The frequency and relationship of flowering plants on the distribution pattern of *Ophiocordyceps sinensis* (Yarchagunbu) in the highlands of Dolpa district, Nepal

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Ophiocordyceps sinensis (Berk.) G.H. Sung, J.M. Sung, Hywell-Jones & Spatafora is a highly valuable medicinal fungus. Biologically it is an entomopathogenic, entomophagous or entomophilous fungus. In order to investigate different floral associations with *O. sinensis*, and to know different threats to pasture biodiversity, research was conducted in three pastures of Raha and Majphal Village Development Committees of Dolpa district. The study revealed that *Juncus thomsonii* and *Bistorta macrophylla* were the principal plant associates with *O. sinensis* as they dominated the alpine pasture vegetation. This research also highlighted the need for some proactive solutions along with conservation awareness program as important management initiatives to ensure ecological balance of *O. sinensis.*

Key words: Alpine zone, flora, *Ophiocordyceps sinensis*, pasture management, Yarsagumba

Ophiocordyceps sinensis is a genus of entomophagous fungi (Pyrenomycetes, Ascomycotina) in the family Ophiocordycipitaceae. This parasitic fungus is variously known as Yarsagumba, Yarchagunbu, Kira, Jeevanbuti, Chyau, Chyau kira and Jara in Nepali, Yartsa gunbu in Tibetan, Dong chong xia cao in Chinese, Caterpillar fungus in English and *Cordyceps* in botanical term (Devkota, 2006, 2008a). Sherpas call them walking herb (Adhikari, 2000). There are about 300-400 species of *Cordyceps* distributed all over the world (Kobayasi, 1982; Sung, 1996). About 68 species have been reported from China and 33 species have been recognized in the Tibetan Plateau and Himalayan region (Zang & Kinjo, 1998). Kobayasi & Shimizu (1960, 1963) have worked on monographic study on *Cordyceps* and its allied species. From Nepal, 21 species of Cordycepioid fungi (*Cordyceps* and its allied species) have been reported (Adhikari, 2008b).

Zang & Kinjo (1998) have described distinct, closely related species (*Cordyceps gansuensis* K. Zhang, C. Wang & M. Yan, *C. kangdingensis* M. Zang & Kinjo, and *C. nepalensis* M. Zang & Kinjo) that in the past had been mistaken for *C. sinensis*. On the basis of molecular phylogenetic analysis Sung *et al.*, (2007) made taxonomic revision of Clavicipataceous fungal group. According to them, the taxa fall in 3 monophyletic (clades) family. The family Clavicipataceae (Lindeu) Earle ex Rogerson (Clade A) includes *Metacordyceps* Sung, Sung, Hywell-Jones & Spatafora; Ophiocordycepitaceae Sung, Sung, Hywell-Jones & Spatafora (Clade B) includes *Elaphocordyceps* Sung & Spatafora and Ophiocordyceps Petch. and Cordycipitaceae Kreisel ex Sung, Sung, Hywell-Jones & Spatafora (Clade C) includes *Cordyceps* Fr.

Ophiocordyceps sinensis fungus is endemic to the Tibetan Plateau including the adjoining high altitude areas of the Central and Eastern Himalayas (Nepal, Bhutan and the Indian states of Uttaranchal, Sikkim, Himanchal Pradesh and Arunachal Pradesh). The significance of the contribution of wild this edible fungus to rural livelihoods is acknowledged, but remains largely unexplored (Christensen *et al.,* 2008). *O. sinensis*, famous as the gold rush of Nepal, has its niche in the alpine meadows/pastures between the altitudinal range of 3540 and 5050m (Devkota, 2008a, 2008b). Its distribution is limited to areas where precipitation is below 300mm per annum (Winkler, 2008).

Ophiocordyceps sinensis is found mostly in Dolpa, Darchula, Jumla, Bajura, Kalikot, Mugu, Humla, Rukum, Bajhang, Manang, Mustang, Gorkha, Lamjung, Dhading, Rasuwa, Dolakha, Sindhupalchowk, Solukhumbu, Sankhuwasabha, and Taplejung districts of Nepal (Adhikari, 2008a,

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Devkota, 2008a). Relative to Mustang district, Manang is far richer in the occurrence and distribution of *O. sinensis* within the Annapurna Conservation Area (Sherchan *et al.,* 2005). Some of the other eastern Himalayan districts of Nepal stricken with acute poverty may also harbour the potential for commercial harvesting of *O. sinensis*; however, detailed exploration of the availability of this species is still warranted.

