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Original article **Biodiversity**

Habitat and diversity of ectomycorrhizal fungi in forests of South Cameroon

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Abstract
Information is lacking on habitat and diversity of ectomycorrhizal (ECM) fungi of African humid forests. For three years, mushroom excursions were carried out in four sites with contrasted soil and altitude characteristics of South Cameroon, during wet seasons. Collected fungi were described in fresh state and dried exsiccates examined for microscopic description before morpho-anatomical identification. ECM fungi abundantly fruited exclusively in mixed caesalp, monodominant *Gilbertiodendron* and *Uapaca* forest clumps, independently of elevation, rainfall, topography and soil texture. 21 ECM tree species in 11 genera belonging to two families, Ceasalpiniaceae and Phyllanthaceae, dominated ECM forest clumps. More than 100 putative ECM fungal species in 27 genera were identified and assigned to seven families and one super family group. Members of Russulaceae and Amanitaceae recruited the highest number of ECM taxa, followed by species of Boletoids and Cantharellaceae. *Paxillus, Clavulina*, *Coltricia*, *Scleroderma*, *Cortinarius* and *Inocybe* genera contained limited species. 12 ECM fungal species were locally edible, including *Lactarius gymnocarpus* and all chanterelles. Though, species richness was very large in a mountainous area, all four sites shared a substantial large number of ECM mushroom species. Low plant diversity but high fungal richness recommends ECM forest clumps for biodiversity sanctuaries.

Key words. Ceasalpiniaceae – Clumps – Ectomycorrhiza – Fungi –*Gilbertiodendron* – *Uapaca*

INTRODUCTION

Little information exists on the habitat and diversity of ectomycorrhizal (ECM) fungi of African humid forests compared with other biomes. ECM fungi are important below-ground components of rain forests. Where they occurred, they form obligatory symbioses with specific plant taxa. Both symbionts are threatened by various scales of deforestation. Lack of data on the distribution of habitat and diversity of ECM tree and fungal species is likely to jeopardize the regeneration of African ECM forests.

Until recently, it has been thought that ectomycorrhizal fungi were uncommon in tropical humid forests [1]. However, recent mycoinventories revealed that native ECM fungal genera in tropical Africa belong, to a large extent, to the same Basidiomycete taxa as those that provide the fungal partners of temperate ECM plants [2, 3, 4]. Common ECM taxa in the tropics belong to the Amanitaceae, Boletaceae, Cantharellaceae, Russulaceae and Sclerodermataceae [5, 6, 7, 8, 9]. However, on species level, hardly any ECM fungal species (except when introduced) is shared between tropical and temperate regions [2, 4]. Moreover, many ECM fungal species are poorly described or have their microscopic details poorly illustrated: a reliable key to all African ECM fungal species is lacking and many species remained unnamed. Various species of Amanitaceae, boletes, Cantharellaceae and Russulaceae have been described from West African rain forests [10], with virtually no members of the Cortinariaceae [1]. Mycoinventories in forests of Korup National Park, South-West, Cameroon yielded close to 40 species of suspected mycorrhizal fungi [10]. In forests of South-West Burkina Faso, 27 ECM fungal species were described beneath indigenous *Afzelia* and *Uapaca* trees [11]. Using a fragment of the mitochondrial large subunit rRNA gene, more than 100 ECM sporocarps were typed from Ceasalpiniaceae in a Guinean tropical rain forest [4]. It was also concluded that in the same Guinean forest, multi-host ECM fungi predominate and are shared between canopy trees and

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seedlings, thereby suggesting the formation of common mycorrhizal networks between trees [12] as found in the rain forest of south Cameroon [13]. Hitherto, the ecology of ECM fungi of African rain forests remains poorly known, with only a small fraction of many thousands ECM formally described. The aim of this study was to provide new records on the habitat and diversity of ECM fungi in forests of South Cameroon.

