Park is one of the largest wildlife sanctuaries in the world covering about 14,763 sq km. The park lies in a high plateau between the Ngorongoro highlands and the Kenya/Tanzania border extending North-west almost to Lake Victoria. It is rich in biodiversity ranging from large mammals such as elephants to small microorganism, all making a complex ecosystem. Being a national park, much attention has been paid to study of large organisms viz: the animals (Sinclair and Arcese 1993, Borner et al., 1996); birds (Schmidt, 1982; Fishpool and Evans, 2001), Amphibians and reptiles (Kreulen, 1975), Vegetation (Schmidt, 1975; Belsky, 1987).

Despite the fact that Serengeti National Park is one of the least disturbed and best studied areas in Africa, mushrooms are among the forgotten taxa within the park which has never been studied although they contributes greatly to balancing the ecosystem in terms of nutrient recycling and symbiotic associations. The aim of the present investigation was to characterise the wild *Ascomycota* mushroom, species of *Sarcoscypha* found in Serengeti National Park.

MATERIALS AND METHODS

Study area: Serengeti national park in Tanzania

A two months field trip was made in the park and covered both the dry and wet side of the park (Figure. 1) in two years (2009 to 2010).

Collection, harvesting and preservation of specimens

Mushroom hunting in the park yielded enormous collections among which this striking red mushroom seems to be unique. On the study site, a transect of 5 x 10 meters was fixed using GPS (Global Positioning System- MAGELLAN EXPLORIST BELGIUM) within which mushroom hunting was done randomly throughout the

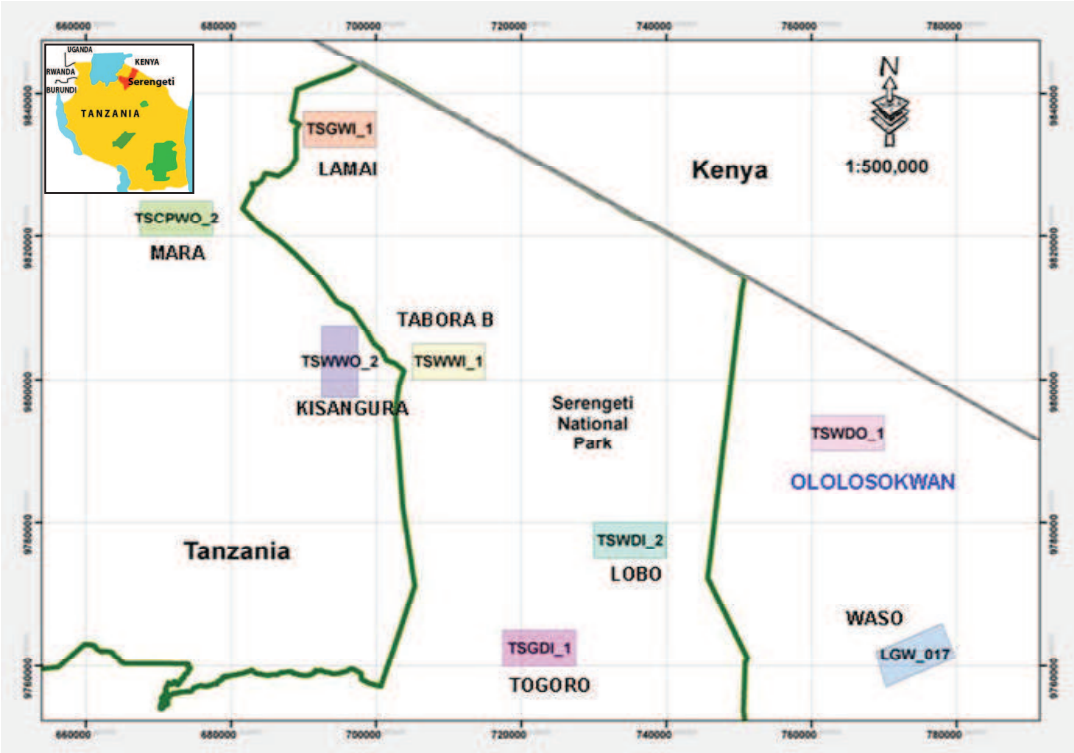


Figure 1. Map of Serengeti National Park, Tanzania showing the study sites, Ololosokwan (in blue color) in the dry side, where the species was collected.