In the Tibetan plateau, the grasslands providing habitat for *Hepialus* moths and thus for *Ophiocordyceps sinensis* are associated with *Kobresia* sedges. The caterpillar of the moth lives in underground tunnels, emerging out at night to feed upon plant roots (Winkler, 2008). Among the sedges and grasses, *Kobresia setchwanensis*, *Poa elanata*, *Festuca rubra* are common. While among forbs, *Potentilla anserina*, *Anemone rivularis*, *Primula sikkimensis*, *Aconitum rockii*, *Gentiana veitchiorum*, *Polygonum viviparum*, *Rheum alexandrae, Nardostachys chinensis, Pedicularis* spp., *Anaphalis flavescens*, *Meconopsis horridula* are common (Wu, 1997; Zang & Kinjo, 1998). Larvae of *Hepialus* also prefer to feed on young roots of plant species of the families of Polygonaceae, Fabaceae, Cyperaceae, and Poaceae (Chen *et al*., 2000).

Although few studies on the wild status of some medicinal plants have been carried out, several others, particularly at the high altitude species, are yet to be evaluated (Shrestha & Joshi, 1996; IUCN, 2000; Lama *et al*., 2001). In this paper, floral associations of *Ophiocordyceps sinensis*, threats to *O. sinensis* in three different pastures and the impacts on the occurrence of this Himalayan treasure due to over grazing in Dolpa, Western Nepal have been analyzed. This research will be useful to know and manage the floral associations of *O. sinensis* in the high altitude areas of Dolpa.

Materials and Methods

Study Area

The study was confined to two Village Development Committees (VDCs), viz. Raha and Majphal of Dolpa district. The district is located between 28° 24'-29° 43' N Latitude to 82° 24'-83° 38' E Longitude. Shey-Phoksundo National Park, the largest national park of Nepal (core park area of 3555 km^2) covers a large part of this district. The first site is the village of Raha located in the buffer zone of Shey Phoksundo National Park (SPNP) and its associated pastures. Some principal *Ophiocordyceps sinensis* collection sites in this village are Palma Ramana, Patauti, Duna, Matey, Sunsey, and Gyalbara. Among these, Palma Ramana was selected for the study. It is located at an altitude of 4260 to 4810m. The main *O. sinensis* sites in Majphal VDC are Ruppatan, Majghari, Saiquarry, Pokeypani and Chinarangshi. Among them, Pokeypani and Saiquarry were selected for this study (Fig. 1).

Methods

Based on Participatory Research Appraisal (PRA) techniques and drawings of participatory resource maps, 80 permanent monitoring plots of size 10x10m were established at different pastures of Raha and Majphal VDCs. The field study was conducted from May 25 to July 15, 2006. A stratified random sampling technique was adopted for inventory of the product. The sample plots were laid at 100m distances along transects. Each individual