MATERIALS AND METHODS

Location, climate and soils

The study was carried out in portions of humid forests of South Cameroon (Fig. 1), located between Bipindi, Lolodorf and Akom II (2°47' – 3°14'N and 10°24' – 10°51'E), approximately 80 km east of the coastal town of Kribi (2°56'N; 9°54'E) and 40 km west of Ebolowa (2°51'N; 11°11'E). The landscape varied considerably. Altitude ranged from 40 m a.s.l. in the northwestern lowland, to more than 1000 m a.s.l in the mountainous southwest region (Fig. 1). The original vegetation consisted of dense humid evergreen forest [14]. The climate was hot and humid, with two wet seasons from mid-March to mid-June and mid-August to mid-November. Mean annual rainfall ranged from 1500 to 2500 mm [15]. Mean monthly temperatures varied from 23° to 25°C in Ebolowa (638 m a.s.l) and 25° to 27.5°C in Kribi (10 m a.s.l.). Relative humidity was generally above 80 % year-round [16]. Geologically, the area consisted of Precambrian metamorphic rocks and old volcanic intrusions [17]. Soil characteristics changed considerably with elevation (Table 1).

Within the study area, four sites were selected in Ebimimbang (low elevation; sandy and near neutral soils), Ebom (mid-elevation; high clayey and acid soils), Nyangong and Bityili (high elevation; very high clayey and strongly acid soils). In each site, one 1-ha permanent sampling plot was selected in near primary forest. All tree species at all growth stages were enumerated and diameter at breast height measured. As trekking towards these plots, observations were also made in other ecosystems including small forest clumps, secondary forests, cocoa (*Theobroma cacao*) plantations, and food crop fields, bush fallow of *Chromoleana odorata*, farmers' trails and homegardens.

Mushroom collection, description and identification

Mushroom excursions were undertaken during the wet seasons, one week per month, with the help of local assistants. Collected specimens were macroscopically described in fresh state before twilight. After description, mushrooms were dried for 2 – 3 days at about 40°C using a locally made drier. Dried mushrooms were temporally preserved in a local herbarium in Kribi before being sent to Wageningen, the Netherlands for microscopic examination and identification.

Several provisional keys were elaborated based on morphological and anatomical characters of sporocarps [18]. Voucher specimens were kept at the National herbarium of the Netherlands (Leiden Branch) and some duplicates were preserved at the agricultural research station in Kribi, Cameroon.

Several manuals were used for the identification of most species such as "La Flore Iconographique des Champignons du Congo", "La Flore illustrée des Champignons d'Afrique Centrale" (Illustrated manual edited by the Belgian National botanical garden), the genus *Lactarius* [19, 20, 21, 22], *Russula* by Buyck B [23, 24, 25] and the genus Amanita [26, 27, 28, 29]. When some taxa were new, they were attributed a new name. Their description will be provided in subsequent publications.

Table 1: Localization, elevation, rainfall and soil physico-chemical characteristics of the four research sites

Research sites	Ebimimbang	Ebom	Nyangong	Bityili
Localization	3°02.67'N; 10°28.25'E	3°04.73'N; 10°41.24'E	2°58.11'N; 10°45.18'E	2°56.07'N; 10°49.55'E
Altitude (m.a.s.l)	0 – 350	350 – 500	500 – 800	>800
Rainfall (mm)	1556	1987	1677	1800
Soil texture	Sandy	Highly clayey	Highly clayey	Very highly clayey
pH	5 – 6	4 – 5	3 – 4	3 - 4
Carbon (%)	1.69	2.26	2.21	5.7
Nitrogen (%)	0.15	0.18	0.19	0.36
Phosphorus(µm/ml soil)	0.01	0.005	0.002	0.000