transect. On spotting the mushroom, the GPS reading of the place was noted. Photographs were taken using a digital camera (CYBER-SHOT DSC-W7 JAPAN) and vegetation around were described by a plant taxonomist. The woody substrates lying on the ground were also identified to the generic level and wherever possible to the species level. Ecological parameters of temperature and relative humidity were also noted. Some of the fresh fruiting bodies were harvested then dehydrated using silica gel and kept in air tight plastic bags until further analysis. The type specimens examined come from Tibuhwa's collections that have been deposited in the mycological herbarium of the Uppsala University (UPS) with duplicates at the herbarium of the University of Dar es Salaam (UDSM) in Tanzania.

Macroscopic study

The collected fresh fruit bodies were examined and described in the field. The macromorphology observed in the field included ascocarp colour, size, shape, exterior surface of the fruit body, presence of stipe and how it was attached to the substrates, cup edge curliness, the fruit body fleshiness when fresh and on drying, developmental stages forms, as well as nature of growth. Other field characters such as spore print odor and taste were noted as in Tibuhwa et al. (2008).

Microscopic study

Microscopic observations were made in Ammonia-Congo red solution (TCI DEUTSCHLAND GmbH GERMANY), after a short pretreatment in 10% Ammonium solution. Observations were made at 20, 40 and 100 magnification of a bright field compound microscope Olympus (OLYMPUS BX50 PHASE POL DARKFIELD MICROSCOPE, JAPAN). Microscopic features of the Asci, Ascospore lipid bodies (guttules), mucilaginous envelop in a living spore and paraphyses pictures were taken using a digital camera (CYBER-SHOT DSC-W7 JAPAN) directly mounted on the ocular lens of the microscope, and measured straight using a graduated ocular lens in µm.

Interview for local people's knowledge

Dietary, Culinary, therapeutic, and other ethnomycological utilization of wild mushroom in the area were investigated. Information was collected by face to face interviews for over 150 individuals; detailed results will be presented in a separate study.

Comparative studies and identification of the material studied

Identification of specimens were done using published works on Ascomycetes mushrooms such as Arora (1986), Baral (2004), Don and Dennis (2002), Härkönen et al. (2003), Seaver (1928), Harrington (1996) and Miller and Miller (2006). The materials studied were as follows: Tanzania: Serengeti National Park- Ololosokwan woodland 36M 0761053 9791554: Tibuhwa 1089.2009 (UPS, holotype, isotype, DSM). Other studied material Serengeti-National Park-Loliondo 36M 0771064 9741252: Tibuhwa 1098.2010.