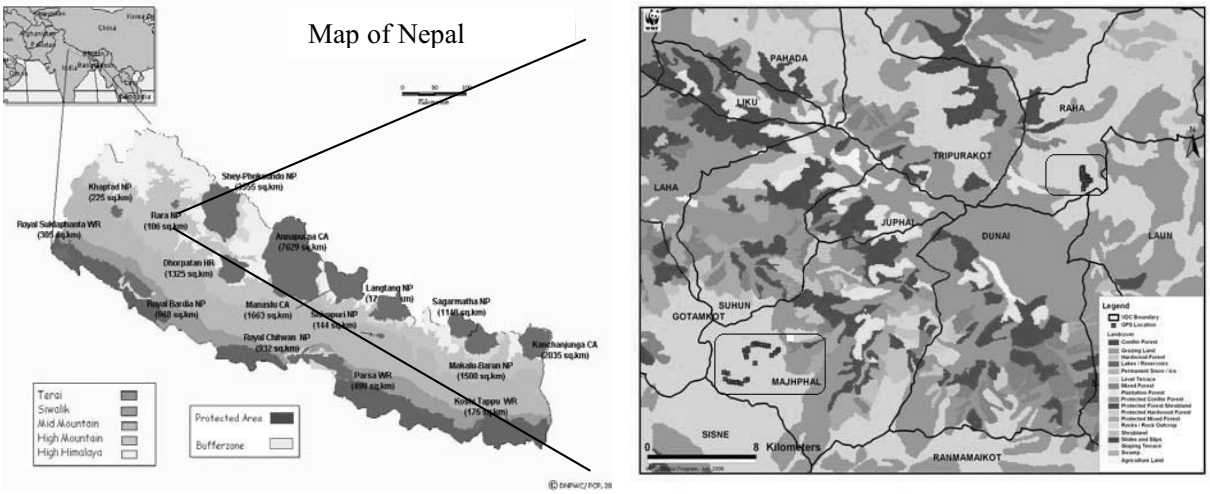

Figure 1: Showing study areas of Raha and Majphal VDCs of Dolpa district.

plot was marked permanently with enamels and stones. Forty plots were established at Palma Ramana pasture of Raha VDC, 10 at Pokeypani and 30 at Saiquarry of Majphal VDCs were established. The plots were established between the altitudinal range of 3905m to 4894m. The floral specimens collected from each permanent plot were recorded. Total numbers of *Ophiocordyceps sinensis* extracted by the collectors from the plots in the entire collection period were recorded.

Herbariums of collected plant specimens were prepared following the standard techniques (Martin, 1995; Lawrence, 1967). The collected plant specimens were identified with the help of standard literatures (Polunin & Stainton, 1984; Stainton, 1972; Lama *et al*., 2001). All the herbariums were deposited in the Tribhuvan University Central Herbarium (TUCH).

Results and Discussions

The frequency and relationship of flowering plants on the distribution pattern of *Ophiocordyceps sinensis* during the collection period were recorded from the permanent plots of Raha and Majphal VDCs. The diverse groups of taxa are given in the Table 1. Altogether fifteen plant specimens belonging to ten families (Primulaceae, Ranunculaceae, Polygonaceae, Juncaceae, Compositae, Ericaceae, Euphorbiaceae, Rosaceae, Scrophulariaceae, Valerianaceae) were recorded from the study area.

The grasslands providing habitat for *Ophiocordyceps sinensis* were found to be predominantly of *Juncus thomsonii* and *Bistorta macrophylla* pastures. *J. thomsonii* covered most of the grasslands between the altitudinal range from 3000m to nearly 5200m, gradually rising from the southeast to the northwest of the pastures. Chen *et al.,* (2000) had also reported a similar distribution for Tibetan *O. sinensis*. Among the associated taxa, the top three plants with the highest frequencies were *J. thomsonii*, B. *macrophylla* and *Rhododendron anthopogon*. These were observed in 79, 68, and 40 plots, respectively. Within the 80 permanent plots, the least frequently associated plants were *Androsace robusta*, *Primula macrophylla*, *Aconitum sp.* and *Rumex nepalensis,* since they cropped up in only one, three, and four plots, respectively. The tender shoot of *B. macrophylla* resembled the fungal part of *O. sinensis* so the collectors were often confused.

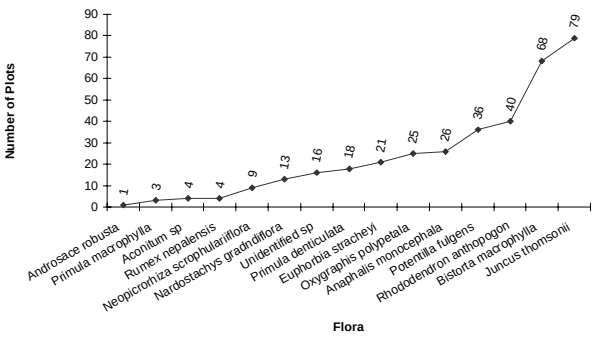

Figure 2: Number of sample plots and frequency of flowering plants.