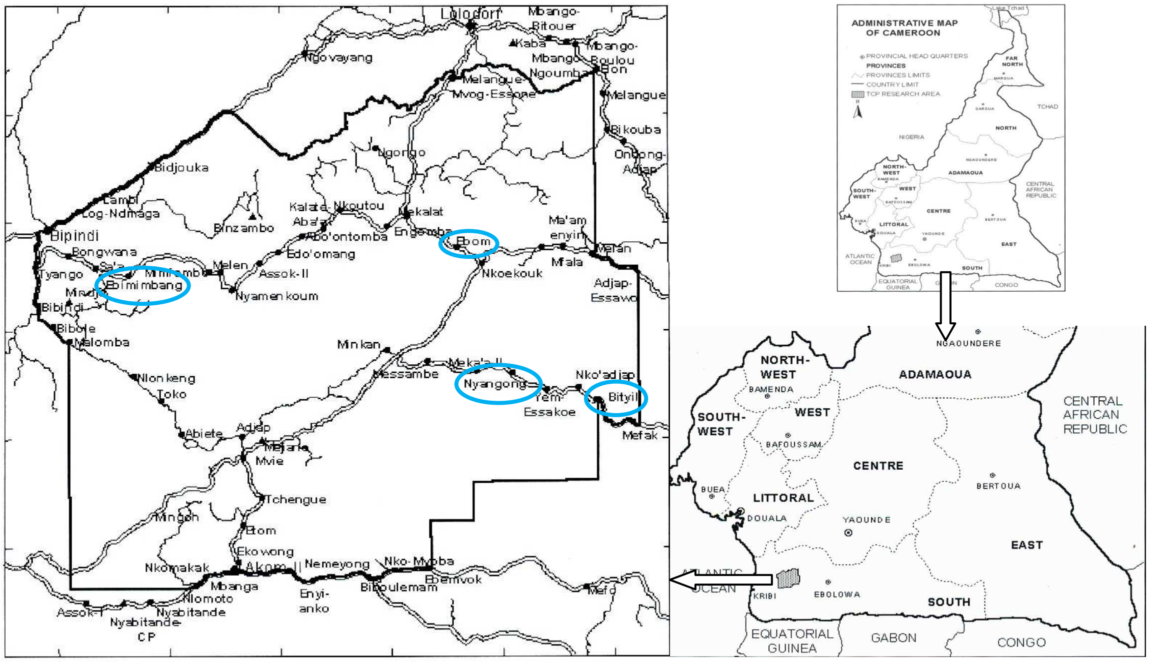

Figure 1: Localization of the villages in south Cameroon where fungal samples were collected for three years

RESULTS

All species of putative ectomycorrhizal mushrooms were found mainly in three types of forests: mixed oligodominant ceasalp, monodominant *Gilbertiodendron* and *Uapaca* forest clumps. ECM forest clumps varying in physiognomy and aspect, recruited at least 10 to more than 100 ECM tree specimens of the same genus or species, simultaneously occurring on a single spot, at different growth stages. ECM forest clumps had low cover values of shrub species and herb without moss. They slightly varied in canopy closure. In general, ECM forest clumps formed closed-canopy dominated by ceasalps and/or *Uapaca* trees. But the commonest ECM tree species differed with site: mixed ceasalp species in Ebimimbang, *Gilbertiodendron* species in Ebom, *Uapaca* species in Nyangong and a mixture of all ECM tree species in Bityili.

Twenty-one ECM tree species belonging to 11 genera and two families, Ceasalpiniaceae and Phyllanthaceae, were recorded. Most caesalp species in tribe Amherstieae of the Ceasalpiniaceae were collectively called “Ekop” by forest prospectors and local populations (Table 2).

Elevation, aspect and landform of ECM forest clumps largely varied with site. Soil humidity was continuously wet during both wet and dry seasons in all sites but Ebimimbang where sandy soils rapidly dried up during the long dry season (mid-November to mid-march). Soil nutrient regimes differed in a relatively narrow range but fell almost exclusively within the range of poor soils (Table 1). Floors of ECM forest clumps were thin, with low litter and little organic matter accumulation at all sites. The most common natural disturbance was wind fall, to a very low extent, though. Ectomycorrhizal habitats were unevenly distributed at the four sites. They were abundant in high (Bityili) and low elevation (Ebimimbang), intermediate in Nyangong and lowest in Ebom. Monodominant clumps of *Gilbertiodendron* tree species were observed only at the Ebom site, in swampy areas, close to small and large rivers, where they stood long periods of inundation.