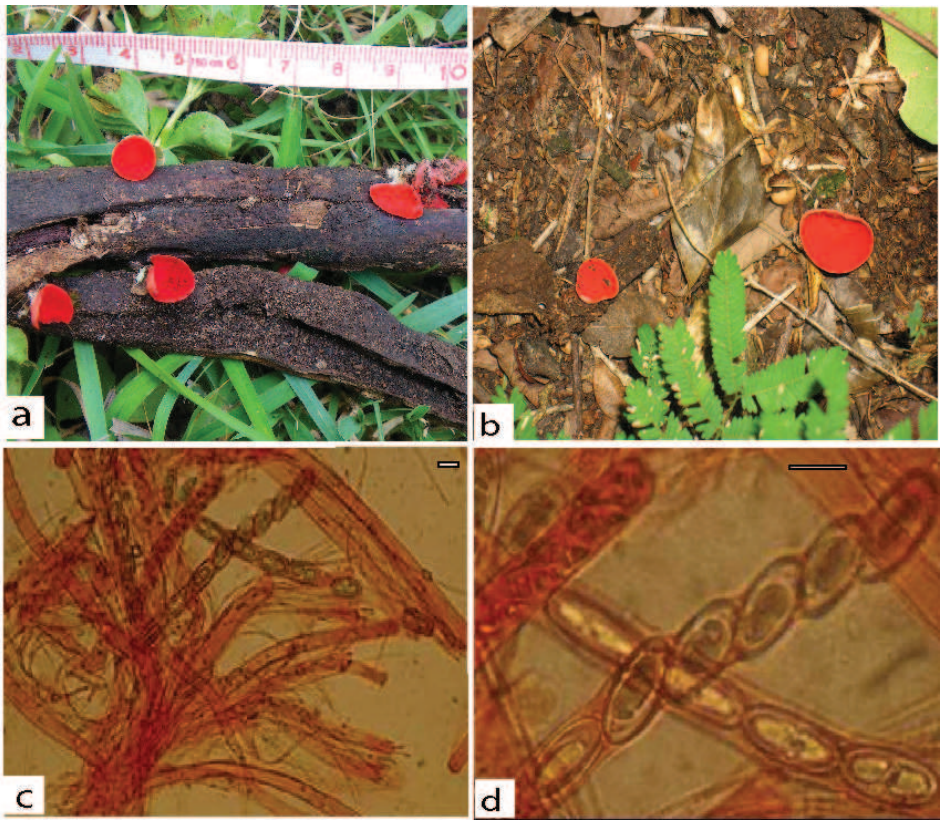


Figure 2. *Sarcoscypha ololosokwaniensis* sp. nov., a and b) General appearance in nature, c) Asci amid the paraphyses d) Ascospores. Scale bar = 11 µm.

RESULTS

Habit and habitat

Fruit bodies of *Sarcoscypha olosokwanii* were found growing singly or in tufts on dead twigs, leaves and fragments of dead wood, usually partly buried on soil and forest litter. The tree associated with them was *Olea europaea* of the family *Oleaceae* although the woodland was also dominated by *Acacia drepanolobium* and *Commiphora africana*. The *Ascomycete* was found fruiting during long rain season of April to June in undisturbed habitat. Average rainfall was 523 mm, Temperature 19 to 21 degree celicius and relative humidity of 32%.

***Sarcoscypha ololosokwaniensis* Tibuhwa, sp. nov.: MB 519507**

Ascocarp 0.5-1.8 cm, sessile or substipitate, smooth, gristly, vivid sharp red inside a saucer which bears the hymenium contrasting a soft paler pellicle which cover the exterior part of the ascocarp.

Holotypus: Tanzania: Serengeti National Park – Ololosokwan woodland 36M 0761053 9791554: Tibuhwa 1089.2009 (UPS, holotype, isotype DSM), April, 2010 on dead twigs, leaves and fragments of dead woods of *Olea europaea* sometime partly buried on soil and forest litter within the vicinity of the same tree species (Figure 2).

Etymology: The *Sarcoscypha* is named as 'ololosokwaniensis' because it was collected from woodland near the Masai village called Ololosokwan under game controlled area of the Serengeti National Park.

Fruiting bodies: 0.5 to 1.8 cm, smooth, gristly, vivid sharp red inside a saucer like which bears the hymenium, contrasting a soft paler pellicle covering exterior part of the saucer and easily removed on holding. The *ascocarp* sessile or substipitate. *Context* very thin and reddish. *Smell* undistinguished. *Ascospores* smooth, hyaline, inamyloid, 28 – 30 x 11 – 13 µm, elliptical with two big oil droplets. *Asci* 8–spored, tubular thinning toward the base, thin walled 120 – 180 x 5 – 9 µm with round end lacking an operculum. *Paraphyses* cylindrical widening toward

the apex filled irregularly with pigments and droplets.

Scientific classification

S. ololosokwaniensis Tibuhwa, sp. nov. belongs to:

Kingdom: *Fungi*;
Division: *Ascomycota*;
Subdivision: *Pezizomycotina*;
Class: *Pezizomycetes*;
Order: *Pezizales*;
Family: *Sarcoscyphaceae* in the
Genus: *Sarcoscypha*

DISCUSSION

This species has numerous striking features, both macroscopically and microscopically. Although macroscopically the species of *Sarcoscypha* look outwardly very similar, thus hardly distinguishable, the small size, sharp red colour of the inner part of the saucer ascocarp contrasting the pale-cream pellicle exteriorly instead of hairy to crenulate apothecial margin, sessile or white substipitite, and microscopically, the small unsheathed ascospore with two large lipid bodies as well as asci which are thin walled without operculum are among unifying characters of this taxa. The distribution of *Sarcoscypha* species is known from tropical Asia, United States east of the Rocky Mountains, Central America, Australia and the Caribbean. Members of *Sarcoscypha* have similar appearance with *Peziza* although these two genera are not closely related Landvik et al. (1997).