Caterpillar fungus thrives in subalpine and alpine grasslands or meadows as well as open dwarf scrublands. In the study area, it was found naturally distributed from an altitudinal range of 3540 to 5050m asl. Since the current actual tree line has been strongly influenced by human activities; wide swathes of forests in study areas have been replaced by pastures. Similar phenomenon was also reported from the Tibetan plateau (Winkler, 2000)

In the case of Dolpa, no clear difference was found in the distribution pattern of *Ophiocordyceps sinensis* on different aspects. Collectors extracted from all sunny aspects. According to Boesi (2003), in Lithang China, caterpillar fungus was mostly found on north-facing slopes. However Winkler (2008) reported its distribution in China on well-drained sunny slopes with lush grass vegetation. In Dolpa, *O. sinensis* was confined to rich pastures and sites which were too wet or waterlogged did not harbor populations of *O. sinensis.*

Over grazing vs Distribution of *Ophiocordyceps sinensis*

Animal husbandry is a vital part of the economy of the local peoples. Almost every household usually maintained a large herd of animals for manure, milk, meat and to plough their fields. These herds included mainly sheep, goats, horses, ponies, ass and zomo (hybrid of yak and cow) that foraged on pastures. People graze their livestock freely in the forest and on grasslands based on customary systems (Ghimire, 2005). Overgrazing leads to the loss of forest regeneration and the loss of grassland vegetation thereby inducing soil erosion (Singh, 2001; Jha, 2006). The animals destroy *O. sinensis* during grazing because they also feed on the host plants. The livestock annually brought onto the pastures by the local collectors destroy the ecological niche of *O. sinensis.*

The collectors also reported that sites with less grazing effect had higher abundances of *O. sinensis*. *Juncus thomsonii, Bistorta macrophylla, Anaphalis monocephala, Potentilla fulgens,* were the plant species most affected by direct grazing and trampling effects. Similar views were also testified by collectors from Darchula district (Chettri & Lodhiyal 2008). This study also confirmed that the permanent plots affected by grazing impact had poorer densities of *O. sinensis* than the plots far from grazing areas.

Threats to the *Ophiocordyceps sinensis* in growing pastures in Dolpa

A number of threats are annually posed to the habitat of *Ophiocordyceps sinensis* growing pastures in Dolpa and these threats are mainly of anthropogenic nature. It was found that more than 50,000 collectors romped around 25 or more pastures of Dolpa during 2006. The major threats were haphazard and unscientific collection of *O. sinensis*; soil and water pollution, excessive use of fuel wood, hunting of wildlife, and intentional fires burns to procure fuelwood and better grass production for cattle.

The collectors burn intentional fires for clearing the sites to facilitate the collection of *O. sinensis* and also to extract fuelwood for the next season. They have figured out that burned areas harbored more *O. sinensis* in the following year. The accidental and intentional fires have, however, had adverse effects on the forest biodiversity in the past. The plant species mainly used for fuel wood by the collectors were *Rhododendron lepidotum, R. anthopogon, Juniperus* sp., *Betula utilis,* and *Quercus* sp. Almost 100% of their energy resources were met from the nearby forests.

The majority of respondents (collectors/users = 74, traders = 25 and local healers = 3) surveyed in this study also complained that thousands of collectors posed negative impacts to the soil of pastures after leaving lots of non-degradable materials like plastics and batteries. Similarly, increasing numbers of foot trails in the pastures were perceived to be deteriorating the virginity of the green pastures.

Illegal hunting of local fauna was also a common practice throughout the collection areas and had become one of the major threats for faunal biodiversity conservation. According to the respondents in 2005, some of the professional hunters hunted the wild animals to exchange the flesh or trophy for *Ophiocordyceps sinensis*. *Pseudois nayaur* (Naur) was the species most targeted for hunting.

Maoists and Pastures Management

The collection and trade of *Ophiocordyceps sinensis* in Dolpa was totally controlled by the Maoist in the study year. Realizing the potential destruction of *O. sinensis*, the local Maoists had drawn up 17 points "Code of Conduct" regarding different aspects, including pasture management. Some of the major codes related to pasture management were a) not to cut green trees for fuel; b) not to make a big hole in the pastures during collection c) not to release cattle in the pastures before collection (there was provision to allow cattle a week after the collection starts); d) not to discard plastics and batteries in the pastures; and e) not to hunt wild animals. Though they have attempted to implement these strict regulations, there was no alternative to fuel except to cut more trees.