Table 2: The ectomycorrhizal species ranged by type of forest habitat

Habitat types	Family	Species	Pilot name
		Anthonotha fragans	Enak
		A.macrophylla	Enak
		Berlinia bracteosa	Ebiara
		Berlinia confusa	Ebiara
		Brachystegia cynometrioides	Ekop naga
		Brachystegia euricoma	Ekop evene
		Brachystegia zenkeri	Ekop gombe
Mixed ceasalp	Caesalpiniaceae	*Didelotia africana*	Ekop rouge
clumps	(Tribe Amherstieae)	*Didelotia letouzeyi*	Ekop
		Julbernadia seretii	Ekop blanc
		Monopethalanthus letestui	Ekop mayo
		Monopethalanthus microphyllus	Ekop mayo ngang
		Paraberlinia bifoliolata	
		Tetraberlinia bifoliolata	Ekop beli
		Touabouate brevipaniculata	Ekop ribi
			Ekop zing
		Gilbertiodendron brachystegioides	Abem
Gilbertiodendron clumps	Caesalpiniaceae	*Gilbertiodendron dewevrei*	Abem
		Uapaca acuminata	Rikio
Uapaca clumps	Phyllanthaceae	*Uapaca buchholizianum*	Rikio
		Uapaca guineensis	Rikio
		Uapaca vanhoutei	Rikio

Notes: Pilot names of "Ekop" were taken in Letouzey and Mouranche (1952).

Most ectomycorrhizal habitats were of small to medium size, with one dominating ECM tree species or one genus. But on the Bityili hill, ECM tree species depicted a distinctive distribution pattern: the bottom abrupt hill was mostly overgrown by *Uapaca* tree species, followed in the middle by *Gilbertiodendron* trees and a mixture of caesalp tree species towards hill top.

Abundance of ectomycorrhizal tree species in 1-ha plot varied in the range of 20 to 80 % with site. It was lowest in the youngest stands of Ebom (19% basal area) and the highest in a nearly pristine rain forest of Bityili (79% basal area). However, it reached almost 100% in monodominant *Gilbertiodendron* clumps. ECM habitats occurred throughout the landscape: along roadsides, on hills, on flat terrain, in swamps, in riverine spots, far in the forests or close to villages, even within agricultural farm holdings. They were not cleared for shifting cultivation, though. Rather, local people claimed that soils under ECM forest patches were very poor and used them only as a collecting ground for ECM mushrooming during wet periods.

In the study area, more than 1000 presumed ectomycorrhizal sporocarps were sampled during three years of mushroom excursions, exclusively in ECM forest clumps during both wet seasons. ECM mushrooms were also observed during the dry seasons, to a much lower extent, though. In the three ECM forest types, ECM fungal taxa were very diverse. More than 100 putative ECM fungal species in 27 genera were identified and assigned to seven families: Amanitaceae, Cantharellaceae, Clavulinaceae, Coltriciaceae, Cortinariaceae, Russulaceae, Sclerodemataceae and one super family group, Boletoids (Table 3).

Among ectomycorrhizal fungal species identified, *Amanita* and *Russula* species were dominant, with 34 and 32 species, respectively, followed by 28 species in 15 genera of the Boletoids, 15 species in three genera of the Cantharellaceae and 12 *Lactarius* species. Both *Russula* and *Lactarius* recruited the highest number of ECM taxa. *Paxillus (*two species), *Clavulina vanderstii*, *Coltricia* (two species), *Scleroderma* (two species), *Cortinarius* (three species) and *Inocybe* (six species) genera contained only limited number of species (Table 3).