It is the first time this species in the genus is systematically described from Tanzania. However, Härkönen et al. (2003) presented similar *Sarcoscypha* from Tanzania, but they did not specify the species name probably due to uncertainties of the species identity. Similar red colored “scarlet cup fungus” species of *Sarcoscypha* have been described from different parts of the Northern hemisphere but with restricted distribution. For example, *S. coccinea* (Scop.) Lambotte, is only found in the New World, Central America and the Caribbean, east and Midwest North America, but not in the far west while *Sarcoscypha austriaca* (O. Beck ex Sacc.) Boud and *Sarcoscypha dudleyi* (Peck) Baral are found in eastern regions of the continent (Denison, 1972).

S. olosokwaan sp.nov. is distinguished from other related ‘Scarlet Cup Fungi’, from north American, *Sarcoscypha coccinea* and *Sarcoscypha occidentalis* (Schwein.) Sacc.; from Hawaii *Sarcoscypha mesocyatha* F.A Harr. and other two *Sarcoscypha austriaca* and *S. dudleyi* in geographical distribution, fruiting season, and macro-micromorphology characters of the fruit body.

While *S. occidentalis* has a vivid differentiated long white stipe (1 to 3 cm), *S. ololosokwaniensis* has none or substipitate. The relatively small size, saucer rather than deep cup and pale pellicle instead of exterior hair, microscopically small size of ascospore, asci, paraphyses and two large lipid bodies also distinguish this species from the closely related *S. coccinea* with numerous small lipid bodies among others. The Hawaiian *S. mesocyatha* differ by having relative large (4.5 cm), shallowly cupulate to flattened discoid ascocarp (Don and Dennis, 2002), while *S. austriaca* and *S. dudleyi* are demarcated by relatively long spores with flattened ends, without full sheath but with small polar caps on either end and typical rounded ascospore in a full sheath respectively (Harrington, 1990). Given the differences in macro-micromorphology, geographic distribution, seasons of fructification, and host differences, this study propose *S. ololosokwaniensis* as a new species (see the key below).

Key to six species of closely similar *Sarcoscypha* species

1. Cup typically < 2 cm across; ……..……..2
1. Cup typically > 2 cm across ……..……..3

2. Short stem present; spores < 21 µ long; numerous lipid bodies; found east of the Rocky Mountains, North America……*S. occidentalis*
2. Short stem absent or present; spores > 21 µ long; two large lipid bodies; found in tropical Africa – Tanzania …….
S. ololosokwaniensis

3. Found in the Pacific Northwest and California; spores usually unsheathed and lacking "polar caps." ……..……*S. coccinea*
3. Found elsewhere; ascospores with or without sheath ……..……..…… 4

4. Found in Hawaii; shallowly cupulate to flattened discoid ascocarp, ascospore unsheathed ……..
S. mesocyatha
4. Not found in Hawaii; deep cupulate ……..…… 5

5. Spores with slightly flattened ends, without full sheath but with a sheath-like covering at each end called "polar caps"……*S. austriaca*
5. Spores with typical rounded (elliptical) ends; encased by a full sheath….....

S. dudleyi.
S. olosokwaan sp. nov. has no cardinal role apart from contributing to the forest ecosystem by degrading complex wood cellulose and lignin since it is a saprophyte. Its small size, insubstantial fruiting, tough texture would daunt most to collect them for food. Nevertheless, the information for the species edibility was also impaired by the fact that the Masai people who live around Ololosokwan village, by their culture never eat mushroom at all.

Conclusions

The new species described in the present study named *S. ololosokwaniensis* sp.nov. is well distinguishable from all known species in this genus macro-micromophologically. With the description of this new species it is obvious that "Vital taxonomy" remains a strong tool in delimiting *Sarcoscypha* species using living material. *Sarcoscypha* species are well known for several applications including culinary use, and ability to produce interesting enzymes with potential uses in biotechnological processes like bioremediation, biodegradation biopulping and detoxification of recalcitrant substances since they have some bioactive compounds. This study thus, recommends a thorough investigation on the possible bioactive compound found in this new described *Sarcoscypha* species for biotechnological applications.

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