Conclusion

From the study it can be concluded that *Juncus thomsonii* and *Bistorta macrophylla* exhibited a wide range of distribution in the pastures of Dolpa during the *Ophiocordyceps sinensis* collection period. It can be speculated that a healthy grassland environment is favorable for the caterpillar development as no informant reported abundant fruiting of the fungus in degraded areas. Plantations of *Betula utilis, Rhododendron* spp*,* and *Juniperus* spp. should be established at lower forests to supplement growing stock depleted by the extraction of fuelwood during collection seasons. To maintain a healthy pasture environment, over trampling effects and over grazing should be minimized and checked.

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Table 1: Frequency in associations of *Ophiocordyceps sinensis* with flowering plants

S.N	Plot No	Plants																	
		Juncus thomsonii	*Potentilla fulgens*	*Euphorbia stracheyi*	*Anaphalis monocephala*	*Bistorta macrophylla*	*Oxygraphis polypetala*	*Primula denticulata*	*Rhododendron anthopogon*	*Unidentified* sp	*Nardostachys grandiflora*	*Neopicrorhiza scrophulariiflora*	*Primula macrophylla*	*Rumex nepalensis*	*Aconitum* sp	*Androsace robusta*	Total plants species	Total frequency of *O. sinensis*
1	RY 01	+	+	+	+	+											5	5
2	RY 02	+	+	+	+	+											5	4
3	RY 03	+	+	+	+	+											5	4
4	RY 04	+	+	+	+	+											5	2
5	RY 05	+	-	-	-	+	+										3	1
6	RY 06	+	+	+	-	+	+										5	2
7	RY 07	+	-	+	-	+	-										3	3
8	RY 08	+	+	-	-	+	+										4	4
9	RY 09	+	+	-	-	+	-										3	7
10	RY 10	+	+	+	-	+	-										4	10
11	RY 11	+	+	-	-	+	-										3	5
12	RY 12	+	-	-	-	+	-	+									3	2
13	RY 13	+	-	+	-	+	-	+	+								5	3
14	RY 14	+	+	+	-	+	-	+	-								5	9
15	RY 15	+	+	-	-	+	-	+	-								4	1
16	RY 16	+	-	-	-	+	-	-	-								2	1
17	RY 17	+	+	+	-	+	-	-	-								4	4
18	RY 18	+	+	-	-	+	-	-	-								3	2
19	RY 19	+	-	+	+	+	-	-	-								4	9
20	RY 20	+	+	-	+	+	-	-	-								4	3
21	RY 21	+	-	-	+	+	-	-	-								3	1
22	RY 22	+	+	-	+	+	-	-	-								4	3
23	RY 23	+	-	-	+	+	+	-	+								5	2
24	RY 24	+	-	-	+	+	-	-	+								4	10
25	RY 25	+	-	-	+	+	-	-	+								4	2
26	RY 26	+	+	-	-	+	+	+	-								5	14
27	RY 27	+	+	-	-	+	+	+	-								5	1
28	RY 28	+	-	-	-	+	-	+	-								3	1
29	RY 29	+	-	-	-	+	-	+	-								3	1
30	RY 30	+	+	-	-	+	-	-	-								3	2
31	RY 31	+	-	-	-	+	+	-	-								3	13
32	RY 32	+	-	-	+	+	+	-	-								4	9
33	RY 33	+	-	-	-	+	-	+	-								3	2
34	RY 34	+	+	-	-	+	-	+	+								5	2
35	RY 35	+	+	-	-	+	+	+	+								6	1
36	RY 36	+	+	-	-	+	+	+	-								5	2
37	RY 37	+	+	-	-	+	+	+	-								5	2
38	RY 38	+	+	-	-	+	+	+	-								5	1
39	RY 39	+	-	-	-	+	-	+	+								4	2
40	RY 40	+	+	-	-	+	-	+	+								5	2
41	MY 01	+	+	+	+	+	-	-	-	+							6	4
42	MY 02	+	+	+	-	+	-	-	+	+							6	14
43	MY 03	+	-	-	-	-	-	-	+	+	+						4	15