Table 3: List of putative ectomycorrhizal mushroom species by family, genus and species in humid forests of South Cameroon

Amanitaceae	**Boletaceae**	**Russulaceae**
Amanita afzeliae nov.	*Afroboletus luteolus* (Heinem) P. & Y.	*Russula afronigricans* Buyck
A. albidodisca nov.	*Boletus boletiformis* nov.	*R. albospissa* Buyck
A.albopulverulenta nov.	*B. macrocystis* nov.	*R. annulata* Heim
A. afrobispora nov.	*B. nyangongensis* nov.	*R. annulatobadia* Beeli
A. afroflavescens nov.	*B. pustulatus* Beeli	*R. apsila* Buyck
A. afrorubescens nov.	*B. suspinulosus* nov.	*R. intrica* Lizoii
A. afrovaginata nov.	*Boletellus sulcatipes* Hein.& Goo.	*R. areolata* Buyck
A.annulatovaginata Beeli	*Chalciporus clypeatus* nov.	*R. aurantiofloccosa* Buyck
A. aureofloccosa Bas	*Gyrodon aberrans* nov.	*R. camerunensis* nov.
A. bingensis Beeli	*Gyroporus microsporus* (Sing.& Grinl.)	*R. cellulata* Buyck
A. brachystegiae nov.	Heinem & Rammeloo	*R. chrysotricha* nov.
A. calopus Beeli	*Leccinum excedens* nov.	*R. declinata* Buyck
A. crassiconus Bas	*Paxillus brunneotomentosus* nov.	*R. diffusa var.fissurans*
A. elegans Beeli	*Paxillus camerunensis* nov.	*R. discopus* Heim
A. flavovirens nov.	*Phlebopus braunii* (Bres.) Heinem	*R. echnosperma* R.Heim & Gilles
A.fulvosquamulosa Beeli	*P. silvaticus* Heinem	*R. fulvoochrascens* Buyck
A. gigavolvata nov.	*Phlebopus sp.* nov	*R. heliochroma* Heim
A. griseofarinosa Hongo	*Phylloporus depressus* Heinem	*R. intricate* Buyck
A. lanosa Beeli	*Pulveroboletus aberrans* Heinem	*R. kivuensis* Buyck
A.leucoagaricioides nov.	*P. viridis* Heinem	*R. lamprocystidia* (Nakasone)
A. luteoflava Beeli	*Rubinoboletus luteopurpureus* (Beeli)	*R.liberiensis* Buyck
A.monopetalanthi nov.	*Strobilomyces echinatus* Beeli	*R.macrocystis* nov.
A. nigropyramis nov.	*S. luteolus* Heinem	*R. mimetic* nov.
A.pseudoafroalba nov.	*S. strobilaceus* (Berk.)	*R. pausiaca* Buyck
A. pseudolanosa nov.	*S. velutipes* Corner	*R. pseudocarmesina* Buyck
A. pustulata nov.	*Tubosaeta alveolata* Heinem	*R. pseudopurpurea* Buyck
A. roseocinnamomea nov.	*T. brunneosetosa* (Singer) E. Horak	*R.speudostriatoviridis* Buyck
A. rubescens (Pers.:Fr) G	*T. goosseniae* nov.	*R. striatoviridis* Buyck
A.strobilaceovolvata Beeli	*Tylopus violaceus* nov.	*R. testaceoaurantiaca* Beeli
A. subviscosa Beeli	**Clavulinaceae**	*R.velutina* (DC per Pers.:Fr)P
A. sulphurea nov.	*Clavulina vanderstii* nov.	*R. yaeneroensis* Buyck
A. virella Beeli	**Sclerodermataceae**	
	Scleroderma sinnamariense Mont	
	S. roseacarneum	
	Coltriciaceae	
	Coltricia spathulata (Hooker) Murill	
	C. pyrophila (Wakef) Ryvarden	
Inocybe	**Cantharellaceae**	***Lactarius***
Inocybe afronodulosa nov.	*Gomphus brunneus* (Heinem)	*Lactarius acutus* Heim
I. afrostellata nov.	*G. clavatus* (Pers.) Gray	*L. annalatoangustifolius* nov.
I. korupensis nov.	*Craterullus crispus* Fr.	*L. claricolor* nov.
I. bipindensis nov.	*C. cornucopioides* Persoon	*L. denigricans* Ver.& Kar.
I. perpusilla Velen	*Cantharellus camerunensis* nov.	*L. densifolius* Ver.& Kar.
I. zingii nov.	*C. cibarius var.roseocanus* Arora &	*L. gymnocarpus* Heim
	Dunham	*L. kivuensis* deWitte
Cortinarius	*C. congolensis* Beeli	*L. medusa* Verbeken
Cortinarius afroconicus nov.	*C. dichrous* (Fr.) Bres	*L. pumilus* Verbeken
C. diobensis nov.	*C.densifolius* Heinem	*L. pulcrispermus* nov.
C. ionopygmaeus nov.	*C. isabellinus* Heinem	*L. sesemotani* Beeli

	C. luteopunctatus (Beeli) Heinem *C. miniatescens* Heinem *C. microcibarius* Heinem *C. pseudocibarius* Hennings *C. rufopunctatus* (Beeli) Heinem	*L. undulates* Verbeken

All four sites shared a substantial number of similar ECM fungal species. But, species richness was the highest at the Bityili Mountain.

Twelve species of ECM fungi were edible by native populations, including *Lactarius gymnocarpus*, locally called 'Nyumelane" and most *Cantherellus* species but *Craterellus cornucopioides*. Several folk names of Bantu and Bagyeli pygmy ethnic groups were used for chanterelles, notably, "Bingõbiñdoñg, Mebeum, Nyavem, Ötoye, Otyetya" and "Ambini, Ampembo, Nyanbembo", respectively.

DISCUSSION

In the Bipindi-Lolodorf-Akom II area, cameroonian ectomycorrhizal mushrooms abundantly fruited exclusively in three types of habitats, viz. mixed oligodominant caesalp, monodominant *Gilbertiodendron* and *Uapaca* forest clumps. Tree-forming ECM clumps belonged only to two families, Ceasalpiniaceae and Phyllanthaceae. Ten genera were ceasalp tree-forming monodominant clumps of *Gilbertiodendron* species or mixed caesalp oligo-dominant stands. *Uapaca* tree species chiefly formed small monodominant stands on riversides. Hence, the presence of such tree species and stands should be made known to all forest stakeholders for their ecological specificity and relevance in the management of African humid forests.

Similar to other sites in the Congo basin, clumping of ceasalp and *Uapaca* tree species with associated ECM fungi is commonplace in humid forests of Cameroon. In Korup National Park (SW Cameroon), ECM fungi were recorded in large groves dominated by *Microberlinia* and *Tetraberlinia* species, on sandy soils with two distinct seasons [30, 31]. In the Dja Faunal World Heritage, in South-East, Cameroun, large forest patches dominated by *Gilbertiodendron dewevrei* developed on swampy areas (14; N.A. Onguene and A. M. Bâ, pers.obs.). In our research area in South Cameroon, the distribution of small and medium-size ECM forest clumps was independent of elevation, rainfall, topography and soil texture: they occurred on both sandy and clayey soils. Our results do not agree with earlier observations of edaphic specialization of ECM trees owing to the competitive ability of ECM fungi on poor soils [30, 32, 33]. The question remains on how ECM clumpiness originated in tropical humid forests and their locally uneven distribution. It was assumed that the gregarious habit of ECM trees could be due to limited seed dispersal of both symbiotic partners [34]. Since both ECM tree and ECM fungus do no possess the capacity to reproduce independently of the other symbiotic partner, establishment of the ECM symbiosis might be a rare chance event [42], which then serve as focal points for subsequent establishment of successive ECM trees, confirming the theory of regeneration niche to explain co-dominance of ECM trees and fungi.

If this hypothesis holds, then it implies that ectomycorrhizal recovery after large scale disturbance (be it natural or manmade) could be very low, requiring special management of native ECM stands of African humid forests. Consequently, ECM forest clumps need a special conservation statute; so far, little information exists on the ecological factors that maintain the current functioning of ECM forest clumps and in general of tropical rain forests.