44	MY 04	+	-	+	+	-	+	-	+	+	-						6	19
45	MY 05	+	+	-	-	-	+	+	+	-	+	+					7	5
46	MY 06	+	+	-	-	-	+	+	+	-	+	-					6	19
47	MY 07	-	-	-	-	-	+	-	+	+	+	-					4	19
48	MY 08	+	+	-	-	-	-	-	+	+	+	+	+				7	20
49	MY 09	+	-	-	-	-	-	-	+	-	-	-	+				3	20
50	MY 10	+	-	-	-	-	-	-	+	+	+	+	-				5	21
51	MY 11	+	+	-	-	-	-	-	+	-	-	-	-				3	18
52	MY 12	+	+	-	-	-	-	-	+	-	-	-	-	+			4	24
53	MY 13	+	+	-	-	-	-	-	-	+	-	-	-	+			4	18
54	MY 14	+	+	-	-	-	-	-	+	+	-	-	-	+			5	11
55	MY 15	+	-	+	+	+	-	-	+	+	-	-	-	-			6	6
56	MY 16	+	-	-	-	+	-	-	+	+	+	-	-	+	+		7	16
57	MY 17	+	-	+	-	+	+	-	+	+	+	-	-	-	+		8	10
58	MY 18	+	+	+	-	+	-	-	+	+	-	-		-	-		6	18
59	MY 19	+	-	-	+	+	-	-	+	+	-	-	-	-	-		5	21
60	MY 20	+	-	-	+	+	-	-	+	-	-	-	-	-	-		4	10
61	MY 21	+	-	-	+	+	-	-	+	+	-	-	-	-	-		5	11
62	MY 22	+	-	-	-	+	-	-	+	-	-	-	-	-	-		3	11
63	MY 23	+	-	-	+	+	-	-	+	-	-	-	-	-	-		4	21
64	MY 24	+	-	-	+	+	+	-	+	-	-	-	-	-	-		5	11
65	MY 25	+	-	-	+	+	+	-	+	-	-	-	-	-	-		5	13
66	MY 26	+	-	-	-	+	-	-	+	-	-	-	-	-	-		3	25
67	MY 27	+	-	-	-	+	-	-	-	-	-	-	-	-	-	+	3	23
68	MY 28	+	+	+	-	+	-	-	+	-	-	-	-	-	+	-	6	11
69	MY 29	+	-	+	+	+	+	-	+	-	-	-	-	-	-	-	6	15
70	MY 30	+	-	+	+	+	-	-	+	-	-	-	-	-	-	-	5	14
71	MY 31	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	2	6
72	MY 32	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	3	5
73	MY 33	+	-	-	+	+	+	-	+	-	-	-	-	-	-	-	5	16
74	MY 34	+	-	-	+	+	+	-	+	-	+	+	-	-	-	-	7	15
75	MY 35	+	-	-	+	+	+	-	-	-	+	+	-	-	-	-	6	14
76	MY 36	+	-	-	-	+	+	-	-	-	+	+	+	-	+	-	7	3
77	MY 37	+	-	-	-	+	+	-	+	-	+	+	-	-	-	-	6	5
78	MY 38	+	+	+	-	+	-	-									4	22
79	MY 39	+	-	-	-	+	-	-	+	-	-	+	-	-	-	-	4	4
80	MY 40	+	-	-	-	+	-	-	-	+	+	+	-	-	-	-	5	12
	Total	79	36	21	26	68	25	18	40	16	13	9	3	4	4	1		

Note:
RY = Raha Yarsagumba; MY = Majphal Yarsagumba; + = Present; - = Absent
Sunbuki, Dhochi = *Juncus thomsonii;* Gorukajera = *Potentilla fulgens;* Peitei Jasto = *Euphorbia stracheyi;* Jhullya = *Anaphalis monocephala;* Nimbu, Nyakuri = *Bistorta macrophylla;* Pahelo tarey = *Oxygraphis polypetala;* Doeli phool = *Primula denticulata;* Paluwa = *Rhododendron anthopogon;* Narku = *Unidentified* sp.*;* Bhutley = *Nardostachys grandiflora;* Kutki = *Neopicrorhiza scrophulariiflora;* Katarey = *Primula macrophylla;* Halhaley = *Rumex nepalensis.;* Bikh = *Aconitum* sp.*;* Begarey Jhar = *Androsace robusta.*