Ectomycorrhizal forest habitats were the only sites where more than a hundred ECM mushrooms species in 27 genera and eight families were collected and identified. It is the first time in Africa that such a highly diverse and abundant putative ECM mushroom collection is reported from the same area. In the Pakaraima Mountains of Guyana, 75 morpho-species of putatively ECM fungi were identified from discrete groves formed by *Dycimbe* and *Aldina* species [35]. In the Miombo savanna and humid forest of West Africa, only five families of ECM mushrooms were recorded [4, 11, 24]. The same high number of members of the Russulaceae (44 species) from this study supports existing data [1]. Species-rich genera included *Amanita*, *Russula*, members of Boletales and Cantheralles. Less frequent ECM fungal species, *Clavulina*, *Coltricia* and *Scleroderma* were also collected for the first time as well as rare ECM mushroom species, viz. two, three and six species of *Paxillus*, *Cortinarius* and

Inocybe, respectively (Table 3), confirming earlier observations elsewhere in West Africa, the Neotropics and the Laojun Mountain region, southwestern China [4, 35, 37]. Lack of frequent inventories and experienced mycologists in Africa could explain the poor accounts for tropical ECM mushroom diversity and their habitat. Only 546 records of wild macrofungi have been made from South Saharan countries [2]. In Benin, one species of *Craterellus*, *Hebeloma*, *Inocybe*, of *Russula* and eight *Lactarius* species were recorded [6]. Five out of six macrofungi in Burkina Faso were identified as *Amanita hemibapha*, *Cantharellus pseudofriessi*, *Lactarius gymnocarpus*, *Phaeogyroporous sudanicus* and *Tubosaeta brunneosetosa* [11, 37] as well as an unidentified Calvatia [38]. No less than 40 ECM fungi have been recorded from the republic of Congo including 15 *Cantharellus* species and dozens of *Russula* and *Lactarius*. The highest number of *Lactarius* species (19 species) has been thus recorded in Tanzania. Other African countries are less prolific in ECM fungi [2].

Ectomycorrhizal forest communities could contribute not only to the preservation of forest refuges of South Cameroon with endemic species, but also to the protection of the biodiversity hot spots of Africa's rain forests. Maintenance of such ECM forest communities with their associated edible mushrooms such as chanterelles also helps to preserve valuable source of alternative protein-rich diet for local communities who depend on non-timber forest products [39, 40], in addition to creating new ecological jobs of mushroom collectors. Thus, ECM forest clumps should be protected as biodiversity sanctuaries, owing to lack of knowledge on their regeneration requirements.

Thirteen ectomycorrhizal genera are worldwide edible including, *Amanita, Boletus, Cantharellus, Cortinarius, Laccaria, Lactarius, Leccinum, Morchella, Russula, Suillus, Terfezia, Tricholoma, Tuber* [2]. In Africa, more than 20 edible *Cantharellus* species were described in dozen countries [2, 5] with various tastes, though [37]. 1998). In our research sites, 12 *Cantharellus* species were edible by Bantou and Bagyeli people but *Craterellus cornucopioides*. Local tribes often seek them avidly, had a very high esteem and gave them no less than eight folk names). Yet, chanterelles can not be cultivated but grow vividly only in the vicinity of ECM tree species in ECM forest clumps. Consequently, growing trees that "produce" chanterelles could serve as a way forward for implicating local populations in sustainable management of remnants of African rain forests. Rejuvenation of degraded forests could be done by mycorrhizal inoculation of tree seedlings with chanterelles.

Cameroonian ectomycorrhizal fungi abundantly fruited exclusively in three types of habitats: mixed oligodominant caesalp, monodominant *Gilbertiodendron* and *Uapaca* forest clumps, independently of elevation, rainfall, topography and soil texture. Though poor in plant diversity, these habitats recruited abundant and various ECM fungal species on which native ECM tree species depend for survival and some others such as chanterelles and *Lactarius gymnocarpus* serve as a ready source of proteins for local people during harsh periods. Therefore, forest clumps should be conserved as biodiversity sanctuaries and the presence of ECM fungi could serve as indicators for sustainable management of African humid forests.

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